

FIG. 1

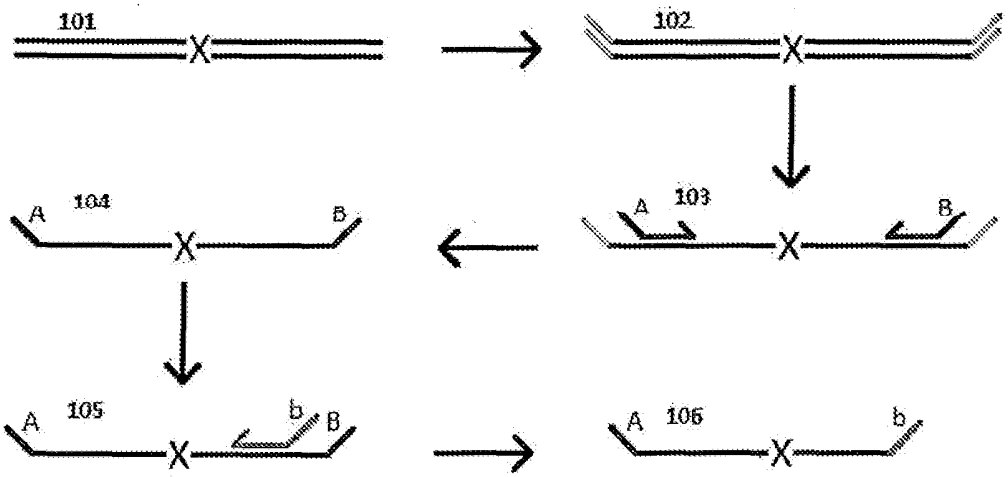


FIG. 2

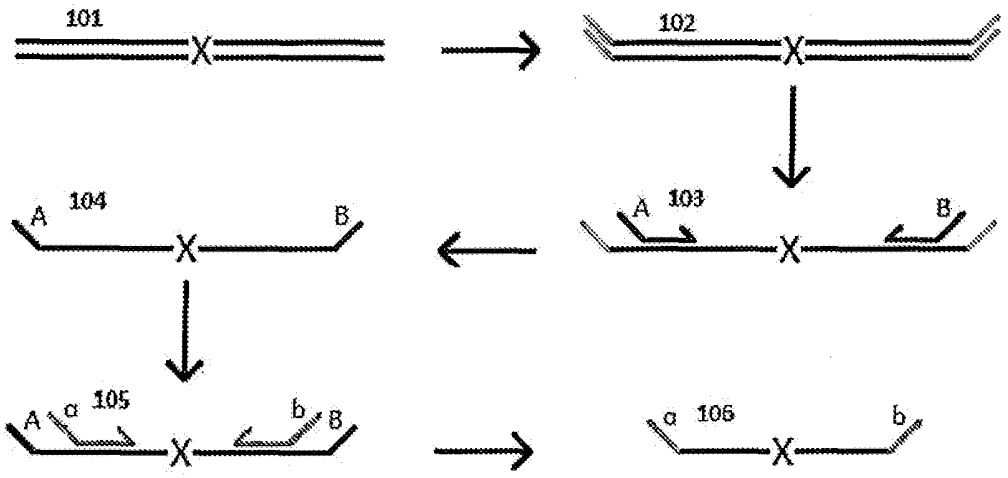

101
102
A 104
B
A 103
B
A α 105
b B
α 106
b

FIG. 3

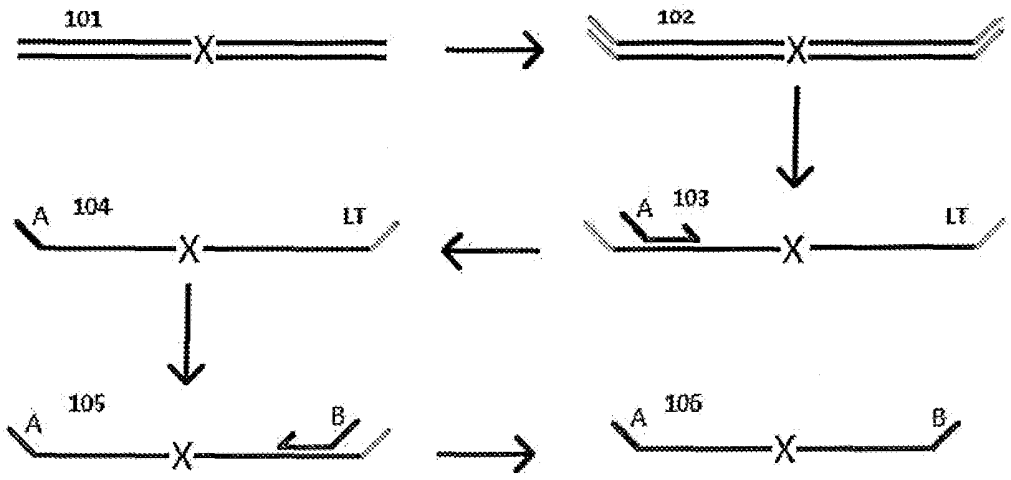

101
102
A 104
LT
A 103
LT
A 105
B
A 106
B

FIG. 4

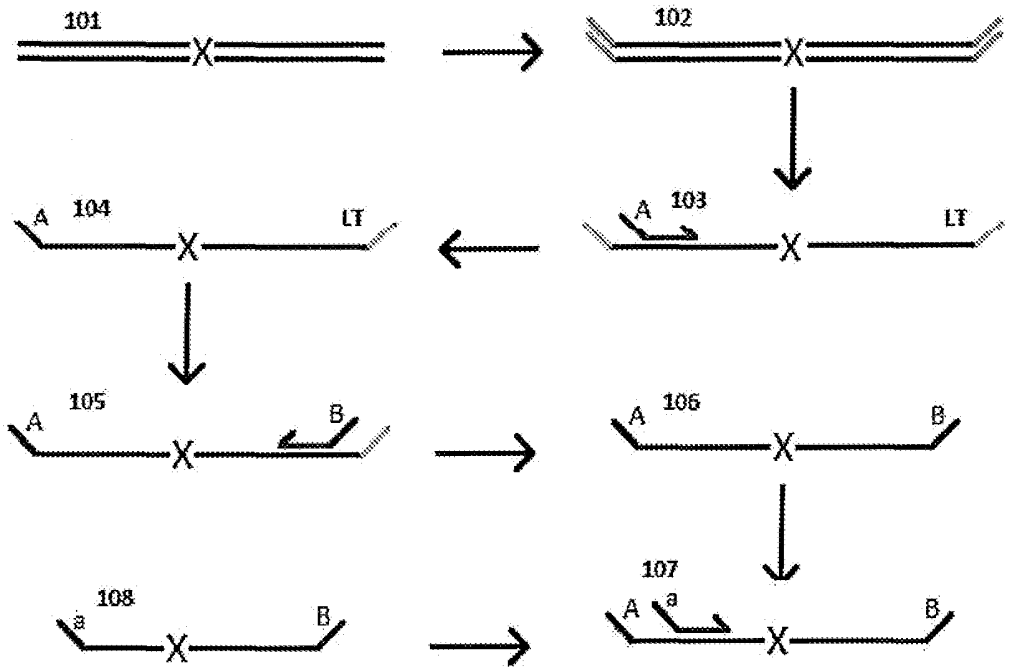

101
102
A 104
LT
A 103
LT
A 105
B
A 106
B
108
a
B
107
A a
B

FIG. 5

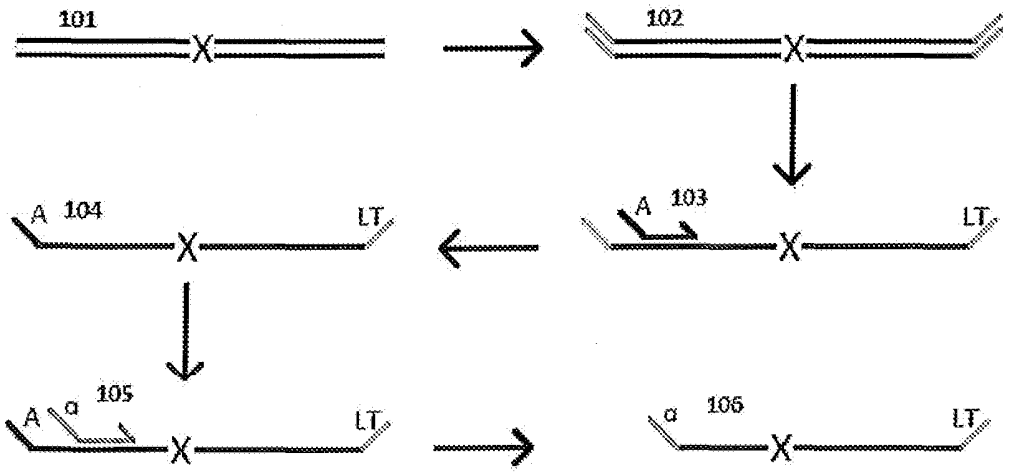

101
102
A 104
LT
A 103
LT
A a 105
LT
a 106
LT

FIG. 6

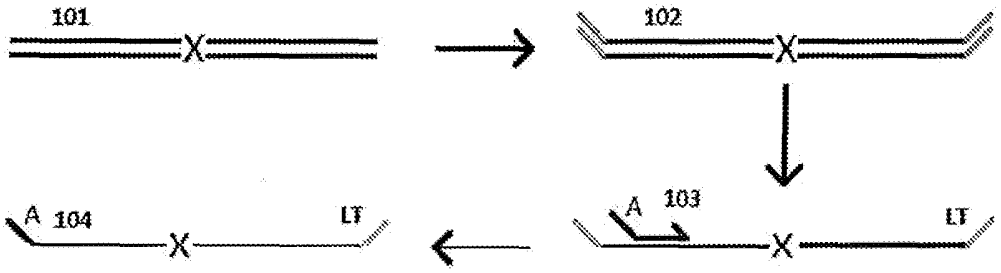


FIG. 7

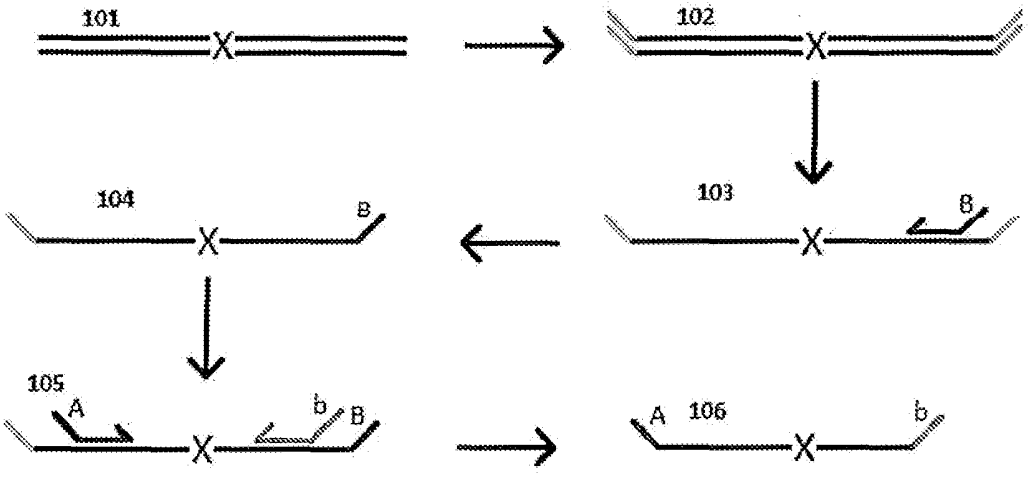


FIG. 8

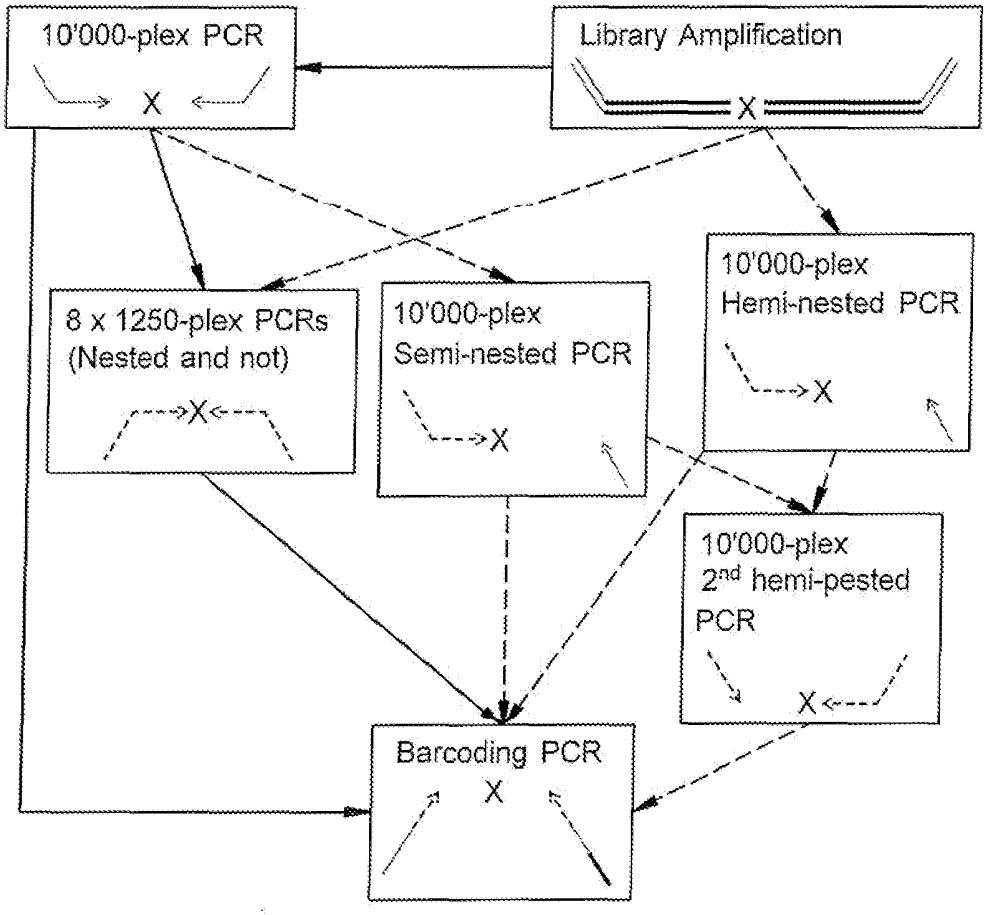


FIG. 9

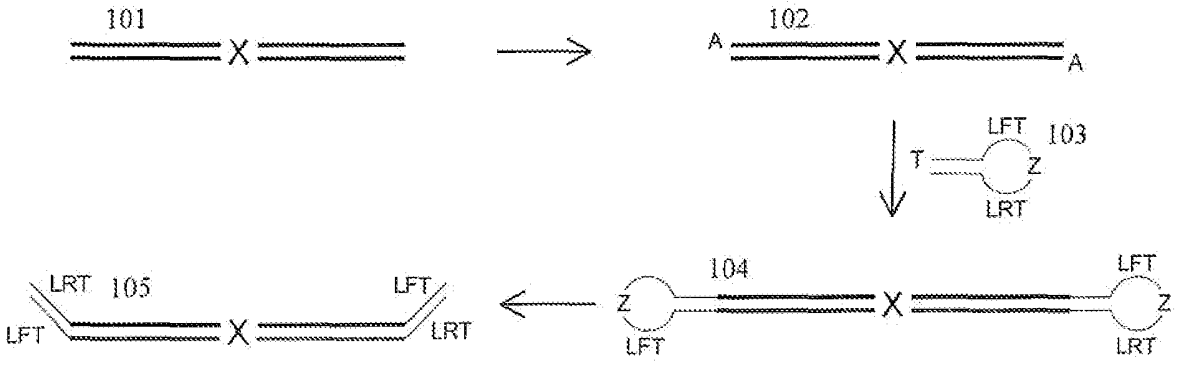


FIG. 10

NEOGENOMICS EX.1013.005

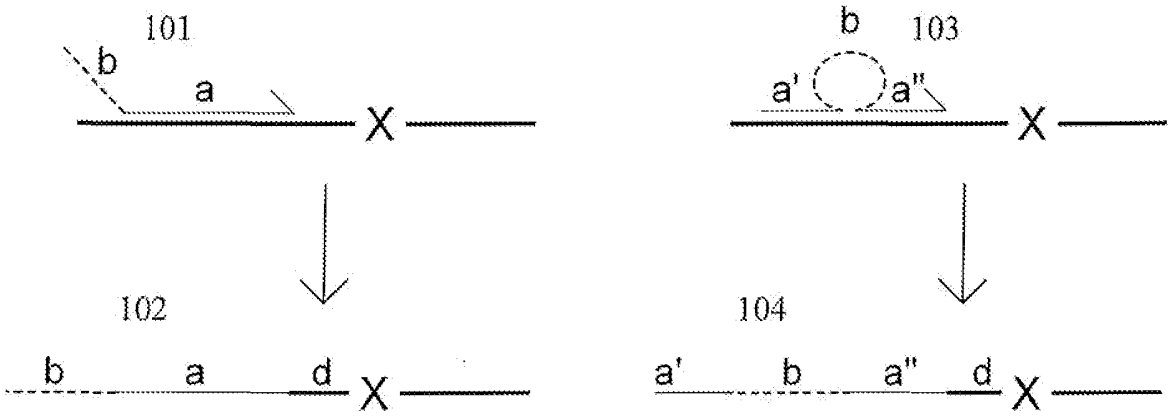


FIG. 11

The sequencing-adaptor sequence is located inside the primer sequence and flanked by target specific sequence on both sides. 10 bases are target-specific at the 3'-end of each primer. Primers were tested succesfully in real-time PCR. For sequencing this reduces the number of primer bases that need to be sequenced.		
rs8130564	int-tag 1.10	AACTCACATAGC *ACACGACGCTCTTCCGATCT* TGCAAGCACA
rs2832093	int-tag 2.10	TCCTCTGTG *ACACGACGCTCTTCCGATCT* CCCTGCTCTT
rs12011281	int-tag 3.10	tcctctctctACACGACGCTCTTCCGATCTcGGGCTGTCA
rs6719561	int-tag 4.10	TACATCCTTGAGACACGACGCTCTTCCGATCTGCTGTGCAGT
rs10187018	int-tag 5.10	tttgcttgagctACACGACGCTCTTCCGATCTcgggagtttc
rs10460481	int-tag 6.10	gtcttatggtggACACGACGCTCTTCCGATCTcaaagccagt
The sequencing-adaptor sequence is located inside the primer sequence and flanked by target specific sequence on both sides. The internal tag is formed into a hairpin structure by 10 complementary bases on either end. This brings the target-specific ends of the primer into close proximity and hinders unspecific binding to the "internal tag". 10 bases are target-specific at the 3'-end of each primer. Primers were tested succesfully in real-time PCR.		
rs8130564	**loop-int-tag 1.10**	**AACTCACATAGC** *tgatcggtACACGACGCTCTTCCGATCT* **TGCAAGCACA**
rs2832093	**loop-int-tag 2.10**	**TCCTCTGTG** *tgatcggtACACGACGCTCTTCCGATCT* **CCCTGCTCTT**
rs12011281	**loop-int-tag 3.10**	**tcctctcttgatcggtACACGACGCTCTTCCGATCTcGGGCTGTCA**
rs6719561	**loop-int-tag 4.10**	**TACATCCTTGAGtgatcggtACACGACGCTCTTCCGATCTGCTGTGCAGT**
rs10187018	**loop-int-tag 5.10**	**tttgcttgagcttgatcggtACACGACGCTCTTCCGATCTcgggagtttc**
rs10460481	**loop-int-tag 6.10**	**gtcttatggtggtgatcggtACACGACGCTCTTCCGATCTcaaagccagt**

Figure 12

NEOGENOMICS EX.1013.007

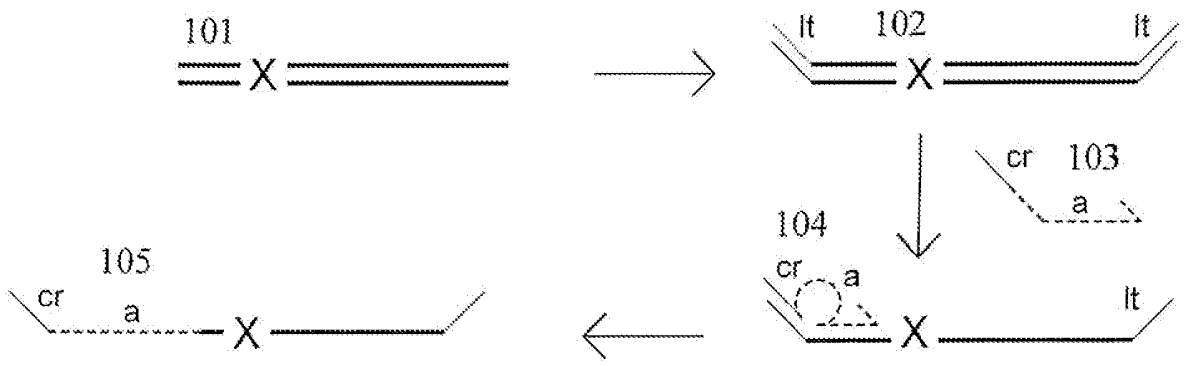


FIG. 13

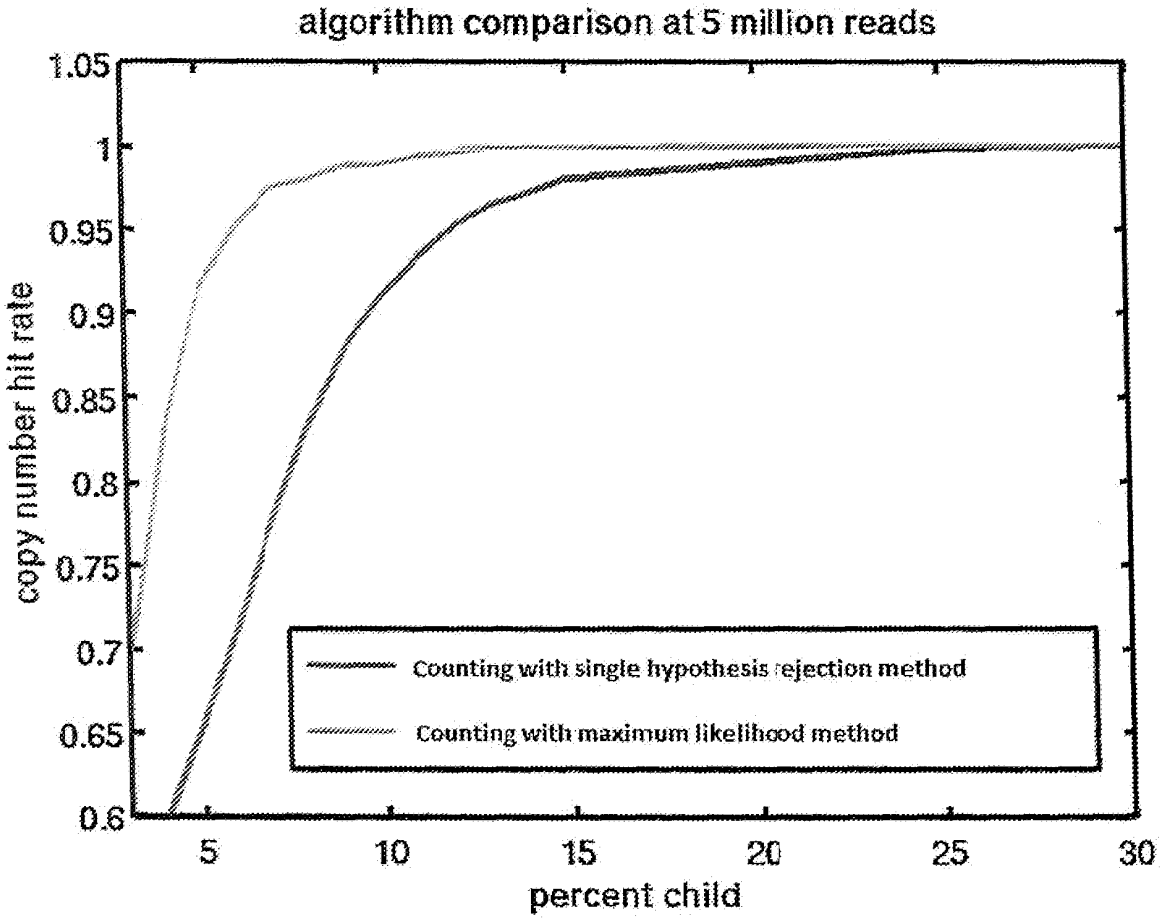

algorithm comparison at 5 million reads
copy number hit rate
percent child
1.05
1
0.95
0.9
0.85
0.8
0.75
0.7
0.65
0.6
5
10
15
20
25
30
Counting with single hypothesis rejection method
Counting with maximum likelihood method

FIG. 14

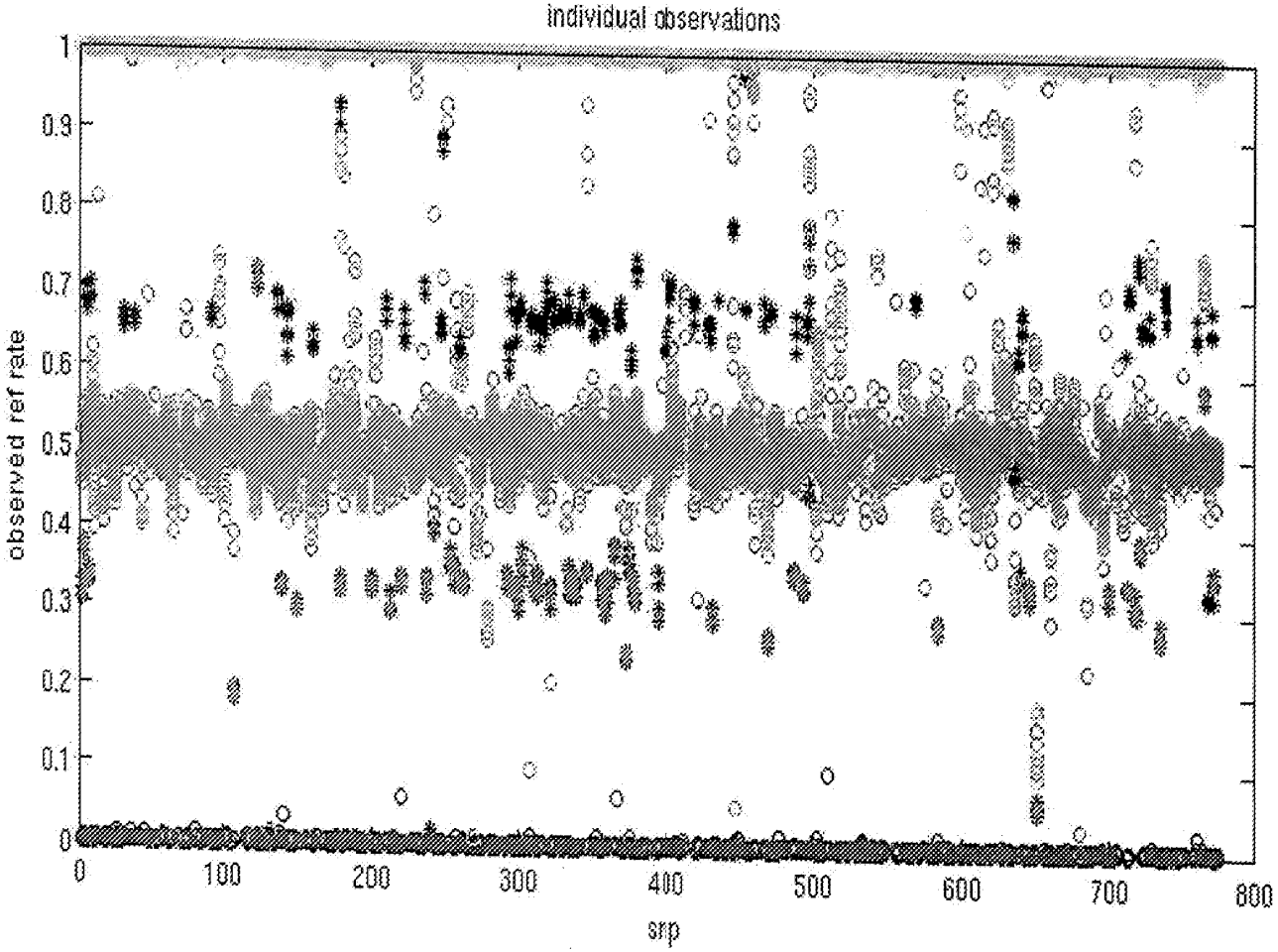


FIG. 15

NEOGENOMICS EX.1013.010

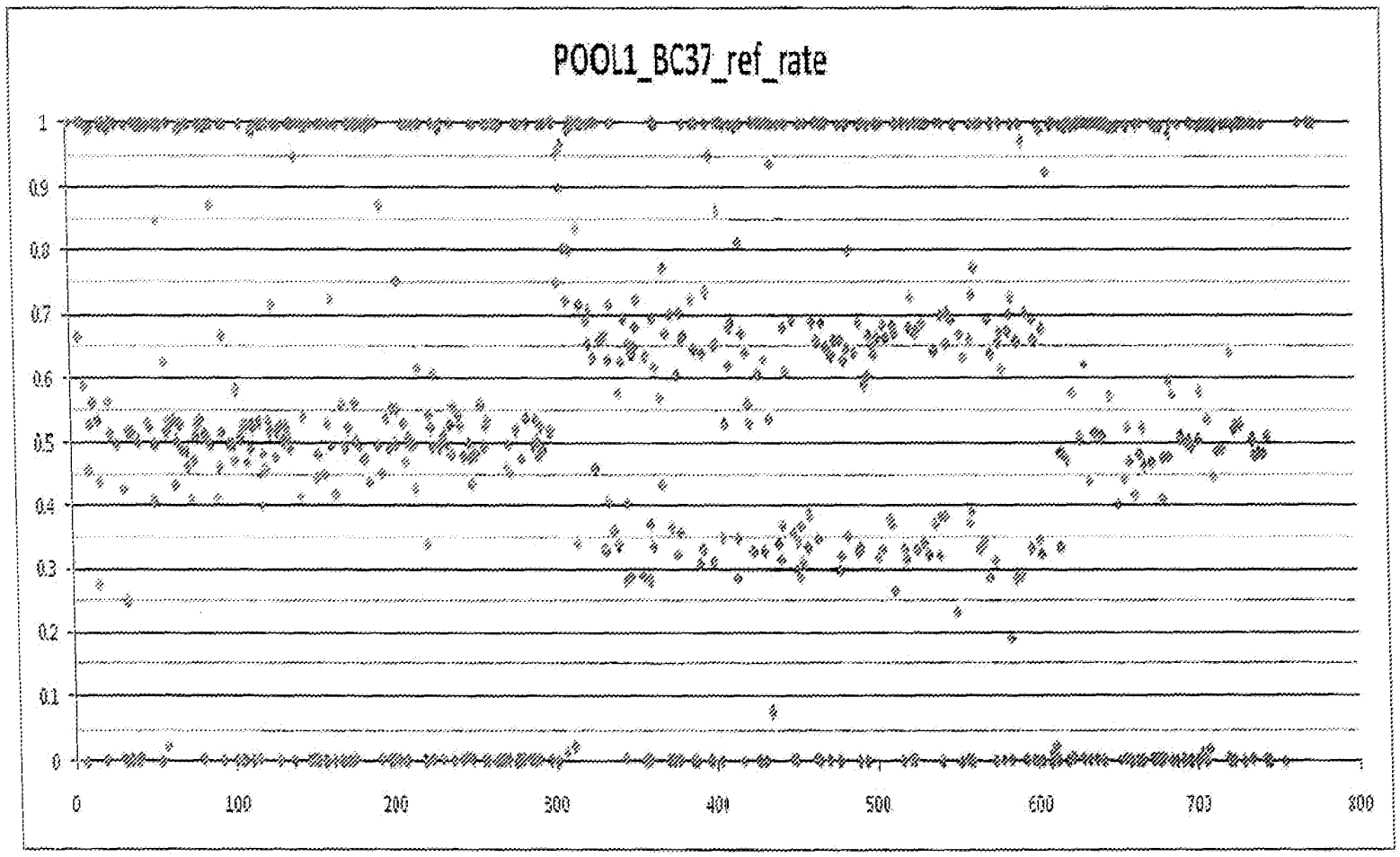


FIG. 16

NEOGENOMICS EX.1013.011

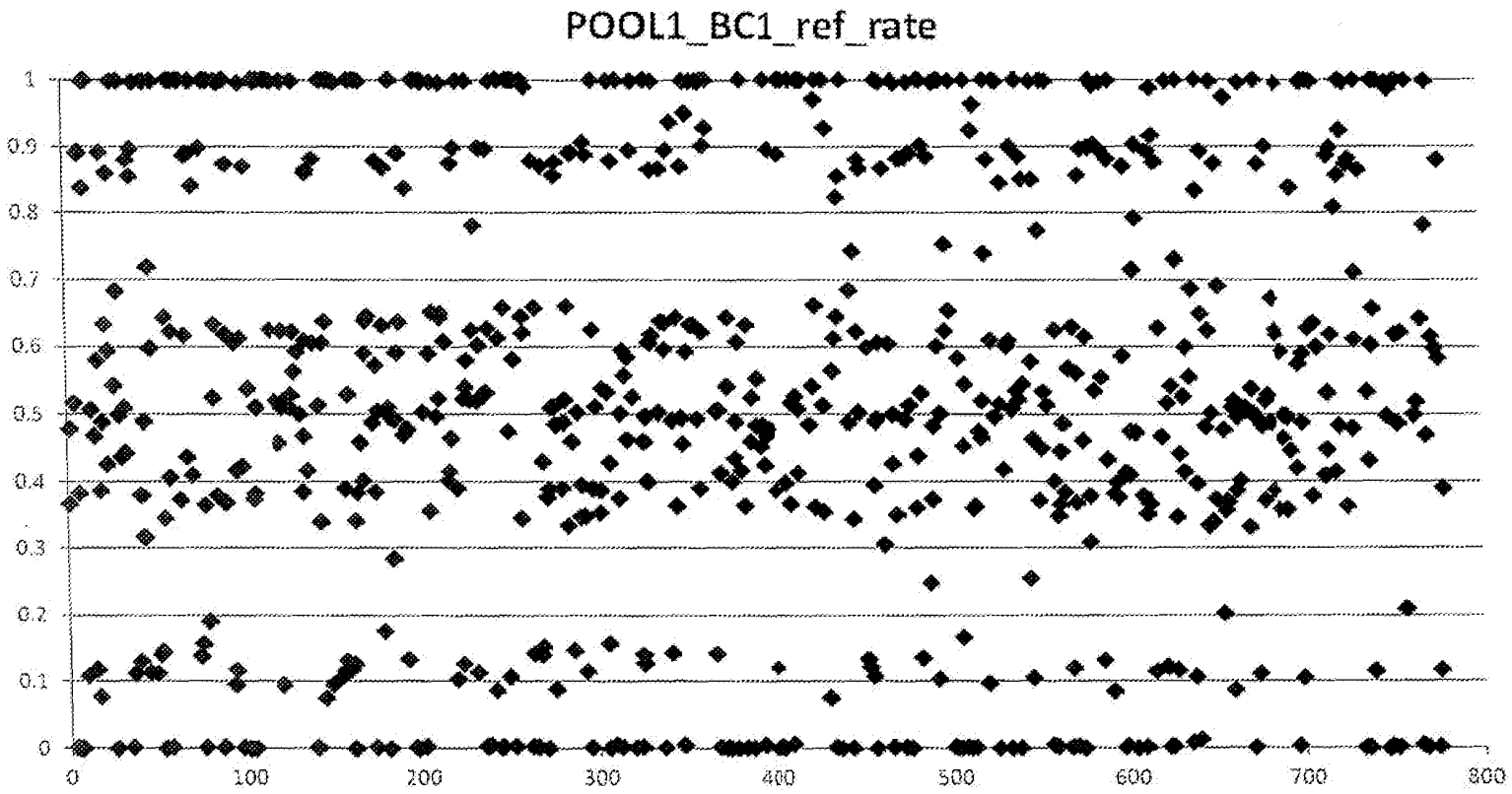

POOL1_BC1_ref_rate
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
700
800

FIG. 17A

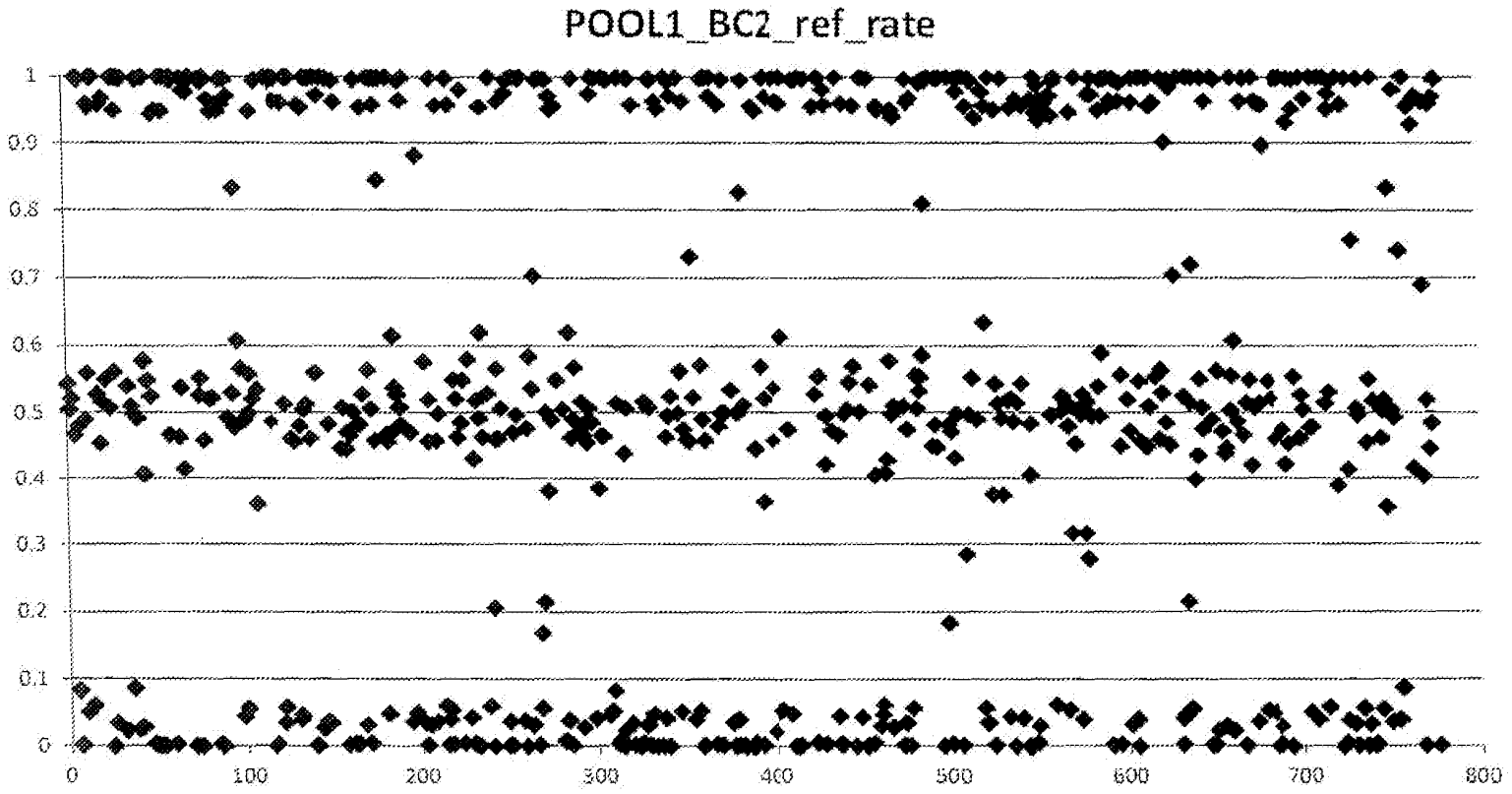

POOL1_BC2_ref_rate
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
700
800

FIG. 17B

No fetal DNA

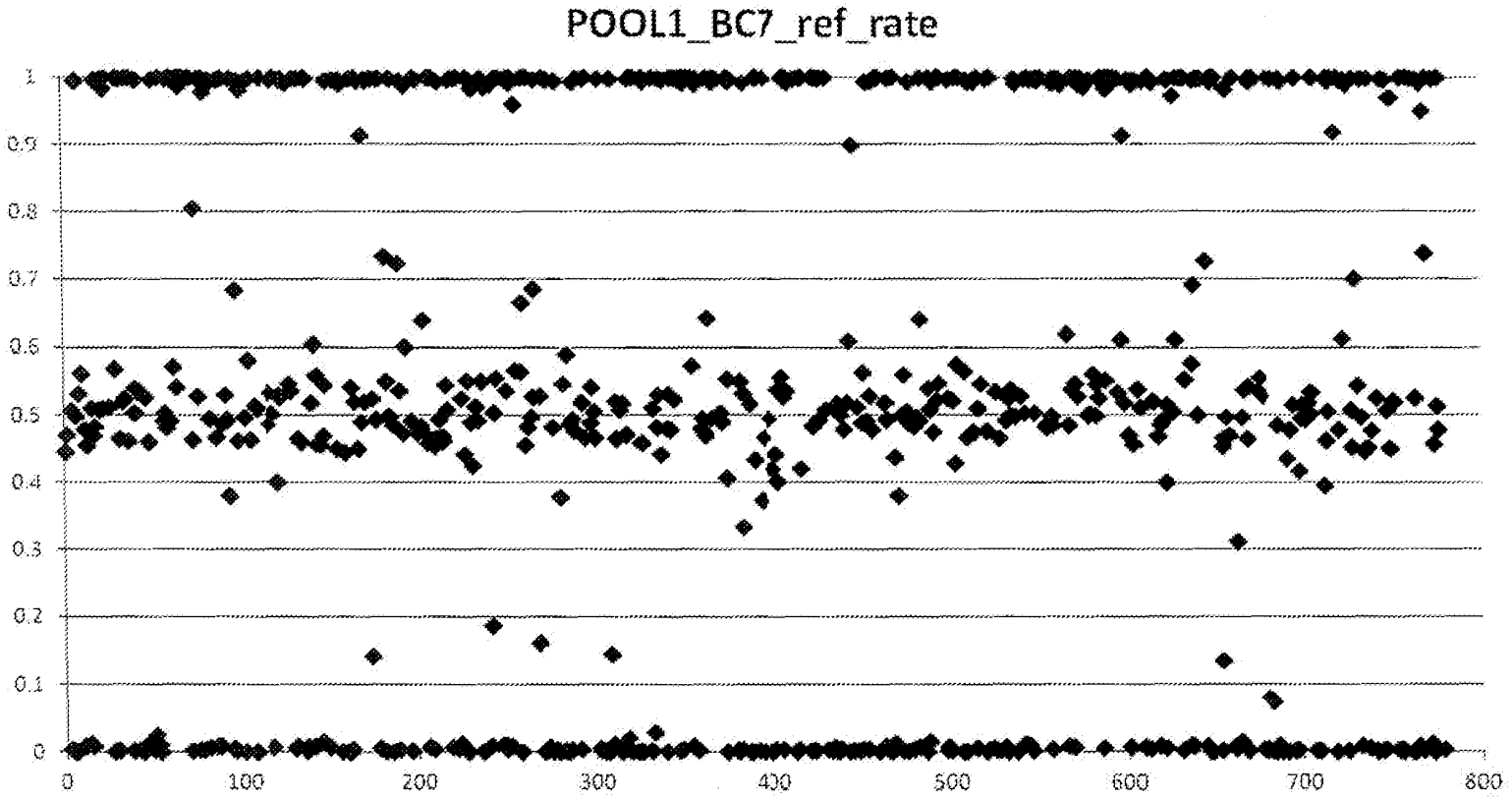


FIG. 17C

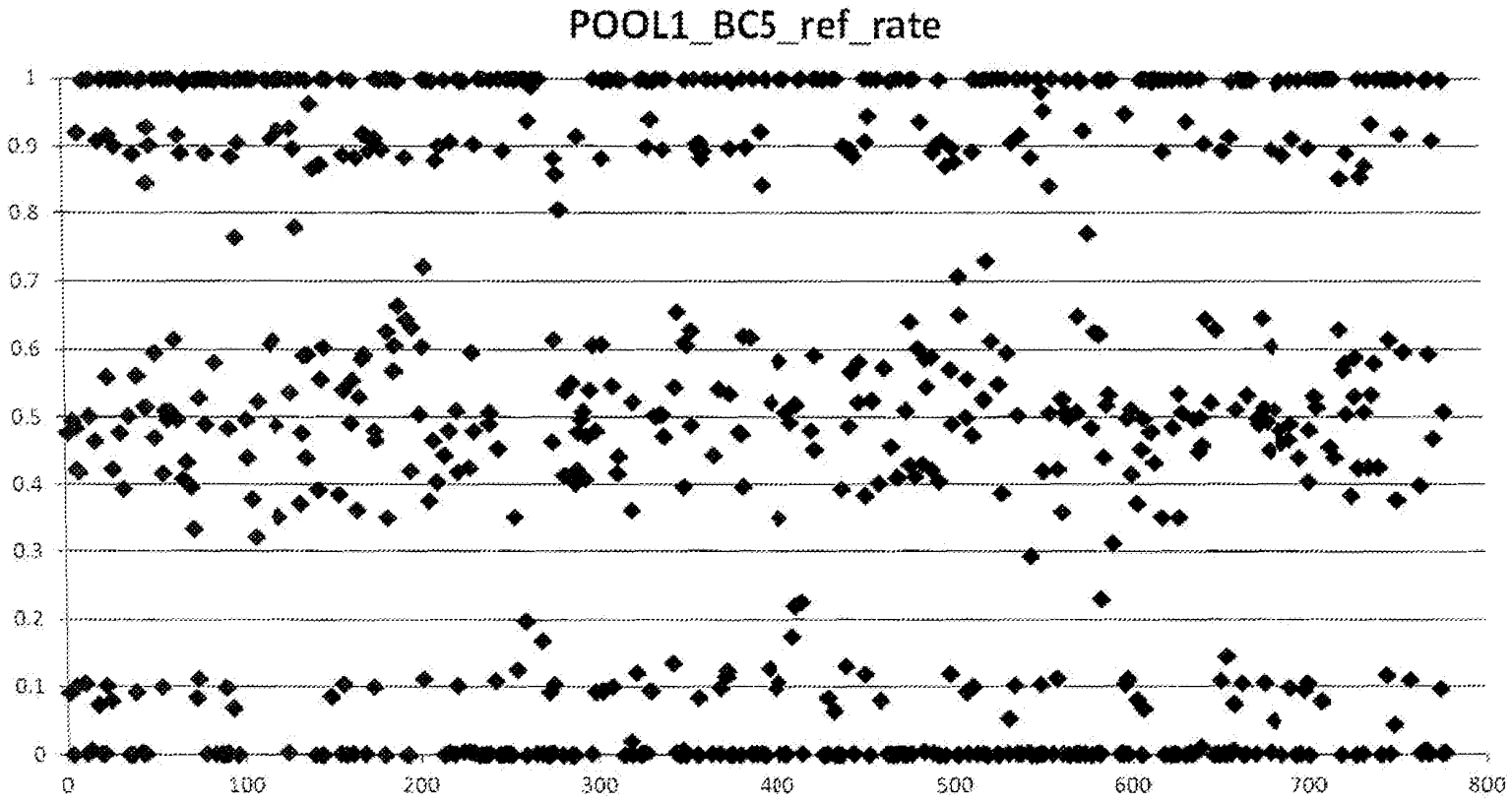

POOL1_BC5_ref_rate
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
700
800

FIG. 17D

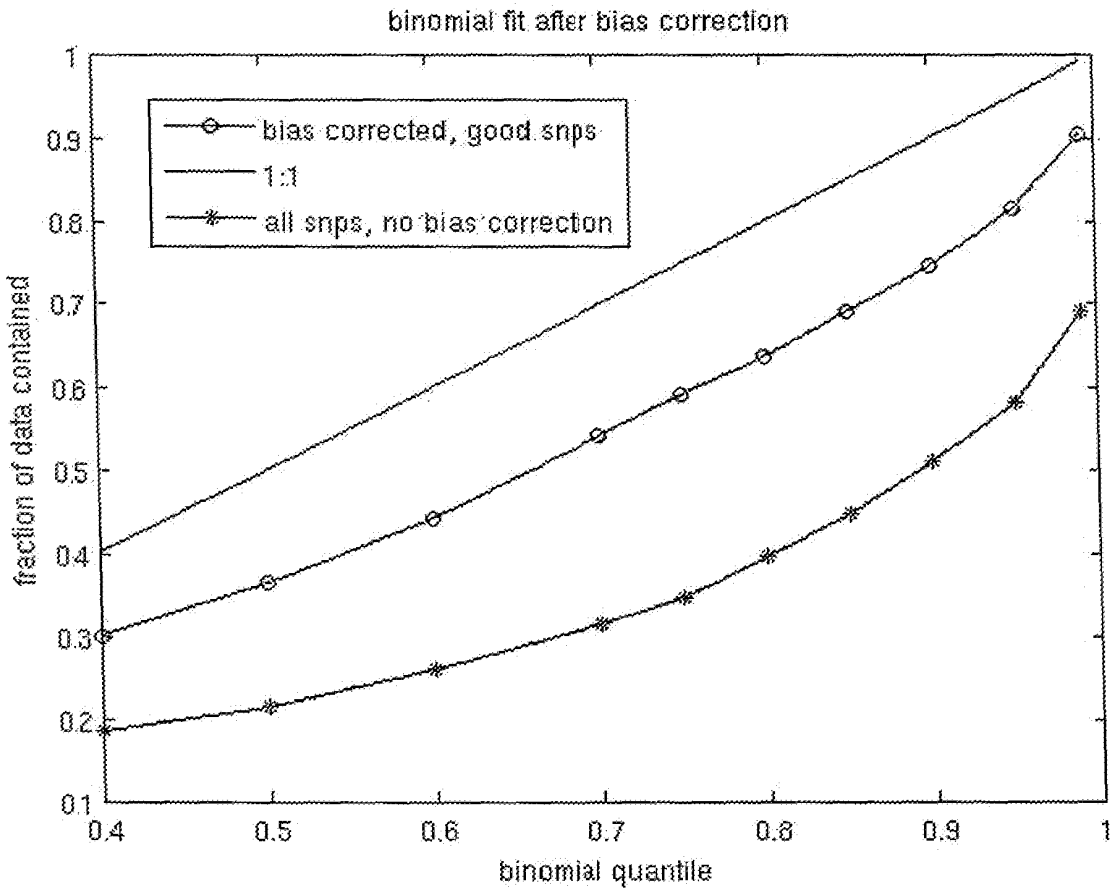


FIG. 18

NEOGENOMICS EX.1013.016

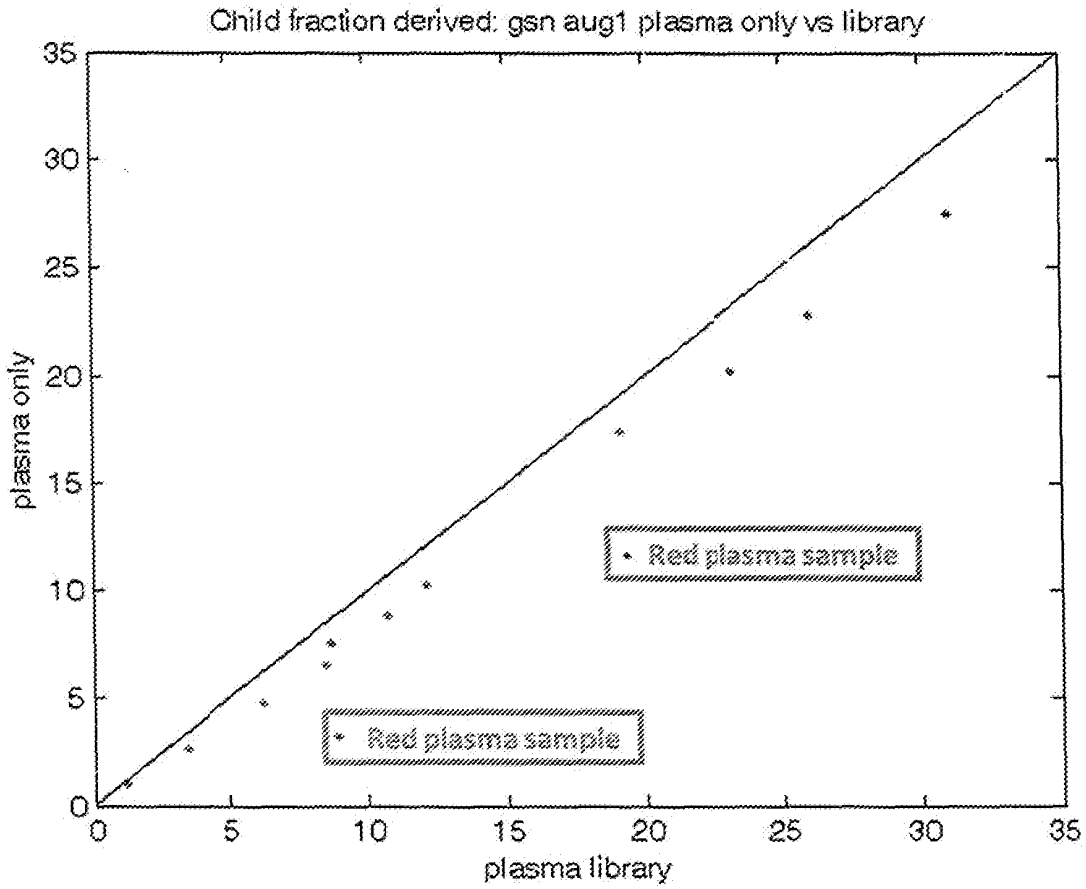

Child fraction derived: gsn aug1 plasma only vs library
plasma only
plasma library
Red plasma sample
Red plasma sample
0
5
10
15
20
25
30
35

FIG. 19

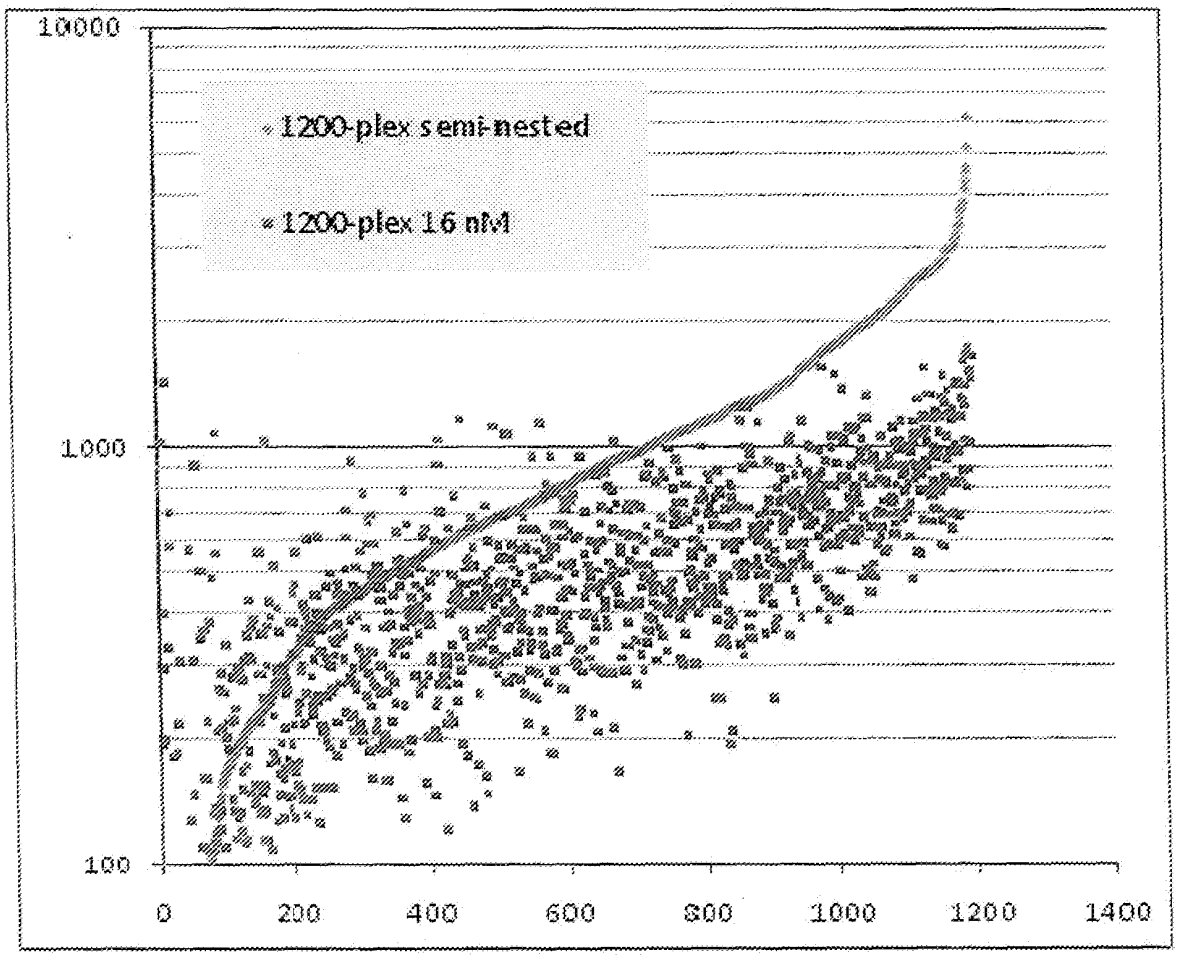

10000
1000
100
1200-plex semi-nested
1200-plex 16 nM
0
200
400
600
800
1000
1200
1400

FIG. 20

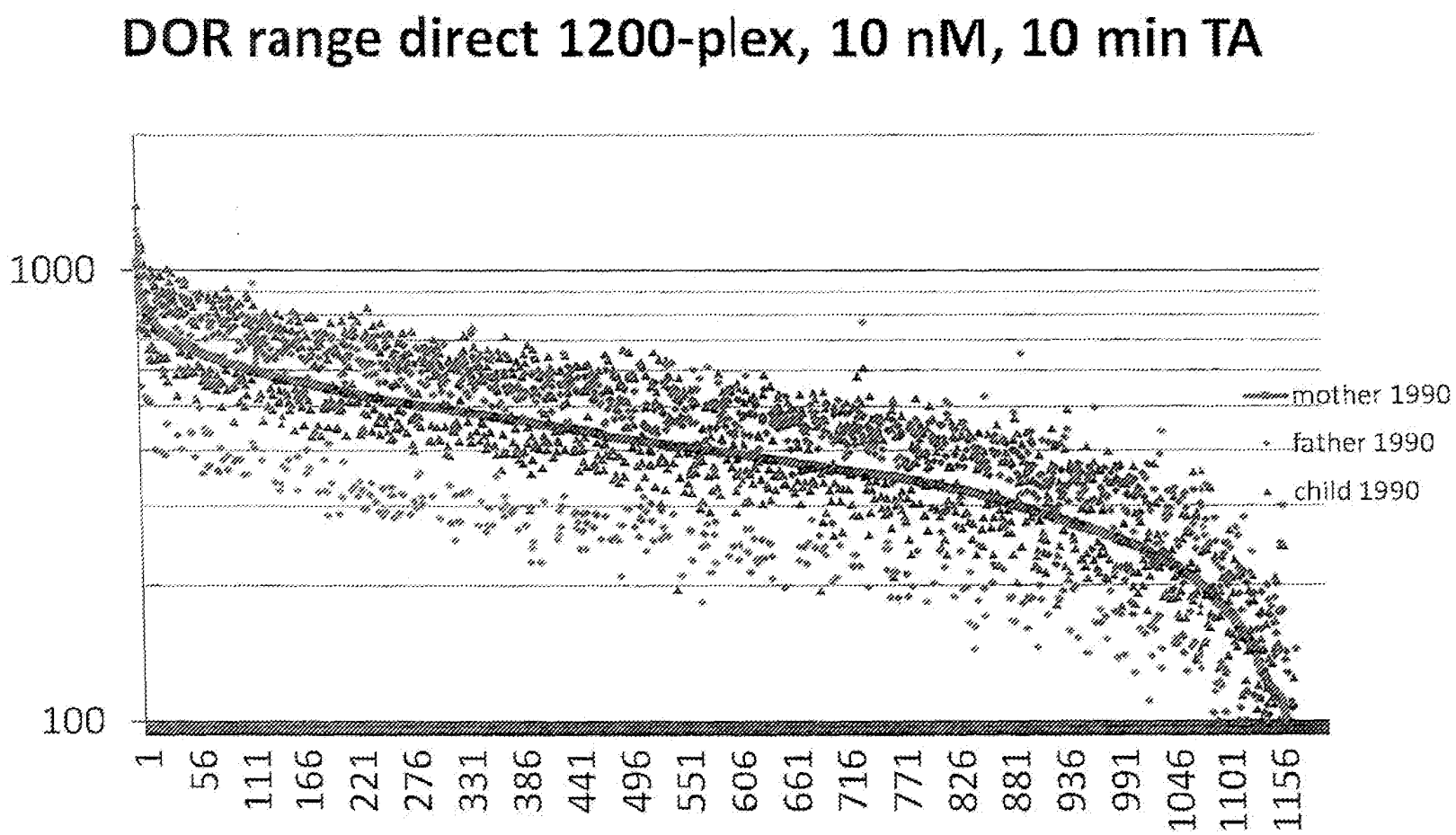


FIG. 21

NEOGENOMICS EX.1013.019

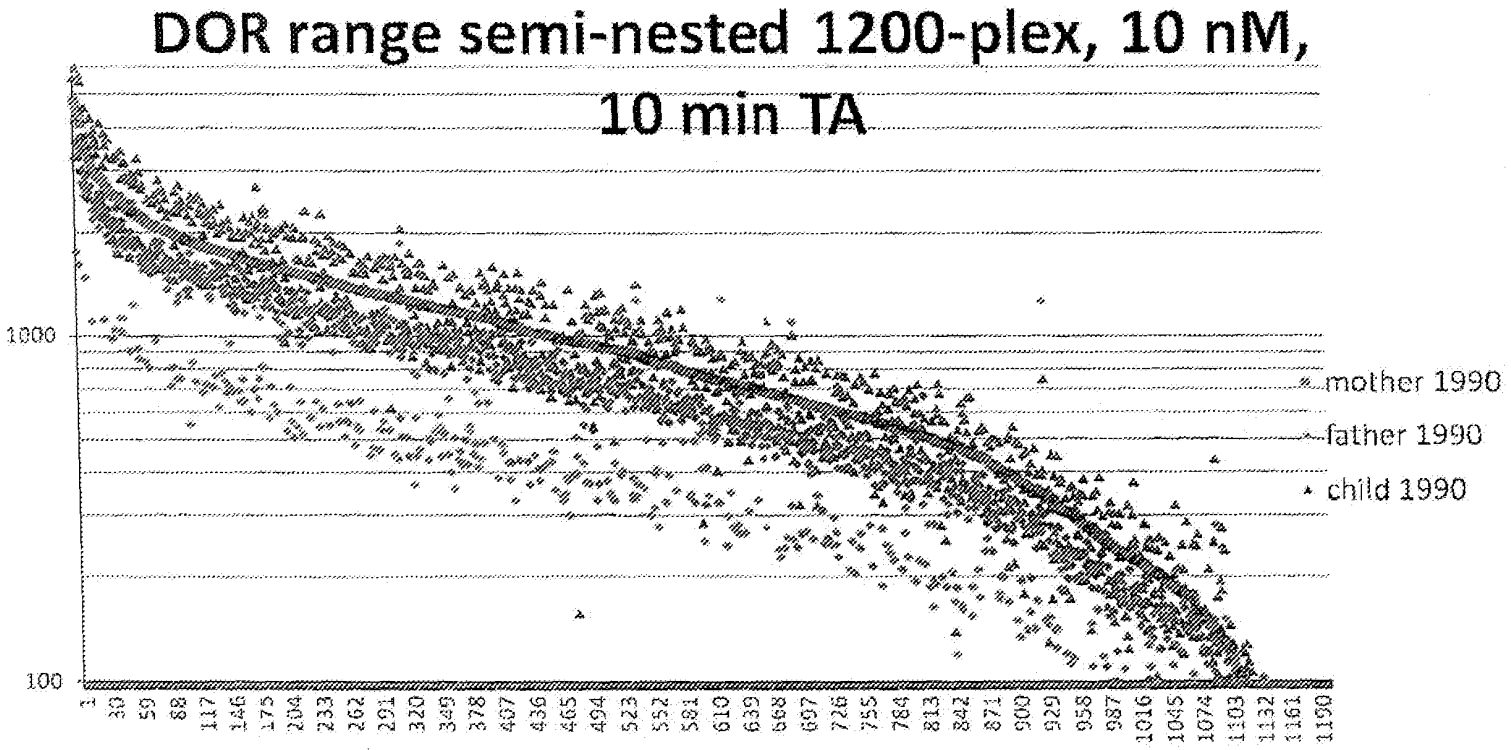

DOR range semi-nested 1200-plex, 10 nM,
10 min TA
1000
100
mother 1990
father 1990
child 1990

FIG. 22

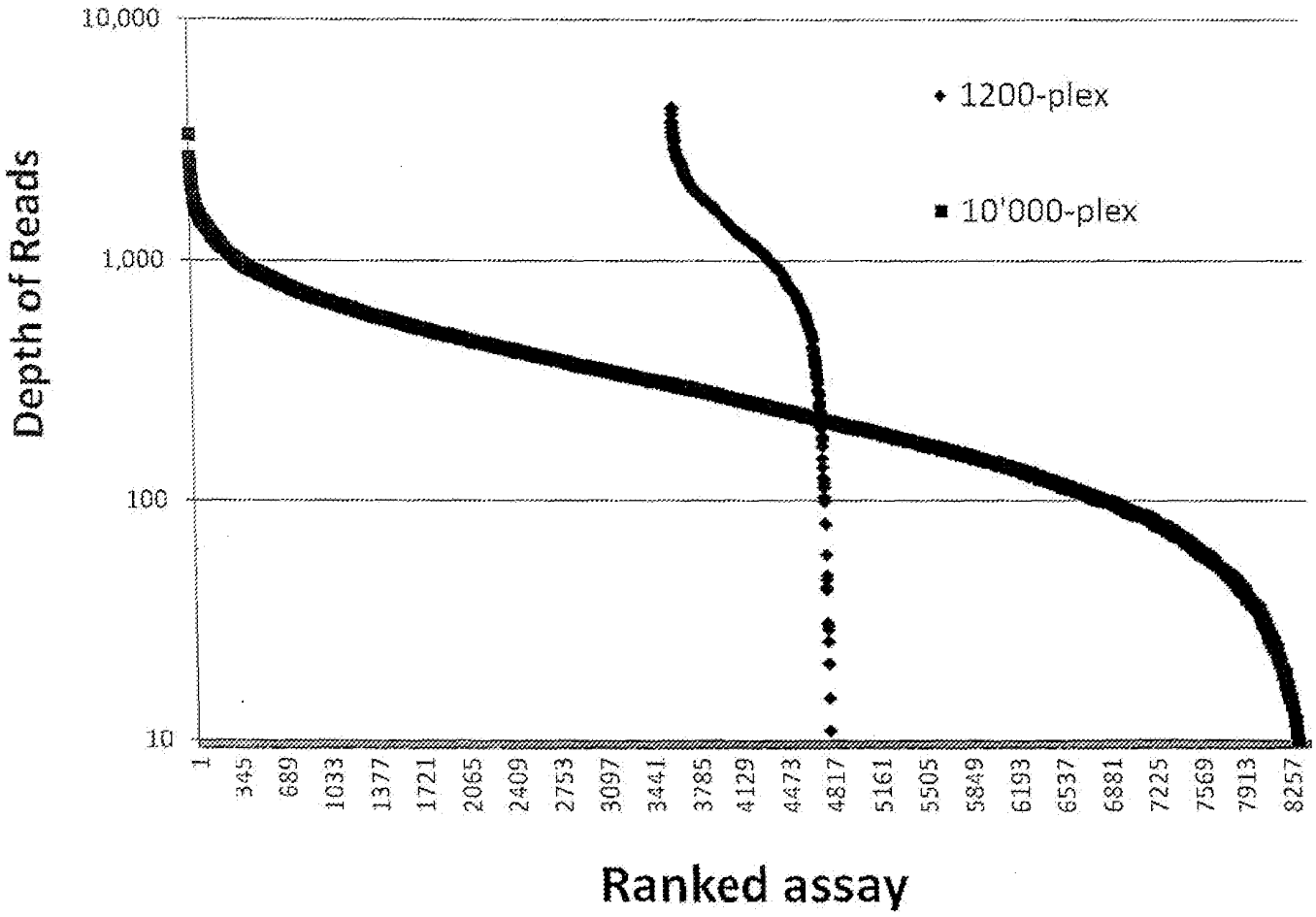


FIG. 23

NEOGENOMICS EX.1013.021

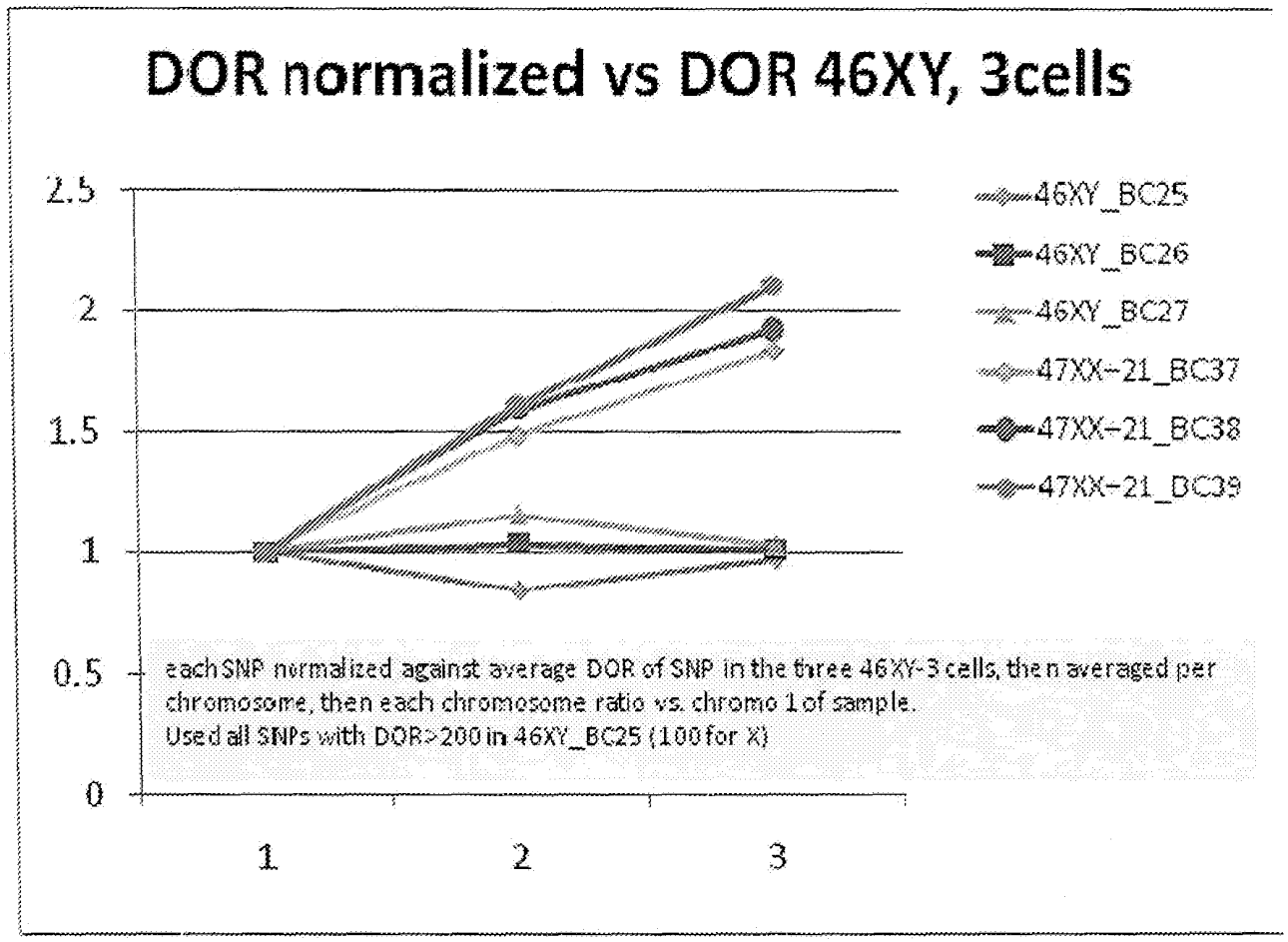

DOR normalized vs DOR 46XY, 3cells
2.5
2
1.5
1
0.5
0
1
2
3
46XY_BC25
46XY_BC26
46XY_BC27
47XX+21_BC37
47XX+21_BC38
47XX+21_BC39
each SNP normalized against average DOR of SNP in the three 46XY-3 cells, then averaged per chromosome, then each chromosome ratio vs. chromo 1 of sample.
Used all SNPs with DOR>200 in 46XY_BC25 (100 for X)

FIG. 24

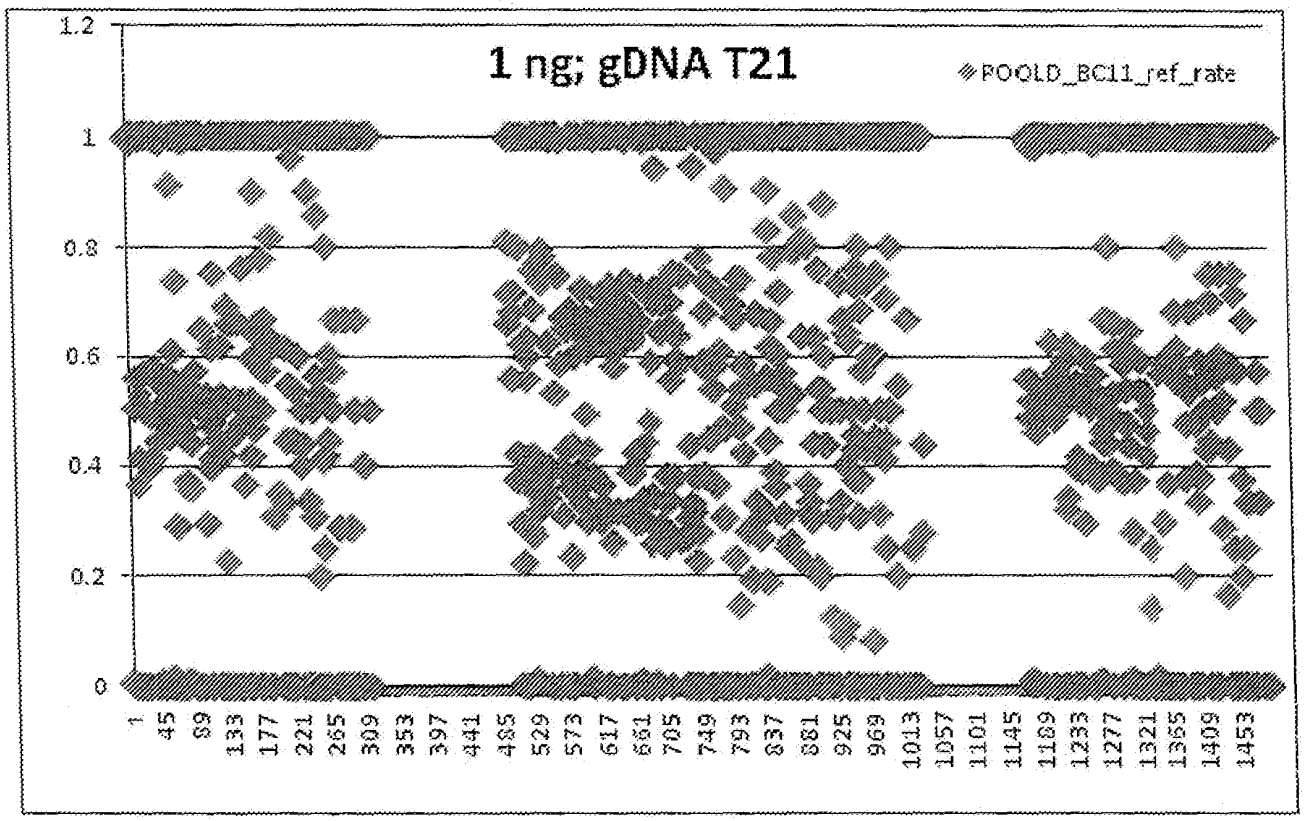


FIG. 25A

NEOGENOMICS EX.1013.023

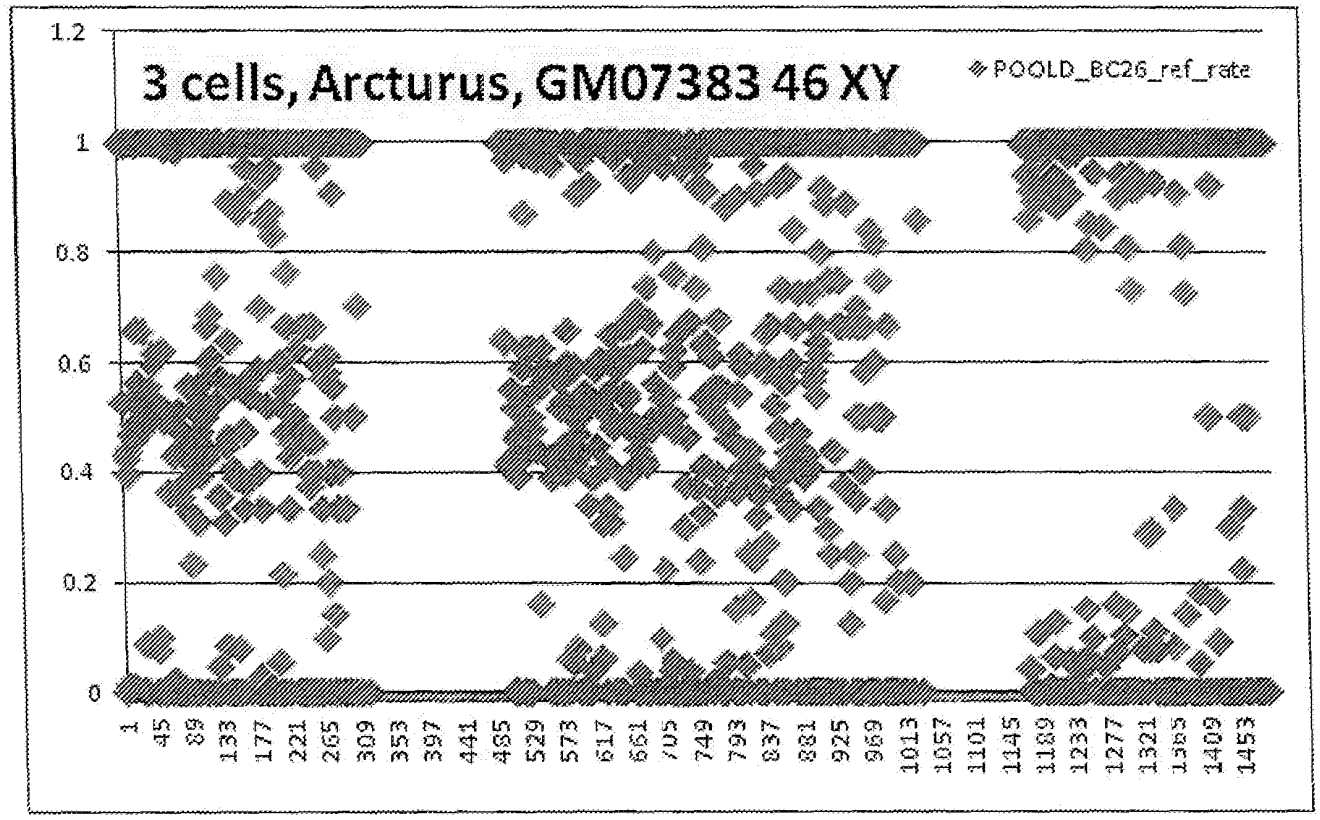


FIG. 25B

NEOGENOMICS EX.1013.024

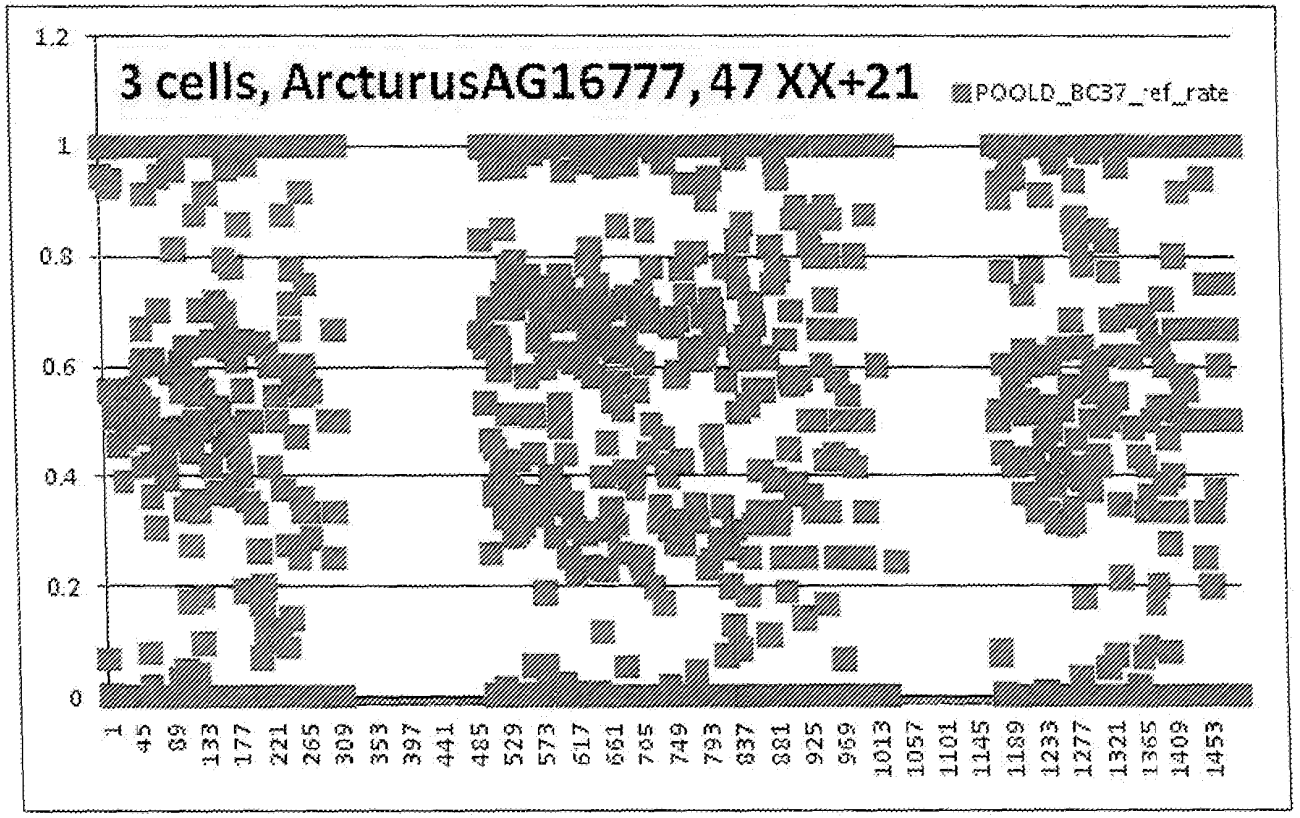


FIG. 25C

NEOGENOMICS EX.1013.025

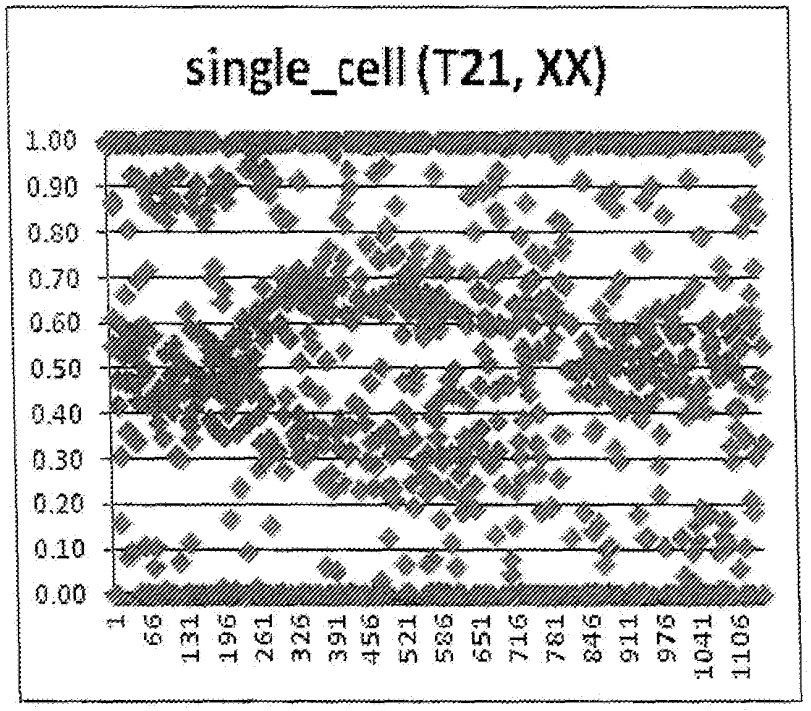


FIG. 26A

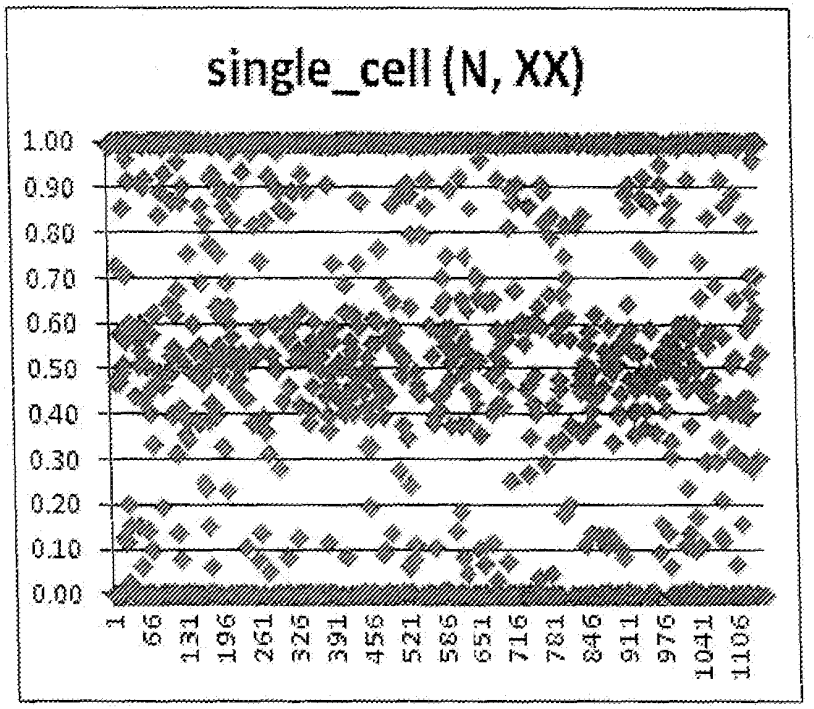


FIG. 26B

NEOGENOMICS EX.1013.026

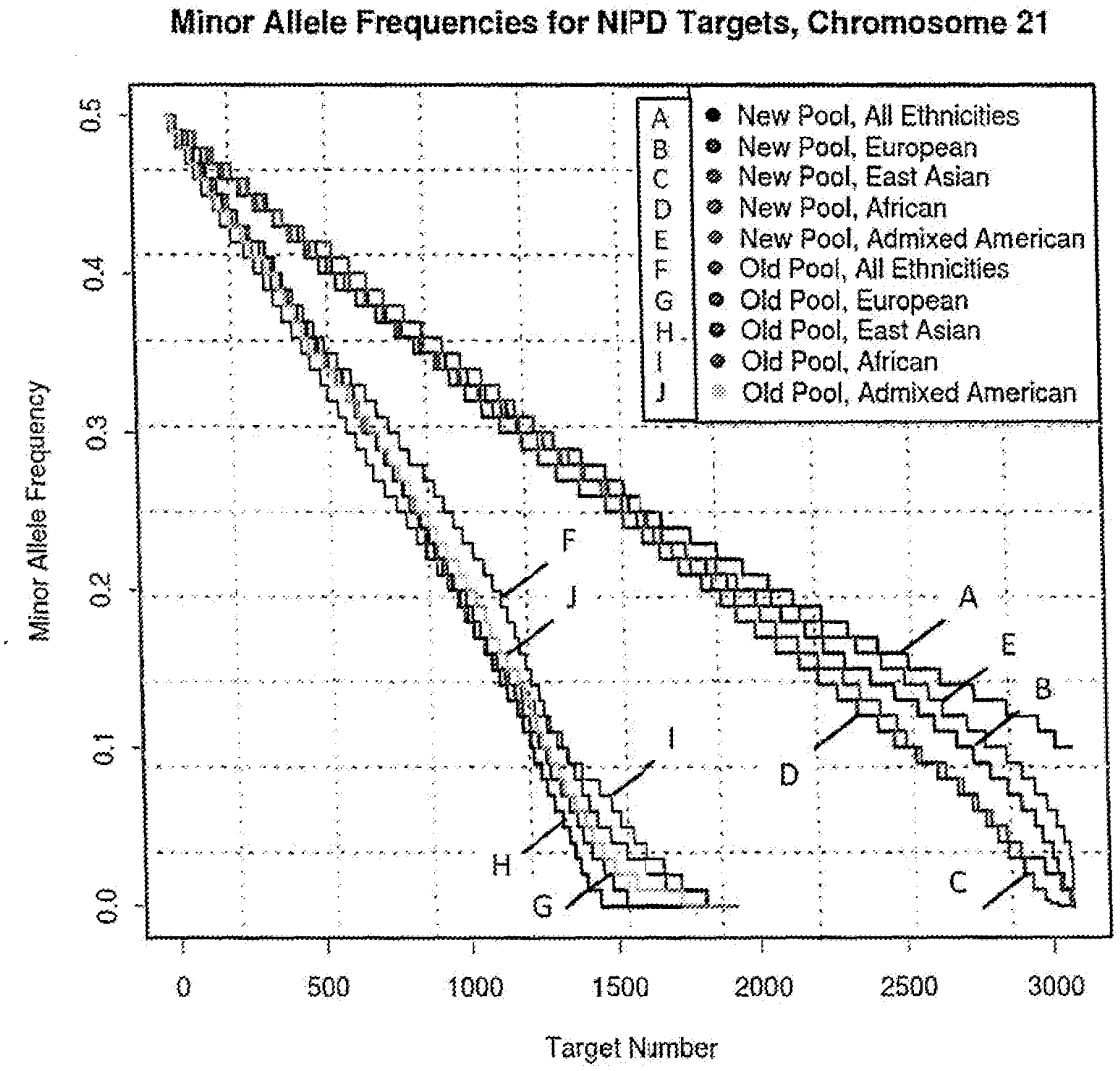

Minor Allele Frequencies for NIPD Targets, Chromosome 21
A New Pool, All Ethnicities
B New Pool, European
C New Pool, East Asian
D New Pool, African
E New Pool, Admixed American
F Old Pool, All Ethnicities
G Old Pool, European
H Old Pool, East Asian
I Old Pool, African
J Old Pool, Admixed American
Minor Allele Frequency
0.0
0.1
0.2
0.3
0.4
0.5
0
500
1000
1500
2000
2500
3000
Target Number

FIG. 27

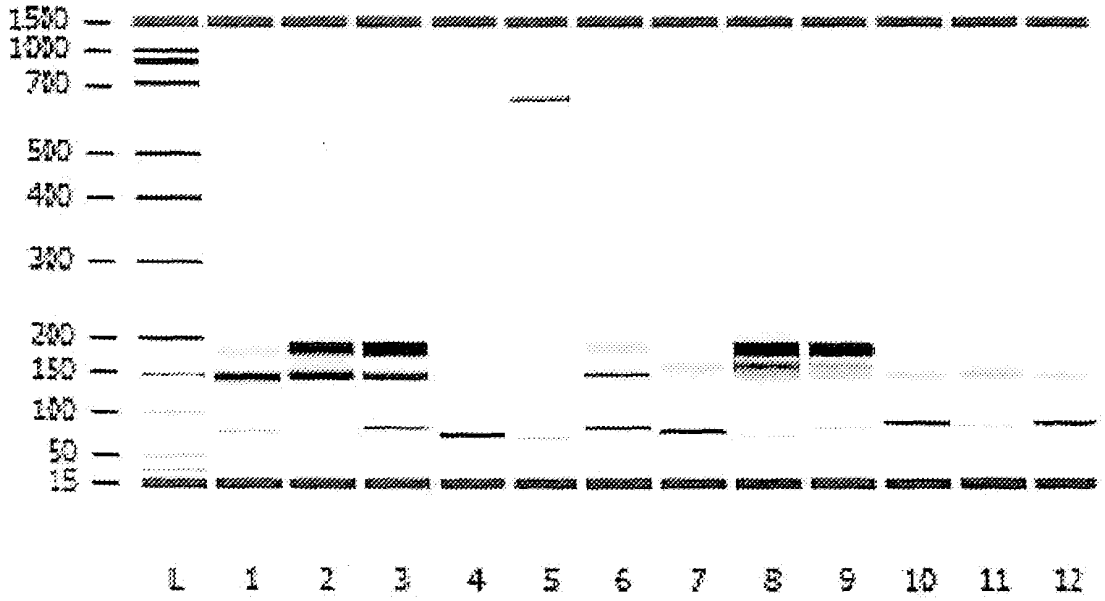


FIG. 28A

NEOGENOMICS EX.1013.028

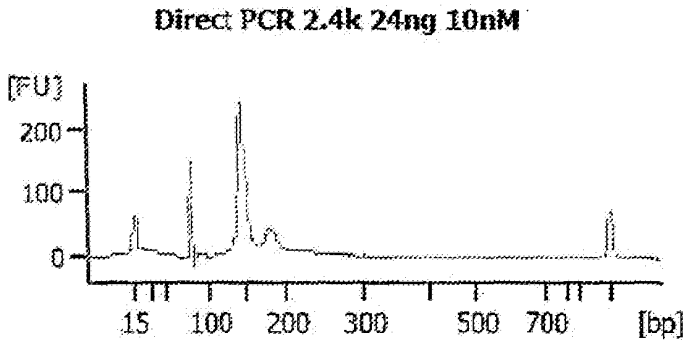


FIG. 28B

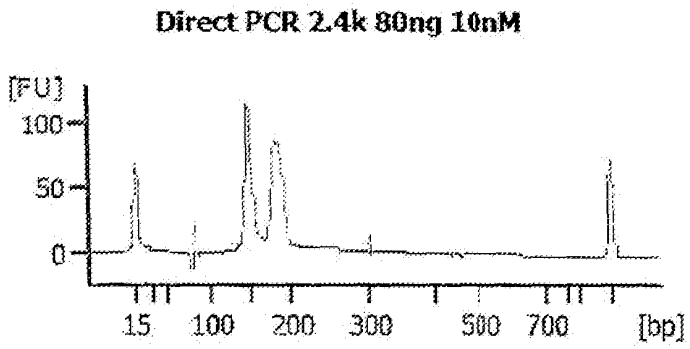


FIG. 28C

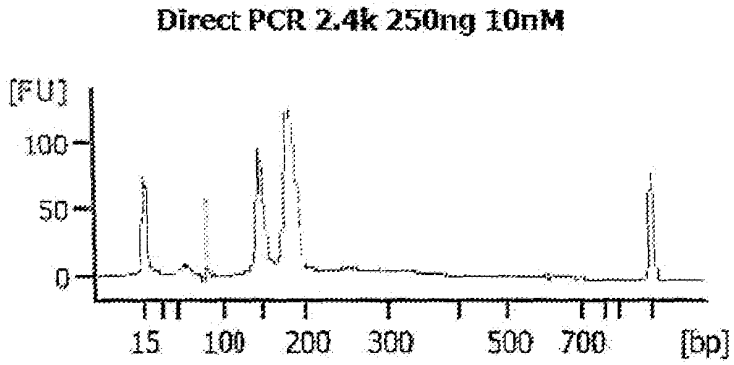


FIG. 28D

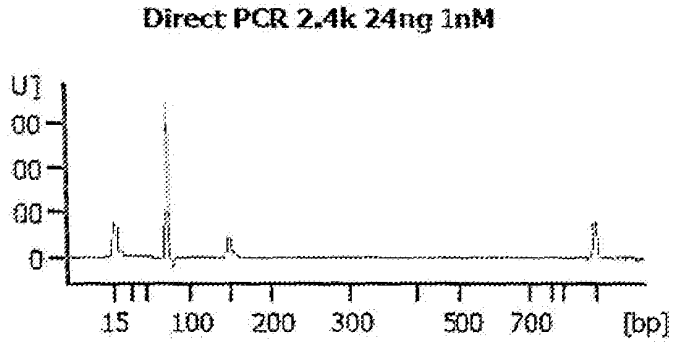


FIG. 28E

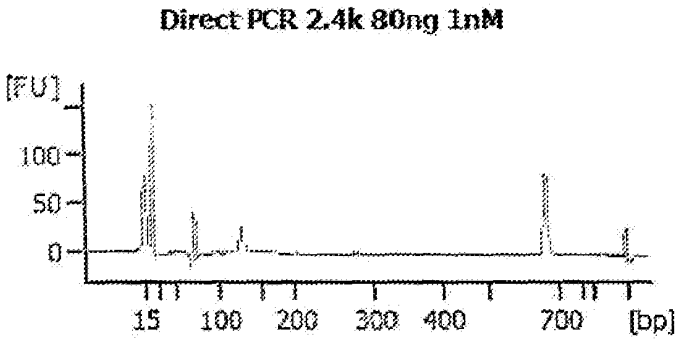


FIG. 28F

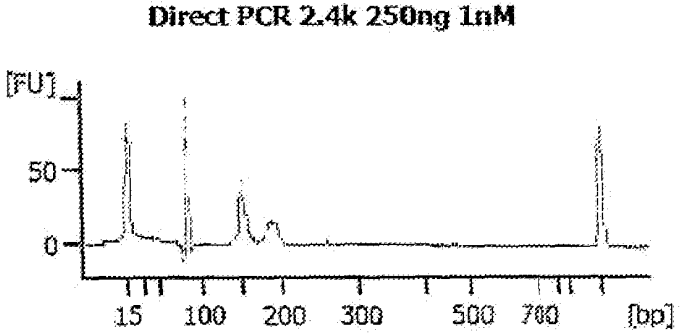


FIG. 28G

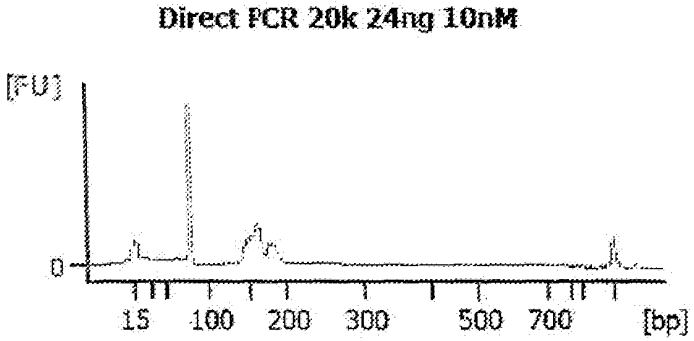


FIG. 28H

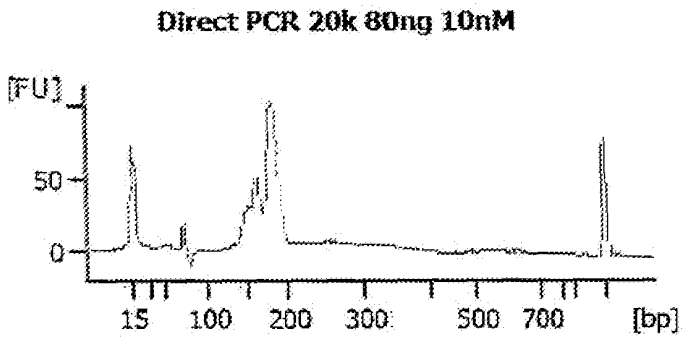


FIG. 28I

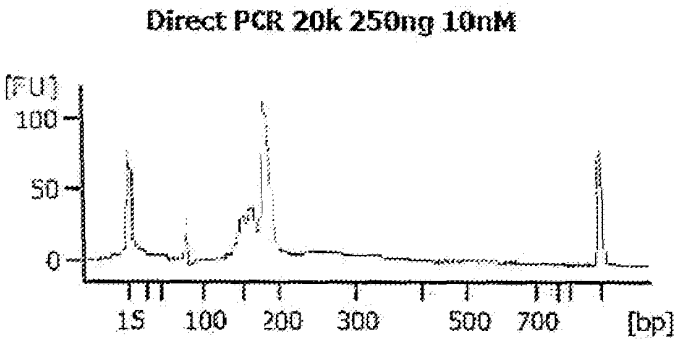


FIG. 28J

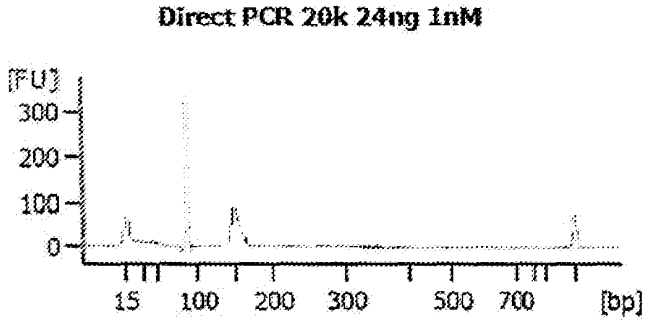


FIG. 28K

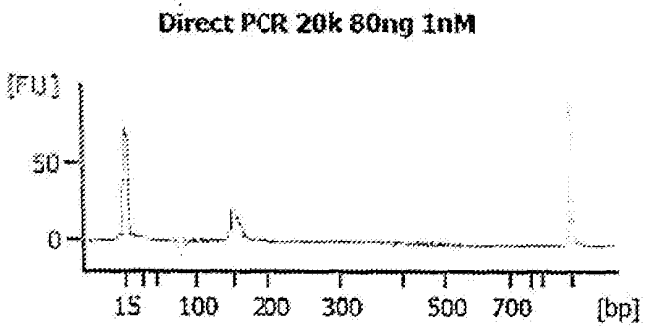


FIG. 28L

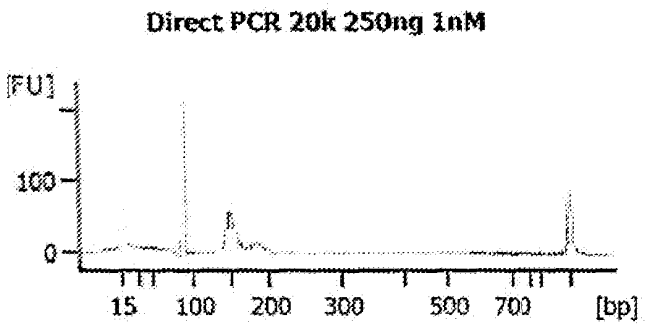
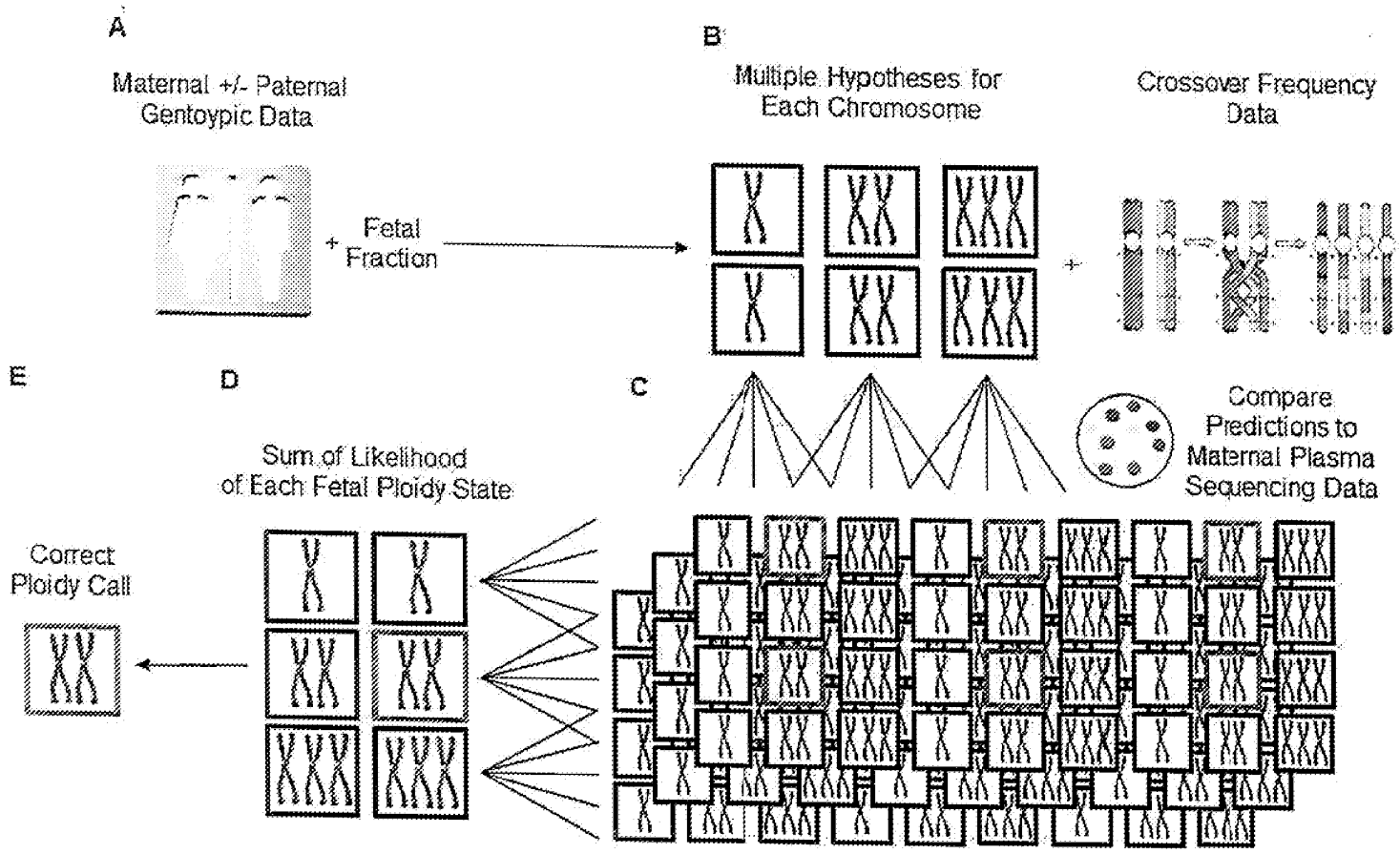
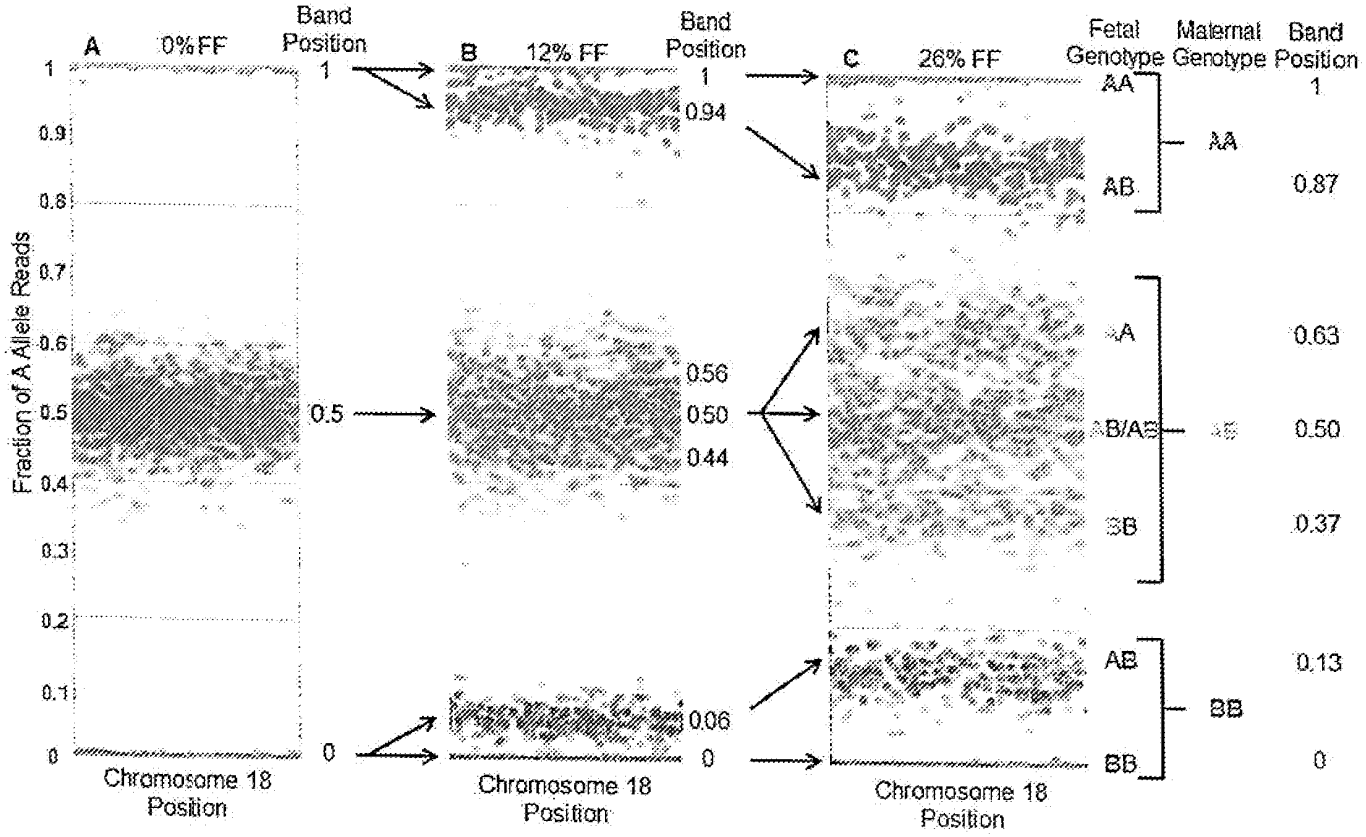


FIG. 28M



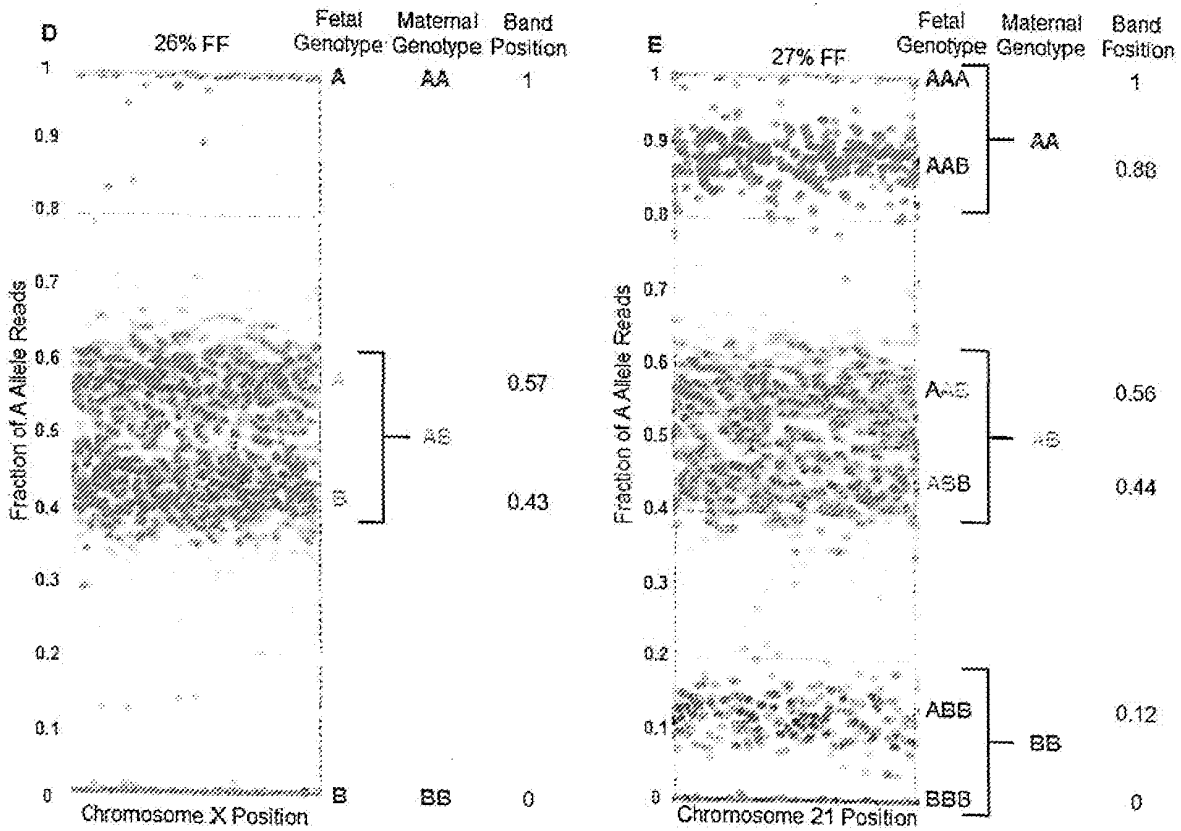
FIGS. 29A-29E

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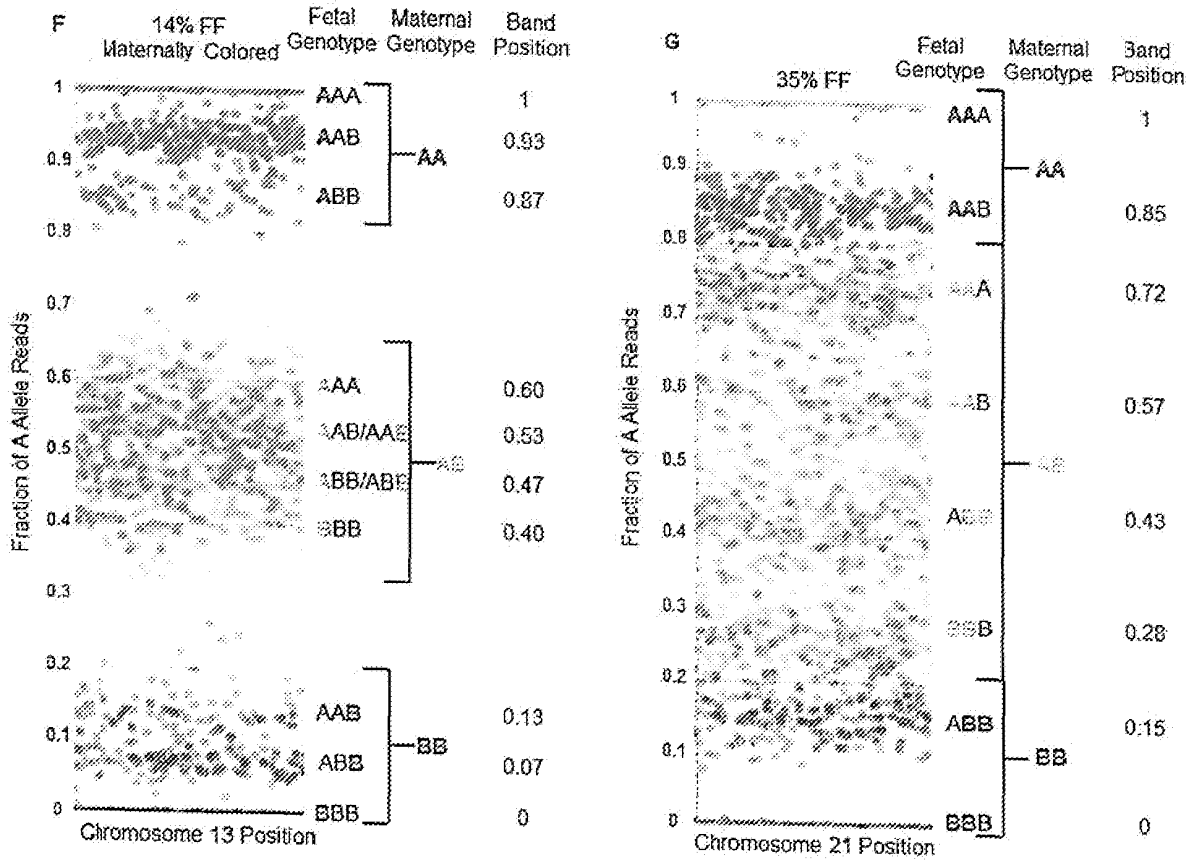
FIGS. 30A-30C

NEOGENOMICS EX.1013.033



FIGS. 30D & 30E

NEOGENOMICS EX.1013.034



FIGS. 30F & 30G

NEOGENOMICS EX.1013.035

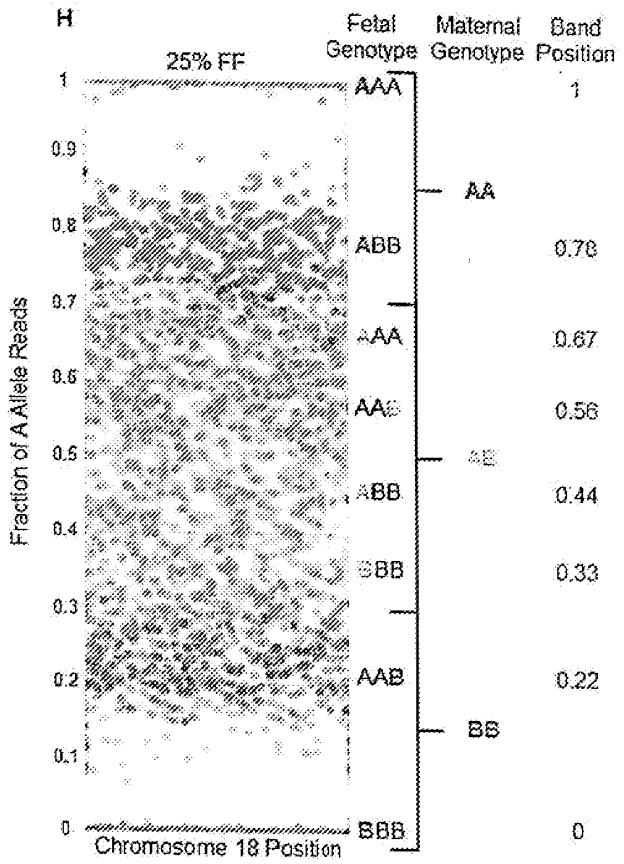
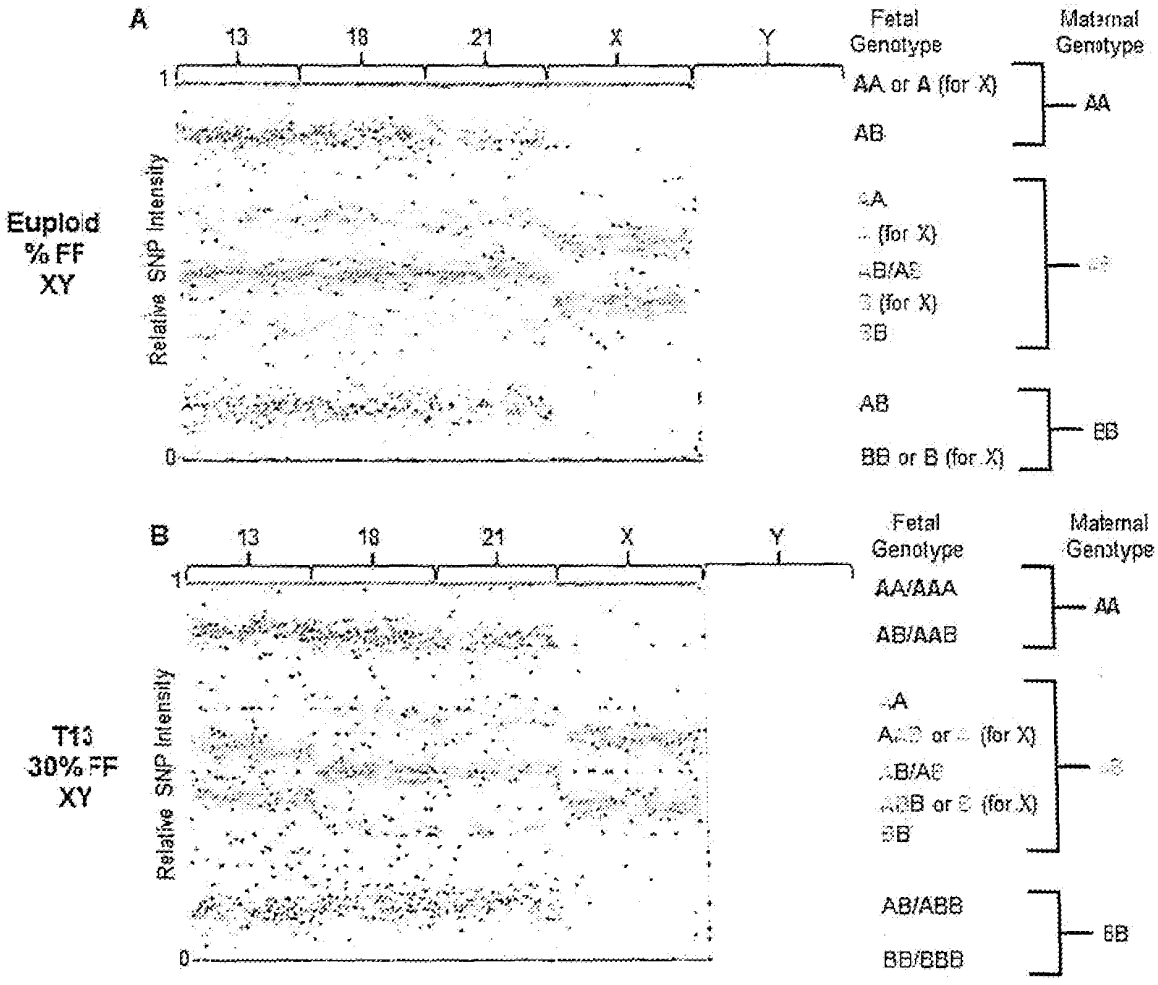
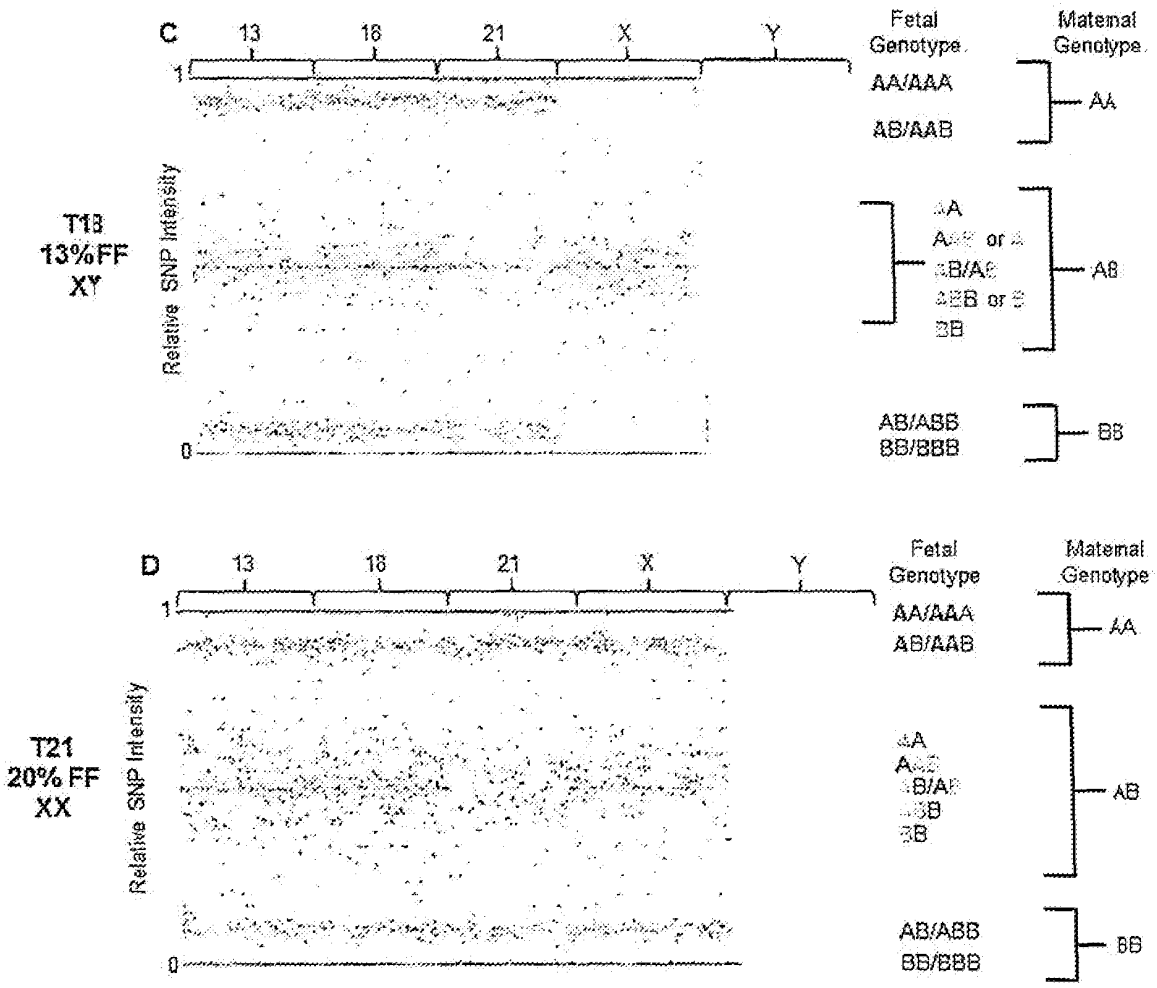


FIG. 30H

NEOGENOMICS EX.1013.036

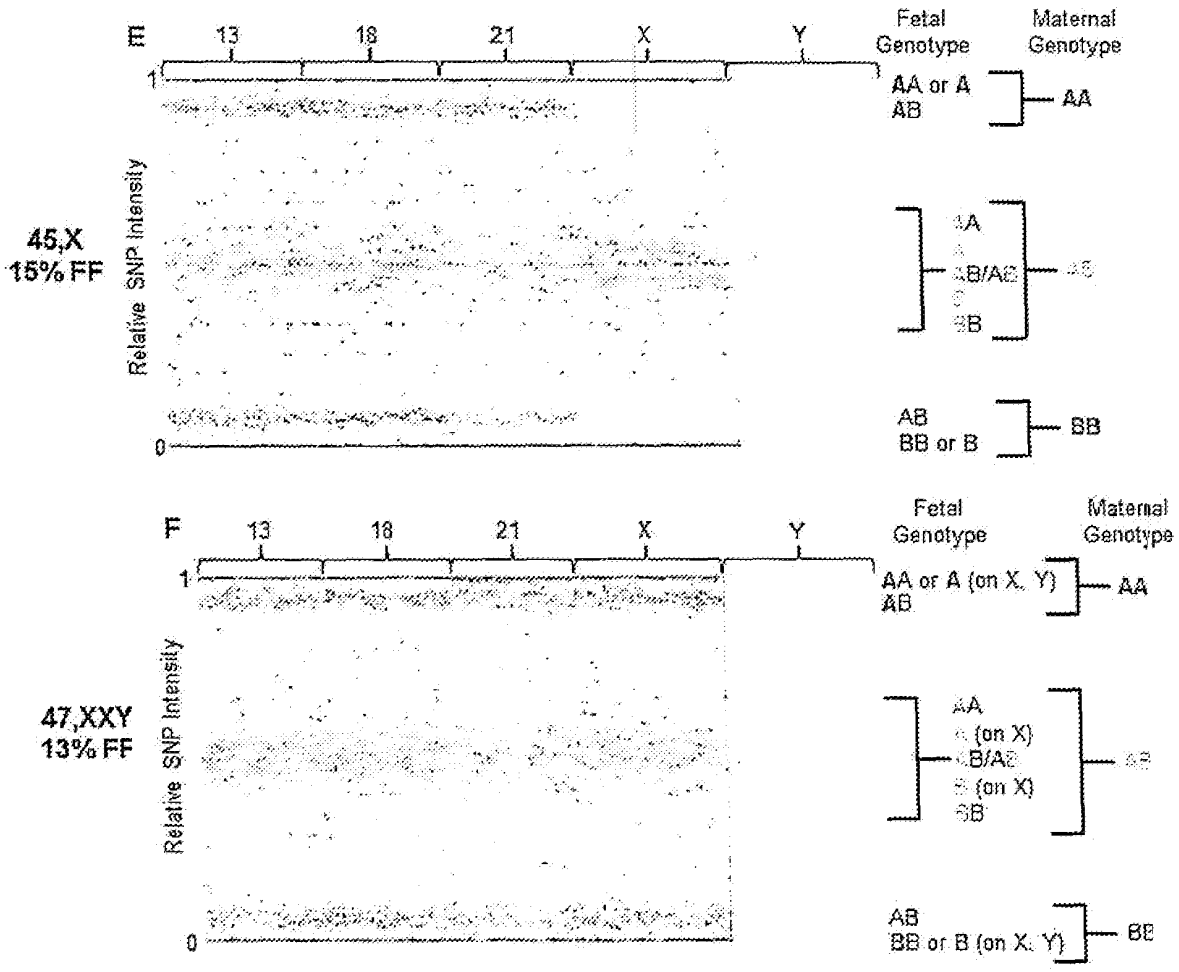

A
13
18
21
X
Y
1
0
Relative SNP Intensity
Euploid
%FF
XY
Fetal Genotype
Maternal Genotype
AA or A (for X)
AB
AA
AB
BB or B (for X)
BB
B
13
18
21
X
Y
1
0
Relative SNP Intensity
T13
30% FF
XY
Fetal Genotype
Maternal Genotype
AA/AAA
AB/AAB
AA
AB/ABB
BB/BBB
BB

FIGS. 31A & 31B



FIGS. 31C & 31D

NEOGENOMICS EX.1013.038



FIGS. 31E & 31F

NEOGENOMICS EX.1013.039

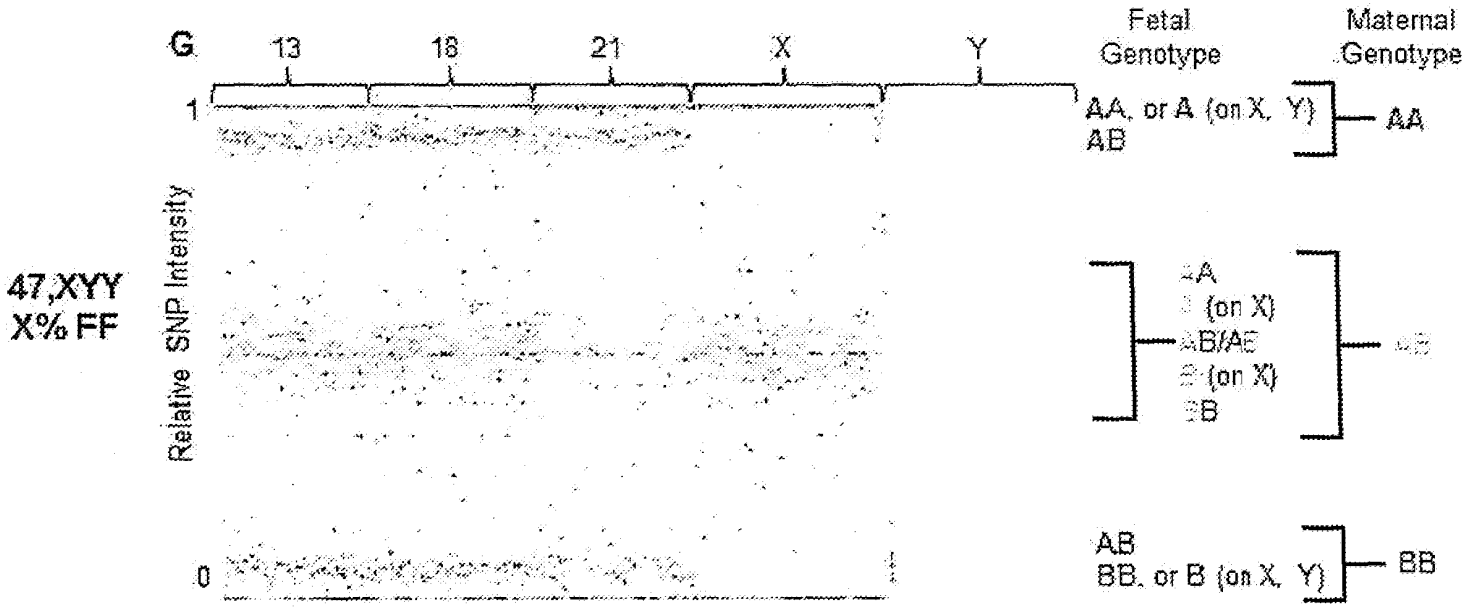


FIG. 31G

NEOGENOMICS EX.1013.040

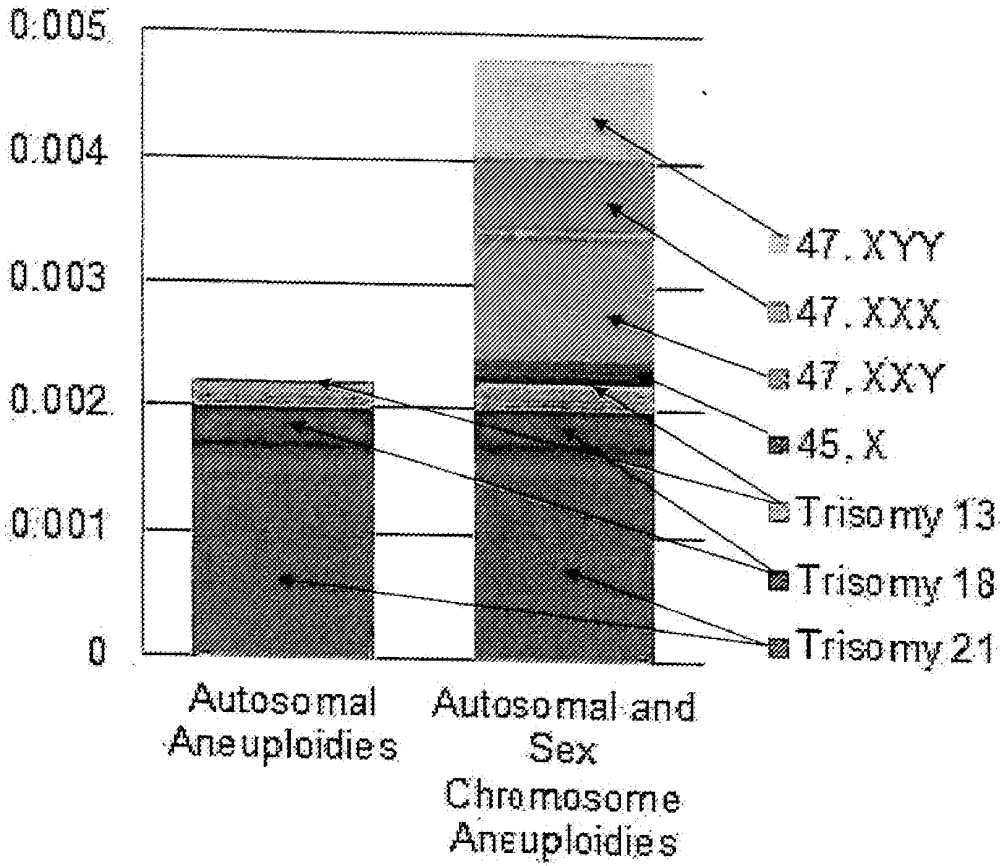


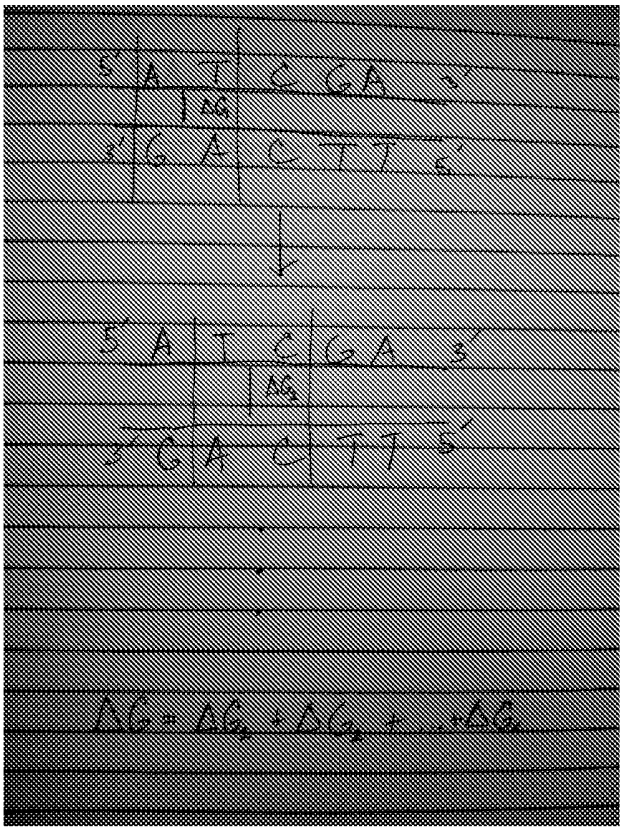
FIG. 32

NEOGENOMICS EX.1013.041

FIGs. 33A and B

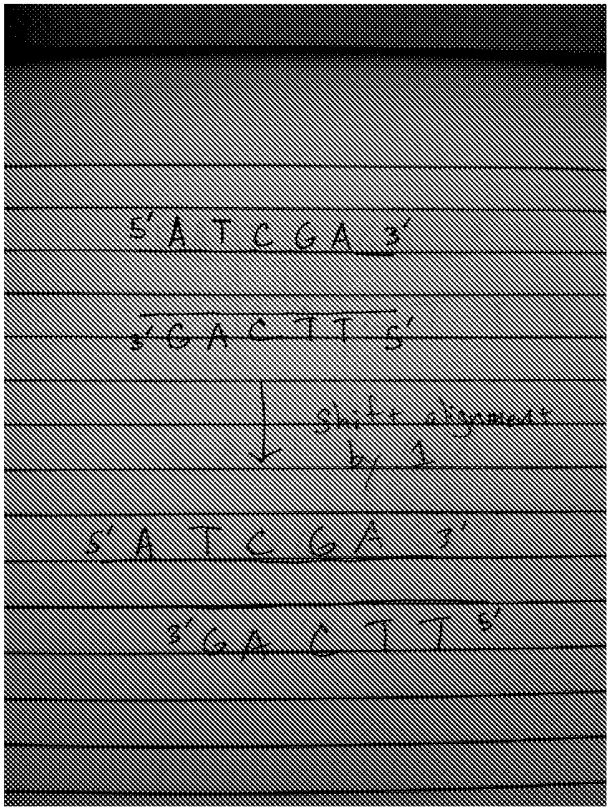
A

B



FIGs. 33C and 33D

C



D

FIGs. 33E and 33F

E

F

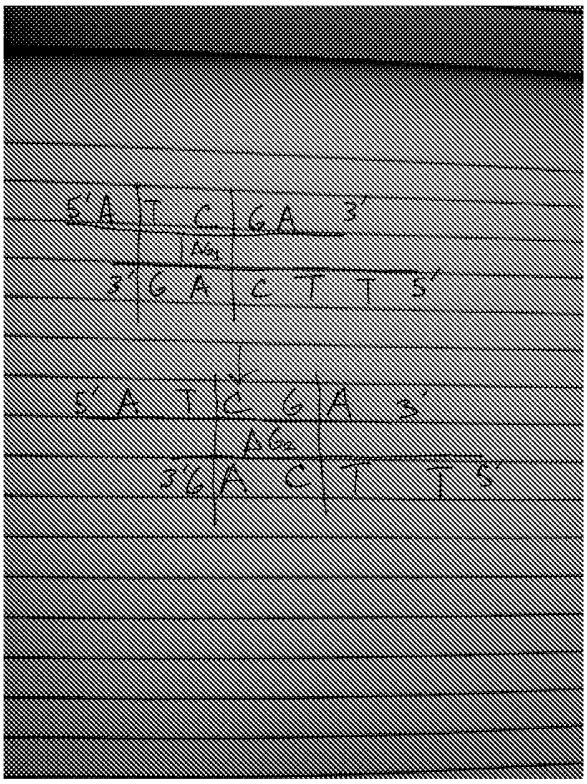


FIG. 34

Target No.	Pool A Sequence (5' to 3')	SEQ ID NO.	Pool B Sequence (5' to 3')	SEQ ID NO.	Pool C Sequence (5' to 3')	SEQ ID NO.
1	GGCACAACATTTGGGAGACAAA	1	CTCACTAAACTTTTCCCCAGAACTCA	1201	GCTTAAGCCACAGAAAGGCACAAC	2401
2	CCTGGAACCTGAAAGCTTGCAAAAAG	2	CCTCTGAAGGGAGACTGAAACAAC	1202	CGAGTGCCCTGGAACCTGAAA	2402
3	ACAGGGTGCTCTCCTCTTGA	3	GCAAGAATGCTTGGCTGCAA	1203	AGCCACCACTCCTGCTTCTGA	2403
4	GGAAGCAAACAGGTAGAGCCTGGTA	4	GTGGCATTTCACTGCCCTTTG	1204	TTGGGGACCCCTGGACTAAG	2404
5	CTACAACAACAGAGCTCCAGGAT	5	GCAGCTTGTTCAATTGTGGTTCA	1205	CCTCCCCAGTATCTTACCACTACAAC	2405
6	TGTCCTTGGTCGTATGTGAGAGA	6	GTGAGGATGAGTTTTCAGTGGCTATC	1206	CACCAAGCCCTCAACTCCTAAA	2406
7	GCAGGAACTGGCTTGCCTTT	7	CCCTGGACAAAATGTAACAGCCAAAGT	1207	GAAGTGAAAGAAAATGGCAGGAACT	2407
8	AGGTTTCGGCTTTAGTTGTGTGA	8	ACCTAGCTATCCTTCCAGCATTTC	1208	GCTGGTATTGTTTTGGGGATAGAGGTT	2408
9	TGTCTGCTTCTGCCGGCATTC	9	GCTGGACAAACTACTGCCTCTTC	1209	GTGGAGGAAATTAGATGTCTGCTTCT	2409
10	GTCCCTCACACCTTTCTACCAGAAG	10	GCCAATAGCTGCTTGATGCAAT	1210	GGGCAAGTCCCTCACACCTTT	2410
11	GGCTGCAGGAGTAGAGGTCTAAATG	11	TAGTTGCCCGGGATTCCCAAGA	1211	TGAGGTCTGGCTGCAGGAGTA	2411
12	GGCAAGAAGAAAACTGGAGCCAAGA	12	GGGAGAGGCTGGTCCTGAGT	1212	CTGAAGCCCAAGTGGCAAGAAG	2412
13	GAGCCTGAGTTCTTGATCACTACA	13	GTTTGTGAAAGGTCAATGTGAGGAA	1213	AAGTCCTGAGCCTGAGTTCTTG	2413
14	CCTGGAAAAAGCCCCGGTAGA	14	TCCTCGGGAGCTCACAACCTTA	1214	TCTCAGGGTGCCTGGAAAAAG	2414
15	GCAATGGTCCCAGTGAGAGTT	15	TCCCACTGATGCCAGACCAA	1215	GCACTGCAAAAGAAGAGGCTATTG	2415
16	GCCCTTTGGAACCTGACCAA	16	CCCAGCTTAAGCAAAATAGGGCAAGTA	1216	TGGGAACCAGCCCTTTGGAA	2416
17	GGCCTTGATTTGACCCTTCTTTTC	17	TGGCCAGTAGTAAGTTGAGAAGACTA	1217	GGCTTCACTATCAAGAAGGGCCTTGATTT	2417
18	CCACCCGCTTCTGAAACACATC	18	CCGGAAGCTTCACGTTCTGT	1218	AAGAGGCCACCCGCTTCT	2418
19	TGGCGCTAAAGCCCAACA	19	GGAGGGAGAGCTCCATCTCTGA	1219	TGGAGCAGGCTGGCGCTAA	2419
20	CCTTCCCCGCTTCTGGATATGAAA	20	CCGGCAATAGGACTCAGCAA	1220	CCGAAAACCTTCCCCGCTTCT	2420
21	GTCAGCGTTTACGGCTATTGAGT	21	TAGGGCAGGTGTCCTCTGCAT	1221	GGAAGGGGAATTTGTCAGCGTTTA	2421
22	GCATCTCCAGTGCCCTCTGT	22	GTGAACCAAGCTATACTACCTGCTTTTC	1222	GCAGTCTCAGCCTTTCCTTTGCAT	2422
23	CATCTTCCTAGACATGCACACAAC	23	GGCTTCTGTGCTCATAGTTCACACT	1223	GTTGGGAAGCATCTTCCTAGACAT	2423
24	GTCCCAAGTCAACTGCCATCT	24	GGGCACACGGAGGTGAAAAAGT	1224	GCCTATTTTGGAGTCCCAAGTCAA	2424
25	GCGTCAGCACAGCTAACACA	25	CGGGAGCCGTTATACCAGCTTAG	1225	TGGGCCACGTTGCCTACT	2425
26	TCACAATGGGAGGGAACATTCAA	26	GCTTCCTATGACCAAGTCCCTTT	1226	CACCTTATTACTACCTTCCTCTCACAATG	2426
27	GGTAAAGGCTGGTTGTAAAGTCCACAT	27	GTGTTGGCCAGACCCACTGT	1227	ACTGGGGTAAAGGCTGGTTGT	2427
28	CCGACTCAATGGGGAACTCCTACT	28	GGGCACTTTTGAGTTGGGCTTTG	1228	AAGCCTCGTGGCCGACTCAAT	2428
29	TCCTTACACCTTGGGGCGTCTA	29	GCAGCTCGGTGCCTAATGGTT	1229	AACAGGGCGTGAGTTTTCCTT	2429
30	GGGCATTTCTAAGACACTTGGACAGT	30	CAGACCTGGCAGGATCAGAATAGT	1230	GGAGAGGCCAGGGCATTTCTAAG	2430
31	CACTGTATCTCAAAGGGCAAAACAA	31	TGCAATGCAGAGGAGAAAGGTT	1231	CAGCCTGCTAGGAACACACTGTA	2431
32	ACTTGTCTCTGCAGACAGTTTCA	32	AGTCACAGGTATCCTGACTCTCATA	1232	GGTGATTCTGGCCGAACTTGTCT	2432
33	GGCCTCTTGGTGCCTCTGTT	33	GGAGCAATCAGCCTTGCAGAGA	1233	TGGATCGGAAACACCAGACTCA	2433
34	GCTAGAGAAACAAGACAACATCCAAGTTC	34	TGGCGCCCGGAAAACCAT	1234	CCTGAGACCGTTGCTAGAGAAACAAG	2434
35	GCGGGCATAGAGAGAAGGAAAT	35	TTCAAGAGCATCCTTGCGCTAT	1235	TGGGATGCGGGCATAGAGA	2435
36	GGCTTCAGTCCTTCACCTCTTTC	36	ACCTTGAGAAGGTGGTATTTGTGT	1236	TCTCCCAGGCTTCAGTCCTT	2436
37	TCAGAGTCCTAGGCCAGCAT	37	AGAAAGACCCCAGGTAAGAACCAT	1237	CCAAGTTATGCCTGCTGCTCAGA	2437
38	GGTTCCTCATGGAAGACTGGTTTG	38	CACTCACACATCCACAGGAGACA	1238	GGGAACCTGGAAAGACCTTTACTT	2438
39	GTTGAGAAGGGAGTAAGATCAGACAA	39	GACGCAGTATCCCACAGAGCATT	1239	GGTGGGATAGTTGAGAAGGGAGTAA	2439
40	CTCCCGCCTCTAAATGAATGGAT	40	ACCAGCTCTGAGTCAGGAAGT	1240	CCTCAGCTCCCGCCTCTAAA	2440
41	CATTCCCTCCCCTACCCTGGAAA	41	CCCACAGTCCGACGAGTGTTTT	1241	GGATGAGCATAAGCAGTGGTTCA	2441
42	GAGACTCAATCCTGGCACTTGT	42	TCACTCCACTTTACAGGCACATATT	1242	AGGAGTGAGAAGGGAGACTCAATC	2442
43	CCCGGCTGCCATTTAAGACCAT	43	ACGAGGATCAGACAAGTCACAGA	1243	TCAAGTCCCGGCTGCCATT	2443
44	GATGAGGGCTGAGTATGGTCAAG	44	CCCATGACCCCAGGTTGGAATTG	1244	GACAGAGAGATGAGGGCTGAGT	2444
45	CCCTTTCCTGAACCCCTTCATT	45	CACTCTGTGCAGTTGCTGGTT	1245	AATTGCCAGCTGTATTCTCCTTCT	2445
46	GGGGCACCAAAGAAAAGACAAG	46	GTCTGTGGAGCACACCTTGGAA	1246	ACAGAGGATGGGGCACCAAA	2446
47	CATCAGTACCCCACATAGGTTTAACT	47	GCCTTTCCACTGAGCCATGA	1247	GCATGAGGATGGAATGGACTCATCA	2447
48	GGAGCTCAGTATGAGTAGAGGGGAAA	48	CAGCCTGGTTGGTTAGATTCTGT	1248	GACCTTCAAGGGGAGCTCAGTAT	2448
49	TTGTGCACGACGCTGGAATG	49	CTTATGTGTGGCTTTGGCTTCTTG	1249	TCTGATATGGAACCCAGGGATTGT	2449
50	TCGCTAGGCCATTCCTCCTGTT	50	GGGGCTGAATCAGACAAGGAA	1250	TGCACCATCGCTAGGCCATT	2450
51	CACGACACCCTTAGATAATGTTGGAT	51	CACAGCTTGGAAGGCCTGAA	1251	GTCAGTTCACACGACACCCTTAGA	2451
52	TGGCACAAGCCCAGGTCAT	52	CTCATGATGTCAAATGCTGCTCAA	1252	AGCCTGGAAAGCTGGCACAA	2452
53	GCCTCCTCTGCATCTCTGAACCAA	53	GTTCTGGTGACAGACAACCTTCT	1253	TTCCCAGCCTCCTCTGCATCT	2453
54	TGCAGCGATCCCTTCACCTT	54	TGAGCAGCACAGTCCAATTCT	1254	CTCAAAGGGGAAGAACGTCAGT	2454
55	TGGTAGTCAGGCCTAGTGACTT	55	CCCCTGGGAAAGTTACTGTCGAT	1255	TCTGCGTCTCACTTCCTGGTA	2455
56	GAGGTCTTCCAAAGGCTATTCAGA	56	GTCAGTGAGCTGCCAGTTTTC	1256	AAGGTGACAGAGGTCTTCCAAAG	2456
57	CCTGAGCCTGTCACACTATTTCT	57	TCCCAAATCCTGAGCCTGTCA	1257	TCCCAAATCCTGAGCCTGTCA	2457

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58	GAGAGCAACAGGCGTTGAGA	58 GCCAACCTATAGACACAACTTTGGTTAC	1258 GGAGAAAGAGTGGAGAGCAACA 2458
59	AGCCCTACAAGGTCAAGTTTTCA	59 GTTGACTTCCGGCAGTAAGTGATGA	1259 GACTATACGATACCAAATAGCCCTACAAG 2459
60	GGAGGCACTTCACTCCTCCTA	60 ACTGGGACATTTCTCACGTTATCA	1260 GTCACAAGGGAGGCACTTCA 2460
61	GTTGGGGTCAACTTTCCCTGAAG	61 GGAATCCAGCCCATCCTCACATC	1261 AGAGCTGCCCTGGAACTGAA 2461
62	GAGGTTTGGCTCCATCATCAGA	62 ACTGCACCACCACACGGAAA	1262 GGAAAGGTACCCCAGAATGAGGTT 2462
63	GAGGCTCATGTCTCTTCAGCCTTAG	63 CCTTCAAGGGCTTTCTCTCACA	1263 GGTGGTAGAGGCTCATGTCTCTT 2463
64	GTTGCTTCCCGTTATCCTCACT	64 ACTTCCACATGGCTTAAACAGAGA	1264 TCCCCAAATCCTTTGGCAATGT 2464
65	GGGCCTGTTCTTGTTGATAGTGTT	65 TCGTGAAGAAGATGGAGAGGAGTTA	1265 GTGTCAAAGGGCCTGTTCTTGT 2465
66	GTTTGCTGTTACTGAATCCCGAATCT	66 CTGGGACCAACCCATTTCACA	1266 CTCTCATCAGCTGTTTGCTGTTAC 2466
67	GCACATGTAAAAATCCTCCCTACTGT	67 AAGGAAGTGGGGTATGGACTGA	1267 GACCTCTTTCTCCCTGCACATGTAA 2467
68	CCTCTGGTAATAGCACCCAGGATAC	68 TGTTGGCCACATACATTGTTCTCA	1268 CCTTCCTTGAACTTGCCTCTGGTA 2468
69	CTGAGGTCCAGAATCCAGATGTTCT	69 CTCCTGCCTTCGGGGATGT	1269 GCTGTCACCAAGACTGAGACTGA 2469
70	GGACAACAGGATCGGGGATGA	70 CACAGGCCAGAGATTCACCAT	1270 TGAAAGCTATTGGGACAACAGGAT 2470
71	CCATGTGTAGGAAGCCCTGTGA	71 TGGGAAGCCTACCTTGACTCT	1271 CAGGAAGATAAGTACTGAGCCATGTGT 2471
72	GCGACCTCACTTTTCCAGTCAT	72 AAGCCCCTGAGAATCTAAAAGCAA	1272 GCACATGTCTGCGACCTCACTTT 2472
73	GTGCTATTGTGGCTCTGTAGGTTGA	73 CGACAGGGCCTCCCAATTC	1273 CAGCAGAGCAGAGTGCTATTGT 2473
74	GTTTCAGCCTGAGAGGCAAGA	74 AGGTAGACACCGGAAGTAATCACA	1274 GCTGTTGCACTGTGAACTTGT 2474
75	GAGATGAAGCGTGTGACCCAAGT	75 TCAGGTAGAGGTCGCCAAGGTT	1275 GCAGCAGACAGCTGGAGATGAA 2475
76	TGCCTCATCTCTGCCCCTCTTT	76 GGAGGCTATGAGAAGTGAGGTTTG	1276 CTCTGCTGTTCCCTCACCTGTAA 2476
77	TCTCAGTGCGTTTGGAAGGAAA	77 GGGCGGTTAAAGAGCTTCAGAGA	1277 TCTGGCTTCTCAGTGCGTTT 2477
78	AGGTTTCCCTGGACACACATTC	78 TGGTCTTCTTTATGCCCAACAAAC	1278 TGTCAAGGGACTAAGTTTCTAGGTTTC 2478
79	AGGAGACAGGGACAGTTTCAATG	79 CTCACACGGGCTCACATTCT	1279 GGCTCATGCTTGTGATAGGAGACA 2479
80	TGGGTTGGAAACACGTCCTTTA	80 CCTGCTCACCGATGCTCAA	1280 GCCGAGGAGAATATGGGTTGGAAAC 2480
81	GCCCTTAGCAACAGCTGCAT	81 GGTCAGAAACAGAGCCTGTAAAAC	1281 GCATCTTGTGGTTGCCCTTAGCAA 2481
82	GCAGACACGCCTCTGGCAAAT	82 CCTGAGACAGAAGCAGTAGCGTCTT	1282 GTCCATTGGGCAGCCTTCT 2482
83	GCCAAAAGTGACAGCAACACA	83 CGCAAAATGGGCTCCTGCTT	1283 CCTGATGCTCCCTCTGCCAAAA 2483
84	GCACACATCCAATCCTTCAGTAAATC	84 GCCGGATTAGGGGTGAACTTTG	1284 GCCTCATTCTCGCACACATCCAATC 2484
85	TCCTCATCCAAGCTCCTTCTCT	85 GGAGGTATTCTGGCTTCCCTAAGA	1285 GCCCTTCCTCTTGCTGAACAGT 2485
86	GGCACTTTCCACCCAGAACTTAAC	86 GGAAGGGTGAGTTTAGCACAATGGTT	1286 ACCTGAAGCCAGGCACTTTC 2486
87	AAGCCCACGTTGTGCTCTTG	87 CCCACTTGCACAAATGCAAAGA	1287 TGGGAGAAAAAGCCCACGTT 2487
88	CTCAGTAGGGTGAAAAGGATGGAAGT	88 AGCTGTGGTTTTCCTGTGTCA	1288 GCAAGGCTCAGTAGGGTGAAAAG 2488
89	GCTGGATCTACAGAAACCAGCAAA	89 TGTGTGTGTTTAGCAAGGGATTTC	1289 GCCAAACTGCTTCTTGCTGGATCT 2489
90	TGGCTGCAGATGCTTGTCA	90 CGTTTGGCAACGGAGATGTGT	1290 GGAAAGGCGGTGGTAATGTGGTT 2490
91	GCCTCTCAGCCTCACGTTCTTA	91 TGCCCACCCTTTTCTTTTGCTT	1291 TCTGACCAAGTCCGCCTCTCA 2491
92	TCCTTGACTCACGCCTCTGA	92 GGGCTATACATTTCTGGGCTCAA	1292 CCTCACACTTTCCTGTCCTTGACT 2492
93	GGAGGACATGGTGTTTGAGGAT	93 TACATTCCCCTCTCCCGCAACA	1293 CAGCTGATCTATGCATTGGAGGACAT 2493
94	CTACAGTTAGAGTCACACGTCACA	94 CCAGCCAATCAGGATCATGGACAGA	1294 GCATCTACCCAATCACTACAGTTAGA 2494
95	CATGCGGAGGGACTTAGCAA	95 GGCCCATGACTCTGCTGGAT	1295 CTCATCAGACACAGGCAGCTT 2495
96	GCTTTCCAGAGTAGAGGCGTTTC	96 GGAGGCATCTCCGGCTACA	1296 AGGGCAAAAGCTTTCCAGAGTAG 2496
97	TGATGGTGCAGAATTGCTACGATA	97 AGTGCAAGGTTGGAGAATGAAAAG	1297 GCAAAGTTTCATGGCCAAGTGTCA 2497
98	GCTGAAATGGACCCACAGAGA	98 CGAGTTGGCCTCGTCTTACCAA	1298 TGCTGCCAGGCTGCTGAAAT 2498
99	TGCTCCAACGATGGCTCTTC	99 CGTGGCAGCTGGCTTTAGAATC	1299 TCCCCAGGCACCTGCTCCAA 2499
100	AGGTGAAAGCGATGTCTGCTTAG	100 TGGTGCAGCCACCTCTCTTATC	1300 TCACCTACCTAGGATTAAAGGTGAAAG 2500
101	GCCCTGACCTCACCATTGGAA	101 CTCTGGCACATGCGTTAGTTG	1301 GGACAGGTTGGTGCCAGATT 2501
102	TGGGGCAATTGCGGGTATT	102 GGGGCTTTGGATAACACCAAGGTA	1302 AAGGAGGTCCTGGGGCAAT 2502
103	GCATTACAGGAGAGTGAAGATGGAT	103 TTGCAACCCCTCCGACATTC	1303 CTTGCCTAGGAAGGGCATTACA 2503
104	GGAGGAGAAAGGTGGTACACTTG	104 AGGAGAAGTTCAGGGGACCAA	1304 GTTGCAGCCTGGAGGAGAAA 2504
105	GGAGACAATACAGGTTGGGTGGTT	105 AGAGCTGCTATCATGATGTGGAAA	1305 GCTGGTCAATTCTGTTAAATCAAGGAGACA 2505
106	GCGGATCTTCCTTACCTAGTCTGA	106 AGGGTGAGTCTGGAAGAGGTT	1306 TGGGTGAGGGCGGATCTTC 2506
107	GGATACTGTGTAGGAAGGCAATACA	107 CCCCACTCCCCTCAAGGTATGT	1307 CCCAGAGAGGCTGGATACTGTGTA 2507
108	CAACTCCAACCCCAGCCTTTTG	108 GTCACCCTCTGAGGTACACACT	1308 CCTGTGGCTCCTGAGAGAGAAATCAA 2508
109	CAGGCCAAGAATGACATACGACTT	109 ATGACAACATCACGGACAGAGAA	1309 TGGGACACTTCAGGCCAAGA 2509
110	GGCGAAAATGAGCACTGTGGAA	110 TGACAAATGCCGAAGGGATTCT	1310 GGCTAGCATTCGGCGAAAATGA 2510
111	GGAAGGCAGTACAAGAGCTTACT	111 CTATGTTTCAGCCACACCACATTC	1311 GCTCAGATGTGTAAAGGAAGGCAGTACA 2511
112	GGCGATTCCTGACATTTGATTGCAT	112 CAGGCTTGTTCGGGAAATACAAG	1312 TTCCCGGTGTTCTGGCGATT 2512
113	GGGGTTTGTTTGGGTCAGAGA	113 GCCGAGGTTTCAAGGTCCAA	1313 GGCATGTAAGGGTGACAAAGGAGTTG 2513
114	CTGCAATATTGGCCATTTCTCTTCA	114 TGCTCAACTCAAACAGGACACT	1314 CCCTCCCAGGCTTCTCATCT 2514
115	AGTGTGCACGCCTGGTTGAA	115 TGCGGCGATGTGGCTGTA	1315 CCTGCCTACGGTCCTAAAGTGT 2515
116	CCCCAGCTCCTTCACAAAGACT	116 CAGAGGGTGGATGGATACAGTTC	1316 TCCCTCAAACCCCAGCTCCTT 2516
117	TGGAGCTGAGGAGGAAAGAACA	117 CGTGGCCTCCTGCACTGAT	1317 GGCATGCTTTTCATTGTGAGTGA 2517
118	TGGGTCATAAAGTGACTCTGTAACATC	118 GTGCAGGCTCAAGTTGCTTCT	1318 GGATCTTCCTTCTGGGTCATAAAGT 2518
119	GCTGTGAATGTTACATGGCAGGAT	119 GCTTGCTTGGAACCGATTTTGGAT	1319 GCTTTTAAAAACCCAGCTGTGAATG 2519

120	CGTCCCTTACCGGAGCAACTTT	120 GGCGGGACCGAGTGCTTTA	1320 TCTTCCCGCTCCGTCCCTTA 2520
121	AGTGAGCTAGAGGCTAGGACTTAC	121 CCTCAATTACTCCCCAGATGTTCAGA	1321 CTGTCCGCAGAAGCCAAAAG 2521
122	TCCTTTCCTCTCATCGCCTTTG	122 TCAGCAGGAGGAAGGATGTTTG	1322 CTGTACTGCCTCCTTTCCTCTCA 2522
123	GGGCACAGAAGGGTGTTCCAAA	123 ACCTTCCGGCCATGTGTGAA	1323 CCTGAGCAAGGGCACAGAAG 2523
124	GGCCCTCATTGCAGTCAGCAT	124 CAAATCTGCTCTGTCTATGCTAGGAA	1324 AGGTTCATGGGCCCTCATTG 2524
125	TCCTGAGATGGGAAAGAGTGAAGA	125 AGCTCCTGACATTATGCCCAAA	1325 GGGCTCCCACTTTCCTGAGAT 2525
126	GAAACTGCACAAACTTCCTCTCCTA	126 GGGAAAGCCAGACATCCTCTCCTA	1326 GCAGGCTCGATATCTAATTCCAGAGA 2526
127	CGCCTGTGGTTGTTGATGAAAG	127 GCCCAGGTAGAGGAACAAATCA	1327 GCAGAATCGCCTGTGGTTGT 2527
128	TCCCCAAGAGCACAGACTCAAC	128 GGAGAATATCAAGCTGTCATGGGATT	1328 GAGAAGCTAAATGGTCCCCAAGA 2528
129	CCTCTTCGCAGACCTTGAGGAT	129 CCAAACGAGGCCCTCTCTCTTCT	1329 GGACTGAGGGGCTCTCTGGAA 2529
130	TCCACACTCTACCATTTGCTGAAT	130 GCCCAGAAGAGTGCTTGACA	1330 GCCCCTGTAGTACATCCACACT 2530
131	TGAATTGTGCAGTGAGGCTATATCT	131 GGCAGTCCTTAACTCCTGGTTTTC	1331 CCTTTCGCATGAATTGTGCAGTGA 2531
132	TGTGAGTAGGATCTAGGCATGTTAGT	132 CCAGCACATTCAGTCCAGAATCA	1332 GAGCTCTAGCTGTGAGTAGGATCT 2532
133	GGTCACACAGAGTTAATGGAGACA	133 CAGCCAAGTGCAGACTGGAA	1333 CCTCTTTGCCAGGTCACACA 2533
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1072	AAGGGGAGCTGTGTGTCCTT	1072 AGCGAGCCCTGCAGCAAGA	2272 ATGCCGGGGAAAGCCAGAA 3472
1073	CCAATCCTACGACGGGACCAAA	1073 CAGAGCAGGGCTGTCATTGTCT	2273 CCATCTGTGCTGGCCAATCCTA 3473
1074	TGCTGATTGCCTGTGTTGCTA	1074 CAGGAAATCCAGGGGACAGAAGAA	2274 TTCCCACCATCCGAGACTTTG 3474
1075	GGGACACTGACCTGCACCAAAA	1075 GGCCAAACACATCAGGGAGCAA	2275 TGTGCCCTGGGACACTGA 3475
1076	GGACCCTTCTAAGCCACTGATCTCT	1076 TGGGGCTGCTGGGAGAGGAA	2276 GCTGGGTCTGGACCCTTCTAA 3476
1077	CTAACCCCAGCTCAACCTCTTG	1077 GGGTGGCAACCCTCAGATTC	2277 GGTGTGCTGGACCGAGATTCTAAC 3477
1078	GTGACACAGGCTGCTTCTCATC	1078 CTTGTTTGCCAGATTTACCTGTGT	2278 TGCACGCACTCTCCCTTATG 3478
1079	TTGGTGTAGGGAGCAGGAGAT	1079 GCTTGTGGTCCTGGGTGTCA	2279 GGAATGCTGCCACTTGGTGTAG 3479
1080	CCTCTTACACCCACTGTTCCACTAAG	1080 AAGGCGAGGTCACCTGAGTAAG	2280 ACACCCACCCATCTCTCCTT 3480
1081	GAAGCATTCTGGGCTGAGAGAGA	1081 CCAGAAGAGGTGTGGCCTGAAAG	2281 TGCCCCAGGCCAGAAGCAT 3481
1082	GGAAACCTGGTCCAAACCACAA	1082 CAGTCAGTAGAGGGCGCTGTT	2282 CCGACGGCAGAACGGAAAC 3482
1083	AGGCACCTGTTTGCCCGAATG	1083 GGATGGGAATGGGTAAGGCACAGA	2283 AGGGCACCAGAGGTGACA 3483
1084	GCTGTCAGAACCAGCCTGAGTAT	1084 GGAGCTTTTGACTGGGGTTCACT	2284 TGGCAGGTGCTGCTGTCAGA 3484
1085	GCGGTAGGATGTTAAAGGCAGCTT	1085 CCAAGGTCCCTGGTCTGGAAGT	2285 TGTTCCCTGCGGTAGGATGT 3485
1086	GGTTCAATCTCAGGCTCACACT	1086 GGGCAGAGCAATAGCCGAAA	2286 GCCCTTGTGCTTTGGGGTTCAA 3486
1087	GCATGGATCTGCGTGGAGACA	1087 GGCCCACGTGGTCAGATTGTT	2287 TCCTTACTGCTCAGCATGGATCT 3487
1088	CAGAACTGGGTGGTGTCGTTTTG	1088 CGAGAGCCTGAGCATGTACAAAT	2288 GGAAACCTCCTATAGTACGGCCAGAA 3488
1089	CAGACCACTTGAGAGCAGGTT	1089 GTTATCTTCTCACTGTCCTGTTCCTTAG	2289 CACCAGCCATGGACAACAAGT 3489
1090	GCTACCAAGGGAGGTCTCTTCT	1090 GCTTCTGCCCAGTGCTCAAGAT	2290 GCCTCCTGGTATTCCTGTGCTA 3490
1091	GAGGTGGGTAACAGACCCATCA	1091 GGCTCTGTAGGGCTTCTAGAATGTT	2291 CCGGGAAATCAGAGGTGGGTAAC 3491
1092	GGAAGCAAGCACATACTCACCAT	1092 GGGTGTAGAGCAGGATGGGTTA	2292 GTCCATCAGGAAGCAAGCACAT 3492
1093	ACTGCGTAAGCGGTGGCATA	1093 GTCCCCGAAACCCACACTGTATTC	2293 CCAGCAAGAGGAAACTGCGTAA 3493
1094	GGTATCAGCAGTGTGGGTTAAGGAT	1094 CCCAAAACTGAGGTGGAGCTT	2294 GGGCAGTAGAAGGTATCAGCAGTGT 3494
1095	CTCGGAAGTCCCAGCAGATCTTTC	1095 GTGACCAGTGCTAGCTGACAA	2295 GTGCCTTACTTCGGGAGCTTTC 3495
1096	GGTGAGGTTCACACCCTCCTTTC	1096 TTCCGGAGGTCAGATCCCAAGA	2296 TGCTGCGGGTGAGGTTCA 3496
1097	CTGCAGTCATGGCTGTGGAA	1097 CAGCTCATCCGCCCACAGA	2297 CCCAATCCGAGCTGCAGTCAT 3497
1098	CCCCTGCCTGTGGACCTTATATT	1098 AGGGAGCAGCGCGACTGAA	2298 TCCTCCATCTAACTCCTGCTCAT 3498
1099	GGGCATTGCCACAATGGTTT	1099 GGCAGTGGGGTCCATTTGAAACA	2299 CTGCCTTGATAACTCTGGGCATTG 3499
1100	GTGGCAACAGGACAGGGATT	1100 TCTCCAGAAGCCTCGCCTTGA	2300 CTGGGGTACCCAGGGATGT 3500
1101	TCTAGGACCAGGAGCCATCAGA	1101 GCCGCATGTCTCTACTTCTTCTT	2301 TGGGCCAGGGCTGTAATCTA 3501
1102	CGAGGGGTCTTTGAGGCAAACTT	1102 AGGCCGAGCAGCTTTAGTCT	2302 ACCGGCCGAGGGGTCTTTGA 3502
1103	TCCCCACCTCTCTTTCTTTAGAGTAT	1103 TCAAGATGGAGCACAACAACCAA	2303 TCCCTTCCCCACCTCTCTTTC 3503
1104	CCAGGAAAGCCACCAATGCAA	1104 GTGTTGAGCAGGCAACTTGTGA	2304 CCGAAAGCTCTGCCAGGAAA 3504
1105	GGGCTGATATAATGGAGGCAGCTTAG	1105 ACTCATCACCTTGGCATGCTT	2305 GTGCCAATTTTTGTGGGGCTGATA 3505
1106	GCCCTGTCCTGACTGACATTCT	1106 TCAGCCAAGAAGGTATTTGTTGGAA	2306 CCTCTCCACCAAGCACAGCAT 3506
1107	TCTTTGGCCACACAGATTTACTCTAC	1107 CAATGCAGGAGCTTCTCCACAAA	2307 CTGCCACTGTCCATCCACATCT 3507
1108	CAGAGTAGGTGTGCCGTAGGAA	1108 CCCACCAAAGGGAGCCAATTCT	2308 TGCAAAGTGCATGGCACAGA 3508
1109	CGCTGTCTCTCCATCCAAGTGTT	1109 CTTTGCCCTGGCATCTGAAAC	2309 GGTGGCGCTGTCTCTCCAT 3509
1110	CGGAGCCTTGGAGCTAGGTGATTA	1110 CTCTGGCACCTGGAAACACT	2310 TCCTGGCATCAAGACTGTGAAC 3510
1111	CAGGGTTCCTAGCTCTTTCCTGTTC	1111 TCATCCATCCTGAGCCAAATTTCT	2311 GTCTTTGTTTACCTCCAGGGTTCCTA 3511

1112	CATTCCCAAATGGATGTGGCTAGA	2312	CCATCTCTGCATTCCCAAATGGAT	3512
1113	GCTCCACTTTCGCCCTCACTT	2313	CCCTCTGTTTTCAGCTCCACTTTC	3513
1114	CTTTGCTCTTGTCTGCACTGAGTA	2314	CAGACTTCACCCACTTTGCTCTT	3514
1115	TTGAGCTGTGCAGGCTTGT	2315	TGGGCTGAGCGGCTTTCTTA	3515
1116	TGTGGCTACTTTCCCACAGTTTG	2316	GGGATAGCCCTTGTGGCTACTTTC	3516
1117	CAAAGGACAATCACAGAGCTGTTC	2317	TCCCATTGGAAAGTATGGACTACAAAG	3517
1118	GGAATGGACCAAGTTTGTCTTGATT	2318	GTTCAGGAGTAAGGAATGGACCAA	3518
1119	TGGATGTGGCCCGCATTTGT	2319	ACTTGGCAGGAAGGGTGGAT	3519
1120	CCCCTGCCCCAAATAGCATTTTA	2320	TCCACCCCTGCCCCAAAT	3520
1121	ACCAAGCACTGGGGAAACTAAA	2321	GGCAGACGGGATTCAGTTGCTA	3521
1122	GGCATGCTCCGCACCAAGA	2322	ACCATGCTGGCCCACAGT	3522
1123	CAGGCCATGTAAACGACTCTCA	2323	GGGACTTTCCCAGGCCATGTA	3523
1124	TGCTGAATGGTTTGCTCAGTTTG	2324	GTCTTGTGCTGAATGCTGAATGGTTT	3524
1125	TCGAGTCACTTACCTGCTCAAATG	2325	CCTGGCTCCATGGGTCACT	3525
1126	AGCAAGCGACGGTCCTCCAT	2326	TCTGGGGTGCCACCAGCAA	3526
1127	ACCTGCAGAACTGAGGCTCTCT	2327	GCCTCTTCATACCTGCAGAACT	3527
1128	AGTGTCAGGAGGGGAAACGAA	2328	GCAGGTGGAGTCTGAAGTGTCA	3528
1129	CCACTCCTTCCCTTATACCCTTGTA	2329	GTCCTCATTATCCACTCCTTCCCTTA	3529
1130	GCAAAGATTGCTGCCCGTTCA	2330	AGGCTGCAGAACAGCAAAGAT	3530
1131	TGGACAAGAATGGAGAAAGACGTT	2331	CTGGTGGGCCTTATGGACAAGA	3531
1132	GCCTTTCCCAGGACTCAACAGT	2332	CTGGGGCCAGAGTCTGATTCTTAG	3532
1133	CTGCTTTGAGGCAGATAGGAGCTT	2333	GCACTGGAGATGAGGCTGCTTT	3533
1134	CCTGCACAAAGTGGCTGTCAAC	2334	TTTCTGCCCCACCTGCACAA	3534
1135	GCAGGAAACCACAAAACATCACCTA	2335	CCAAAACTATCTCAGGTGCAGGAA	3535
1136	GCTTTCTCTCCCCTAGGACATACAGT	2336	CCTCTGGCAAAGCACCCATTCA	3536
1137	AGCCTGGCTCCCTACACATT	2337	AGAGCCAAGAGGCTGCAACA	3537
1138	TGCTATTGGGGCATTGGTGTT	2338	GCAGTGAGCCAGTAGCCTTTTG	3538
1139	TGCATCCCGTTGCTGTCAAG	2339	GCTCCAAGGTACTAGTGCTGCAT	3539
1140	GACCTGTGTCCCTTTGTCCTTAG	2340	GGCCAACCTCTGTACCTTCAGT	3540
1141	CCTTTGCCCTTCCAGAGTCTCCTT	2341	CACTTCTCAGTCTCTCCCCTTTG	3541
1142	GCCTGGTACTCTCCCAGTTTTAG	2342	CTCCTGGTTTGCCTGGTACTCT	3542
1143	GGGACCAGTCAACTTTGCCAGATT	2343	AGGGAGGGACCAGTCAACTT	3543
1144	ATTCCCATTCCTAAAAGCCGTGTA	2344	GCCCTACAGGTTTACATGCAAAGATATTC	3544
1145	TCCAGGCACGCACCTCTGAT	2345	GCTGTCCATGCAGAGCTAAGT	3545
1146	GGCAGGCATCTGTGCAGTCATT	2346	GAATGAACTGGCAGGCATCTGT	3546
1147	TGCCGACATAACCCCTTCCTT	2347	GGCTAAGGGAATGCCGACATAA	3547
1148	CGCCAATCTCAGTGGTTGGAAT	2348	GCCAGAAGGGAATCGCCAAT	3548
1149	CGGAGCAACTTGGCATGTTAATACA	2349	TTCCATCCCCGGAGCAACTT	3549
1150	GTGGCAGCCTTTCTGTAGTTGA	2350	TTGAAGGAAGTGGCAGCCTTT	3550
1151	GCTCGTTAAGAATTAGCCCCACATC	2351	GCCTTCCCTGGAGCTCGTTA	3551
1152	CACCAGCTAGAACCTCCTGTAGT	2352	CTGAAATGGCAGCCTTTATGTTCATC	3552
1153	GCTGCTTTGCGGGACCTT	2353	TTCCTCGGAGTGCTGCTTTG	3553
1154	GGCAGGAGTCAGTGACAAGTGGTA	2354	TGGAGCTGGCAGGAGTCAGT	3554
1155	TGTGTCAAGCAGATTTGAAGCTAAC	2355	GGGCTGTGAGGTTTGTGTCA	3555
1156	TGCACATTCCTTCCTTGGTGAT	2356	GCTCAGGGCTTGCACATTCCTT	3556
1157	CCTGCATTGCAAGCCGTTT	2357	TGCCCTAACTTCTCCTGCATTG	3557
1158	ACCTGCCTGTGTGGTATGGAA	2358	ACCAGGCCTTCCTTCTAGTCA	3558
1159	GAGCCGAATGTTCACTTATGGAGTT	2359	GGGTGAGCCGAATGTTCACT	3559
1160	CTGAAGCCTAATGGGGATCCAGAA	2360	CCCAGCCAGAGAGGTATCAACT	3560
1161	AAATAGCTGTCCCATTCTGGTTGT	2361	GCTACTTTGCAGCTACCACTGAA	3561
1162	TCCCCGGATTTCCACAGTAGT	2362	CTGAAGTATCATCTCTTCCCCGGATT	3562
1163	GGGTAGGATGCATGAGAAGAACTGGAT	2363	CAGAGGGGTAGGATGCATGAGA	3563
1164	GCCAGCTGAGGAACTATTAGACACT	2364	CTGATGCTAGATCCTCCTTCTACTTG	3564
1165	CTGCAGATCTAGCCAATGAGGTT	2365	GGCAATAGGAGCCTGCAGAT	3565
1166	GGCCAAGATTTTGGTGTTCAAGTAG	2366	GGGCTGTTGGGCCAAGATTT	3566
1167	ACAGCTGCGAATGGCCTAAT	2367	CCCAACTATTTTTGGGTTAGGAAGAAC	3567
1168	GATCTCATGGCTACGGAGATCAAG	2368	GGGTGACACCTGAGGTTCTTGA	3568
1169	CCTCCTGGGAAACTGGCAAGTAAAG	2369	CCCAAAATGCCTCCTGGGAAAC	3569
1170	GTACATGGTTATGGCTACCAGGAA	2370	GGAACATCTCATGGTCACGTATTTG	3570
1171	TGGTCAGTGCTCCCAGAGTT	2371	GGTCTAGGGTCTCAATTTGGTCAGT	3571
1172	CGCACATGTCTCGTGTCACT	2372	CCCTTTGGATGACTCTTCGCACAT	3572
1173	ACCACTGACAAACCTGTGCAT	2373	CCACTGCCACCACTGACAAA	3573

1174	CAGGGATGAATAAGCCTCATGAAAGA	1174	CACCGTGCCCAGCCTGTAA	2374	GAGTGGTTTCCCATCAGGGATGA	3574
1175	ACTGCAACACTGTGATTGTCTTCA	1175	GGCATGTTAGCGAGCATTTTACA	2375	GGCAAAACTGCAACACTGTGA	3575
1176	TTGGCTCCCCAGAGTGTTGT	1176	GTTCTCGTTCAGACAGTGGTTCT	2376	TGCAGGTTTCTCACATAACTCCTT	3576
1177	CAGTCGAAAGAGTGGAAAGGATGA	1177	CAGGAACCTCATCCTGAGTTGATTT	2377	TTCTCTCCAGCAGCACTCTTTATT	3577
1178	TGAAGCATCTGGCCTGTCTGA	1178	TCTTGCTGGACCAGAGTGTGA	2378	CTGTGGCCCATGAAGCATCT	3578
1179	TTCGGGGTGCATCCTCACAT	1179	AGGCCCGAGGGAGTTTGTTTG	2379	TGTCTGCTTCCTAGCGTCTTC	3579
1180	CATCCACAGCTGTCTCCGTGTA	1180	GGCCCTCAAAGTGGTGAAGAGT	2380	CACACATTTCAGGAGCTTGATCTCATC	3580
1181	TTCCAGGTTGTTTTCTGCAAAGTAG	1181	CCTTGCCTGAGCTGGTATTTCT	2381	GGAACCATGTTCCTTTCCAGGTTGTT	3581
1182	TCCCAGAGACACAGCTAGTTAAAAAC	1182	TGTGGCTACAACTCAGGCAAA	2382	TCATCATCCCAGAGACACAGCTA	3582
1183	TGCTGCTCGTACAGTGGAAAG	1183	GACATTGCTTGACTTGGGTGTGT	2383	CCTTGTGCTGCTAACCATGAGACT	3583
1184	GGAGCAGTATCATTTTGGTGCATTG	1184	CTCACCGCTCCCATGAATGAATC	2384	GGACCCTAGTGATTCTCCCATCAT	3584
1185	CGGTGGTGCCTTTGTTTTAACT	1185	TGTGTGCTGACAGGTTTGTGA	2385	CACACTTTTCGGTGGTGCCTTT	3585
1186	GGAGAAGGAATGTCAGGAAGCAAAC	1186	GCAACAGGAGCTACTTGCCAAA	2386	AGGAGCCAAGAACAAGGAGAAG	3586
1187	AGTAGGTGTGATGGCCAGGAT	1187	GCCCCTGTTGTTGTGCTGAT	2387	TGGCCATAACAGAGTAGGTGTGA	3587
1188	TGTCTTCCTAGCCTCATCTCTCA	1188	GGCTGCAGCATAAGGAATGTGA	2388	TAGCCCCACGTGTCTTCCTA	3588
1189	GCTAGGGGATACTGAGTCCTGCAT	1189	GCAGACCATTGCTTGGAGGAGATA	2389	ACTTGTTCACTTGCTAGGGGATAC	3589
1190	CCCAAAGAGTCCCCATCTGGTT	1190	CTGAGCTGGGAAGACTCAAACT	2390	CAGAGAGAAGGAGGCCCAAAGA	3590
1191	AGACCTAGGCGGCAAACAAA	1191	GCAACTTTAGGCCAGCTGAGTT	2391	TGTCAGTAACGGTGGAGACCTA	3591
1192	AGTTTCCTGGAAGCCAGCTATTAAA	1192	AAGACAGGGTGGGAGATGTAGT	2392	GCCTTTTAATTAGCCTTGCAGCTT	3592
1193	CGTAAGCATAGGGGCTGCAGTA	1193	CCTGGATTTGGGCATACGTGAA	2393	AGTGGGAGCCTCGTAAGCAT	3593
1194	GCAGTTGAGGGAGTTCAACTAAAGA	1194	TTCCCTGAACTCTTGCTGGTTT	2394	GGAATAGGTAGGTGCAAGCAGTTG	3594
1195	CCTAGGCTAGAAGCCCAGAAAAC	1195	GGGTTGAAACTGGAGGCTTGTTC	2395	ATCCACAATTTCCCTAGGCTAGAAG	3595
1196	CCTTCCCTTTGTCACACCACATC	1196	ACTCTGTACCGGGTATTTCACACT	2396	GCCTAGGGTTCTCTTCCTTCCCTTT	3596
1197	CCTGTGTTCAGGAAGAATGAGGAT	1197	GCCCATCCAGACCCTTCTAATTG	2397	TGGTTCCCCGAGCTCTTTTC	3597
1198	GAAGGAAACTGACTTTGCAGCTTAAC	1198	CAGCCTCGTGCAGCAGTTG	2398	TGGGTCTAGAAGGAAACTGACTTTG	3598
1199	AGGGAGAAAATCACACTCTTGCAT	1199	AGAACCCCATACCTGCATTACAAA	2399	GGGCTCAGGGAGAAAATCACA	3599
1200	GGGGACGAAGTCAAGGTGTAG	1200	TCCCCTGGAACCTCCAAAACT	2400	TGAGTCTTACGGGGACGAAGT	3600

FIG. 35

Target No.	Pool A Sequence (5' to 3')	SEQ ID. NO.	Pool B Sequence (5' to 3')	SEQ ID. NO.	Pool C Sequence (5' to 3')	SEQ ID. NO.
1	GTGTCGTGCCTGCTAGGTATT	3601	TCGGGAATGCTGAGGAAAAGAG	6287	CCTCTGCTGAGCTCCTGTGT	8973
2	TGTGCCAGGCCCAAAAGCTT	3602	GAGCCCTCTCCGTTTGTCACA	6288	GCCTCCACCACCACTACACA	8974
3	TCTGCTTCTGCCGGCATTC	3603	GCTGGACAAACTACTGCCTCTT	6289	GTGGAGGAAATTAGATGTCTGCTTCTG	8975
4	AGGAACTGTCAGATTGCAACCA	3604	CAGCTTCCCTCTTTTGCTCTGT	6290	GACACACACAAAAGGAACTGTCAGAT	8976
5	CCACCAGGTGCTCTCAACTCT	3605	GAACTCAAATGGCCTGTGAAGTC	6291	AGGGCCTGCTGACTCGTTCT	8977
6	TCCCACACACGTGTCTGTATCT	3606	CAACGGATGGAGATGCAACCAT	6292	AGTTCTGTCCCAATCCCACACA	8978
7	AGAAGGGCAAGGAAACATAACCTT	3607	ATCTGGGGCCCATCCTTTGG	6293	GCTTGAAAGAAGGGCAAGGAAAC	8979
8	CCACTCCTGGCCCTTTTCTCTTTT	3608	GCCTGGAGAAGAGGTAGATGTG	6294	TCCCCACTCCTGGCCCTTT	8980
9	AACTTGGGGCCAGGCTCTA	3609	CGATCTCCTTGTTTCACCTCTTTCC	6295	ACTGGGATTTGACTCTGGAACTTG	8981
10	CCACGATGAAAAACAGAAACCAAAC	3610	AGCCCCATGTCTGTGATGGT	6296	CAGCTAGAGCAACCACGATGAA	8982
11	GGTTGCAAACCCCTCCTCTTT	3611	CTCGCCAGCATGGTGACA	6297	GCCTGAAAGGTAGGTTGCAAACC	8983
12	GCCTTTGTGTAGACAGCCACTT	3612	TCCACTGGGCCCATCTCTCT	6298	AGAGTGATAAACCAAGCACCAACA	8984
13	TGTGTGCATAAAGGGTCTTGAAGA	3613	GGTGGACAAGGTGGAAAAGTCT	6299	ACAGCCCTTGTGTGCATAAAGG	8985
14	ACCCTTCCCTGCCACTGTTT	3614	CTAGTCTTAAGAGTATAAGGTAGGGTGAGA	6300	TCTGCACCAGTCTCAGGTCTTC	8986
15	TAGCCGTAGCTCAGATCCTAGCT	3615	TTTGCCCTTCCCAGACAAGACA	6301	TTTGCAGAAAGGGCTCAAACG	8987
16	TCATGGTGCCCTGGCCTCACT	3616	CCACCGAGCTCCTCCTGTCT	6302	GCCAGGCAGCCTCATGGT	8988
17	CTCCAAGCCACAGAACTCAACATG	3617	TGCAGGGAAGGACTGTTGTC	6303	TGCCTCCAAGCCACAGAAC	8989
18	GCCTCACCATGGCTCTGATC	3618	TCACCCACCATTCATGCAAGAG	6304	GGTACCAGGGGCAGAAGGT	8990
19	CCAGGTCCCCCCAAACAC	3619	GGGAATACAGTGCAAAGCAAGGA	6305	AAAGCCCAAGCTCCTCAACAAG	8991
20	TCCAGTGCTCACTCACTCTCCTT	3620	GCGCAGTAGAGAAGAGGCTTTTAG	6306	TGAAAACCTAAACCCTTCATCAACCT	8992
21	GAGTAGTGCATGCACACAGTGA	3621	CAAGCTAATTCTGCAGCAAGTATGA	6307	AAGCCCAGAGTAGTGCATGCA	8993
22	TGGGCCTCATTCCCAAGTTC	3622	ACTCTTAGTTAACACAGCCATGGTAT	6308	GTTGACAAAGTCTAGATAACAAGGAGTCA	8994
23	GGCTTCTGCAGAATAACATCCTGATT	3623	ATTGTGGAGGCCTGAAATGCT	6309	TCAGTGGCTTCTGCAGAATAACA	8995
24	TCCAGCCTCAGCCTGGTT	3624	GGGCTGTTTCCATAATCATCCTACCT	6310	GGAGTCAGACACAAATAAATGGGTTCAG	8996
25	TGCATGGCCAGACAATATGGT	3625	TCACAACCAGTTTTCACCAACCA	6311	GGCTGGGAAACTGCTATGCAT	8997
26	GAAAGTTGTGTTCCCAAGGCTTTG	3626	GAGCTGAGTAAAGCCATGTGTCA	6312	CAGATACATGGAGGTGAAAGTTGTGT	8998
27	GTATGACTCCCCTTAAGGATGAGAAG	3627	GTCACCCATCCCACCTATTTACTTG	6313	CACTGTGTATGACTCCCCTTAAGG	8999
28	AGCAGCATGATGCCAACGAA	3628	AAGGAAATAGACCCATTTAGCTTCAGT	6314	GCTCCAAAATAACTAGCAGCATGATG	9000
29	GGCTCATCTTTGCCAGTTATTTCCA	3629	GGAAGTTCTGGCTCTGTACCTGAAA	6315	ACAAGACTACAAAGAGGCATTGAACT	9001
30	GCTGTTCCATGGAGCAATCACTT	3630	TCCGTGTGCTCAGCTTGACT	6316	AATGTGAGGATGGCAGGCATTG	9002
31	AAAAATGGCCCAGTTTTGCAGAA	3631	GTTTTAGTCCTCCTTATAGCCCATTGG	6317	GGCTGAAACTAAAAATGGCCCAGTT	9003
32	AACAATCCCCGGCCCAATCCT	3632	TTTGGTCACCAGCAGTGGAAGT	6318	AGGTCAGGGATGACAACAATCC	9004
33	TGCCCAGGCTTTCCCCAAT	3633	GCCAGTGGTGGATTCAAAAAGCA	6319	TGCCTGGCACACATGATGT	9005
34	TGCTGTCTGCCTCAGGATGA	3634	GCTGCTGGCCCTTATGCCTGAAG	6320	TGTTCCCATCTCTTGCCATCTC	9006
35	GTGGGACAGACAGCCCTTGTT	3635	AGTCTCTCAAAGCCTGACGTTTC	6321	TGCCAGGGTGGGACAGACA	9007
36	GTTTTCCTGTTCAGGTGACCTAGTC	3636	GTGGAATAAGATGGAAGCCACACT	6322	CTGGTGAACCACAAACAATTAAATGTTT	9008
37	GAGAGCTATACAGAATTGTGGCTTTTC	3637	AATATTACTAGCCACACTGCTATTCCTT	6323	GGTTGCCTGAGAGCTATACAGAATTGT	9009
38	CCCGCAGTGATTCCAACATAGA	3638	CGAGGACCCACCACAGTCT	6324	AGGCACCCGCAGTGATTCC	9010
39	TGCAAACTGCGACCTGAACT	3639	GAGTGGGATAAATAAAGGCTGTAAAGAG	6325	ACTGGCAGCAAAACTCAACCT	9011
40	AGCTGGGGCTCTGAATGTGA	3640	TCCAATCCCACCTCTGACAATG	6326	TCCTCTACCCTGGAGGCAGTAAA	9012
41	CCCAACTGTCCCCTCTCACTA	3641	CCTGCTTAGTGGCCTATGTGATCA	6327	GCTGCAAGGCCCAACTGT	9013
42	CTGGTTTGAATGTCTTGGATTGCTTAG	3642	TTGTCCCCCCAACCCATTCA	6328	GAGGACTGACAGGAGATGTTAGTAAGA	9014
43	TCAACAGGCTACCATTCTTTGGAT	3643	GGCAACACTCCGCTTCACTATT	6329	AGTACGTATTCAACAGGCTACCATT	9015
44	TCCCCCAAAGAGGAACTGCTT	3644	GGTAGTAAGATTAAGAGCTCTGATTGCA	6330	ACTGGAGGCATTTTCACACCTT	9016
45	GGAATCCCCTCCCCTGTATTTATTG	3645	CCAGGAAAAGACACCCCCATACA	6331	TGTGCTAGCGTACCCAATGG	9017
46	GAAATGTCCTCTCTGGTCCAAGAT	3646	GAATGGTTGGTATCTAGGCATCACT	6332	GGAGAGGACCACACAAAGAAATGT	9018
47	TGGGCTACTGGCACTGAGT	3647	GAGTGCCAACAGCTCAGCTAAT	6333	CCTAAAGTGGGTGGGCTACTG	9019
48	CACGGTTAAGTTGCACCCAAAC	3648	GCATCAAACTCAACTCAAATGGAATGT	6334	TGTCTCAGATAATGTCACGGTTAAGTT	9020
49	AGGAGGTGGTGAGTGCAGAGAA	3649	TCCACATCCCCTTTCCACCTT	6335	AGACCCCTGGTTTCATAGAGCTT	9021
50	GCTGGGCGAGCTCTTTGG	3650	AGGCACCCACCTCTAGACAGTA	6336	AGCACACAGGCAGGAAGGT	9022
51	AGAGTGAGGAGCAGAGACTTCA	3651	GAGTGATGGTCAGGCAGTTCATAT	6337	AACCACCCTGGGAGTAGGAAT	9023
52	TAGGCAGAGGCATGCCTGAAT	3652	TGCTTCCTGGGCCTGTTTTGT	6338	CATGAGTCCGAGGCCACCTA	9024
53	TTGGGTGCCAGTTTGGACCAA	3653	TCGGCAGGGAGGGAAGCTTT	6339	AGGGGTTGGGTGCCAGTT	9025
54	CCCCAGCTGTCTTCACTGTT	3654	CCAGGGTGCACTCTCTTCCT	6340	TGATGGCCCCAGCTGTCT	9026
55	GTGTGCTCAGTGGACAAGCA	3655	AGCCCCTGCTGAGCTTATCCA	6341	GCCCCAGGTGTGCTCAGT	9027
56	AGGACAGGGGCAGGGAGTAAT	3656	CACACCTGTTATCACAGTCTTCCTAGT	6342	GTGGAGGCCTCGGGAGAT	9028
57	GGAACCACTTATCCAGAACCAGAA	3657	AAGTAGCCTAGATTTGCCTGCTT	6343	ACTCGAGATGGAACCACTTATCCA	9029
58	GAGGCTGACTCCTGTTGCAT	3658	ATTTCCTCGAACCACTCGCTAAA	6344	TTGGTGTGACTGCCGCTTTT	9030
59	TGGAAAGCTACATTCAATTCAAAGACAAA	3659	CAAAGCCCTCCTTGACTTGCTT	6345	CAGACTTTAAGAATGGAAAGCTACATTCAA	9031
60	CTCCAAAACTGATCCCGGCAAA	3660	TTGGCTGACCCCTGGTGTAGT	6346	AACCGGGGTGTCCTTGGAATT	9032
61	TGATAAGGGCTGTGCTCTTCTAAG	3661	TGCCCTTAGTCTAGCACAGCTT	6347	CCACACTTGCCCCAACTTGATAAG	9033
62	AGATACCGAGTGGGTTTATTCTTCTC	3662	CCCATCAGGAAGGCTGAGATG	6348	GCAGTTGTAGATACCGAGTGGGTTT	9034

63	TGTTTCCCCATCCATCAACCATATC	3663	GCTACTTCCCCTGCATATCTATTAGG	6349	CCAAGATGTTTCCCCATCCATCA	9035
64	TGCCCATGCCCACATCCAT	3664	GGAAATTAGGTAAGCATTTCCTGAATGG	6350	TCACCTGCTCCCTTCCTTTCT	9036
65	AGGCTATGAACCAGCTCTCCTT	3665	CCAAGGAACAATTTTCTGTCAGTGAGA	6351	GGCTGCAGGCTATGAACCA	9037
66	CCACACGATTCAGAAAGAGCCTTA	3666	GTGATCATCAACTGGATTGCACAAAG	6352	AGTCCTGAAAGACAAACTACCAACAT	9038
67	CACCTCTACTTCTTCAGGGACAAG	3667	CAGGCATGCTCTCCAAATTCTG	6353	CAAGTGTTCAGTGTGAATTTAAGGAAATC	9039
68	GCTATGCCCACACAAGTACAGA	3668	TGGGAGTCTTTGCCAGTGGACTA	6354	GCACATTCAAGATCAGAGCAAAGAT	9040
69	GCATCTTCATTGATGGGAACTGTAAACC	3669	CTGGGGGAATAGCTATATCTAGTTGGT	6355	TTGTCCTCAGACCACTCTCTTTTG	9041
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2666	GGCAGAAGAGTAATGGACTGCAT	6266	TGGCCCACTGTGACAGCTT	8952	AGCTTGAAGGTAGGAGGCAGAA	11638
2667	CTCAGTTCACACAAAGGATCTTCCA	6267	CTGTTGCCTAAATCCCTTTCTACATC	8953	GCCATATTCTCTCAGTTCACACAAAGG	11639
2668	GTGATTACCACACCAGTTAAAGAAAGAAC	6268	ACATTTGCCTTCAGGAAGATCCTTAG	8954	CCTTTCAGGAATAAAAGTTGTGATTACCA	11640
2669	AACTGAGCTGATGGCAGTTAGG	6269	ACAAGTGGATTTCTATGTGACTCTATCA	8955	ACAGGACAGGGAGATTATTTCACTAAC	11641
2670	CTATTGGCAGGATCTTTCTGTTTGTAG	6270	TGGCCTCAAACGCACACTGT	8956	CCTGAAAAGGAAAATCTGGCCTGAT	11642
2671	TTAATCTGTCTTACACTCTGCTGACA	6271	CGGTGATCCTGTACATGGCTAATC	8957	GGGATGCTAACTGCTCTTCTCTTTGG	11643
2672	GGAGGCCCTCCATTCTTTGTT	6272	GCCCCATGGAGAGGCCTATA	8958	TGCACGGAGGCCCTCCAT	11644
2673	CACAATAATAGCTTCTTACTTGTGCATTTC	6273	CAAGTAAGGTTTAAGCCAGTGATCAG	8959	GCTGAACTCACAATAATAGCTTCTTACTTG	11645
2674	AGCCTTCCAGATCCCCTTGTT	6274	GCAGGAATGTTCAATCGCTTCCT	8960	AAGCTCTGCAGCCTTCCAGAT	11646
2675	CCAGTTGACTACCATCACAGATACAAGATG	6275	GCTTTGTTACCCATATGGTCTAGTTG	8961	ATCTTTCTCTTCCTCCAGTTGACTAC	11647
2676	GCATATAACTGTGGTGGGTAATTGTG	6276	CAGTATTTCAAGTGACTGCAGTTATACAG	8962	CCAGTTATAAGCAGATGAGGTGTATTCAT	11648
2677	CCACTAGAATACAAATCCACTTCTGACA	6277	TGTAGCCATGACTGAGGCCTAT	8963	CCCAGGAAGCCACTAGAATACAAATCC	11649
2678	AGGGTGACATGCTTCACAGATC	6278	ACTGATTACTGAATACTGGCCTTAATCT	8964	ACCATAATGGCAGGGTGACATG	11650
2679	AGGTTAGTAGTCAAGGCCTATGTTTTAC	6279	AGGAAGGGATATGAAACATGGCTTAA	8965	CTCCAAAGCTTGTTCACTTCCTACT	11651
2680	GCAACCTATAGCAGCCTGGATT	6280	CAGGGTTCCAATATGGCCAACA	8966	GCTTGCCCTTTTTCGTTCATTGT	11652
2681	AAAGGCCCCAAACCCTTTGCT	6281	CCAACATATGGTTTCAGAAGCACAAA	8967	TGGTGAAAATTAACACGGAAATTCTAAAGT	11653
2682	CCCTCATTCTATGGTATTGATTCCACAA	6282	ACATGTTGGTGGGCACAGAA	8968	TCTGTCTACCCTCATTCTATGGTATTGA	11654
2683	GGGGATCACAAAACTGTCATTCTG	6283	CCCTGGCCATCAAGGAGCTA	8969	ACCTACTGGGGATCACAAAACTG	11655
2684	GCATTATTCGGATACTACAACCAGACGAA	6284	GGATATTGGCCTGTAGTTTTCTCTCCTT	8970	TCCAGGACCTGATGACTTCACT	11656
2685	TGAAAGACAATTTGCCGGTATGTTAC	6285	GCAATTGCTGGACCATATAATGAGACAA	8971	GCATAATGTTGTAGGCACTTTGAAAGA	11657
2686	CCTGAGGTCCCCATGAAGAGCTATAA	6286	GGCAGAGTGATCATTGATTGATAACCA	8972	AACACCCATTAATCCTCATGTTTTCC	11658

FIG. 36

Target No.	Pool A Sequence (5' to 3')	SEQ ID NO.	Pool B Sequence (5' to 3')	SEQ ID NO.	Pool C Sequence (5' to 3')	SEQ ID NO.
1	GGCACAACATTTGGGAGACAAA	11659	CTCACTAAACTTTTCCCCAGAACTCA	22643	GCTTAAGCCACAGAAAGGCACAAC	33627
2	CCTGGAACCTGAAAGCTTGCAAAAAG	11660	CCTCTGAAGGGAGACTGAAACAAC	22644	CGAGTGCCCTGGAACCTGAAA	33628
3	ACAGGGTGCTCTCCTCTTGA	11661	GCAAGAATGCTTGGCTGCAA	22645	AGCCACCACTCCTGCTTCTGA	33629
4	GGAAGCAAACAGGTAGAGCCTGGTA	11662	GTGGCATTTCACTGCCCTTTG	22646	TTGGGGACCCCTGGACTAAG	33630
5	CTACAACAACAGAGCTCCAGGAT	11663	GCAGCTTGTTCAATTGTGGTTCA	22647	CCTCCCCAGTATCTTACCACTACAAC	33631
6	TGTCCTTGGTCGTATGTGAGAGA	11664	GTGAGGATGAGTTTTCAGTGGCTATC	22648	CACCAAGCCCTCAACTCCTAAA	33632
7	GCAGGAACTGGCTTGCCTTT	11665	CCCTGGACAAAATGTAACAGCCAAAGT	22649	GAAGTGAAAGAAAATGGCAGGAACT	33633
8	AGGTTTCGGCTTTAGTTGTGTGA	11666	ACCTAGCTATCCTTCCAGCATTTC	22650	GCTGGTATTGTTTTGGGGATAGAGGTT	33634
9	TGTCTGCTTCTGCCGGCATTC	11667	GCTGGACAAACTACTGCCTCTTC	22651	GTGGAGGAAATTAGATGTCTGCTTCT	33635
10	GTCCCTCACACCTTTCTACCAGAAG	11668	GCCAATAGCTGCTTGATGCAAT	22652	GGGCAAGTCCCTCACACCTTT	33636
11	GGCTGCAGGAGTAGAGGTCTAAATG	11669	TAGTTGCCCGGGATTCCCAAGA	22653	TGAGGTCTGGCTGCAGGAGTA	33637
12	GGCAAGAAGAAAACTGGAGCCAAGA	11670	GGGAGAGGCTGGTCCTGAGT	22654	CTGAAGCCCAAGTGGCAAGAAG	33638
13	GAGCCTGAGTTCTTGATCACTACA	11671	GTTTGTGAAAGGTCAATGTGAGGAA	22655	AAGTCCTGAGCCTGAGTTCTTG	33639
14	CCTGGAAAAAGCCCCGGTAGA	11672	TCCTCGGGAGCTCACAACCTTA	22656	TCTCAGGGTGCCTGGAAAAAG	33640
15	GCAATGGTCCCAGTGAGAGTT	11673	TCCCACTGATGCCAGACCAA	22657	GCACTGCAAAAGAAGAGGCTATTG	33641
16	GCCCTTTGGAACCTGACCAA	11674	CCCAGCTTAAGCAAAATAGGGCAAGTA	22658	TGGGAACCAGCCCTTTGGAA	33642
17	GGCCTTGATTTGACCCTTCTTTTC	11675	TGGCCAGTAGTAAGTTGAGAAGACTA	22659	GGCTTCACTATCAAGAAGGGCCTTGATTT	33643
18	CCACCCGCTTCTGAAACACATC	11676	CCGGAAGCTTCACGTTCTGT	22660	AAGAGGCCACCCGCTTCT	33644
19	TGGCGCTAAAGCCCAACA	11677	GGAGGGAGAGCTCCATCTCTGA	22661	TGGAGCAGGCTGGCGCTAA	33645
20	CCTTCCCCGCTTCTGGATATGAAA	11678	CCGGCAATAGGACTCAGCAA	22662	CCGAAAACCTTCCCCGCTTCT	33646
21	GTCAGCGTTTACGGCTATTGAGT	11679	TAGGGCAGGTGTCCTCTGCAT	22663	GGAAGGGGAATTTGTCAGCGTTTA	33647
22	GCATCTCCAGTGCCCTCTGT	11680	GTGAACCAAGCTATACTACCTGCTTTTC	22664	GCAGTCTCAGCCTTTCCTTTGCAT	33648
23	CATCTTCCTAGACATGCACACAAC	11681	GGCTTCTGTGCTCATAGTTCACACT	22665	GTTGGGAAGCATCTTCCTAGACAT	33649
24	GTCCCAAGTCAACTGCCATCT	11682	GGGCACACGGAGGTGAAAAAGT	22666	GCCTATTTTGGAGTCCCAAGTCAA	33650
25	GCGTCAGCACAGCTAACACA	11683	CGGGAGCCGTTATACCAGCTTAG	22667	TGGGCCACGTTGCCTACT	33651
26	TCACAATGGGAGGGAACATTCAA	11684	GCTTCCTATGACCAAGTCCCTTT	22668	CACCTTATTACTACCTTCCTCTCACAATG	33652
27	GGTAAAGGCTGGTTGTAAAGTCCACAT	11685	GTGTTGGCCAGACCCACTGT	22669	ACTGGGGTAAAGGCTGGTTGT	33653
28	CCGACTCAATGGGGAACTCCTACT	11686	GGGCACTTTTGAGTTGGGCTTTG	22670	AAGCCTCGTGGCCGACTCAAT	33654
29	TCCTTACACCTTGGGGCGTCTA	11687	GCAGCTCGGTGCCTAATGGTT	22671	AACAGGGCGTGAGTTTTCCTT	33655
30	GGGCATTTCTAAGACACTTGGACAGT	11688	CAGACCTGGCAGGATCAGAATAGT	22672	GGAGAGGCCAGGGCATTTCTAAG	33656
31	CACTGTATCTCAAAGGGCAAAACAA	11689	TGCAATGCAGAGGAGAAAGGTT	22673	CAGCCTGCTAGGAACACACTGTA	33657
32	ACTTGTCTCTGCAGACAGTTTCA	11690	AGTCACAGGTATCCTGACTCTCATA	22674	GGTGATTCTGGCCGAACTTGTCT	33658
33	GGCCTCTTGGTGCCTCTGTT	11691	GGAGCAATCAGCCTTGCAGAGA	22675	TGGATCGGAAACACCAGACTCA	33659
34	GCTAGAGAAACAAGACAACATCCAAGTTC	11692	TGGCGCCCGGAAAACCAT	22676	CCTGAGACCGTTGCTAGAGAAACAAG	33660
35	GCGGGCATAGAGAGAAGGAAAT	11693	TTCAAGAGCATCCTTGCGCTAT	22677	TGGGATGCGGGCATAGAGA	33661
36	GGCTTCAGTCCTTCACCTCTTTC	11694	ACCTTGAGAAGGTGGTATTTGTGT	22678	TCTCCCAGGCTTCAGTCCTT	33662
37	TCAGAGTCCTAGGCCAGCAT	11695	AGAAAGACCCCAGGTAAGAACCAT	22679	CCAAGTTATGCCTGCTGCTCAGA	33663
38	GGTTCCTCATGGAAGACTGGTTTG	11696	CACTCACACATCCACAGGAGACA	22680	GGGAACCTGGAAAGACCTTTACTT	33664
39	GTTGAGAAGGGAGTAAGATCAGACAA	11697	GACGCAGTATCCCACAGAGCATT	22681	GGTGGGATAGTTGAGAAGGGAGTAA	33665
40	CTCCCGCCTCTAAATGAATGGAT	11698	ACCAGCTCTGAGTCAGGAAGT	22682	CCTCAGCTCCCGCCTCTAAA	33666
41	CATTCCCTCCCCTACCCTGGAAA	11699	CCCACAGTCCGACGAGTGTTTT	22683	GGATGAGCATAAGCAGTGGTTCA	33667
42	GAGACTCAATCCTGGCACTTGT	11700	TCACTCCACTTTACAGGCACATATT	22684	AGGAGTGAGAAGGGAGACTCAATC	33668
43	CCCGGCTGCCATTTAAGACCAT	11701	ACGAGGATCAGACAAGTCACAGA	22685	TCAAGTCCCGGCTGCCATT	33669
44	GATGAGGGCTGAGTATGGTCAAG	11702	CCCATGACCCCAGGTTGGAATTG	22686	GACAGAGAGATGAGGGCTGAGT	33670
45	CCCTTTCCTGAACCCCTTCATT	11703	CACTCTGTGCAGTTGCTGGTT	22687	AATTGCCAGCTGTATTCTCCTTCT	33671
46	GGGGCACCAAAGAAAAGACAAG	11704	GTCTGTGGAGCACACCTTGGAA	22688	ACAGAGGATGGGGCACCAAA	33672
47	CATCAGTACCCCACATAGGTTTAACT	11705	GCCTTTCCACTGAGCCATGA	22689	GCATGAGGATGGAATGGACTCATCA	33673
48	GGAGCTCAGTATGAGTAGAGGGGAAA	11706	CAGCCTGGTTGGTTAGATTCTGT	22690	GACCTTCAAGGGGAGCTCAGTAT	33674
49	TTGTGCACGACGCTGGAATG	11707	CTTATGTGTGGCTTTGGCTTCTTG	22691	TCTGATATGGAACCCAGGGATTGT	33675
50	TCGCTAGGCCATTCCTCCTGTT	11708	GGGGCTGAATCAGACAAGGAA	22692	TGCACCATCGCTAGGCCATT	33676
51	CACGACACCCTTAGATAATGTTGGAT	11709	CACAGCTTGGAAGGCCTGAA	22693	GTCAGTTCACACGACACCCTTAGA	33677
52	TGGCACAAGCCCAGGTCAT	11710	CTCATGATGTCAAATGCTGCTCAA	22694	AGCCTGGAAAGCTGGCACAA	33678
53	GCCTCCTCTGCATCTCTGAACCAA	11711	GTTCTGGTGACAGACAACCTTCT	22695	TTCCCAGCCTCCTCTGCATCT	33679
54	TGCAGCGATCCCTTCACCTT	11712	TGAGCAGCACAGTCCAATTCT	22696	CTCAAAGGGGAAGAACGTCAGT	33680
55	TGGTAGTCAGGCCTAGTGACTT	11713	CCCCTGGGAAAGTTACTGTCGAT	22697	TCTGCGTCTCACTTCCTGGTA	33681
56	GAGGTCTTCCAAAGGCTATTCAGA	11714	GTCAGTGAGCTGCCAGTTTTC	22698	AAGGTGACAGAGGTCTTCCAAAG	33682
57	CCTGAGCCTGTCACACTATTTCT	11715	TCCCAAATCCTGAGCCTGTCA	22699	TCCCAAATCCTGAGCCTGTCA	33683
58	GAGAGCAACAGGCGTTGAGA	11716	GCCAACCTATAGACACAACTTTGGTTAC	22700	GGAGAAAGAGTGGAGAGCAACA	33684
59	AGCCCTACAAGGTCAAGTTTTCA	11717	GTTGACTTCCGGCAGTAAGTGATGA	22701	GACTATACGATACCAAATAGCCCTACAAG	33685
60	GGAGGCACTTCACTCCTCCTA	11718	ACTGGGACATTTCTCACGTTATCA	22702	GTCACAAGGGAGGCACTTCA	33686
61	GTTGGGGTCAACTTTCCCTGAAG	11719	GGAATCCAGCCCATCCTCACATC	22703	AGAGCTGCCCTGGAACTGAA	33687
62	GAGGTTTGGCTCCATCATCAGA	11720	ACTGCACCACCACACGGAAA	22704	GGAAAGGTACCCCAGAATGAGGTT	33688

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63	GAGGCTCATGTCTCTTCAGCCTTAG	11721	CCTTCAAGGGCTTTCTCTCACA	22705	GGTGGTAGAGGCTCATGTCTCTT	33689
64	GTTGCTTCCCGTTATCCTCACT	11722	ACTTCCACATGGCTTAAACAGAGA	22706	TCCCCAAATCCTTTGGCAATGT	33690
65	GGGCCTGTTCTTGTTGATAGTGTT	11723	TCGTGAAGAAGATGGAGAGGAGTTA	22707	GTGTCAAAGGGCCTGTTCTTGT	33691
66	GTTTGCTGTTACTGAATCCCGAATCT	11724	CTGGGACCAACCCATTTCACA	22708	CTCTCATCAGCTGTTTGCTGTTAC	33692
67	GCACATGTAAAAATCCTCCCTACTGT	11725	AAGGAAGTGGGGTATGGACTGA	22709	GACCTCTTTCTCCCTGCACATGTAA	33693
68	CCTCTGGTAATAGCACCCAGGATAC	11726	TGTTGGCCACATACATTGTTCTCA	22710	CCTTCCTTGAACTTGCCTCTGGTA	33694
69	CTGAGGTCCAGAATCCAGATGTTCT	11727	CTCCTGCCTTCGGGGATGT	22711	GCTGTCACCAAGACTGAGACTGA	33695
70	GGACAACAGGATCGGGGATGA	11728	CACAGGCCAGAGATTCACCAT	22712	TGAAAGCTATTGGGACAACAGGAT	33696
71	CCATGTGTAGGAAGCCCTGTGA	11729	TGGGAAGCCTACCTTGACTCT	22713	CAGGAAGATAAGTACTGAGCCATGTGT	33697
72	GCGACCTCACTTTTCCAGTCAT	11730	AAGCCCCTGAGAATCTAAAAGCAA	22714	GCACATGTCTGCGACCTCACTTT	33698
73	GTGCTATTGTGGCTCTGTAGGTTGA	11731	CGACAGGGCCTCCCAATTC	22715	CAGCAGAGCAGAGTGCTATTGT	33699
74	GTTTCAGCCTGAGAGGCAAGA	11732	AGGTAGACACCGGAAGTAATCACA	22716	GCTGTTGCACTGTGAACTTGT	33700
75	GAGATGAAGCGTGTGACCCAAGT	11733	TCAGGTAGAGGTCGCCAAGGTT	22717	GCAGCAGACAGCTGGAGATGAA	33701
76	TGCCTCATCTCTGCCCCTCTTT	11734	GGAGGCTATGAGAAGTGAGGTTTG	22718	CTCTGCTGTTCCCTCACCTGTAA	33702
77	TCTCAGTGCGTTTGGAAGGAAA	11735	GGGCGGTTAAAGAGCTTCAGAGA	22719	TCTGGCTTCTCAGTGCGTTT	33703
78	AGGTTTCCCTGGACACACATTC	11736	TGGTCTTCTTTATGCCCAACAAAC	22720	TGTCAAGGGACTAAGTTTCTAGGTTTC	33704
79	AGGAGACAGGGACAGTTTCAATG	11737	CTCACACGGGCTCACATTCT	22721	GGCTCATGCTTGTGATAGGAGACA	33705
80	TGGGTTGGAAACACGTCCTTTA	11738	CCTGCTCACCGATGCTCAA	22722	GCCGAGGAGAATATGGGTTGGAAAC	33706
81	GCCCTTAGCAACAGCTGCAT	11739	GGTCAGAAACAGAGCCTGTAAAAC	22723	GCATCTTGTGGTTGCCCTTAGCAA	33707
82	GCAGACACGCCTCTGGCAAAT	11740	CCTGAGACAGAAGCAGTAGCGTCTT	22724	GTCCATTGGGCAGCCTTCT	33708
83	GCCAAAAGTGACAGCAACACA	11741	CGCAAAATGGGCTCCTGCTT	22725	CCTGATGCTCCCTCTGCCAAAA	33709
84	GCACACATCCAATCCTTCAGTAAATC	11742	GCCGGATTAGGGGTGAACTTTG	22726	GCCTCATTCTCGCACACATCCAATC	33710
85	TCCTCATCCAAGCTCCTTCTCT	11743	GGAGGTATTCTGGCTTCCCTAAGA	22727	GCCCTTCCTCTTGCTGAACAGT	33711
86	GGCACTTTCCACCCAGAACTTAAC	11744	GGAAGGGTGAGTTTAGCACAATGGTT	22728	ACCTGAAGCCAGGCACTTTC	33712
87	AAGCCCACGTTGTGCTCTTG	11745	CCCACTTGCACAAATGCAAAGA	22729	TGGGAGAAAAAGCCCACGTT	33713
88	CTCAGTAGGGTGAAAAGGATGGAAGT	11746	AGCTGTGGTTTTCCTGTGTCA	22730	GCAAGGCTCAGTAGGGTGAAAAG	33714
89	GCTGGATCTACAGAAACCAGCAAA	11747	TGTGTGTGTTTAGCAAGGGATTTC	22731	GCCAAACTGCTTCTTGCTGGATCT	33715
90	TGGCTGCAGATGCTTGTCA	11748	CGTTTGGCAACGGAGATGTGT	22732	GGAAAGGCGGTGGTAATGTGGTT	33716
91	GCCTCTCAGCCTCACGTTCTTA	11749	TGCCCACCCTTTTCTTTTGCTT	22733	TCTGACCAAGTCCGCCTCTCA	33717
92	TCCTTGACTCACGCCTCTGA	11750	GGGCTATACATTTCTGGGCTCAA	22734	CCTCACACTTTCCTGTCCTTGACT	33718
93	GGAGGACATGGTGTTTGAGGAT	11751	TACATTCCCCTCTCCCGCAACA	22735	CAGCTGATCTATGCATTGGAGGACAT	33719
94	CTACAGTTAGAGTCACACGTCACA	11752	CCAGCCAATCAGGATCATGGACAGA	22736	GCATCTACCCAATCACTACAGTTAGA	33720
95	CATGCGGAGGGACTTAGCAA	11753	GGCCCATGACTCTGCTGGAT	22737	CTCATCAGACACAGGCAGCTT	33721
96	GCTTTCCAGAGTAGAGGCGTTTC	11754	GGAGGCATCTCCGGCTACA	22738	AGGGCAAAAGCTTTCCAGAGTAG	33722
97	TGATGGTGCAGAATTGCTACGATA	11755	AGTGCAAGGTTGGAGAATGAAAAG	22739	GCAAAGTTTCATGGCCAAGTGTCA	33723
98	GCTGAAATGGACCCACAGAGA	11756	CGAGTTGGCCTCGTCTTACCAA	22740	TGCTGCCAGGCTGCTGAAAT	33724
99	TGCTCCAACGATGGCTCTTC	11757	CGTGGCAGCTGGCTTTAGAATC	22741	TCCCCAGGCACCTGCTCCAA	33725
100	AGGTGAAAGCGATGTCTGCTTAG	11758	TGGTGCAGCCACCTCTCTTATC	22742	TCACCTACCTAGGATTAAAGGTGAAAG	33726
101	GCCCTGACCTCACCATTGGAA	11759	CTCTGGCACATGCGTTAGTTG	22743	GGACAGGTTGGTGCCAGATT	33727
102	TGGGGCAATTGCGGGTATT	11760	GGGGCTTTGGATAACACCAAGGTA	22744	AAGGAGGTCCTGGGGCAAT	33728
103	GCATTACAGGAGAGTGAAGATGGAT	11761	TTGCAACCCCTCCGACATTC	22745	CTTGCCTAGGAAGGGCATTACA	33729
104	GGAGGAGAAAGGTGGTACACTTG	11762	AGGAGAAGTTCAGGGGACCAA	22746	GTTGCAGCCTGGAGGAGAAA	33730
105	GGAGACAATACAGGTTGGGTGGTT	11763	AGAGCTGCTATCATGATGTGGAAA	22747	GCTGGTCAATTCTGTTAAATCAAGGAGACA	33731
106	GCGGATCTTCCTTACCTAGTCTGA	11764	AGGGTGAGTCTGGAAGAGGTT	22748	TGGGTGAGGGCGGATCTTC	33732
107	GGATACTGTGTAGGAAGGCAATACA	11765	CCCCACTCCCCTCAAGGTATGT	22749	CCCAGAGAGGCTGGATACTGTGTA	33733
108	CAACTCCAACCCCAGCCTTTTG	11766	GTCACCCTCTGAGGTACACACT	22750	CCTGTGGCTCCTGAGAGAGAAATCAA	33734
109	CAGGCCAAGAATGACATACGACTT	11767	ATGACAACATCACGGACAGAGAA	22751	TGGGACACTTCAGGCCAAGA	33735
110	GGCGAAAATGAGCACTGTGGAA	11768	TGACAAATGCCGAAGGGATTCT	22752	GGCTAGCATTCGGCGAAAATGA	33736
111	GGAAGGCAGTACAAGAGCTTACT	11769	CTATGTTTCAGCCACACCACATTC	22753	GCTCAGATGTGTAAAGGAAGGCAGTACA	33737
112	GGCGATTCCTGACATTTGATTGCAT	11770	CAGGCTTGTTCGGGAAATACAAG	22754	TTCCCGGTGTTCTGGCGATT	33738
113	GGGGTTTGTTTGGGTCAGAGA	11771	GCCGAGGTTTCAAGGTCCAA	22755	GGCATGTAAGGGTGACAAAGGAGTTG	33739
114	CTGCAATATTGGCCATTTCTCTTCA	11772	TGCTCAACTCAAACAGGACACT	22756	CCCTCCCAGGCTTCTCATCT	33740
115	AGTGTGCACGCCTGGTTGAA	11773	TGCGGCGATGTGGCTGTA	22757	CCTGCCTACGGTCCTAAAGTGT	33741
116	CCCCAGCTCCTTCACAAAGACT	11774	CAGAGGGTGGATGGATACAGTTC	22758	TCCCTCAAACCCCAGCTCCTT	33742
117	TGGAGCTGAGGAGGAAAGAACA	11775	CGTGGCCTCCTGCACTGAT	22759	GGCATGCTTTTCATTGTGAGTGA	33743
118	TGGGTCATAAAGTGACTCTGTAACATC	11776	GTGCAGGCTCAAGTTGCTTCT	22760	GGATCTTCCTTCTGGGTCATAAAGT	33744
119	GCTGTGAATGTTACATGGCAGGAT	11777	GCTTGCTTGGAACCGATTTTGGAT	22761	GCTTTTAAAAACCCAGCTGTGAATG	33745
120	CGTCCCTTACCGGAGCAACTTT	11778	GGCGGGACCGAGTGCTTTA	22762	TCTTCCCGCTCCGTCCCTTA	33746
121	AGTGAGCTAGAGGCTAGGACTTAC	11779	CCTCAATTACTCCCCAGATGTTCAGA	22763	CTGTCCGCAGAAGCCAAAAG	33747
122	TCCTTTCCTCTCATCGCCTTTG	11780	TCAGCAGGAGGAAGGATGTTTG	22764	CTGTACTGCCTCCTTTCCTCTCA	33748
123	GGGCACAGAAGGGTGTTCCAAA	11781	ACCTTCCGGCCATGTGTGAA	22765	CCTGAGCAAGGGCACAGAAG	33749
124	GGCCCTCATTGCAGTCAGCAT	11782	CAAATCTGCTCTGTCTATGCTAGGAA	22766	AGGTTCATGGGCCCTCATTG	33750
125	TCCTGAGATGGGAAAGAGTGAAGA	11783	AGCTCCTGACATTATGCCCAAA	22767	GGGCTCCCACTTTCCTGAGAT	33751
126	GAAACTGCACAAACTTCCTCTCCTA	11784	GGGAAAGCCAGACATCCTCTCCTA	22768	GCAGGCTCGATATCTAATTCCAGAGA	33752
127	CGCCTGTGGTTGTTGATGAAAG	11785	GCCCAGGTAGAGGAACAAATCA	22769	GCAGAATCGCCTGTGGTTGT	33753

128	TCCCCAAGAGCACAGACTCAAC	11786	GGAGAATATCAAGCTGTCATGGGATT	22770	GAGAAGCTAAATGGTCCCCAAGA	33754
129	CCTCTTCGCAGACCTTGAGGAT	11787	CCAAACGAGGCCCTCTCTCTTCT	22771	GGACTGAGGGGCTCTCTGGAA	33755
130	TCCACACTCTACCATTTGCTGAAT	11788	GCCCAGAAGAGTGCTTGACA	22772	GCCCCTGTAGTACATCCACACT	33756
131	TGAATTGTGCAGTGAGGCTATATCT	11789	GGCAGTCCTTAACTCCTGGTTTTC	22773	CCTTTCGCATGAATTGTGCAGTGA	33757
132	TGTGAGTAGGATCTAGGCATGTTAGT	11790	CCAGCACATTCAGTCCAGAATCA	22774	GAGCTCTAGCTGTGAGTAGGATCT	33758
133	GGTCACACAGAGTTAATGGAGACA	11791	CAGCCAAGTGCAGACTGGAA	22775	CCTCTTTGCCAGGTCACACA	33759
134	GGACCCAAGAGGCTGAGAAAG	11792	TCCTGGCAAATCTTGCACAAAG	22776	ACTGGGTGAATAAAGGACCCAAGA	33760
135	CTCGTGGGTGTGATGGAACAGA	11793	GTGGGATTACATTCACAGATGCAGAA	22777	AGAGGCTCGTGGGTGTGA	33761
136	TGTTGAGCGTGGACCAGTGA	11794	AGACCTTCACCTCTGCCACAGT	22778	TGGGCTCTGCTGCCTGTTG	33762
137	GGCTGAGGCTGTAGCCTTGAT	11795	GGAACAGGAGAAGAGCAAGGAA	22779	GATGGTCCCCAGAGGGATGA	33763
138	GATGGGCTCAAGTCAGAGGTTAAG	11796	ACTGATGCTACCAAAGAGGCTAGA	22780	ACCCCTGATGGGCTCAAGTCA	33764
139	GACAGCTGCTTCTCGGCTACAA	11797	CACAACCGCTTTCCCCTTGA	22781	GTCAATACTGTGGACAGCTGCTTCT	33765
140	ATCGCACGTACTGCCATGT	11798	GGGAGACACTGCAGGACAAAGT	22782	GGAAGCATCCCGCCAGCAT	33766
141	CCAGACCATGCATCCATCCACAT	11799	GTGCCTGAGAGTTTCCTGGTT	22783	ACTAGGATCTATTTCAACCCAGACCAT	33767
142	GCAAAGAACCATCAGGGTAGACATC	11800	CACAGTGCTGTAGTTGCCTGTT	22784	GCCTCTGCCTGCAAAGAACCAT	33768
143	AAGGGAGGCCTTCCACTGTCAT	11801	GCCACAATCTAGGACCTGCAGAA	22785	GCTCACCATCTTCCTCATCAAACAGA	33769
144	AAGAGGCCATTGCCATAGTTCA	11802	TTTCTGCCCTAGTTCAGGTCATC	22786	GGGAGAGACAGGTTAAGAGGCCATTG	33770
145	TGGGGTTCAACCGGACAGT	11803	CCTTGAAGATGCAACAGACATTCTTCTAC	22787	CCAGCAAGCATGGGGTTCAA	33771
146	CGTGCCTTAAGCCCAGGTATT	11804	GGATGCAGGCAGGCAAGT	22788	CGCCAGGAATTCGTGCCTTA	33772
147	GGTCTCAGGAATCCACCTCCAA	11805	GTGCTAGAGAGCGAAATACCTGTT	22789	GGCTCTCAAAACCCTCAGGTCTCA	33773
148	ACACAGGGAACGCTCTGTAAATG	11806	CCACAGAACTCCCACTCGAATAAG	22790	ATGCTTAGTGCCTGACACACA	33774
149	CCTGTCCCTCTTTGGTTGAAGGTT	11807	CCCCAGACCAATCCGAAGTGT	22791	ACCGACTCCTGTCCCTCTTTG	33775
150	CAGGCATTATCTACATGTCTGCTTGTCT	11808	AAGCAACCCAGTTCCTTCCAA	22792	CAGGAGGTGGTCTTTACCTCAATTC	33776
151	CGTCCAAGCCTCAGCTGTGTAA	11809	CAGCTGCTGCCTCATTTGCAT	22793	CACCTTAGAAAAATGCCTACCCTTGA	33777
152	CTAGCCAAAAGCCATTCTGGTAGT	11810	AATGGTAGCCTTTGAGACGATGT	22794	GGAAATAGATTTCAGGGACTAGCCAAAAG	33778
153	TGTTATCTGCCTTCTCCCAACAAA	11811	GCCCCTTCTTTTCTATGAGGATGT	22795	GCCTTCTCACTGCTGAACCTT	33779
154	CCTCACTGCCAAATCTGATACAGACA	11812	AGCATGGGAACCAGTGTTCATC	22796	AGCCTCCTCACTGCCAAATC	33780
155	CGAGAATTACACCCATCTGATCCTCTTTC	11813	TGACAGGACGAGGAGACTCAA	22797	CCCACTACACGAGAATTACACCCATCT	33781
156	GGCAAGGGCTAAGACGTTTGAA	11814	TCTGCCTTGCAGAGTTACAGAAT	22798	TTTAGCAGGGCAAGGGCTAAG	33782
157	TCCTAACTCCTACCTCATACCTCAAC	11815	AACCTTTTCGCTACCCATGTACT	22799	CAGGGAAAAAGACTGAATCCTAACTCCTA	33783
158	CCGGGACTAGGGTGGAAGTCA	11816	CCGAGCTCGTCTGGGTTTGAA	22800	ATGAGGACGAGGCCGGGACTA	33784
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161	GTGTGCCCACAGAGACTTGGAA	11819	CCCACGGGTTTGGGTAGCTT	22803	TCCTGGCTGTCACTACTGTGT	33787
162	AAGCCGCCTAATAATGGAAACCTA	11820	CTTGACCTTGCTCTAGCAGTTTTG	22804	CTGGGAACAAAGCCGCCTAAT	33788
163	ATGTTCTGTAGTCGTTAGGCTTGT	11821	CTCACGTAGTCTCCACCGTCAT	22805	CGTCTCCGCTGATGTTCTGTAG	33789
164	TGAAAGAGGCAGGAAAAGGTTGT	11822	AACCCTTTCAACCCTCCACATT	22806	GTGCCAACTGTGGCTGTGA	33790
165	AGCCAGATACCGTGAGCCAAGA	11823	AGTTCTCAGGCCACCTTGTTC	22807	TGGCTAGTGGGGAAGCCAGATA	33791
166	CAGAAGTGGGATTCAAATCCACAGAGA	11824	CCCGCAGGACTGAGCACAA	22808	TTGGTACCAGAAGTGGGATTCAAA	33792
167	GGCCCAAAGTGCTTTGAGAACA	11825	CATCCAGGCACACCGTTAAGA	22809	CTGTGGTTTGTGTAGGCCCAAAG	33793
168	GTCACGGAAGAACTGGTGGATCA	11826	TCGGGAGCACGTTTGCAGAT	22810	GGGCCAACACATGAGGGATGT	33794
169	GCTTAATAGGCGCCAACCATTT	11827	GGTTGCTCTCACGGGAATAAGA	22811	CCACTGGATCACTGGGTGCTTA	33795
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172	GCTGCCTCCTAGAGCCACTACA	11830	GCATGCTTCACAAACATTATCCCATTC	22814	TTATAGCCGCTGCCTCCTAGA	33798
173	TTTACGGGCCTCCCAAGTCA	11831	GGGCATCCAAGGAGAGAAGAGA	22815	GCTCATCCAGGACCCCATCTTT	33799
174	CGTCAGCCGATAATAGCTGTTGCTT	11832	TGTCAGCCTGGCTTCTGTAAG	22816	CTCATTGGCGTCAGCCGATA	33800
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177	CAGGGCTTTGTGACCTAGACAGA	11835	CGTGACAGCTAATCACCCACAT	22819	AGGCCACTCAGGGCTTTGTGA	33803
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181	GTTGTCTTGGTTGCCGTGTTC	11839	CCAGACTAGCCGAATTTTCCAGGAT	22823	GCCAACTGCTGTGTTGTCTTG	33807
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184	GCCCTTTTCCTTAAAAGCAGGAAGA	11842	CACAACCTCACATTCTCCAGGTTA	22826	AGTGCGCAGCCCTTTTCCTT	33810
185	GCGCAGGAAGAGCAGTTTGATG	11843	GAGCCCACGTATCAGCTCAACA	22827	ATGGCGAAGCGCAGGAAGA	33811
186	GGTTCCCCAGATTGGCTCAAAAG	11844	ACTGCAACCTTTTTGCCTGAGA	22828	TTGCCAGAGGTTCCCCAGATTG	33812
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189	CACACTGGGTTTTCTGGTTTCAGT	11847	TGAGAGGTCACCACACACAGA	22831	GCATCTGTATGCACACTGGGTTT	33815
190	CAGGGCAACTCAACGGCAAAAG	11848	TCATCCCAGCATCATTTGTTGAGAA	22832	AAGGTGCCAGGGCAACTCAA	33816
191	AGCTGGTCACTGTGGGAGAAGA	11849	CCGCCTCTCCACCTTTGAACTT	22833	GTTCTGCAGCTGGTCACTGT	33817
192	AGGCTCAGCTCTGGGGATTG	11850	CGACAATTTAAGGCGAGACATGGAA	22834	TGCTGCAGCCATGCTTCCTA	33818

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197	GGAACGGGAGTCAGGTCAAACA	11855	GCTGTGTATTTGTTGAGCCTTGT	22839	GCTACCAGGAACGGGAGTCA	33823
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212	CAGCTCTACAAATTGTTGCCATCTTC	11870	TCAGTAGAGCCTTGAGGTAGCTT	22854	ACCACGATCCACACCTCCTAAC	33838
213	GGCTCTGGAAGTTGCCAGTCA	11871	CCTGCCCTACCAACCCACATT	22855	TTGGCTGGAGGGCTCTGGAA	33839
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219	TGCTCTGCTTTCTTCCAGTTTCA	11877	TCAACACCTTTTTCGGTGACAGT	22861	GGGCATTATTGAATGCTCTGCTTTCTT	33845
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882	GCCCTGGTGAATGCACATTTTG	12540	TTGGATATTGTTTGTCACCCCTACA	23524	GGCTAACTGAGCCCTGGTGAATG	34508
883	TGGCATCGAGGATGGCATGA	12541	GGCCCACCTCGTCACTATG	23525	CTGGAGACGTACATGCCAGTCA	34509
884	TCCTGTTTGTTATGGGAACCAAGT	12542	GCTTACAACGCAACTTCACACA	23526	CCGCCCATCTCTCCTGTTTGTT	34510
885	GCATCCAGCCCAAATCCTCACT	12543	CCCACCCTGAGCCTTCACTTC	23527	GGGGAACAGCTGGAAAAGCAT	34511
886	TCCTCCCTGAGACCACAGCTT	12544	TTGGTGGCGCCCATGAGTCT	23528	AGTCTGGCCTGTGGTGTCTGT	34512
887	CCATCACCCATTTACCCCTACTTG	12545	TTGAAAGCCTCGTGCCATTTG	23529	AGGGACCCCATCACCCATTTAC	34513
888	ACTGGCTGTCAGGCTTGCTT	12546	TTTAGGTGGGATGTGAATGTGTCA	23530	GGAACTACAATAGACATAAACTGGCTGTCA	34514
889	GCACGAGATGAACTAAGACACCATT	12547	CACATAAGGGAACAAGCGCACAA	23531	TGTTTTAGCAGCACGAGATGAACT	34515
890	GAGGGAATGGTACCTGGATTAGCAA	12548	CCTCCAACCTAGACTGGATGTCA	23532	AGGCAAATGGCAGGAGGGAATG	34516
891	TGCCACTTCACCCATACTGAATTT	12549	GACTTGGTCGTGGGTTTTGAGA	23533	GAGATTACAGGAGCATGCCACTTC	34517
892	CACACTGCCACCTGCTTGAGTA	12550	TGACTGCTGACACGGGAGGAAT	23534	TGCTTTCGAGAAAAGCCACACT	34518
893	TGATGAGTGGAAGCCCATGTTG	12551	TGGTGGCGAGGGTAGAAGAT	23535	GCAGCGGCTATAGACAGATCCAA	34519
894	TGCTCATCATGGCTATGCTTTCT	12552	ACTCACAGTAATCCAGGGGAAAAG	23536	GCACTGCCTCTGCCTCAAAA	34520
895	GCTGATCCAGAGATGGACACAA	12553	CTGCAAAAATACCAGGGAGGAACT	23537	TACCACCAGGGCTGATCCAGAGA	34521
896	GCAGTGCTGTCTATGCCAATG	12554	ACTCTCACACCCTTAGGCCTTT	23538	GTGGTCTGCAGTCACATCTTGA	34522
897	GCCAGATGTGGAGTTAACAAGTCA	12555	ACCGCGCCCCATCACTTACT	23539	GTCCATCAGCCAGATGTGGAGTT	34523
898	TGACTTAACAGGTGACCTAGACACA	12556	CTGACCCTGGGCAAATGACTCT	23540	GGGTCTGTCGCCTGACTTAACA	34524
899	CCTTGGAGGCCAGCAACAA	12557	CGCCACGGACTAATGCTGTT	23541	AGAGGTCAGGGTGAAGCACTA	34525
900	GAGGCACTGATTCATTCAACAGGATT	12558	TTGGCTTTGTATCTCCAGCAACT	23542	GGAAAAAGTGGAGGCACTGATTCATTC	34526
901	CCCATGGGTAAACTGCTGTAAGTA	12559	CATCGCTACCGGGTTATGTTTTC	23543	AGAGAATAGTACCTCCCATGGGTAAA	34527
902	CAGGCCTCAAAACACGAAGAAG	12560	AGAGGCAAAATCCGCACCTAA	23544	GCAGAGCCAGGCCTCAAAAC	34528
903	GGGGAGAAGTGTCACCAGATTCA	12561	CCCTGCCCTTCTCACTCACAAA	23545	TGAGTCCTGCCGCTTCACA	34529
904	GCGGGTTAGGAAGATTCCCTGAAG	12562	CATGCATGACCCTGCTCACTTT	23546	GTGGGAAGCGGGTTAGGAAGAT	34530
905	CTTCCTCACCCGTCCTTAATAACA	12563	GGCCTGCCTTAAGTTCTGCTAAAG	23547	GGAGCCAGACTTCTTGGTCCTT	34531
906	GGTGCTTTCCTTTCCACCGAAGT	12564	GGAGTTCTGCACTTGTGATGTTC	23548	GCGTTGTGGTGCTTTCCTTTC	34532
907	GGGAGGAACAGTGATCTGAGCAT	12565	GCGTCCAAGGCTGGGTAAAGA	23549	AATTTTCAGAGGAAAGGGAGGAACA	34533

908	GAGAATTAAACAGGGCTTTGAGGGATT	12566	GGCAGCCTGGGTATGTCTTG	23550	GCACCACATAGGTGACAGCAA	34534
909	CACCGAGCACTCGGGTGAATAA	12567	TCAACTTGATTGTCTCCACACTGTA	23551	AGTGCACGTCCGCCGTAA	34535
910	TTGATGGCTCCCATGTAAGTTTGA	12568	GGCCGGCCATCCCAGTATAATTT	23552	TCCTCAGGTTGTTAGGCATTTTGA	34536
911	CGAAGAAGCAAGACCGCAGTAAC	12569	GTGCGGGGCTTGAAGGAT	23553	GGCACTGGGGAAGACGAAGAA	34537
912	CTCTGGGGAATGCCTAACAATGA	12570	TTGTTCCTATCGATTACCCACCAAA	23554	TCCTGCTTGCCTCTGGGGAAT	34538
913	CAGCAATGAGGCCAGCGTAGTT	12571	CTAGTACCACTTTCTCCTTGCTCATT	23555	ACCCTGGTGGGATCCAGAAA	34539
914	CCCTATCCAGAAAGAAAAAGCCAAGTGA	12572	CTGCCAGACCACACCAATCTCA	23556	CGAGCCCCTATCCAGAAAGAAAA	34540
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917	CATCCTCTTGCCGTGTGTGACT	12575	GGAGGAGTGCGTGCAGAAGTATCA	23559	GGGCTGTTACCTCATCCTCTTG	34543
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920	GCCACAGGTCGCCTTCTGAAC	12578	GCCCACGTCTTGGCCTTGT	23562	ACACCGGCGACTTCTCCAAT	34546
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922	AGGCCCTTCAAGAAGTTGAAACA	12580	CCACTTTGCCAAATTCCCTTTCATC	23564	CTCAAGGCTAAAGTAACTCCTTGGAAAG	34548
923	CCCGTGTAGCCAAGAGACTGAAG	12581	ACGAGGACCCGCGATGTCT	23565	AGTCTGCAGAGACCCGTGTA	34549
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925	GCTCTCAAGGACTTTGCAGTCT	12583	ACCCACCAACACCACATTGAT	23567	AACACAGGCCTGGCTCTCAA	34551
926	GAGGGAGGTGCTTTGCTGTCAA	12584	TCCCTTGAGCCTCCTCTGTTCT	23568	GGAAAGGGAGGGAGGTGCTT	34552
927	GGCTCTGTGAGGATGGAGAGAGT	12585	GGGGACAGTGGGAGGATGTCA	23569	GCTCTCCTGGCTCTGTGAGGAT	34553
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935	CAGGAGGGAGAAAGCTTTGGAAGA	12593	TCCATCACATGCTGGTGAAATGT	23577	TCTGGTAGCAGGAGGGAGAAAG	34561
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938	GCTGTACTCCTGGGTGACATTC	12596	TGAGCTACCCATCAGGGTTATTTTT	23580	CCTGGTACCTGATATGATGTTAGCTGTA	34564
939	AGCCCTCTGATTCCAGGTAAGA	12597	CCATTGAGTTTCAACTGGGGTACA	23581	GGTCCACCCATAGCCCTCTGA	34565
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943	GCACACCTGAAGGTCAAACGAACA	12601	TGGTTTACCCTGAATCCACTCTGA	23585	CTCTTCCCTGAAGACAACCAACTT	34569
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945	TGTGGCCTGGCTCAGACATA	12603	CCAGGGGTTGGCGTGAAAG	23587	CACTGCCCAGATGGCAGAAG	34571
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948	TGCCCTGATGGATAGGTGGAT	12606	GTAGCCAGGCATGCATTTTTGA	23590	GGAAATGTTTGACTCATGCCCTGATG	34574
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965	AGCCATGCCGATTGCCTCTT	12623	GGTGGCGAAGGGGTAGTGAAA	23607	GCAGGAGCAGGTTTAGCTGACA	34591
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971	CCTGCCTCAAACAGAGGAACTCTA	12629	GCTGAAAGCTTTGCTGGCTTCT	23613	ATAATCAGCCTGCCTCAAACAGA	34597
972	CACTGAGCAATCATGGCATTTGT	12630	CAATGGGTAACTAGGCAAGGGAATTAT	23614	GCTGCCAAGAGAAATGGGACACT	34598

973	TCCATCCCTGTCCCAGCTCCTA	12631	CCCGGCCTCCTTATAATGGAAAGA	23615	GCCATCTCAAACTCGGCCAATG	34599
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975	GTAAGAAGGAGGCGTCGGAATC	12633	TTCCCTACCCCTTCCCCAGAAC	23617	GTGAATGGAAGGTGCATGTAAGAAG	34601
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985	CCCTGAAGAGCTGAATCACAGACA	12643	ACAGACTGCGTGTCCTGCAA	23627	CCCTTTTTCCCTGAAGAGCTGAA	34611
986	AGGAAGGTGTAGGAACTGCAAATAC	12644	GATTACGAACGTAGGGCACAAAC	23628	GGACTGGTGAGGAAGGTGTAGGAA	34612
987	CCTCAACATACGGGCTACCAAA	12645	CCCAGGGGTTCCTGAAGTGAAAA	23629	GCAAAACTCCGGGATCCTCAAC	34613
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993	GGGAAGGAGCTCTCGACCTA	12651	GGATGTCTGCTGTTGCCTCTTC	23635	CCCACTTCGGTACAAGTCAGACA	34619
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997	CTCGGGTGCTTGGATTGTGT	12655	CCAAGATGCTCATCCAAGAATGGAA	23639	AGGGTCAGTTGGGAGTAGAACA	34623
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999	CTGTGTGTCTGGCTGCTGACAT	12657	GAGAGGGCCAAGATGGGAACAT	23641	TCAGCGGGCTGTGTGTCT	34625
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1006	GGGTTGTGTGGCAAATGGAAGT	12664	TCCCCAGGTGTCTGTGGAAA	23648	GCAAACAGCTGGGCAAGAGA	34632
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1012	CCATGCGTGCAGGGGAGAATTA	12670	ACAGCGGCCTCCTTCCTTTT	23654	GGACGGATGCACCAAACGTGAT	34638
1013	TGTGCTCAGTAAGTGCTTGTTAGA	12671	TTGCATGGTTGAATGCACTGTAG	23655	CCAATCAGCCATTTGGTGAATAGTT	34639
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1040	GCTGTCAGCCCCACAGATG	12698	ACCCTCATCCAGCACTCCACTA	23682	TTCGGTGGGAGGGCTGTCA	34666
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1042	GTCTAGAAGCTGTATCCCCAGAGTCA	12700	ATCTCCAGACGTCGGCAATG	23684	TGCTGCTTATTGTCTAGAAGCTGTATC	34668
1043	CGCTTCCGTAAGGGAGCATCT	12701	GCAGGGAAGAGGCAGCATTGA	23685	ACATCCCTGCCTCCCACAAGT	34669
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1049	GAGACTTTGAGGCAGACTGTCA	12707	ACACACAGCTAAAAGAAAGCAGTTG	23691	GCTTGTGGGTTTGACTGAAGAGACTTT	34675
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1057	ACCCGATGCCCCATGACT	12715	GGGGACCATCAGTGCCATCT	23699	TGAGGGAGGTCGGCACCTAAA	34683
1058	CCCAGTGAAGCCCCACCTTTAG	12716	CCGCCAGGCTTCAGACCTT	23700	GGAGCAGGTCCCCAGTGAA	34684
1059	GTGGCAGACAAGGACAGTGAGT	12717	TGGGCCTCCCCTGAACAA	23701	AGTGCTGAGGGTGGCAGACA	34685
1060	CCATGGCAACTCCATGCTTCCAA	12718	GAGGAGTCAAGGAATCGAGGATGA	23702	GTTCTCAAATCAATCCATGGCAACT	34686
1061	GCTTCCCAGGATACCCACCCATTA	12719	GGTAATGTGTAGACCCCACAGTTCA	23703	CCTTCTCCAGCTTCCCAGGATAC	34687
1062	GCTGTCTCGGTCCTCGTTAGT	12720	TGAACCCACAGCCCCATCTCA	23704	AGGCGATGGCACGCTGTCT	34688
1063	GAGCCGATTTTATATCCATGCCTGTAG	12721	ACCTGCGGCAAGATCTCAGT	23705	CAACATCCAACAGAGCCGATTTT	34689
1064	CCCAGAAACGAGCCATGGAAAG	12722	TGCTGGGTCCCTGGATGGAT	23706	ACACAAGGGGCACCCAGAAA	34690
1065	AGGTCGCCAGAGTGCTGTGA	12723	ACTCTCCACCAGGCATGCAGAT	23707	GCTTCAGGGGAGATAAGGAAATCAAGA	34691
1066	CCAAACCCAATTGCAGCTGTGTAAC	12724	TCTGAGAGAGGCTGGCTACCTT	23708	GCAATCACCCAAGACCAAACCCAAT	34692
1067	GCTGCCTTCTTGAGCTCCTGATTT	12725	AGCCTGAGGACTTGGCAGTCA	23709	CAAGGGGCTGCCTTCTTGA	34693
1068	CAGCCTAGAGGCAGCAAAACTGT	12726	TCGGGCAACTGCTCCTCTGT	23710	TGCCCCATTTCCCTCAGAGT	34694
1069	GCAAGTGTCAGGTCCCTCTAGTCA	12727	GGGACAACTTTGGGGCTTGA	23711	TTTGTGGAGAAGGCAAGTGTCA	34695
1070	AGCCAACTGAGCCAGGAACA	12728	GGCCAAGCTTTCAGCCTTTC	23712	GCCTGTGCCATCAGCCAACT	34696
1071	CCTGCATGCTTGGGTTAATTCTTG	12729	CTGGATGAGAATGGTTGGCCTAAT	23713	AACCAGATACACCTGCATGCTT	34697
1072	AAGGGGAGCTGTGTGTCCTT	12730	AGCGAGCCCTGCAGCAAGA	23714	ATGCCGGGGAAAGCCAGAA	34698
1073	CCAATCCTACGACGGGACCAAA	12731	CAGAGCAGGGCTGTCATTGTCT	23715	CCATCTGTGCTGGCCAATCCTA	34699
1074	TGCTGATTGCCTGTGTTGCTA	12732	CAGGAAATCCAGGGGACAGAAGAA	23716	TTCCCACCATCCGAGACTTTG	34700
1075	GGGACACTGACCTGCACCAAAA	12733	GGCCAAACACATCAGGGAGCAA	23717	TGTGCCCTGGGACACTGA	34701
1076	GGACCCTTCTAAGCCACTGATCTCT	12734	TGGGGCTGCTGGGAGAGGAA	23718	GCTGGGTCTGGACCCTTCTAA	34702
1077	CTAACCCCAGCTCAACCTCTTG	12735	GGGTGGCAACCCTCAGATTC	23719	GGTGTGCTGGACCGAGATTCTAAC	34703
1078	GTGACACAGGCTGCTTCTCATC	12736	CTTGTTTGCCAGATTTACCTGTGT	23720	TGCACGCACTCTCCCTTATG	34704
1079	TTGGTGTAGGGAGCAGGAGAT	12737	GCTTGTGGTCCTGGGTGTCA	23721	GGAATGCTGCCACTTGGTGTAG	34705
1080	CCTCTTACACCCACTGTTCCACTAAG	12738	AAGGCGAGGTCACCTGAGTAAG	23722	ACACCCACCCATCTCTCCTT	34706
1081	GAAGCATTCTGGGCTGAGAGAGA	12739	CCAGAAGAGGTGTGGCCTGAAAG	23723	TGCCCCAGGCCAGAAGCAT	34707
1082	GGAAACCTGGTCCAAACCACAA	12740	CAGTCAGTAGAGGGCGCTGTT	23724	CCGACGGCAGAACGGAAAC	34708
1083	AGGCACCTGTTTGCCCGAATG	12741	GGATGGGAATGGGTAAGGCACAGA	23725	AGGGCACCAGAGGTGACA	34709
1084	GCTGTCAGAACCAGCCTGAGTAT	12742	GGAGCTTTTGACTGGGGTTCACT	23726	TGGCAGGTGCTGCTGTCAGA	34710
1085	GCGGTAGGATGTTAAAGGCAGCTT	12743	CCAAGGTCCCTGGTCTGGAAGT	23727	TGTTCCCTGCGGTAGGATGT	34711
1086	GGTTCAATCTCAGGCTCACACT	12744	GGGCAGAGCAATAGCCGAAA	23728	GCCCTTGTGCTTTGGGGTTCAA	34712
1087	GCATGGATCTGCGTGGAGACA	12745	GGCCCACGTGGTCAGATTGTT	23729	TCCTTACTGCTCAGCATGGATCT	34713
1088	CAGAACTGGGTGGTGTCGTTTTG	12746	CGAGAGCCTGAGCATGTACAAAT	23730	GGAAACCTCCTATAGTACGGCCAGAA	34714
1089	CAGACCACTTGAGAGCAGGTT	12747	GTTATCTTCTCACTGTCCTGTTCCTTAG	23731	CACCAGCCATGGACAACAAGT	34715
1090	GCTACCAAGGGAGGTCTCTTCT	12748	GCTTCTGCCCAGTGCTCAAGAT	23732	GCCTCCTGGTATTCCTGTGCTA	34716
1091	GAGGTGGGTAACAGACCCATCA	12749	GGCTCTGTAGGGCTTCTAGAATGTT	23733	CCGGGAAATCAGAGGTGGGTAAC	34717
1092	GGAAGCAAGCACATACTCACCAT	12750	GGGTGTAGAGCAGGATGGGTTA	23734	GTCCATCAGGAAGCAAGCACAT	34718
1093	ACTGCGTAAGCGGTGGCATA	12751	GTCCCCGAAACCCACACTGTATTC	23735	CCAGCAAGAGGAAACTGCGTAA	34719
1094	GGTATCAGCAGTGTGGGTTAAGGAT	12752	CCCAAAACTGAGGTGGAGCTT	23736	GGGCAGTAGAAGGTATCAGCAGTGT	34720
1095	CTCGGAAGTCCCAGCAGATCTTTC	12753	GTGACCAGTGCTAGCTGACAA	23737	GTGCCTTACTTCGGGAGCTTTC	34721
1096	GGTGAGGTTCACACCCTCCTTTC	12754	TTCCGGAGGTCAGATCCCAAGA	23738	TGCTGCGGGTGAGGTTCA	34722
1097	CTGCAGTCATGGCTGTGGAA	12755	CAGCTCATCCGCCCACAGA	23739	CCCAATCCGAGCTGCAGTCAT	34723
1098	CCCCTGCCTGTGGACCTTATATT	12756	AGGGAGCAGCGCGACTGAA	23740	TCCTCCATCTAACTCCTGCTCAT	34724
1099	GGGCATTGCCACAATGGTTT	12757	GGCAGTGGGGTCCATTTGAAACA	23741	CTGCCTTGATAACTCTGGGCATTG	34725
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1101	TCTAGGACCAGGAGCCATCAGA	12759	GCCGCATGTCTCTACTTCTTCTT	23743	TGGGCCAGGGCTGTAATCTA	34727
1102	CGAGGGGTCTTTGAGGCAAACTT	12760	AGGCCGAGCAGCTTTAGTCT	23744	ACCGGCCGAGGGGTCTTTGA	34728

1103	TCCCCACCTCTCTTTCTTTAGAGTAT	12761	TCAAGATGGAGCACAACAACCAA	23745	TCCCTTCCCCACCTCTCTTTC	34729
1104	CCAGGAAAGCCACCAATGCAA	12762	GTGTTGAGCAGGCAACTTGTGA	23746	CCGAAAGCTCTGCCAGGAAA	34730
1105	GGGCTGATATAATGGAGGCAGCTTAG	12763	ACTCATCACCTTGGCATGCTT	23747	GTGCCAATTTTTGTGGGGCTGATA	34731
1106	GCCCTGTCCTGACTGACATTCT	12764	TCAGCCAAGAAGGTATTTGTTGGAA	23748	CCTCTCCACCAAGCACAGCAT	34732
1107	TCTTTGGCCACACAGATTTACTCTAC	12765	CAATGCAGGAGCTTCTCCACAAA	23749	CTGCCACTGTCCATCCACATCT	34733
1108	CAGAGTAGGTGTGCCGTAGGAA	12766	CCCACCAAAGGGAGCCAATTCT	23750	TGCAAAGTGCATGGCACAGA	34734
1109	CGCTGTCTCTCCATCCAAGTGTT	12767	CTTTGCCCTGGCATCTGAAAC	23751	GGTGGCGCTGTCTCTCCAT	34735
1110	CGGAGCCTTGGAGCTAGGTGATTA	12768	CTCTGGCACCTGGAAACACT	23752	TCCTGGCATCAAGACTGTGAAC	34736
1111	CAGGGTTCCTAGCTCTTTCCTGTTC	12769	TCATCCATCCTGAGCCAAATTTCT	23753	GTCTTTGTTTACCTCCAGGGTTCCTA	34737
1112	CATTCCCAAATGGATGTGGCTAGA	12770	AAATCCAGCCACAGGATGATACAA	23754	CCATCTCTGCATTCCCAAATGGAT	34738
1113	GCTCCACTTTCGCCCTCACTT	12771	GCACCAAGGAAGCATCAGGTACT	23755	CCCTCTGTTTTCAGCTCCACTTTC	34739
1114	CTTTGCTCTTGTCTGCACTGAGTA	12772	CTGCTCAGGGGCCAGACTTC	23756	CAGACTTCACCCACTTTGCTCTT	34740
1115	TTGAGCTGTGCAGGCTTGT	12773	GAAGAGCAGTTTCACTTATCTGTGTCA	23757	TGGGCTGAGCGGCTTTCTTA	34741
1116	TGTGGCTACTTTCCCACAGTTTG	12774	GCCTCCGGATCTGTTGCTAGTGA	23758	GGGATAGCCCTTGTGGCTACTTTC	34742
1117	CAAAGGACAATCACAGAGCTGTTC	12775	AGGGGAGCCTCAGCATCCTAAA	23759	TCCCATTGGAAAGTATGGACTACAAAG	34743
1118	GGAATGGACCAAGTTTGTCTTGATT	12776	CGGCTGACTGCAGTTGTCT	23760	GTTCAGGAGTAAGGAATGGACCAA	34744
1119	TGGATGTGGCCCGCATTTGT	12777	TCGCTTCGGGCTTTCAGCAA	23761	ACTTGGCAGGAAGGGTGGAT	34745
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1121	ACCAAGCACTGGGGAAACTAAA	12779	GAGAGCCCCTCAGGCTCTTG	23763	GGCAGACGGGATTCAGTTGCTA	34747
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1123	CAGGCCATGTAAACGACTCTCA	12781	ACTGTCCTGTTCCACCCTTCA	23765	GGGACTTTCCCAGGCCATGTA	34749
1124	TGCTGAATGGTTTGCTCAGTTTG	12782	AGGGCCCCTACTACCACTTTAG	23766	GTCTTGTGCTGAATGCTGAATGGTTT	34750
1125	TCGAGTCACTTACCTGCTCAAATG	12783	GACCAGGGAAGAAAGACCTCTGAA	23767	CCTGGCTCCATGGGTCACT	34751
1126	AGCAAGCGACGGTCCTCCAT	12784	TGGACACTGCCGTAAGGTGAAG	23768	TCTGGGGTGCCACCAGCAA	34752
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1128	AGTGTCAGGAGGGGAAACGAA	12786	TTCCCATGGCAGATGCTGTT	23770	GCAGGTGGAGTCTGAAGTGTCA	34754
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1135	GCAGGAAACCACAAAACATCACCTA	12793	CATCACTCCCCTCTGCTGCTA	23777	CCAAAACTATCTCAGGTGCAGGAA	34761
1136	GCTTTCTCTCCCCTAGGACATACAGT	12794	AGGCCACTGCCTTCTACCTT	23778	CCTCTGGCAAAGCACCCATTCA	34762
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1138	TGCTATTGGGGCATTGGTGTT	12796	GACAGCTTGCAGTCAAGACCTTCT	23780	GCAGTGAGCCAGTAGCCTTTTG	34764
1139	TGCATCCCGTTGCTGTCAAG	12797	CATGGGGAATGGGTCAGTACAGT	23781	GCTCCAAGGTACTAGTGCTGCAT	34765
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1143	GGGACCAGTCAACTTTGCCAGATT	12801	AGCGGCTGTGTGCAGAATCA	23785	AGGGAGGGACCAGTCAACTT	34769
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1183	TGCTGCTCGTACAGTGGAAAG	12841	GACATTGCTTGACTTGGGTGTGT	23825	CCTTGTGCTGCTAACCATGAGACT	34809
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1185	CGGTGGTGCCTTTGTTTTAACT	12843	TGTGTGCTGACAGGTTTGTGA	23827	CACACTTTTCGGTGGTGCCTTT	34811
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1194	GCAGTTGAGGGAGTTCAACTAAAGA	12852	TTCCCTGAACTCTTGCTGGTTT	23836	GGAATAGGTAGGTGCAAGCAGTTG	34820
1195	CCTAGGCTAGAAGCCCAGAAAAC	12853	GGGTTGAAACTGGAGGCTTGTTC	23837	ATCCACAATTTCCCTAGGCTAGAAG	34821
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1198	GAAGGAAACTGACTTTGCAGCTTAAC	12856	CAGCCTCGTGCAGCAGTTG	23840	TGGGTCTAGAAGGAAACTGACTTTG	34824
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1203	GAAAGTACAGGCTATAGCTGATGGAT	12861	GTTTGTGGCAGAGACGCTATTG	23845	CCTGTGAGCAAAAGAAAGTACAGGCTAT	34829
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1207	GGGTGGGAGCAGAAAGTATGTTG	12865	CAGGGCAAAGCCACGAAGTT	23849	AGGTGGGTGGGAGCAGAA	34833
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1213	CGTCTGCGCCGAGACCTTT	12871	CTCCAGTGCTTACTCCGTCAGT	23855	CGCGTCCCATGACGTGAAC	34839
1214	GGCCTGTCATCTCAGCATGT	12872	GGCACAACCAACAGTGGACCAA	23856	TTTCCGCATGGCCTGTCATC	34840
1215	GAGGAGTCCTAACTGCAGGGATAC	12873	AGGGCAAGCTTCCCATTAAACT	23857	TGGGGACAGAGGAGTCCTAACT	34841
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1217	GACCCTTGCATAGTTAGAAGAGTGGTT	12875	GGGGCCTGTGTGGACTGTAG	23859	CCATCTGTGACCCTTGCATAGTTAG	34843
1218	GCCCTAAATAACCTCTACTCACGGGATTT	12876	CCTCCTGGCTTGCCTGGTTT	23860	AGAAGTTCTGCCCTAAATAACCTCTAC	34844
1219	ACCCAGTGCTTTGGTGTTCA	12877	CTGATCCATTTGCCCACACACA	23861	TCTCACTACAAAAACCCAGTGCTT	34845
1220	GCCCCATCCATGACTTGGTGAA	12878	GTCCTTAGCTGGACGCATAGTTG	23862	GTTGAACAATGAGCCCCATCCAT	34846
1221	GGGGCCTGGGGTCAAAGAA	12879	GGGACTTGGAAAAGGCCTCATAG	23863	GGCCATGCAGACCCTCTGT	34847
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1223	TTCCCTGGCCCTGTTTCTCT	12881	CCTGGGAGCCAAAGAAGAAAGTTAATGA	23865	CCCCTGCTACAGAATGCAGCTT	34849
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1225	CCTGAGCATGTGGTGTGGTTT	12883	TGGGAACCACTGGACCTTCA	23867	GCATATGTCCTAGAACAACCTGAGCAT	34851
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1229	GGCAGGTAGAAAGGCACACCCTAT	12887	TGCCATTGGGCCTGGTTGAT	23871	CCATGTCTAGTCAAGGGCAGGTAGA	34855
1230	TGACTCCAGCTCCAGGGTTACA	12888	CAGTCCTGCCTGGGATAGCAA	23872	GTGTTCAAATTGCAGTGCTCTGTT	34856
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1410	GCGAGCAAACCTTTGACTTTTCCTT	13068	CCTGCTCCCCTGTCCTTATAGA	24052	TGCGTGCGAGCAAACCTT	35036
1411	AGCCATCATGTCCACATTCCAT	13069	TGCTCTTCCCCTCACTCAATTTT	24053	GGCTGTTGCATACTCAGCCATCA	35037
1412	GTTCTGTCAGAGGGTGTGCTTCA	13070	GTGAGGCCAGTGCTTGTGGAT	24054	TCTTGACCATGGAAAGTTCTGTCA	35038
1413	GGAAGGCTTAGTCAACAGACGACATTT	13071	GCCGCTGAAAACCACGACATTC	24055	GCCAGCAAGAAAGGGAAGGCTTAG	35039
1414	GCTGAAAGGGCCAGGTTGT	13072	GCAGTTGGAGCCTTAGCAGTCA	24056	CTCTGGGCAGTGTTGCTGAAAG	35040
1415	CATGACCGGCTTCTGTCTAGTT	13073	GCAAGAGGGAAATGGCTTAAGATG	24057	CAGCAGAACTTACAAAGCTTCCATGA	35041
1416	CAGACCCTGCTATCACCAGTTT	13074	CCAGTTCAGTTGGGGCTTGTT	24058	TCAGCCCCAGACCCTGCTAT	35042
1417	TGAGAGTCAGCAAGTGCCTTAG	13075	CCTCTTACACCTGGTGGCAAT	24059	GGGAGGCCAGCCTGAAGTA	35043
1418	GCAGATGCACTGTCAAGCTGTACT	13076	TCATGGACGGCCTCCGTATG	24060	GGAACAACACTGACCCTTGCAGATG	35044
1419	CGATGGGTGTGGTTCAAGGTCAAA	13077	AGCGAACTTCGGCAAAACTTTC	24061	TGGGTCGATGGGTGTGGTT	35045
1420	GGCTCCCACCCACTGAGTTTT	13078	TGCCTCTTCCAATTGAGGTGAAA	24062	CAGACTTCCATAGTTGCCTTTCTCT	35046
1421	CACCCTGTAAACATCATTTTCGCATCA	13079	CACACACACGCTCTGTCCTT	24063	CCCCGAAATTCACCCTGTAAACAT	35047
1422	TTCCTGACCTCGCGATCCTT	13080	GGCCAGGGGAAGTACAGGTAGA	24064	GGCTGACTCTGAAGAGCCTTT	35048
1423	CCTTCATTCAGCCACTGTCCTGTA	13081	GCTGCCAATGTCCTGAAGCCATA	24065	CCACACAGGCACTTGTCCTTCA	35049
1424	GCCCACCTCAGTCAGGGAAGATA	13082	CCTGCTAGAAGCCGTGGCTAA	24066	GGAGAAATAGGCCCACCTCAGTCA	35050
1425	GGAGTCAAGCAAAGGCAATAGTGA	13083	GTAGTTTTCCTTGTGGAGGGCTAA	24067	TGGGTTTTAAAGCAATGGAGTCAAG	35051
1426	GGGCTGTGGAAACCACAAATG	13084	GAGCACACCCCTACCAAAAGGAAA	24068	GGGTCAAATGGGCTGTGGAA	35052
1427	ACGAGAGAGGTGAAGTCACAGA	13085	TCCCTGAAGAGGGCCAAGGAT	24069	GCCTTACCATTGCCTCCCAAA	35053

1428	AGCCCTGAAGGTCGTCCATA	13086	GGAAATAAATGGCCTGTTAGGCTGAA	24070	GACCATCTCCAAAGCCCTGAA	35054
1429	CCTTGAAGCTGAGCTGAAGCAGTT	13087	GCTCCGTGTTTCCACAGAACCTT	24071	AGGCAGCAGGGTCACCTTGAA	35055
1430	TCAGGCTTAGATCCCTGGAGTAG	13088	GGCAGCAACGTCAGGGAAAA	24072	GGCTTTGTCAAGCAATCAGGCTTAGA	35056
1431	GCTGCTTATAGCACAGGCACCAA	13089	TGCCAGTACAGTGCAATTGGAAA	24073	CCTCCCACAAAATCTGCTGCTTA	35057
1432	TGTTGGGACCCACCTCTGCAA	13090	GACGTGAAGTTGCCCATAGCTACA	24074	GGCCTCACAGTCATGGTGTTG	35058
1433	GCTGATCTGCTGGCGAGGTT	13091	GGCGAAGAAGGAATGGAGTGTGA	24075	TGTCTCCCATATTGGGCTGATCT	35059
1434	CACATGGCCTTGGATGGTATTCT	13092	CCAGCTCACAGACTCCTGTAAC	24076	TCTGGCCCTTGGTGCACAT	35060
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1440	GTGTGGAAAATGGCAAGACAGTTCT	13098	CTATGGACCTGTTCCAACCACAT	24082	TGAATGATGCCTATCAAGTGTGTGT	35066
1441	CCTCACCTTTCAGGCTCAAGT	13099	GCAGGACTAAAGCCACAGGTAA	24083	GCTGGCCTTCCTCACCTTTCA	35067
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1584	AGGCTTTTCTCCCTGGGTTGA	13242	GAGCTACAAAATGGAAGGAGACTGA	24226	CTTGGGCAGTGAATAAGAGGCTTT	35210
1585	TGGTGGCACCCAAAGGCATTC	13243	ACCAGGGAGGAGGCTTGTGTT	24227	TGTGGTTTAGCGTCTGACCTAGT	35211
1586	GATGGCTTGGACATGAAGGATGGTA	13244	GCCCACAGTTATCCTTGACTCTCTT	24228	CCCCATTGATGGCTTGGACATGA	35212
1587	CCCAAAATGCAACACACCATGA	13245	GGCTGACCATTAGGTTTTACTGTGA	24229	GTGCCTCATCTCACTGACCCAAA	35213
1588	GTGTTGGCCTAAGTTCCCGAGTTC	13246	AGCACGGGCAGCAGCAGAA	24230	GGGTCAGAGTGTTGGCCTAAGT	35214
1589	GGGAAGCTGACTTCACATCCAT	13247	CCTGGTGCACACTAGGAATAACA	24231	CTGCTGTGAAAGGGAAGCTGACT	35215
1590	TTTGGTCCCAGGCTCCTCCTA	13248	CCCTCAGCTACATGCCGAGAAA	24232	GTCCAGGGATAGTAAGGCAGTTTG	35216
1591	CTCTCACGCCCAGCACTCT	13249	AGCAGGCGGATGTCAAGGAA	24233	ACCCACCGGCTCTATCTCACA	35217
1592	CGTGGCAACAATTCCCTCTATCTCA	13250	TGGGTCTCTGGCCTCACTT	24234	TGGAGGAGCGTGGCAACAAT	35218
1593	GGCAGTGGTACAATCATAGCTCCAT	13251	GGTCACCTGTGCCTAGGAGTT	24235	CTGGAGGGCAGTGGTACAATCA	35219
1594	GATCCCTTAGGCTGTGCGAAAG	13252	GTCTAGGGGCGGTTAGGATACACT	24236	GGCCTTGTGCAGAACTGAGA	35220
1595	CACTGTTTTCTAGTCCCAGTTCTGT	13253	GGAACGGGGTGGAACTTTCCTT	24237	GTCACTTGACTGCTTTCCTAATCACT	35221
1596	GCGCAGTACCTCGGGCATA	13254	AGCCAGGCAGCAAGCATGAA	24238	TCAGTGCCTGGCGCAGTA	35222
1597	CCTGTGGATTCTGGCTGAGGATTTG	13255	GGCATAGCGTGGTGAATGACT	24239	GCCCTTCTTAGTCCTGTGGATTC	35223
1598	GGGCTCCACACATTCCACCTTAG	13256	GAGCAACTCAGACTCATGGAGCAA	24240	CCAAAATCAATATGGGCTCCACACA	35224
1599	GTCAAGAAACTGACTGTCACTCCTA	13257	CACCAGCGGGAGCATCATTA	24241	CCCTGCCTGTCTCAATGAGGTCAA	35225
1600	CTGAATCACCATCACACGAACCAT	13258	CCCGGCAAAGCCTCCCTAAT	24242	GGGGAACAGGATATAGACCCATGA	35226
1601	CGCTGTGTATCAGGCATACCATA	13259	GAGTCTGAGCCAAAATCCTTCACT	24243	CCTGATCAGAAGCTACGCTGTGT	35227
1602	AGAGGGAAAGACTCTGAAATCTGAAC	13260	ACTGAAAAGGCGGTTGCTTATAGA	24244	TCTTCACCCAGAGGGAAAGACT	35228
1603	CCAGCGTGTTTCAAAGGCAAATG	13261	TGTAGGCAGGGTGGTCGTGATA	24245	TGGCATACCAGCGTGTTTCA	35229
1604	AGGGCCTAGAGGACGATGCTT	13262	GCACAATCGATAGGCACACCACAT	24246	CCCAGAGGAAGCATGGAACTGAAG	35230
1605	CCTCCTGCCATGTGGTTAGAGAGT	13263	GCGTCTGTGGGACTAACCTCTT	24247	GCCACACCCCTTCCTGTTCTT	35231
1606	GGATTAGTTACTGTGAGAGTGGGTTGT	13264	ACATGAGGGGCAGCTTCACT	24248	GTGTTCTTGCTTTTGAAGGACAGGAT	35232
1607	GCAAGACATTTGGTAGCCTTTTCAGA	13265	AGGGTATGCCTGAGCACACA	24249	CCCTGCCCAAGCAAGACATTTG	35233
1608	TCCCAATGTAATTTGAGGCAGAACA	13266	GCCCTCCAGCTCCCCATATC	24250	GCTCAGAGAAGCAGTCCCAATGT	35234
1609	GGGGTGAAAACAACAACCACTAC	13267	GGCTTCCACACAGGAAATAGTGT	24251	TGGGGTCATGTGGGGTGAAA	35235
1610	GGAACTTCGGGAGGATGTAGGTA	13268	GGGTGAGGTTGTGGGAAGCATAAG	24252	TTATGGCAGCTAGATGAAGGAACTT	35236
1611	CGCACAAAAGACCTGGCACAGA	13269	GCTCGCAGGCACCCTTTATTGA	24253	AGCCAGCTGCGCACAAAA	35237
1612	CCCAGTCTGCTCCTTTGGAATCTTG	13270	CCTGCTCCCGGATACACAGAAAC	24254	TCCTCCCCAGTCTGCTCCTTT	35238
1613	TTCCAAGTGTAAGGCTGAAGAAGT	13271	GGGATTCTTCCTGAAATTGGCTTTG	24255	GCTAACGGTCCATCAAGACTTCCAA	35239
1614	GGCTGGAGAAAGTAGTCCACAGA	13272	GGCAGAAGCCGAGTCAATCTCT	24256	ACACATTTGACTTTGGCTGGAGAA	35240
1615	GTGGACCACGTGCAGAAGTCA	13273	ACATGCGGCTTCGGGTGTT	24257	TCTGCCTAAGCTGGGGATCA	35241
1616	GCATGGTAGGACAGTGGACGAA	13274	AGACAGCGGCCAGGTTGAAT	24258	AGTGAAGACGCTCCCTGCAT	35242
1617	GGGATGCAGAGGCTGTTTCA	13275	CCAGGAAGCTTCTCAAAGTCAAGA	24259	AACCTGGCAGCTGGTGTTAG	35243
1618	CCCATGCCTACCAAGAGCTAGT	13276	TGCTTCCTTGCGCACACA	24260	TCGCACTCCCAGCCTGAAT	35244
1619	AGGGCTCTGACCTTGAAGAATTTG	13277	CCTAAGACATGTCCCCAGGAAAAA	24261	CCTTGTGCAGTGGCCAAAAA	35245
1620	TTCTCCATCAAACTCGGGATGAAT	13278	ACAGCCTGGAGACCCTGAACA	24262	GGTGATGAAGTTTTGTGCTCTTTCT	35246
1621	CCTTCTACCAGGTTGGACAAAGTCT	13279	CCTTCGAAACCCAGACGATCTCA	24263	CCACTTCAAACTCTGCACTTTTATCCTTCT	35247
1622	CAGGAATCCTCAGATAGGAGGTAGA	13280	GACTAGGACCTCTGCTAGGATTGTA	24264	CCTATGAATGGACTTTGTTCCTGTTC	35248

1623	ACCAGGACGAACCTTCCCATTC	13281	TTCCCTCCGGGGTCATGGTT	24265	TCGGCTGCACCAGGACGAA	35249
1624	CTGGACTCTGGGACTTCTTTTTGAGT	13282	CTGCACCCAGCCCAAATGAT	24266	TCTCTAACTGGACTCTGGGACTTC	35250
1625	ACCAACTTATACGAGCTTCTTGGAT	13283	GCTGGGCAATTCACGAGGTTTTC	24267	GCTGAACTGGAAGATCCAACCAACT	35251
1626	AGCTTCAGGTCGCCCCACTA	13284	GTGATTTTTAAGGCCAGCACCAAT	24268	AAGAGGCTCCCCTAGTCAGCTT	35252
1627	TGTACAGATACTTGGTGGAAGCAAT	13285	GGCAGATAGTGCCAAGAGAGGTT	24269	GCAGCCTCTGCACTCTGGATTT	35253
1628	AGTGATGCAATTGAAGGCACTGA	13286	ATGTTAGTGTGCTGTTTCCAGAGA	24270	AGTGTGCCACAGTGATGCAA	35254
1629	AGACGTGTGCCCTGTTCATC	13287	TCCAGGGCCTTCTGGTCATGT	24271	ACCCCAGTTCTTTAGCTGAGATTG	35255
1630	ACTGGACCAGCACCTTCTCTTC	13288	TGCTGCTGGGGCATGAATAA	24272	GCTGGTGATCCCACACTGAA	35256
1631	GAGATTGGCCCAGTCCTGTTTG	13289	GTGTCCTCGGTGTCAATACATAACAT	24273	TGACTCGAGGAGTGACTAAGAGAT	35257
1632	AGCAGCAGTACCACGTCTGA	13290	AAGCGCCAGCCGACACTGAT	24274	TCCAGGTCGTAGCAGCAGTA	35258
1633	TGGTGCTGTTTCAGGACTAGGAT	13291	GGGGAGGAAAGAGTGAGCTGTAAAA	24275	GGCCTCTGTGTTTGGTGCTGTT	35259
1634	TGCAGGCACGTGACGAAT	13292	CCTGCCATACAGCACTGACAGA	24276	CGTCATCAGCCAGAAGGAATGTCA	35260
1635	CCTGGGAAGGATCGGCATTATTTG	13293	TGCAGAGCCCAGGACAAAG	24277	ACCTCTGCCTCTTGCCTTTC	35261
1636	GCTTTCAGCAGCGTCCCAGAT	13294	CCCTCCAGCACTTTGGAGGAA	24278	CCCTTTTGGTTGCTGTGCTTTC	35262
1637	AGGTCGTGCTTTTAGTAGCAATGT	13295	AGGAGACAGTGGCCCTATTCA	24279	TGGCTTTCTTAGGTCGTGCTT	35263
1638	GCTTCGCACTTGAATCCTGACT	13296	CCTTCTCAAGGCCACTGAGCTA	24280	GACATGGTGGGAGAGCACAA	35264
1639	GGGACAGGCTTCCTGTGAACT	13297	TCCCACATCTGGGGACAGCAT	24281	AGGCAGGTGTGCACTGTATG	35265
1640	GTCATCCAAGCTCAAAATCCAAGAGA	13298	CCCAAGCTCCTCCTCCTTGAAA	24282	CCAGGCCTTTCTCACTCTGTCA	35266
1641	CCCACTTCCCGTGTTTCTCA	13299	CCTCTGCTTCTCTCCAGCTTCA	24283	GGCTCCTCCAACCCCACTTC	35267
1642	CTCAGCCTGCTAGGGAGTGA	13300	GGGAGAAGGGGTGGATCAAGAA	24284	CCACCGAGCTGCTGTGTCA	35268
1643	CCTCTCTGTGTGGGCTAGAATGCTT	13301	GTCTTTCAGCTCAGCCACAATG	24285	TCCACGTGACCTCTCTGTGT	35269
1644	GCACCTGGTGTTTTACAGTTTGACAAT	13302	TGGAGGCGTACCCAACTGT	24286	GGCCAGCACCTGGTGTTTTACA	35270
1645	GCACAGTGGGTGAGAGAGAAGCAT	13303	GCCTGCCTACCAGTCTGTCT	24287	TGGAGCACAGTGGGTGAGA	35271
1646	GTTGTCCATTGCCCCTTTACTGA	13304	GTGACTTGTAGGCATTGGTGTCAA	24288	GGTCATTCCCTTTGTGCCAAGT	35272
1647	TGGTGGTGCCACTCTTGGAA	13305	CCTTTGTATACCAGTTCCTTCTGCAT	24289	GCTGTCATCCTTCATTGGGTGAATG	35273
1648	CTGCATAGCTTTGCTTCACTTCTTC	13306	ACTCAGCCTCACAGGTGTTG	24290	CTGACCCGAGACTGCATAGCTT	35274
1649	GTGAGAGCTGCTAGAGGCTGAT	13307	CCAGCAGATCCTGCCATCCTT	24291	ACTGCAGTGGTCCTGGTGAGA	35275
1650	ATCTTAGGCCTAGGGAGCAAGT	13308	AGGAGCTAAAACACTCTTTACCAGATG	24292	TGTGCAGTGATAGAATGGCATCTTAG	35276
1651	GCTAGAAGGTGATGCTACAGGACGAA	13309	ACTGCAACAAGGGCAAGGAA	24293	AGTACTGTGCTAGAAGGTGATGCTA	35277
1652	CTTTGAAGTCTCTGCACCTCAGTT	13310	GCATGAATCACGCTTGTTGTACT	24294	GGGCCCTGACCAAGTCTTTGAA	35278
1653	TGATGCCTGCTCGAGACATTG	13311	AGCCAGCTGCAGTTCCAAA	24295	TCTCCCCACCACCCATCTTGA	35279
1654	TGTCGGCCGAGCCCATTTT	13312	CAGGCTACAGAAAGCATTTACAACA	24296	TGGAAAGGGCCGGTTGTGT	35280
1655	TCCCTGCAAAGGGGTCAAGGTA	13313	GGCAAGTGGGCACTTATCCAGTAG	24297	ATTGGGCCTCCCTGCAAAG	35281
1656	CCCTAATCACAGCAAATCTGCTCCTT	13314	CCCCTAAGGGTGAATGCAGAAT	24298	GAGTAGACCCCTAATCACAGCAAA	35282
1657	GCTTGCGGGATGTATCTTCCTT	13315	CCACTAAACCCACAGAACCCAGAGA	24299	CCCAGTTATCTGCTTGCGGGATGT	35283
1658	GGTGAGGCTCAGCCGTCTATACT	13316	CAGCCAGACCATCTGTGGAACAA	24300	GCAGACTTGCCAAATCCCTCTCA	35284
1659	CTGCCACTTGCTCTGTCTCA	13317	CAGGTGTGCATGGAGATGTTTGAAG	24301	TCTCCTAGGCCTCTGCCACTT	35285
1660	GCTTGGCTCAAACATCACCTCCTA	13318	GAGGATGGGGCAGGTCAAAGAA	24302	CCTTGAGGGCTTGGCTCAAA	35286
1661	ACAGGCCTACCAACCACAGA	13319	ATCACGTGGCTGCTGCTT	24303	TCCCACTGGGGCCCTTGTAA	35287
1662	TCAGGTGGACTGCGAGTCTT	13320	CCACCCTCCAGGTGTTTGCTTT	24304	ACCCACCCTGGCTGTGTTCA	35288
1663	GGTAAGGTATCCATCCTTATGGAGTTT	13321	ATCAGTGTTGGCGTCTCCTTT	24305	CATGGTGAGTAAAAGCTGGTAAGGTA	35289
1664	CCTGCCCTAACCTCCGTTTACA	13322	GAGGCCTGAGAAACTCGTGATG	24306	ACCCGAGGTGCCTGCCCTAA	35290
1665	TGCCCTCTGGGCTCCCCTTT	13323	GCCTCATGTGAGGGGCTTTTC	24307	TCCCTCCTCACAGCCACTTG	35291
1666	GCATGCTTATTCAAGCGAGAGATGAT	13324	TCTACTGCTCCCACCTTAGTCAA	24308	ACTAAGAGGCTGCATGCTTATTCAA	35292
1667	GTAAGCCAGGGAAGAGTGGAAAG	13325	GCAGCCACTGATGCGAAGAAC	24309	CACCCAGTGTGACTGAACAATGTAAG	35293
1668	GACAGAGTTGATGTGGGATTACCTT	13326	CCCTTCAGGTGGTCCTGTTTTG	24310	GCCCTGTTGTATGCAGGACAGA	35294
1669	CAGGCCTCCCCTTAAAATGTCATC	13327	GGCTGGGGTTCAGGCCATA	24311	TGTCTTTCAGGCCTCCCCTTA	35295
1670	AGGGCTGTGCTGATGGAACA	13328	AGGCCCGGAAAAAGCACAGT	24312	AAGCCAGGGATGCACTGAAG	35296
1671	CTCCAACTGTTGCTGAATATGTCTAC	13329	TGAGTGCCCAACGGCAAGAA	24313	TGGGCGGATGTCTCCAACTGT	35297
1672	GACAACACAGTTCCCTTTCCATTTTCA	13330	TGGGGACTGCCCTATAATGTCT	24314	CTGCAGACAACACAGTTCCCTTT	35298
1673	GTGGACCTCTCAGGGCTACATC	13331	GGCTGGAACAGCAAGTCTGCTA	24315	CCCTACAAGTGTGGACCTCTCA	35299
1674	AGTCGATGGGTGGTGGAAGA	13332	ACCACCAATCCTAGGCAACTTAC	24316	GGAGATGGCGTGAAGTCGAT	35300
1675	TCCAGGAAAGTCAATTCATGGAGTAG	13333	CACAGGGGAGGTTTCCTGGTTA	24317	GCTGTGTCCAGGAAAGTCAATTC	35301
1676	GGGCCAGGTAAGGAGCAGTAG	13334	CCCTCTCACCAGCTCCTCTCT	24318	GGGAAATGCCTGTGCATCCTTCA	35302
1677	CTGTGAATGCAGGTCCTTGTTG	13335	CACCACAGAGCCCAAGAACA	24319	GGCAGCAGTGTCAAGCTTCTGT	35303
1678	CTCAACATTGCATGCTACAACCTGAA	13336	GTGGAAATTGCCTGCGTGTCT	24320	GTCCACCCCTCAACATTGCAT	35304
1679	CTCACAGAGCTATCAAGGTCATTCACA	13337	TCATGTTGGCCGGTGGAAAT	24321	GGCCCGAAATCAATACTCACAGAGCTA	35305
1680	GGCAGAGGAGTGACATAAGTTGA	13338	GTGCAGCCACAGTAATGCTTTT	24322	CCAGGAACCATGGCAGAGATGTT	35306
1681	ACCTGCGGTTCGTTTAACACA	13339	AACGGGTCCTGAGACCAAAC	24323	GGCTTGCCTGGTGAAGAACA	35307
1682	CTGGAGAGCCACCAATACCCTAGA	13340	CCTGAGCAATTCCCAACCTCCTCTT	24324	GTGATTCCTGGAGAGCCACCAA	35308
1683	GGCCTGGAGATGGACAGCTT	13341	TTCACTCTCCCTCCTGTGTAGAA	24325	ACTGCAGAGGGCCTGGAGAT	35309
1684	ACCCCGTCTTGGTTCTTACCTT	13342	GGTCCCTGGCACGTACTTCT	24326	CTCTCCTGCTTGTGGCACTT	35310
1685	CTCGGATTTCCATCCAGCGTTGA	13343	TAGCAGGAACAGTGGCGTGTCA	24327	GGCTCTGTAGCCTCGGATTTCCAT	35311
1686	GGCTCACAGCAGCTTCAGTCT	13344	GGCTTAGGTGGGAGGATCACTT	24328	TGGTGCAATCCTGGCTCACA	35312
1687	CCCGTCCCACCCTTTGTTTAGAAG	13345	ACGCAGAGACCCACAGCAATAC	24329	GCACTTTGGGAATGCCAGTGA	35313

1688	CCCGCATGGCTACAGAGTTAATA	13346	TGCTTCTCCCATTTTCCATTTCCTT	24330	ACCTCCCGCATGGCTACA	35314
1689	TGCCTTGTTGAACTTAGGCCATT	13347	GTTCCCAGAACTGGTGTGGTT	24331	CTCCGGTGCCTTGTTGAACT	35315
1690	GGGCTGGCAGACAGGAAAACA	13348	TCTGAAGCTCACATGGATTCCTTAG	24332	GTCATCCCCAGTTGTGTTGAGA	35316
1691	CCAGTTCCAGAGTCCCTCGTTTTC	13349	AGTTTTGTCAGAGGTGTTGAGTGA	24333	TGCAGCCCAGTTCCAGAGT	35317
1692	GACTCGGTGCATTCACTGTTTATC	13350	GCAGCAGGGACTAACGACAA	24334	GGGTTAAAACTGTGACTCGGTGCAT	35318
1693	GTGTTTGCAAGTTATAGGGTCTCACAA	13351	TCGCACTGCAACGGCTAA	24335	GCACTCGTCACCGAACAAGT	35319
1694	CCATCCCCATCACCTTATTTTGACAAG	13352	CAGGTGGCACATAGGAGCTGAA	24336	ACTCCCATCCCCATCACCTT	35320
1695	GCGCAAGTCCTATACTGGTCTGT	13353	CTGCCTGTTCCCAATCCAGGAA	24337	GGTTAACCAAGCGCAAGTCCTA	35321
1696	CTCCCTGCCAATATGCCAGTTC	13354	GGGGTCTGAACCCAGCTCCATA	24338	GGGCATTAAGTCTCCCTGCCAAT	35322
1697	TGGATGCTCAAGAGTTGGATCTTC	13355	AGCACCTCTACTTGGTCACAGA	24339	GGTGATGTGCCTGTGGTGGAT	35323
1698	TGAGCTGAAGTTCTTGCTGAGATT	13356	GGTTTCCCCAATACCCAGTTCA	24340	GGAACCATGATGAGCTGAAGTTCT	35324
1699	CCCTGAGGCTATCCAGGGAAGAT	13357	CCTCTGACGTGCCATTTGTGT	24341	GCCACTGCCCTGAGGCTATC	35325
1700	TGGCTCATAGGCAGCAAATTATTCA	13358	GGCCTGCATTCCCGAAACT	24342	GCTAATCTGTGGATAACTGGCTCATA	35326
1701	CCAGGTTGGTTAGGCTCTGCTCAA	13359	TAGCAAGGCCTGCCCTCAA	24343	TCCCCTTCTCCAGGTTGGTTAG	35327
1702	CACCAAAGGCATACTCCCATCAGT	13360	GGCTCAGAAGGAACTCATGCAAA	24344	GCAGGGCACCAAAGGCATACT	35328
1703	TCTTCCCTGCTGCCCACCAA	13361	GCAAGGAGGAACAAAAGTTTCCAT	24345	AGCTGCTTTAGAGGCTGTCTTC	35329
1704	GCAAAGAGGCCCCATGATCCTA	13362	CACTCCTATTCCCAAGCTTCCAGTTC	24346	GAGACTCAGACGTGAGCAAAGA	35330
1705	TCTTCTGCTGGAGATGCGATTC	13363	ACCACACCTGGGCAGAATAAG	24347	GGTGCTAGGTCGTGTTCTCTCTTC	35331
1706	GATGGCCATTCCTTACTCTCAGTT	13364	GCCGCCATGAACTCTACTCA	24348	CCTAGTGTTTGATGGCCATTCCTT	35332
1707	GGTGCTTTATCCCAGGGAGATG	13365	CCCCAGTCAGGGGCTTATACA	24349	TCCCTTCCCCTAGGTGCTTT	35333
1708	CACAAGGGTGGAGGTTACCTTT	13366	TGCCTCTCCTAATCTGTCTTTCTACT	24350	CCAAGGCTGGCCTCACAAG	35334
1709	TGCGATGGCATTCGTCACA	13367	CTGACCTAGGATCTGACTCTCTCTGA	24351	GCAGGTGTGAACGGTTGCAT	35335
1710	TGCATGAGGAGAGGTGTCCAA	13368	GCCACCAGAGACGTCTTTCCTTT	24352	GTTGGACCTGAGACTCCACCAT	35336
1711	CTGCACATCAGTTTGCTTGCAT	13369	GTCTCAGGATCTGCAGTTGACAAG	24353	TTTCCAGCCTTGGGCTCAGA	35337
1712	GGTCCCAAGTTACAGCCAACCAT	13370	ACCATCACCCTATCCCACAGT	24354	CGGGAAGGAGGTCCCAAGTTACA	35338
1713	GGGGTCATCCACAAATGTCTTCT	13371	CGCCAGTATCCTTCAAGGTGGAGTT	24355	TCCAGAGGGGTCATCCACAA	35339
1714	ACACCATCCCCTGAGTGACA	13372	GGAGCTAGGGAAGTGGAGGAT	24356	AGGACCAGGGCCCACACCAT	35340
1715	GCAGGACTTTGGAGGCTGACTA	13373	GGAGGCAGCTGCGGTAACTT	24357	GACCGTGCCTTCCTGATTTACA	35341
1716	AGCTCAACCTGAGCCCTTCCTT	13374	GGCACAGACAGGAACTCACA	24358	TTTCCCTCCACTGTAGCTCAAC	35342
1717	AGTGGCCCAGGTGAATACAGA	13375	GGGGCTCCTCTGCTGTGAT	24359	ACCACAGGGTGATGCAGAGT	35343
1718	CAGGTCAGGAACGGCAGGTA	13376	CAAACAGAGCAGGTCCGTGGTA	24360	ACAACCGAGCAGGTCAGGAA	35344
1719	GGAACAGTATTCTGACTCCAGATCCTA	13377	AGGCAGCAGAGAGTAGTGGTTTA	24361	CCCAAGCTTGTAAGTGGAACAGTA	35345
1720	TCTTTGCACCCACCTCACATC	13378	TAGGCCCATGCCACTGCAGAA	24362	ATGCCCGGCCAGTCCTTTAT	35346
1721	GCAATGACCTAGCAAGGTGTATGTCT	13379	GTCTTCTGAGTGGTGCAAGGAA	24363	GCAGCTAGCAATGACCTAGCAA	35347
1722	CCAAGCCCCAAGTCAGAAAAGTT	13380	GTCATAGCTGGCTGCAGACTT	24364	AACTCCAGGGGTGCCTTCCAA	35348
1723	GCTTTAGGTAATGAGCCAGACTGA	13381	CCACTCCCTGCCCATCTGT	24365	GGAAGACCCGGATGAACAAACAAAG	35349
1724	TTTACAGGACCCCTTTTTCTGTCTT	13382	TGCCTTCCAGGAGCACAGAGTT	24366	CCCCTGTTCTATTTACAGGACCCCTTT	35350
1725	GTCTGGCTCTCGGGAAAGAGAA	13383	GGCAACCCTTCCCTTCAACCTT	24367	CAGCACCAGGATGTCTCAGATTAC	35351
1726	GCCTGCTAGACAGGAGAGTTGCTT	13384	TCAAGCCAGTGACCGGAGGTTA	24368	TGGGAGCCCAGCCTGCTAGA	35352
1727	CCTGGCCTTAGGATTGGGAGATGA	13385	CCAAACCTTTCACACTGTCTTTCAA	24369	AGGGCCCTGGCCTTAGGATT	35353
1728	GAAGCTGCAGGTGCTGTACTCT	13386	CCTTGAGCAGATCCATCCCCATGA	24370	TTCAATGCCAGCCCACGAA	35354
1729	TGGGTGGACTGGATTCCTGCTT	13387	GGATGCTCTTCCAACTGGTTCA	24371	GCCTCTTCCCCATCCTCACTTCT	35355
1730	GGAACCCCAGTAAAGATAGAGCAGAA	13388	GCCCAGTATCCCTAGCTCCATAC	24372	CCACTGGGCAATTGAAGAGAGGAA	35356
1731	GGTGGGAGTAGTGGGGAGAAA	13389	TCCTAGTGGAATCTTCTGATGCATTG	24373	TCCCTTACTGGGGTGGGAGTAGT	35357
1732	GCCGGAGTCAGGTATTGGCAAAG	13390	CAGGTCCAGGGCCCCTTTT	24374	AGTGGGCCGGAGTCAGGTA	35358
1733	GGCAGGCCCCAAATCACTTACA	13391	GGCTTCCCATTGCCTTCCATTG	24375	AGAGTTAGGCAGGCCCCAAA	35359
1734	AGAGCCAGCCCAGTGGTCTTA	13392	GCAGGGTCTCTGCTCAACTT	24376	CAGCCTTCCAGGCCAAGTATGT	35360
1735	TGGGTCACGCCAACCTATTC	13393	GGAGAGTAGTTGCAAAGCTCGAA	24377	TCTCCAGCACACCCTCTCCAT	35361
1736	AAGCTGCAGCCCTCCTTTTG	13394	AGCCCTGCTGCTGCTTCTT	24378	TGGGGTCGAAAGGGGTTGTAAG	35362
1737	ATTAACGCTGGGTACCTGAAAGT	13395	ATGGGCCTACCTGAGTGACCTT	24379	CCAAGGGACAGGACCATCTCT	35363
1738	CTGCAACAAACGCCACTTATCA	13396	CTGACCCGGTGACCGAATG	24380	TGCTCCTCCCTGCAACAAAC	35364
1739	GCTTCTGAGGGTTTCTACCACTAACT	13397	GCTCAGGCCTGCTTTCTTCAA	24381	CAGCCTTTGCTTCTGAGGGTTTC	35365
1740	CAAAGAAAGGAAAGCCAGGTAGCAT	13398	TGCCCGGCCGGTACATTTTT	24382	GCAAGCAATGCACAAAGAAAGGAAAG	35366
1741	CACTTGCTGTTGGCAGAGCTA	13399	AAGGTAAGGGCTCGGGAACT	24383	GACCTAACTTTATGTCACTTGCTGTTG	35367
1742	CACCTGAAGGCAAGAATTGCTACAAA	13400	AGCCTCCCTATCATTGTGGATGA	24384	CCTGTTGAAAGGCATTTCTCACCTGAA	35368
1743	CCAACACGGAACGGTGCTGATA	13401	GCAGGGCAAACGTCCTGAAGAA	24385	TCTCACAGCCAAGCCCAACA	35369
1744	GCACGGGCCACTTTTCTAGATG	13402	ACTCAGAGGGACTCCCGGTAT	24386	AGACAGCACGGGCCACTT	35370
1745	GCAACACTTGAGAAATGCTGGTTCTCT	13403	TCTCCTGCTGCCCCACCAAA	24387	TGAAAACTTGGTGGCAACACTTG	35371
1746	TCTATTGCTGGCCCCTCTATCT	13404	TGGGGTGAAAACGTGAAGTGA	24388	GCCTGCCCCAGCTTTTCTTCTA	35372
1747	AAGAATCCAGAAGGAAACCAGTGT	13405	AGCCGTGGAGTCCATCCTCTT	24389	TGAGGAGCCTGCCTGCAA	35373
1748	TAGGAGAGCGCCGTGCAT	13406	AGCTCTCATGGAAGCCTGTATTG	24390	GCTCACTACTTAAGGGGATGTCAGT	35374
1749	TGAGGCTAGCTCGGTGAACT	13407	GCACCCAGACAGACAGAACTGA	24391	CTGTGTTGAATGAGTAGGGTGGAA	35375
1750	CTCTACTGGTCTGGGGTCCTAGT	13408	AAGCCCCACTAGGTTGGTGATG	24392	CCCAGGCTGAAATTGCTTTCCTA	35376
1751	GTGGCAGCCTCGTGCAGTA	13409	GCCAGGAGGGTGCAAGGATTT	24393	GGCAGAGACTAGGGTTCCAGGTT	35377
1752	GCTGACTTGCTGTGGGTCTCT	13410	AGGGAGGGCTGCTCCTTTAGAT	24394	ACGGCTCGCTTGCTGACTT	35378

1753	GCCCATCAGCAAGTCTTATTGACTCT	13411	GGGCAGACTCAGGATGGGTTTT	24395	ATCCCCAGCCCATCAGCAAGT	35379
1754	CCTTCCCTGTGTGTAAGACTGATCT	13412	AGTGGCAGGTCAAGGCATCT	24396	AGCTCTCCTTCCCTGTGTGT	35380
1755	CTCGGGTTGGCTGTTACAATCGAA	13413	TGGCGGCTGCTCAGTCTTG	24397	ACAGACTCGGGTTGGCTGTT	35381
1756	TGGCCGGTGGGGTCTAAGT	13414	GGGAATGAAGCGGGGAGTGA	24398	TGTCCCCTCAGGCCTTCTGTA	35382
1757	CCTGGCAGAGTAGCTTCCCATTC	13415	CCTCATCCTGCAGAGGAGCAAAC	24399	TCCGGAGGTGCAGGTTTTG	35383
1758	AGCGGTGTTTGAGGAGACATT	13416	GGGAGGGATGTTGAAGATAATTGGAT	24400	CTGGGTAGTAAAAGCGGTGTTTGA	35384
1759	GCCTCTAGGAGAACTGGCAACA	13417	AACCGGGCCCAGAGGTTAAG	24401	GCAGCTACACTTGCCTCTAGGAGAA	35385
1760	GAGGGGAAATCAGAGCTTTGTCA	13418	GCATAGCTTGGCAGTGAACTTG	24402	TCCTGCCCCTTTCCTTCTAGT	35386
1761	CGCCCCAACACTTCCAGTCT	13419	GCTGAATGGATCACGGAAATGATCT	24403	AACCAGCTGCGCCCCAACA	35387
1762	CACTCAGATCTCTGGTTCCAATGT	13420	GACAGGGTCAGTATCCCAGTCA	24404	GGTCTTGTTACCATAATGTGAGTCTTCACT	35388
1763	CAGGAAGCCCCAAAGAGTTGAAGA	13421	AGTGGGAAGGTGGGAAGCAT	24405	TTTGGCAGGAAGCCCCAAAG	35389
1764	TCGGACTCTGTACCTCTCTTCTTT	13422	GGATCCCCAGACAGAGGATTCAA	24406	GCTGAGTTTCAGTCGGACTCTGT	35390
1765	GCAGGAACTTACTAATGGGATCCAGAA	13423	CCTCTCACCTTGAGCTCCTGATA	24407	GAGGATGTGAGCAGGAACTTACTAATG	35391
1766	CGGTGGGAATCCCTGACTTTC	13424	GCCTGGGCAACCCACATTTTG	24408	TAAGAGGAGGGCGGTGGGAAT	35392
1767	CAGATGTATCTTTTCAGGGCCATTAC	13425	ACACAGGTGGAGCAGACCAGTT	24409	GTGTGCTGCTTGCAGATGTATCTTT	35393
1768	GGACGGTTTGGCCAGGTTTCT	13426	CTCATGGGCACCTTTGACTTGA	24410	CTGTAGTGCCAAAAGGACGGTTT	35394
1769	AGGAGGGCTGCACCAATCCTAT	13427	CCTTGCAAAGCGATCCAGACCTT	24411	GCCTTCCCTGAGATGGCAAA	35395
1770	CCCCTGTGAATTCTAACCTAGGAAGT	13428	GGAGTCAAAGCTCCGGCTTCAT	24412	GGCTCAGAAATGCAGGGCTAT	35396
1771	GCTTCTCCCAAGCTGATCTCTGT	13429	GTGCAGCCACCAAACTTTACTGA	24413	GCGCAGGCCATTTTTCTGCTT	35397
1772	GGAAGTGACCAGGTGCCATGAT	13430	GGTGGCCTTTGTTCCCTTGCTA	24414	GGCAAAGCATCGCTGAGGAAGT	35398
1773	CACCAGACCTTCCCTTCAGTGT	13431	CTGTGACCAGAATGAGACATGAGT	24415	GCTGTCACATTCCACCAGACCTT	35399
1774	AGGGAACAGAGAGGCAGTACA	13432	CACCACCTTCTTAGGTTTACCAGAT	24416	CCCCATACAGTCTTAGGGAACAGA	35400
1775	CCCCAGTTGCACATGACTGT	13433	GGCATTGCAGATGGAGAATTGACT	24417	CTCATTCACCATTTCTCCCCAGTTG	35401
1776	AGGCTGGAGCGAGACACTT	13434	GCACCCCTAGGTTGAACCATTTTC	24418	TGGTGGGACTGTGCTGTTAC	35402
1777	GCCAGGGATAAGTTCCTTCTCTAAC	13435	GGCAGAAGTCACCACATCAGTT	24419	CACCATCATGCCAGGGATAAGTTC	35403
1778	CTACTTCTGCCATTCTGGTCCAA	13436	TGGCAATTGTCCAGGCAAGA	24420	TCCACCTACTTTCTCAAACTCTACTTC	35404
1779	GCTTCCATCTGTACCCTGTTACCTA	13437	TGTGGCCTTCGAGCATGTGA	24421	TGCACACTTTGCTTCCATCTGTA	35405
1780	CAGGAGGCCAATCCAAATCACA	13438	ACCTAACATGAGCAGAGGCTCTAA	24422	AGAAGCACAGGAGGCCAATC	35406
1781	CACCCTCCCAGATCTGACGATCTAA	13439	CGATGAAGGCACCCTAGAGCTATT	24423	CCTGCATGCACTGGAAAGGATGA	35407
1782	CAGTCACAACAGGGGCATCTCA	13440	CAGGGCCAAGAAACACAGCAT	24424	GGGAGGGTCCACAGTCACA	35408
1783	GGAGCCCTTATCTGGAGACAGAAC	13441	AGCCCAACCCACCAGCCTAA	24425	CTGGTTTTGAAGGGAGCCCTTATCT	35409
1784	TGGTCGTCCTGATGGAACTTG	13442	GCAGGAATGGAAGATGAGGCACAA	24426	AACCCACCCTCACTGCATCT	35410
1785	TCATTGAGGCTGGATGTGTATCAA	13443	GCTAGGATGACCATATCCCTGTGA	24427	GGCAGAGAGGAATAAAGACCCATCATTG	35411
1786	GGGGTCAGCTGAGCTAATGTGT	13444	ATCCTCTAGGCCCACCTTCTTC	24428	CCTTCCTACCCTAGAGCCACAT	35412
1787	GTCGAAGTTGGCATCCGTTCA	13445	AAGGGGCATGTGCCCAGTAA	24429	GGTTCCAGAGACCCAAGTCGAAGTT	35413
1788	CTTCCTAAGAAGGCAAAGACTGAGA	13446	CCCCTGGACTGTGTGCTTCA	24430	CCTTTAGTTCACCCTGTCCTTCCTAAG	35414
1789	GGCCTGTCTGGATGGTGGTT	13447	TCCTGAGACACTGGCCTAACT	24431	TCCTGACCTCTGGCCTGTCT	35415
1790	CCCCAAGCCCTGTCATCCAATAC	13448	AAGTGATGGCTAAGTGCCTTGT	24432	GTGTTGGCGGAGATGCAGAA	35416
1791	CAGAACCGTCTCTGCGTCCAA	13449	CCACGCTACTGTCCCGCTTAG	24433	CTCGAGTTTGCCAGCAACAGA	35417
1792	GGATTGGCTAAATTGATAGCTCCTCAT	13450	TTTCCTGCCCACCCAGTGACA	24434	CCCCTAATGCCTCGCAGTTCTT	35418
1793	CAAAGCAGGCGGGAGAACTAGA	13451	GCGTGGGCCTGGATGTTCA	24435	AGGCTAGAGCGCGGCAAA	35419
1794	CAGCTATCTCGCTCTTGACACT	13452	GGAGCCACAGGGACATGAATAG	24436	AGTCCTGAGAGGGTCAGCTATC	35420
1795	CCTCCTACTCACCAAGCAGGAA	13453	TAGCAGCATGGAGTGGCTGAGT	24437	TAGCTGTGGCTCCCTCCTACT	35421
1796	CAGCCTCTTCACGTTGCCTTGT	13454	GTGCTGCTACCGTTACTGAACA	24438	ATCCTCACCCCAGCCTCTTC	35422
1797	AGCATGGCAACACTCAACCAA	13455	CTGTGGTAGAAGCAGGAGGACACT	24439	GTGAAGGAAACAGCATGGCAACA	35423
1798	CTCAGGGACCTACGGTGTCATTTG	13456	AGAAGCAGTGTTGGGGAGCTA	24440	AGCCTCTGGAGGCAGCAT	35424
1799	CCCCGTGATCCTGGTCTACACA	13457	GGCACAGGGCTGCAACGTA	24441	AAGCCTCCCCGCCTCTTCT	35425
1800	CACAAACCTGGCTGTCTTTGTGA	13458	ACTCAAGGTCCTAGTGCTTTACAATC	24442	CCGCCCTGAAGAGAAGAACACAAAC	35426
1801	GTCGCTGGGATGCATAGGAACA	13459	GCTCAGCGCTCAGTAGTCTCT	24443	AGGCATGGCAGCAGTGTGTA	35427
1802	GTGACTGTTTGCCTGCCTTTC	13460	AAGGCCCTCCAGGGACTCACA	24444	GCATGTAACTTACCGTACTGTGACTGTTT	35428
1803	GCTGACAGGTGAGACTCTGAAG	13461	CCAGCCTGTCAATAAAGCTGAGAT	24445	AAGCAGGGTCTGTGCTGACA	35429
1804	GTCGCGGGAAGCTAAAGATAACAT	13462	CCCCAAAACAACTCAGGGACATA	24446	TCGAGTCGCGGGAAGCTAA	35430
1805	GGGGTGGAATCAGGACTGTGA	13463	GGCCACGTGACTCATAGATGCTT	24447	CACTGATAGCAAGGGGTGGAATC	35431
1806	GGAGGCCTTAACATACGAGGTGAGA	13464	CACGAAGCTCCACACACTACA	24448	GAGGAGATGGAGGCCTTAACATAC	35432
1807	GGTTGTGGGCTACGCTTGTCA	13465	GCACAGGAGCTGCAGAGTGAAA	24449	TTCCCCTTGCCAGGAGGTTGT	35433
1808	CATCAGGCCCTACTTCCAGAGATG	13466	GGGTCAGAGGGAAAAGCTCCAT	24450	CTGCCTTCATCAGGCCCTACTT	35434
1809	CGGGTAAGCCGTGAACAGTAGT	13467	ACAGCGTTACAATGACACACTTG	24451	TCGCTGTGACGCGGGTAAG	35435
1810	GCCTTGGAAGGGAGTCTGGGAAT	13468	AGAGACGGCTGCCTCGTTGA	24452	AAGTGGCGTGGCCTTGGAA	35436
1811	CAACACTAAGACATGCCCTTGAAGAAG	13469	CCCGGTGAACCGCACTCAA	24453	GGGCACAAGCGATTTCCAATCAA	35437
1812	GCATACAATATGACCCACTCAGCTT	13470	CCTGTGTGCCTCAATCTTCCAA	24454	CAGTGGGATTCCAGACGGCATA	35438
1813	GAGGTGCGGCAGCTACTTTTTC	13471	CAGAAACAGGCCCTCAACCATGA	24455	GGGCTGCTTTACCTCTAATGCCAAGT	35439
1814	CGTAGCCCGTCCTTCAGAGA	13472	CAGGGCGATTCTTGCTCATCA	24456	CGAATCTAAGACAACACTCCGACGTA	35440
1815	GAGCTGTGTATGTACTGAGAGATCAA	13473	CAGGGCTTTAGGACTGCCATT	24457	GAGGTGAGGGAATGAGCTGTGT	35441
1816	GAGAGACCGCTTCACCATCTTC	13474	GTTCCAGGGATTTGACCCTTTTCA	24458	CCTGAACACCACAGCCTTAGAGA	35442
1817	CTGTGCCTTTCCACCGAAGA	13475	GCCATCCAGCACCACAGCTT	24459	GGAAGGAAGGGCTATGCTCTGT	35443

1818	GGAAATACGACTCAGGGAAAATACAGT	13476	TCGCTCCTGACTCTGCACCTT	24460	GCACCTGACAGGAAATACGACTCA	35444
1819	TGCTCCAACCCCACAGTAAAG	13477	TCTCACCTCACTGTCATCTCTGT	24461	CACTTATCAGCAGGTACTATGCTCCAA	35445
1820	GCCATGGGCCATCCTGACAT	13478	TGGAGTGGGGACTGAGTTCAT	24462	TCACCATGGCTGCTGGGATTG	35446
1821	CTGGTGGATTTTGCGGAGAGTAG	13479	GCAAGTCCCCACCTGTCTGT	24463	GCTCTCACTTATTGGAACTGGTGGATT	35447
1822	TCCCAGGTGTGTTCAAAGATTGT	13480	TGAGCCAAGCACCGTCCTAA	24464	CCCAGAATGTTCCCAGGTGTGTTC	35448
1823	CCTCTGCCCTTTTGCCATTTTT	13481	GCTGGAAAGGGTTTGATTCAATGGATGT	24465	CTCATGCTAATCCTCTGCCCTTTT	35449
1824	CTTCAGGTTGGGGAGATGCCTTAG	13482	GATTGCAGGCCTCCATGCAAAG	24466	ACGGCCAGTGTCTTCAGGTT	35450
1825	GTGTTGGTCATTCCTGGAGAGT	13483	ATTCCTGCTGCCCTCGCATT	24467	CCAAGTTGGGAAAGCTGGTGTTG	35451
1826	GCTGAGAGCTTGGGTTATTCCCATAC	13484	CAGCCTCAGGGGTGAGAAATGA	24468	CCATGTTGCTGAGAGCTTGGGTTA	35452
1827	CTTTTGTGCCAGACACCAATTCT	13485	TTGCCTGAAGTCTCTGCTCTAAG	24469	TCTATGAGACTCTGGAACTGTCTTTTG	35453
1828	GGACTGTGTACTTGCTGAATGAGGTA	13486	CCAGCTAGGACATGGCAGTTC	24470	CAGAGAGGAAAGGACTGTGTACTTG	35454
1829	CTTCAAGGAGGAGAAGGCACCTA	13487	GCCCAAATCCTACCCTTTGTTTAC	24471	CTGGAAGCATCAAGAAAGGCTTCA	35455
1830	GCTGAAAATTGCCCTGCACACT	13488	CCCCTTTGCCAGAGCACATCT	24472	CCCAAGTTGAGGACAGCTGAA	35456
1831	AGTGAAGGGACGCCACGTTT	13489	GCAGCTGGGCACACAGAA	24473	AGCAGGGCAGGGCAGTGAA	35457
1832	AGTGAGAGACCCAGGGACCAA	13490	AGAGCGCTCCGCCTGTACT	24474	TCATCTCGGCCCAGTGAGAGA	35458
1833	GCAACACTGGATCCTGGAGAGT	13491	CACGTCTGCGTGAGTACTGT	24475	TGAACAACGTAGCAACACTGGAT	35459
1834	TGGTCGAGAAATGTGACCCAAA	13492	CCAGTCAAACTGGTCTCTTCAGT	24476	GCAAAGGCTTGTGGTCGAGAA	35460
1835	CCAGGACCAATGGGTAAAAGTAACA	13493	AGCTCTTCCTCAAGCCAAAATCT	24477	GCTACAAGGGTAAAACCAGGACCAA	35461
1836	GCCTTTCCAAGGGCATGACA	13494	GCGGTAGTGTTTCCCTCTCTGTAG	24478	TGCCATTTGGCCAGCCTTTC	35462
1837	CCTGTTTGGCCAGTAGACAAATG	13495	TTGCAGGGAAAGGAGAGTACAAG	24479	AGAGCTCTGGCACCTGTTTG	35463
1838	CGTCATTGGATCTGTCTCCTTTTTC	13496	GAGGGAGAACACCTGCCTTGAA	24480	GCTCCCAGACGTCATTGGATCTGT	35464
1839	CTGACCGCATTTGAGGAGTCT	13497	GTCATTTCAGGCCACGTGCAA	24481	CCCTGGAACTTCTGACCGCATTT	35465
1840	GGCCTTCCTGGAGCGAGTTAAT	13498	GCAATCCCGAGCTTGGTTGACA	24482	GCTAAGCATCCCAGAAACCCAAATG	35466
1841	TGCACCATGGAGACCTGGAA	13499	ACTGCAACCCGAGGAAGCAT	24483	ACCCCGTGCAGCTGGTCACT	35467
1842	AAATGGGTGACCAGCAAGTGA	13500	AATACATGCCTGGATTGCTCTGT	24484	GGCCCTCCCTGAGGAAGTAAAATG	35468
1843	GCATCCAGTTGCCTGACACT	13501	TCTGCTGGGTGGGACCATTC	24485	TTCTGGGCAGCATCCAGTTG	35469
1844	GGCACTGACTTGAGTGGAAAGT	13502	ACCATGGCCTTGGAGAGAGATG	24486	CCAGGTTCGAGGCACTGACTT	35470
1845	CGAATCCATTCTACCCGCCTAATTTC	13503	CCGGATGGGAGCGGAGAATC	24487	GTGCTTTGGCTTCCTCGAATC	35471
1846	AGGTGGTGACCTTAGCCAATAAC	13504	GGATCGGGATGCTGAAGCAATAG	24488	TCCTTGCAGGTGGTGACCTT	35472
1847	AACACGAGGGATTTCGCAGAT	13505	GCATCCCATCTTTTGCCACTCT	24489	GGTGTGCGTGCATTAGCAAAC	35473
1848	GGAATCTCCCTTCGTGGACTGT	13506	CACATCATGGAGCAGCTATGTCT	24490	GGACAATGACCTTTAGAGAGAAGGAATCT	35474
1849	TCCCGGTCTCTGGCTTTTCA	13507	CCCAATCTCCTTTGAATGGCAAAC	24491	GACTCAGGTATCCCGGTCTCT	35475
1850	TGGTTTTATCAGCTCACCCAAACT	13508	GCATCACGAGGAGCCAGTGTT	24492	CCTCCTTGCCTCTAAAGCTCTTGGTT	35476
1851	TGGGAGATTGCAAAGGTCACAAT	13509	CCGGGGCCATTCACTGGTAA	24493	GCCCACAGAACTCTGGGAGATT	35477
1852	AGAGCTCCATGCAAATTCCAGAT	13510	CAGAGCCTCACACAAGCAATGAA	24494	TGAACAGAAGAGCTCCATGCAA	35478
1853	TGGCACATTCCTCCCTGATACA	13511	GGCAAGGAAGGAGTGCTTGGAT	24495	GGGCTGGCTTCCCAAAGTGATT	35479
1854	TGGGGCTTTGGGCTGACAA	13512	TGAGGGAGCTGCAAGGAAGA	24496	TCTCTCTAGCCTGGGGCTTTG	35480
1855	TGTGGGGAGAAATGGCAAAACT	13513	GCCATGGCTGACAAGTCTATCA	24497	GGAGCATACTGTGTGGGGAGAA	35481
1856	GTTCGCACCTTGGCTTCTGT	13514	TGCATCGAATTTGAGAGGCAATG	24498	AGTCGGGGCTGCAGGACTAA	35482
1857	CCGGTGCATCTCTCATGCCTTA	13515	AACAACCCTCGTGCCCCTAGA	24499	CCTCAAAGCCGGTGCATCTCT	35483
1858	TCCGGTCACTTGGCCTTCTTG	13516	CCGCCTTGGAGTCCCTGAAA	24500	TCGCACCTCCGGTCACTTG	35484
1859	GCCTGGAATACCCTTTGCCTGTCT	13517	GAGTTCCGCCAGGATTTCACT	24501	TGCGTCTGCCTACGCATGTT	35485
1860	GATAAGGCTCAGGGCTTAATGATGA	13518	CTGGGGCTGGCTGAGATGT	24502	AGTCAGATGCAGAATAGGCTGATG	35486
1861	CCTCCCCAAATCCTCACTCCATTG	13519	GCCAGGTCTCGCTTTTGGTGTT	24503	AGAAGGCGACCTCCCCAAAT	35487
1862	CCTTCCTGTAGGAGACCCAGCTA	13520	GGCAGGATAAAAGCCCCTAAGAAG	24504	CCTCCCAGCCTCTCTACCTCTTT	35488
1863	CAGCTTAAGCCTGGTGAACTCT	13521	GGAGGAAGCCCAGCATCAAGAA	24505	CATGTGTTGACCCTAAACTTGACAAC	35489
1864	GCCCATTTTGCCCTCTGTTG	13522	AAGGAGAACAGATCATGGTGCAT	24506	GCCTCCCTTTGGCCCATTTTG	35490
1865	GTCTCGCCCAACCTCATTCTTC	13523	GCACATCCCTGCCTTCCCTAT	24507	CAGGCCTTGCCTAACTCAGATG	35491
1866	CACTCCATTCACAGCTAATGTTACGAT	13524	AAAAGCAGACACAGACCCAACT	24508	GGGTTGTTCACTCCATTCACAGCTA	35492
1867	GCACTAGCAGTTGGGGCAAT	13525	CGATGCGAGTTACCGAGTAGAAAAT	24509	CAGCAGTGCACACAGCACTA	35493
1868	CCCCTACCCCAAAGCCAAAA	13526	GCCACGGGAATGTTTACTCTGT	24510	GCCATTGGTGCTGCATGTCT	35494
1869	GCTGTCGCTCAGCCAGTTCTT	13527	AGCTTTGACGCCACCGCTTT	24511	TTCCGCTTCCCTGCCAAGAT	35495
1870	GCAGGCCCTGTGATGGATGAT	13528	GGTAGCACCATGTGCCACTGA	24512	CTCACTCTGGGACTATGCGAGAT	35496
1871	GCCAATATCCATGGCATAAATGAGGTT	13529	CACCTGGCAGTAGCCTTCGATT	24513	CCCTTTTCATGGACAGAGGACAT	35497
1872	GCCAGTTGCCGTTGATCTAAGACT	13530	GCTGCTGGAACCCCAAGTGAT	24514	TCTTGCCAGTTGCCGTTGA	35498
1873	CAGCAGGATGACTTCTGCGTTA	13531	GGCTCTAGTGAAGTGTGGTGAAC	24515	TGCCGGAACAGCAGGATGACT	35499
1874	CTTCCTGAGTGGATGGTTGACTCT	13532	GGCCTCAGGAACTTGAGTCCTTT	24516	GAGAGTTCTGTACCTGTGTGTATCTTC	35500
1875	TGCGTGCAGTGCAGACAGAT	13533	CCCCACCGTGCCACCTTTT	24517	ATGGCCAGGGCAGTGCAT	35501
1876	AGGACTCCTGGAAGCATGAGA	13534	GCTTGGCCTGGGAATTGGGTTT	24518	TGGCTCTCCCTTGGGCAGTAAG	35502
1877	ACACTGTCCCTTCCCTCAGCTA	13535	CAGGGCAGGTCAGACTGAGAAG	24519	GGGGCATTAAAGCTAGTGGAAAAC	35503
1878	TGACGGAGGTAGACGGTCAGT	13536	ACAGCAGCACCGCACTGTA	24520	CCCTATGTGCTGACGGAGGTA	35504
1879	GTGGAAAAGGGTAGCAGTGATATGT	13537	GTCAGGCGTCTGCCTACTTCT	24521	GTCTGCCTATCATTTGTGTAGTGGAA	35505
1880	ACAGGCTGGGAAGTGTGCTT	13538	CACTTTCCAAGTGCCTGGTAATGGTT	24522	GCAAGGTGGCCATTCTGACA	35506
1881	CTTCCCCTGTCCTTGGCATAAC	13539	AGGTGGGAGCTGACACGTATT	24523	TTGATCAGGGTCTTGGCTCTTC	35507
1882	TGAGTTGAGGCAGGGACATCA	13540	GCCCTGAATCCCAAGGAAACAATC	24524	GAAGCCCAAGGGTTGAGTTGA	35508

1883	CCTGGGAGACCATTTGGAAAACT	13541	GGCTTCTCATCCAACTCAGTACAA	24525	GGGGACCTGGGAGACCATT	35509
1884	TCCCTCCTGTGTCCTGTTGGAA	13542	CCCAAGCCTACGTGACACCAAA	24526	CCCTCTCAGCCTCCCTCCTT	35510
1885	CATTTGCCCAGCTGGCCTTT	13543	GCCCAGTACTGAGACCAAGAAA	24527	CAGTCCAGAGAGTGGGCATTTG	35511
1886	AGGCCCCTGTCTCCTAGTCT	13544	GCGCTTATCCAAGCTAGGTTCT	24528	TTCCAGGCATCACCTGAAGATAAG	35512
1887	CAGGGTAGGGAGGTGGAGAAG	13545	CACATGGTTTCCCTCCTCCTTTTT	24529	CCCTCTGTACCCTGAGGACAAACA	35513
1888	TGTAGACAACCTGGAAGAAACAGATG	13546	TCGTGGCACCATCTCACAAA	24530	GGGCCTTGTGTAGACAACCTGGAA	35514
1889	CAGAAAAGGCTGGTATCCCAGAAA	13547	GCCAGGGAGCAGCACAAAG	24531	GCGATGCACAGTGCTTTCTCAAAC	35515
1890	AAACCAAGCAGGTGGAATCAGT	13548	CACCCTCCTGTCACCTTCATTC	24532	TCGCACGTGGACCCAACTAA	35516
1891	GGTTTAAAGACCGTGAATGATCCCAAA	13549	CCCCTGCTCCCATGACACAAT	24533	GGTTCCTGAAAGGTTTAAAGACCGTGAA	35517
1892	GTGCCTAAGGCTAGTGTCATTCT	13550	GCAGTGAGTGGCAGCTATTGT	24534	GTGATCCTGTGAACTTTGTGCCTAA	35518
1893	GACAGAGCATTTCAGGTGGAGAGT	13551	GTTCTTACCTCTAGACCTGTGCAA	24535	GGTGCATGAAAGGGACAGAGCAT	35519
1894	GAGTCTCCCCAGGCCTATTAGGTT	13552	TTGGGCATGGAGGGAGGTAACA	24536	ACTGCCTGAATCTCTGGAGTCT	35520
1895	CCATTCCTCCCGGTACAGTCT	13553	CTGGGAAGTGCAAGGGGTCAAA	24537	TGGGCTGGAGTGCACCATT	35521
1896	TTCTGGGGCTGCTACTCACA	13554	TGCTAAGAAGGCTATGCTGCAA	24538	CCTGGCCTCATAACGTCCATTC	35522
1897	GCACTTCTGCCATTCAGTGTGTA	13555	ACCTGAAGGAGGGAGTGTGT	24539	CCCCTTTCCCTTCGCACTTCT	35523
1898	GGGTGGTTCCAAAGAAGGGATCA	13556	GCCCAGATTCCTGAGGGCTTTAG	24540	GGAAGAAAAGATCTGGGAGGGTGGTT	35524
1899	TCCACCCGCTTCTCCACATGA	13557	GGCTCTTTGGTGGTTAGCTGGACTA	24541	CAAACACAGATCTGCCTCTGGAT	35525
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1901	CTGAGACTAGAGTGCCTCCTTCA	13559	CACCAGATGCTCCTAGGTGTAGA	24543	CACACTCGACATCAACTGAGACTAGA	35527
1902	GGGGTTTTGCCAGTCATCTAAGT	13560	GAACCGTCTCCTTCTCCAAGTTC	24544	GCAGCTACAAGCCTTCACACT	35528
1903	GCATGGTGGTCTCTGGGTAGTTG	13561	GCCCTGTGAAGCCAGTCTCTAT	24545	GCTTGGGCTTCCTCACAGCAT	35529
1904	GGGATGGGGACAAGGGAAATCA	13562	ACACCTGAGAGACCCTCGCTTT	24546	TAGGGAGTGGGATGGGGACAA	35530
1905	CTCTCTGGGTTTCTGTCTGCATTC	13563	CCGTCACCCCAGTCTCACCTAT	24547	GACCAGAGAACCCTTGCGATTC	35531
1906	CACCTTGTCTTCAGGTGAGTGCTA	13564	GGGAAGCTGTAGTTGCAGTGAT	24548	AGTAGGTGTACCCACCTTGTCT	35532
1907	GGCACGCTGCACACTCCTT	13565	GCCCTCTGCTGACAGTGTGAAA	24549	TCCTTCTGTATGATCAACGGACTTG	35533
1908	GGGAATGGGCCAAGACTCAA	13566	GAAGTAAAGTGGACCTTGACATCTGA	24550	TCACAAGGCAGTGGGGAATG	35534
1909	GGGCAGCGTCCTGACATAGT	13567	CCTGGTGGCTCAAACCGTTAGT	24551	GCGGCTTGGTGACCGTGAT	35535
1910	GGGAATGGAGACCAGCAGAGACTA	13568	CACAGAGTGTGACTCAGGTCAAG	24552	GGATGTTGTTTCTAGGCTGTGATG	35536
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1912	AGACCCCAGGGCCACCATGA	13570	CAAGGTCCAGAAGCCAAAGCTA	24554	GCCGAGTCCAGCCCAATCA	35538
1913	GCCAGTCTGTCGATGCTCTTGT	13571	CCCTGGCTGATTTATGAGAAAGGTA	24555	ACCCCAGGAGCCAGTCTGT	35539
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1915	TGGGCTGTACAAGCTGCTTTC	13573	TCTCGGTTAACTGCAAAAGGACAA	24557	TGCCTCAGAGACTTGGGCTGTA	35541
1916	CTGTAACACAAGAATGCGTGCATCA	13574	TCTGGCCTTCCGGTCTTTTG	24558	GACATGCCTATTTGACCCAAATTCTGT	35542
1917	GGCAGATGGGACACTTTTTCTTG	13575	GCCAGAAGTCACAAACCAAAGACA	24559	GCAGTGCTAACAGTTTGGCAGATG	35543
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1919	CACTTAATCTCCACCACAGTGCTA	13577	CACAGGAGGGTGATGTTATCTGTCT	24561	TGTGCCTCGTCTCATCTCACT	35545
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1921	GATGCACTGGAGTGGGCATTT	13579	CTGGCATGTGAATTGCTGTGGAT	24563	CCGAAGGATAATGTAGGAGGTGAGAGATG	35547
1922	CAGGCTCACTTTTGGATCAGGCAAT	13580	GTAGGCCAAGGTTGCCTTAAAAC	24564	GCTGATCCCAGGCTCACTTTTG	35548
1923	CAGCTGGTATCTGGTGGAATACTT	13581	CCATCAGCAGCCTGAATGCTT	24565	AGGGAACTGGCTCAGCTGGTAT	35549
1924	ACCCCTCTCACTCAGGAGACA	13582	TGATGAGCACGTGAAAGTTTGAGA	24566	GTGGAGCAAAACCCCTCTCACT	35550
1925	CCACAAAGGGGTTGCCTTGAGA	13583	GCGTGAAGCCAGGACTTCCTAA	24567	AAACACCCCTCCCCACAAAG	35551
1926	CAGAACTGTGTGCTGCCTGAA	13584	CGCAAAGCCCCTTTAACTGCTGAT	24568	GGTGGCTCTCCAGAGATTTTAGACAGA	35552
1927	GACTCAGAGGAGACTCCCTTCATA	13585	CCTCCCTCACCAGTGGGTCTT	24569	CCACTTCCCGGCTGTGTTCTT	35553
1928	AGGTCTGCCCCAGAGGAAAT	13586	CCCTGCAAGACTGGTTGTGTGA	24570	AGACCACCGGGGACCTTGA	35554
1929	CCTCTGTGCCTTGGTAGAGGAA	13587	GCCTTAGCCACAGAATCCATTCACA	24571	GGGCTAGACACAGGATGAGTTTCT	35555
1930	AGTACCAGCCCTGCCACTGA	13588	ACCACTGGGATGAACTCCAACA	24572	GGGCCCTGTGAAAAGAGACTGA	35556
1931	GTTGGCCCTGCTTGGTACTT	13589	TCAGGCAAGCTGGAAATGAGTT	24573	CCAGCAGCTGAGGCAGAGA	35557
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1933	CTGGCATTGTGTAGGAAAGGAGAGA	13591	GCCCTAATGATGGGCACAAGGTT	24575	GGGGTGCGTGTGTTGGATTTCA	35559
1934	CTCAGGCTTCGCACTTGATCT	13592	TGCCTTCTGAGACCGGCAAGA	24576	ACGAGATCCAGGACTACATTTCTCA	35560
1935	CCCAATTCCTAATGCGGCAAATGT	13593	CCAGTGCTCACTGTCCAGTTCA	24577	GTCAGCTTCAGCTCCCAATTCCTA	35561
1936	GAAGGGAGCAGGAATTCACTCAATC	13594	GCACATGGCCTGGAAGAGATGA	24578	CTTTTCCATGAAGGGAGCAGGAA	35562
1937	GGTGGCTATCCAGAGTGTGTCT	13595	CCTCCTGATTTCGCCTCTCTTC	24579	ACTCGCAAAATGGGTGGCTATC	35563
1938	GGGGCAGAGTGGTGATGGATAACA	13596	TAATGCAGCCCACTGGCCTTAC	24580	AAATGGGCATGGGGCAGAGT	35564
1939	CATCAGAAGACCTGCATAGCTACA	13597	AGGAGTGACCAATGCCATGAAT	24581	GTGGTACCCTTAGTCCCCAAGA	35565
1940	GTGCTGGCACTAAAAGGATACAATG	13598	GCAGACGGCCCTGAGACAA	24582	GGTGCTGTGCTGGCACTAAAAG	35566
1941	GCAAGCGGTTTCCACTCTGA	13599	GCTTCCAGGCTCCATTGTGT	24583	TGTGGCCAGACCAGGCATACT	35567
1942	CTTGCTCACTGCTAAACTCTCAGAT	13600	CATCAACCATGTGAGGGACACTCT	24584	TCCCTGAAGCAGAGAGCTTGT	35568
1943	GGCATCCAAGGAGCAACAGA	13601	ACCCAGCTTCCAGAGACTGCTT	24585	CTGCATGTAAATCAGGGCATCCAA	35569
1944	CCGTCCATGTGCTGTCATCTCT	13602	GCAATGAAGGCCATGGAGACA	24586	GGAATTGGGTCCGTCCATGT	35570
1945	TCCGGTACCTGGCTCTTGGAT	13603	ACCCCGGAGACAATGTTCATC	24587	GCTAGAGAGGCTGTCCCTAGTTGT	35571
1946	GTTAGGTGGCATAACAAGCTGATG	13604	TTGGTAGGCTTGGGTCTAGTCA	24588	CACAACCTATGAGGTTAGGTGGCATA	35572
1947	CAGCCCTTTAGGGTATCTGCACTTG	13605	AGGCACCAGGCAAGAGTTTG	24589	GGCCAGTGTGACAGCCCTTTA	35573

1948	AGGTGGTCTGACCTTCCTCCTT	13606	CCCTCTGCTTCCTCCTGGTGAT	24590	ATGGGCAACAGGTGGTCTGA	35574
1949	GTCCCTACTCCAGGGCATATTG	13607	TGAATGCCTTTGCTTATGGCTCTA	24591	CTTCTCCCTCATTTGGCATTTTGTT	35575
1950	GGGACTGTGCAGACAAACCAA	13608	GCCAGACGAGCTGCACACT	24592	TGAAGACAAAACTGTGGGACTGT	35576
1951	CAGTGAGAAGAGAGTGCAAAGTCATT	13609	TGCGGGACACCAGACTGATT	24593	CAGGGTAGGAACAGTGAGAAGAGA	35577
1952	CACAGTCACAGACATGGATGGTA	13610	GGAATCCCGTCACTTACAAGTACAA	24594	TCCCACTGTCACCACAGTCA	35578
1953	GAGGATAACCTTTGCTTGGGTACA	13611	ACAAGGGGAGTGGCATTTTGT	24595	TTCTGCAGGTAGCAGTTTGTGA	35579
1954	AGTGTGATGTGCTGTTGTGTTG	13612	AACCATGCCCCTAGTCCACAGT	24596	GGGTTCTCAGGCGAGTAGATGT	35580
1955	GGGTTTCAGCAGCATATTCCAGGTA	13613	ACAGGTGATCAGCTCTGGATCTT	24597	GGCTTATTCAAGGGTTTCAGCAGCAT	35581
1956	AGGCCTGGTTGTCATCCCTCAA	13614	AGTGGGGAGGGTGTTACAGAGA	24598	TGGTGAGCAGGCCTGGTTGT	35582
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1959	AGGCTGCTGGCACAGACCTAT	13617	AGGGTGGGTGCTGGAGTACA	24601	TCCCTGGAGGGGTGAGGTCAT	35585
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1961	AGGAGCAGATCTGGCTGAGTTG	13619	AGAGCAGCCCCAGGTTCT	24603	AGGAGTCCGGAGGAGCAGAT	35587
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1963	ACTGGAGTCAGGAAGGGTAATCA	13621	GCATGGGGCCTTTTCCCACAA	24605	GTCGTTGAAAACTGGAGTCAGGAA	35589
1964	ACTGCAAGTGGGTGATTTGCTA	13622	CAGGCTCAGGCCCTCCTTTTAT	24606	ACTCCAGCTGGTGGGAGACT	35590
1965	ACAGTGCTCACCATTGGAAACA	13623	TCCCCTGCAGAACCACAGA	24607	GACCTCTGCTACAGGCAAACT	35591
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1967	TGGGCCTTTGAGGACACAGA	13625	TCCGCGCAGAGGAAAGTTGT	24609	ACCATTGGCTGGGCCTTTGA	35593
1968	CTGGCCTCGCAATAAACCTTTC	13626	GCCAATACTATAGCACCAGCTTTTGA	24610	TTGCCAACTGGCCTCGCAAT	35594
1969	GGGAGCAGGAAGGCAAGAACATTA	13627	GCCACTCAGGGGTGGATCTTTT	24611	AAGGTGCTGGGAGCAGGAA	35595
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1971	TGCTAGACCCGGAGTCACTTG	13629	GTGTGTGGCTGGATGCTCAGAA	24613	GCTTCCCTCAGAACAGAAAAGCAA	35597
1972	CCACTGGCGGCAGGAATGAAT	13630	CGTAGAAGGGCTGCGGGAAAAT	24614	CCAAGCTGTGGGCAGTGATG	35598
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1974	TTCCTGGCTTTGCTGCTTTTC	13632	GCACCTGTGTTCACGCATTTC	24616	CAGGATTGTGATTCCTGGCTTTG	35600
1975	GGTGACTGTTTTAATCCGTGCAA	13633	GGGCTCATTCACTAGGAGTGTTG	24617	CGAGATTAAGAATGATCCAGGTGACTGTT	35601
1976	GAACCGGGACTTCCCAGCTAAT	13634	GTTAGGGCTTAGGGGTCTGAAC	24618	CCAGTAAGCTGAACCGGGACTTC	35602
1977	CAGGTGCTATGGCTGTGAACTTG	13635	AGCAGCCAGGAAGGCTGATG	24619	GGCATTCTCTGCAAACAGGTGCTA	35603
1978	CCACCACCTCAGTTCCACACAAA	13636	GCTTAGCTGCACCGGACATCTT	24620	ACAGTGCCACCACCTCAGTT	35604
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1980	CTGTCCTGGTCTTCATTGACCAAA	13638	GGAGTTGTCCGTGGTACTGATTG	24622	TGTCTTGCTCTGTCCTGGTCTT	35606
1981	GCTACCTCATTCGGGCGCATT	13639	ACACGTCCCTCCTGGAGTTTC	24623	AGAGCGGCGCTACCTCATT	35607
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1983	CAGAAGGAAGCCTTCAAAGACACT	13641	GCTTCCCCACTGCTACTTCCTATG	24625	CAAATGCAGCCGTGTGAAAACA	35609
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1985	GTGCCTTGCCAAACAGGGAAGA	13643	TGCAGGCTGTCGCGTACT	24627	GGGAAAGCCTCATGGGATGGAA	35611
1986	CTCAGAGGGCTCTCCTTCCAT	13644	CCAACTGCCACTGACCAAAAG	24628	CCCGCATGCCTGTGTCTCA	35612
1987	GTCCAAGCAGGGCCTTCTGATT	13645	GCTCACTTGCCAGAACAGTTAAG	24629	GAGTTGTGCTTCCACAGTCCAA	35613
1988	GCGAAGAGGAATACGCTCAAAG	13646	AGCAGCTGGACTGCTTGACT	24630	GGAAAACAGCAGCGAAGAGGAA	35614
1989	GTCTTGGGCTCTATTTGCCAGGTTT	13647	CAAAGGAGCCACAATGGTAGGTA	24631	CTGAGAGCTGTCTTGGGCTCTA	35615
1990	CAGTGGAAATCTGCCCATTTTGT	13648	GCATTTGTGTACCCTGTTTCCTTTC	24632	GGGGCTAAATGTGTTGCTCTTCAGT	35616
1991	GAGCCTGTGGACAGAACCACTA	13649	TGTGTGATTTTCAGGGCTAAGAAGT	24633	AAACCAGGCTCCCCAGCTTCA	35617
1992	GGATGAGTCCTCTAGTTTTGGTGTT	13650	TCTAGGCCCCATGACTGAAGTT	24634	AGCAACGGGAGAGGATGAGT	35618
1993	CCCTCAAAGCAACATTGGCTACACT	13651	GTTGCTTGCCCAAAGTCAGAAG	24635	CGTTCTCCTCCCTCAAAGCAACAT	35619
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1998	CCAAAAGCAGGAGAATGGGGTTCT	13656	CAGGCATATAGAACTCCTGCCATT	24640	GGTAGCCATCCAGGTAGACCCAAA	35624
1999	CGGTTTCCTCGTAGCCCTAAAC	13657	TGGGTGGAAGAGAGCCTTGAGT	24641	AAGGGGTCTCGGTTTCCTCGTA	35625
2000	AGGCAGCTCTTACCTCCCCATT	13658	AAGGACTGCCCCTCCTCAGAAA	24642	TTTGCAGCCTTCACCAGGAA	35626
2001	CAGGTCAGAGAACCTGTGTGAA	13659	TGGCATGCCGGCACATTGA	24643	CAGGCAGAACTTTTCAGGTCAGAGA	35627
2002	GGCAATGACACTGGAAAAGAATCAA	13660	GCACCACCATGGCGACACA	24644	CCCAGGCAATGACACTGGAA	35628
2003	AGCTCCTCCATGGCCTGTCT	13661	GGAGAAAAACCCTTCTCACGCATCA	24645	GTGGAATTACACACAGCTCCTCCAT	35629
2004	GAATGGTGAGAGAGGAGGTCTGA	13662	GGGGATCAATGCAAACTCTGTAGT	24646	GGGGCACAGTTAATGAATGGTGAGA	35630
2005	AGCATGGGCCTTGGTGACAT	13663	TGCCTACCTTGGGAGGGTTCA	24647	ACCTGCAGGGACCTGTTAGCAT	35631
2006	GAGAAGGGTGGCATCTAAAACAGAGAA	13664	CACCACACCTCTCCCTTCTGA	24648	GAGACAGAGAAGGGTGGCATCT	35632
2007	CTCTGCTTTGTGACTGTGCTCCTT	13665	CCACTTTCGATGACCGCCTTGT	24649	GGCGGTAGCAATCTCTGCTT	35633
2008	ATCCGAGAGCTTCCTAGAATCAAC	13666	TGTGGCACCTCACAGCTATTTT	24650	CCCATTGACATCATCCGAGAGCTT	35634
2009	TTGGAGTGCCTCGCCAGCTTT	13667	GCCCGAGCAAGTCATATCCAAAC	24651	CACAGAATTGTGCCAGTGTTTCTCT	35635
2010	CCGTGTCTCTAGTCTCTTCCCATCCTAATA	13668	TGCCCCTGGCCTTCATGT	24652	CTAATATTCTCCGTGTCTCTAGTCTCTTC	35636
2011	TCCTCCCTTCCTCTGCCTGAAG	13669	CTGCCTCACTCCAGATCTCACA	24653	CTTCCAAACGCTATAGGAAGAGACT	35637
2012	CACACCAGCTTCTCCTTCATTCT	13670	GAGGAAGGACACCTCTTCTGTTG	24654	TCAGCTATAGACACACCAGCTTCT	35638

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2015 GGTGGTTGCGTGGCATTGAA	13673 AAAATCAGCTGACTGTATGGAGACT	24657 AGGGGAACTTCAGGGGTGGTT	35641
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2017 CTCCCATGCACAGTGTCTCACA	13675 GCAGGCAAGGGCTTCAGAAAGA	24659 TCCCCGCTCTGTTCTTCCTTTG	35643
2018 ACGGCTCAGCTTTTCCATTAGT	13676 TGCAGCCTGGGTGGGTTTT	24660 TCCATCCACGGCTCAGCTTT	35644
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2063 CTTGCCACCAGATCCACTCAGA	13721 CCTCCCTACCCAAGTGGCTACA	24705 GTGTAGTCAAGCTTGCCACCAGAT	35689
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2085	CACAGACGCACACGTATACACCATAG	13743	CTCACAGCACAGAAGTGGGATGA	24727	TGGAGATGACACAGACATAAACACA	35711
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2099	GCCACTGAGGTTCTGGTCGTT	13757	CCCGCCTTTCCCATCTCCTACT	24741	GGGTTTCATGCCACTGAGGTT	35725
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2110	CAAAGGCTCAACTTTGCACCAA	13768	CGAAACTTCCAATGGACACCAGAA	24752	CCTGCAGAATTCAAAGGCTCAACT	35736
2111	CAGAGACAATGATGGGGCTCTTC	13769	AAGATGGCCAGAGCCCCTGTAG	24753	CACCAGGGAAATCAGAGACAATGATG	35737
2112	GCCAGGCCACTGTGTGAT	13770	CCAGAGGAGCTCAGGGATGTAT	24754	CCCCTTGGGCATTGGCTGAT	35738
2113	GGTGTGAGTTTTGTTCCCTGACTA	13771	CTGAGGCCCATAAGCCTATCAGT	24755	CCCTGCTGAAACCAGGTGTGAGTT	35739
2114	GGGGCCTTTGGGAAAGACACT	13772	ATGTCAGCCGATTCTCCTTTTCT	24756	TCTGCTACATGGGGCCTTTG	35740
2115	CCATGAGTTGAGTGCCTTGTAAAATGT	13773	CCTGCCACTCCCCAAGAGAT	24757	GGTGGAAGGAATCCATGAGTTGAGT	35741
2116	GTTGGATGCTGGTACTCTCAGCTA	13774	GAGTGGAGTTCGGTCCTTCTCTGA	24758	GCTCCCAGTTGGATGCTGGTA	35742
2117	GACTTACCCAATAGAAGGGTGAGTACA	13775	CCCAGTCCGGGAGGTTGTTAC	24759	GGGCTATAGTCATTGGGTGGATGA	35743
2118	CTGGCATTGTGGGGACAGTCAA	13776	GCGGAGGGAGACTCAGAATTGCAT	24760	CATCAACACAGCCCTGGCATT	35744
2119	CACCAGTGGGAAGAGACTGAGATG	13777	GCAGCTACCTCCCTGCAGTTTT	24761	ATGGCCACCAGTGGGAAGA	35745
2120	GGTGTCGAGATCTCTGGCCATT	13778	GCCAATGCCCACCTAACACACT	24762	TGAGTGAGGGGTGTCGAGAT	35746
2121	GCTGGAGGCAGGTTCACATTTTG	13779	TGACAGACAGGCATGAATCACA	24763	TAATGGGAGGCTGGAGGCAGGTT	35747
2122	ACTCCACCCAGCGTGTCCTATT	13780	GGCCGTTTTGCCCAAAGTAG	24764	GTCTAAAATGAACCCTGCCCAACT	35748
2123	GTATAGGAAGTCACTGGTCAGCAA	13781	TTCCGGAGCCACTTGCATCA	24765	GGCTAACAGTGCCATTTGTGAAA	35749
2124	CAGACCCTAAGTGGCATTTTTGAGAA	13782	CACTGTTCCAGAGCACATTCACT	24766	AGGAATGGGACAGACCCTAAGT	35750
2125	CAACAGGTTCCATAGGCCAGAATTAG	13783	GCTTGCAGCTGAGGAGTGAGT	24767	AGCCCCTCAACAGGTTCCAT	35751
2126	CATCCAGCTAGCAGACACTGAAG	13784	CCCCTGAGTTCATGGGCTTGAT	24768	GCAATTGTCTTAGTGTCATCCAGCTA	35752
2127	TCAGCTTCCATCTCCTGCTTTG	13785	TGCAGAGAGGCCCGTGATG	24769	CCTTCAGCCCTCAGCTTCCAT	35753
2128	TCCTTTTGCTACCTGAGCTTGTT	13786	GCAGTTAAGAGGGTACTCTGCTGTTG	24770	GTCTGCAAGAAACTGGCTTAACAA	35754
2129	GGTTGAAGAAGGGGATGCGATGAA	13787	GGCCACACACGAACATTTACAA	24771	CAGTTGTAGCTGAAATCAGAGGTTGA	35755
2130	GTGAGCCATTACCTGGGTTTGA	13788	GGGAAACACACATGTACTTTGAGCAT	24772	TGTGACAGAAGTTTGTGAGCCATTA	35756
2131	TGGATTCTGGCAAATGCATGTCT	13789	GCTCATGGCTCTCTGCACTTC	24773	CTCCTGCCTGGGACAATGTAATG	35757
2132	CCAGAAATAGCTCCTGCTCCAA	13790	CCTTATCCTTGAACTGCCGGTAA	24774	CATCACAGCACATGACCCAAAG	35758
2133	AAGTGAGACGGGTGCCAACT	13791	ACCGCTGCACTTCTGTGACT	24775	GGTCAGGACGTACGCATTTGGATTA	35759
2134	TCCTCTCTGGCCAGTGGTTT	13792	ACAACAGACGAGTTCATGTGAAGA	24776	TGGTCAGCCCAGGGTGATTT	35760
2135	GCCACAGTTCGGGCTTGACT	13793	GTCAGAAACAACTGGGGCATCA	24777	TCAGGTAACTCTGGTGACAGGAA	35761
2136	CCTTCTGGATCACCAGGTCACT	13794	GGTTGGCTCTTGAGGACATTGA	24778	GGCTTTCCTCTCTCCAGGTTTCCTT	35762
2137	AAGCATGCCGAGCTGTGGTT	13795	TCCATGAGGCACAGCGAAGT	24779	GCGCTGCGTGGAACTAAAAAG	35763
2138	CCTGGCAGTGAAACTATGGGGATT	13796	CGTGGAGGACCTAGGCTGAAG	24780	GCTGGCCTGGCAGTGAAACT	35764
2139	CCCGACTAAGTGTAAGCACTCAA	13797	AGGCACCACGTACAGAAGCTA	24781	TCCATGGCCCCGACTAAGTGTA	35765
2140	CCCTGATTATGCCACAAGTCATCT	13798	CTCAGTGTCTGTGTCTCATGGTT	24782	CCTGCATCGGAATTCCCCTGATT	35766
2141	TGGAAGAAACTGCTGTGCAACT	13799	GATCCAAGCATTCCTGCTGATAATAGA	24783	GGTCTGTGTTTTCAGTGGAAGAAACT	35767
2142	CTACAGTTTGGATGTGAGGCATTG	13800	ACCTCATGCCATCTCCCCACTT	24784	GTCCCTGAAACTACAGTTTGGATGT	35768

2143	GGAAGCAAAGGTCTGGTTCTGA	13801	GCAGAGGCAGTTAAGCAGGAGGTA	24785	GCCTGGCTGTTTGGAAGGAA	35769
2144	GCTTTGCATATCCCTACTTACTGCAT	13802	CCACTGTTCCTTAGCTATGTGAGT	24786	GCACCTCCACAGAGTGCTTT	35770
2145	CAGCTTGTACCCTGGTAGGGAAATG	13803	TGGCTCAGCCTCCCCTTTCA	24787	GAGCACCAGGAAACAGCTTGTA	35771
2146	CTCTGACTGCCTTGTCTCCATCTTC	13804	AAGCCAGGGAGGTCTGCAGCTA	24788	GGCTCTGGCTCTGTCTTCATTG	35772
2147	GGGCTGCAGGAATACACAACA	13805	GAAATCTGCCCAGCACCAGACA	24789	GGCATTCTAGGGCTGCAGGAA	35773
2148	CAGGGCTTTACAACACCCCTGAA	13806	CCCAGCACTGAACTGACACA	24790	ATGGCCCAGGGCTTTACAAC	35774
2149	CCTGCAGCAAGAGCAGTCCTA	13807	CGCCCACTCTGAACCACGTAA	24791	TTCACTGCCCTGCAGCAAGA	35775
2150	CCATTGGGCCCTACGGCTTT	13808	ACTGGAGAAGCCGCCATGTAG	24792	CAGCTCTCAAACCCTCACCATTG	35776
2151	CCTGCCTCCAAGTGAGAATCAGA	13809	GCTGTCTCCTTTTCTGCCAAGA	24793	GCTTCTCATTCCTGCCTCCAAGT	35777
2152	GCCCGCCATGGAATTGATTTCT	13810	GGGAAGCAGGTAGGGAGCTT	24794	CACAATTGCCCGCCATGGAA	35778
2153	GCTGGCGACTCTAAAGCCATCTT	13811	GGCTGTGAGAAGCATTGGGAAA	24795	ACCCAGGCTGGCGACTCTAA	35779
2154	CCCCTGACATCTGGGAGTGA	13812	GGGGCTGGGCAAGTCTTAGAA	24796	ATGGCCACACACCCCTGACAT	35780
2155	GCACTGTCCCCAACCCTCAAT	13813	CCCCAGAACGTGGCCTTTG	24797	GAAGGGTGGCCATTGTCTGA	35781
2156	GGAGCCATTGAATCACGTTCACA	13814	TGTCCAAACCTGCCCTGAAAG	24798	CCAGGAGAGGGAGCCATTGAATC	35782
2157	GAGAGACAGGAAGGACCACTGT	13815	TGGGAGGCCAGTGTGAAGT	24799	GAGCTGGGAGAGAGACAGGAA	35783
2158	AGTAACTTGCCAAGGGTTTCTGA	13816	CCTCACTCTTCAGGGACAATAGACT	24800	GAGAAACTGAGTTATAGAGCCATCGAGTA	35784
2159	AGGCAAACGTGGATCCCTTAC	13817	CTGTAGCGAAGCCTTTGGATTCT	24801	CGAGACTCAAACGGAGAGGCAAAC	35785
2160	GAGTGTGAGCCCATTGCTGTA	13818	CTGACCAAGGAACTCACTTCACA	24802	AACCAGTGAATGCCATGAGTGT	35786
2161	AAGCACCAGCATGCATGTGTAA	13819	TGCTGAGCCACTGGGATACA	24803	GTCTGCAACAAGCACCAGCAT	35787
2162	GACATCCCAAGAAGGTCTACAAGT	13820	GAAGAGTAAGCAGAGAGCAGGTTGA	24804	GGCGACATGGTCTGAGGATGAA	35788
2163	CCTGGGTGAGACCTTCCCTTCA	13821	GGGACCACGAACGACACAACT	24805	TAGAGGCGTCCCTGGGTGAGA	35789
2164	CCCAGCATGGTTCCAGAGTCA	13822	GCAGGATTGCCAAACTCTGTGT	24806	AGAGCACCCCAGCATGGTT	35790
2165	AACCGGAAAAGGAAGCATACTCA	13823	CGGCCTGGTTCCAACTTCTGA	24807	ACCTCCAAAACCGGAAAAGGAA	35791
2166	GTGACACTGCCGTCATCATTCA	13824	GCAGGTGGCATGACTGGACTTT	24808	TTTGGCAAGCCCACCAGTGA	35792
2167	GACCTTTCCAGGAAGAGCTTGT	13825	CTTTGTTCAAACCCTGAGCCCATA	24809	CGGGACAGAAAATTCCGTAAGAAAGA	35793
2168	AGGGCCAGGTAGAGGTAGGAGTTA	13826	TCTGCTATGCTGCTTTCTCCAA	24810	AGGATCAGGAGGGCCAGGTAGA	35794
2169	CGTGCCCAGACTGCTGCTTTAT	13827	GCCCAATGAGGAGGAATGGGATCA	24811	TACCACAACGTGCCCAGACT	35795
2170	CCAGCTCTTTCTCTCGATCTTTGT	13828	AATGCCGTGCTGAGTGGAA	24812	CAGCTCTGAATGTATTCCAGCTCTTTC	35796
2171	CTATCAGATTGCTTGCTGTTTGCTT	13829	GTGTGTGCTTCTGGTCTCGAT	24813	GGTAGCCTGGGAAGCTGCTAT	35797
2172	GTCAGGGCCTTACATTCTCGACAT	13830	GGACCCTGAGAGTACCTGCACTT	24814	AGAGTGGTCAGGGCCTTACAT	35798
2173	CACCTCTAGGCATCCCCACAAA	13831	CTCGGGTGCAATTGGGTCTGAA	24815	TGACTCCCCAGGCACCTCTA	35799
2174	GAGCCACAAGCGATAACTTTGAAC	13832	GCACTAAAAATGCCTCCTTGCAT	24816	CCTCAGTGAGAGCCACAAGCGATA	35800
2175	CAGCCACACTCCTCATGCTT	13833	TGGGAGGGTAGGAGCGTGTA	24817	CTGCTTTCCAACAGCCACACT	35801
2176	GTCCAGGCTTGATTTCCTCAGTCT	13834	AGCTTCACCAGAGTCACGTTTC	24818	CCCACGTGTCCAGGCTTGATTT	35802
2177	CCCTGTTTCCTGCTGCCTTGAT	13835	ACACCAGCCATGCGCCTAAT	24819	TGCTCAGGGTCTCCCTGTTT	35803
2178	AAGCCAACTCAACTCGCTGTA	13836	CCTACATGCTACAAGCCACCAT	24820	GACTGTGGGAGAAGCCAACTCA	35804
2179	CCTGATGGAAGCATGGGAATGT	13837	GCTGTTTACGTGGCTCTGCAT	24821	GCGGAAGGATAAAGGGTCCTGATG	35805
2180	TGTGGCTAAGACGTCTGTGTTG	13838	GGAGTCACAGCTGGTCCTAATG	24822	CGGAACCAGATAGTTTTGTGGCTAAG	35806
2181	ATGGTCACCTGGACCGTGCTT	13839	TTCCTGGCCAGTGCTGTGT	24823	ATCGGCCTCCACCCACCAT	35807
2182	AACTGGGTTCTTTGCCTGTCA	13840	CATAGGTCGGGTGGGTGGAA	24824	GGAAGTGGTGCTGTGGCTTCAT	35808
2183	CCACCATGCCCAGCTAGAAA	13841	GGCCCCTTTCCCTGCTCTTATT	24825	GGATTATAACTGCGTGCCACCAT	35809
2184	AGGCCAGGCCAATTCTTACCTA	13842	GGCCAGTGGGCCTGTTTGA	24826	GCTTTGCTAAGGCCAGGCCAAT	35810
2185	GCACCATGGTAGGTGTTGAGGAT	13843	GCAAGAAACAGGTTGGTTGTGT	24827	GCACATCTACAATATGTTTGGCACCAT	35811
2186	AAGTCAAGGCTGGGTAGGTAGA	13844	AGAGAAGCCATTCACAAGCTGAT	24828	GCACGGGCACTGAGAAAAGTCA	35812
2187	CAGCTACTGGGAGGTGGTTTCT	13845	GGCCAGACAAAGGCAGGACATT	24829	TGGCCTTTGCCCTCAGCTACT	35813
2188	CACCCACTCACCAAGCCTAGTTC	13846	CAGCCTTGCCCTGTGTCATACT	24830	TAGAGGCACCCACTCACCAA	35814
2189	TGTCCTGCGGCCTCCTAAGTT	13847	CCCAGAGTGGTTCAGAGAGCTA	24831	CGGGCAATTCCCAAGGCTTAGT	35815
2190	AGCCCCAGTTGCTCACAGT	13848	GAAGCCAGGAAATGACGTAGGAT	24832	TGAGGCCTGGGTCCTCAGT	35816
2191	CTGTTTACTCAGAGGCACTTGCTA	13849	CACACGGTGGATCAGCCTTT	24833	CCTGCTTCCCTCTGAGCTGTTT	35817
2192	GTTTCAGGGGTTAGGTGACAGTCT	13850	GCGCTCCAGGGATTTTGATTG	24834	CTCCCAGTGTTTCAGGGGTTAG	35818
2193	CCCCTGAACCCAATTTGCAGAGA	13851	ATCTGGGCCCCTTCTTCTGA	24835	ACCTGTCCCCTGAACCCAAT	35819
2194	TGAGAAAGGGGCCACCCATCA	13852	TTCCTGCTACCTCTTCTCCTCAA	24836	GGTGACCCTCATTGGAGTTGACCTT	35820
2195	GTGGAGATTCCGCCAGGTGTT	13853	CCTCCTGATGGACTGCCAGGTA	24837	GGCTAGAGGAGGTGGACAGGAT	35821
2196	ACCTCCAACGCCGAACACT	13854	GACGGGCGTTTGTTCTCCTTGA	24838	AAGTCCCGCCTGGTGTTCACT	35822
2197	GTCAGCTCCACCTGGGTCTTTT	13855	TGCAGTACTGGAGAGGTGCAT	24839	CTGAGCTGTGTCTTCCCAGATG	35823
2198	TGCTTAGGCCATTTGCCATTTC	13856	TGCTGGTGCGAGGAGAGA	24840	GCAGAGCGTTGCTTAGGCCATT	35824
2199	CTTGAGAGTGGCCTTGGAAGT	13857	GACAGCCCCAACACTTCACTCT	24841	GCCCTTCTGGATGACAATAAGGTCTTGA	35825
2200	CCCTCGCACATGGCTGAAGAAT	13858	ATCCATCTCCTGCTCCCGTTTC	24842	TGACTGTGTCCCTCGCACAT	35826
2201	TCTGGGCGATTTGGCAGTT	13859	GCTTGGTGTGTTACTGAACCTCTA	24843	TTTTTAGTGCCACAGGTTGGATTG	35827
2202	GCATGCCCATGCTCCTGTGT	13860	CAAGTTCCTTCAGTGAGGCCATT	24844	GAGTAAAGCAGGGAGCAGCAT	35828
2203	GGATACCATGGCTTTAGGCAAGT	13861	GCTGCAAGCAAGAGACCATTTC	24845	CCTGAAAGCCTCGGGATACCAT	35829
2204	GCTGTCTGCTTAGGGTTGGTCTCT	13862	TGGCTTCCCCAGAAGGACAA	24846	GCTCGCACATTGCTGTCTGCTT	35830
2205	GACACAGCTAAAGCCCCATTAAAC	13863	GGAAATGTGTGTCTCCCAACCAT	24847	CGGCCAACCTTAGACACAGCTA	35831
2206	GGTTTCCCGCCTCACAGATTGA	13864	CCTCTCCTGTTCCGGAGTGTTC	24848	GCAAAGCGAGAGTCCTGCTA	35832
2207	TGTGACGATGGCCTCAGTTC	13865	GCAGTAGCGACTTACCCACAGT	24849	CTGGGCATCTTTCCTCACCTT	35833

2208	GGGCTCCACTACTTACTGGGTTA	13866	CCTCCTTGCAATCAGCTTTGGTA	24850	AGCAAGCATGGGCTCCACTAC	35834
2209	GGGTCCCGGAGTTAGTAATGTTG	13867	GTAGACTTACTCTGAAGTCCACGTT	24851	TGCATAGGGTCCCGGAGTTA	35835
2210	TTGGACCTCACCTTCTGGTTTTT	13868	GATGAATGGGATGGTGACCTTTCT	24852	TCTGGCTTTGGACCTCACCTT	35836
2211	CCTCTGCTAAAATGCACTCCCTTCA	13869	CGGAGGCCCCTCTAAGTCAGT	24853	CAGTTCAGCTCCTTCCTCTGCTA	35837
2212	GCCTGTGGGAGTCAGGTGAGA	13870	AGTGGTCACCTGGGGCAGAT	24854	CTGGGAAGTAAGCAGGCTGAGA	35838
2213	TTCATCCCCACCTCCCCATCA	13871	TGGGACATAGAGTGACAGGACTT	24855	TCCAGGCTCACCCACTTCAT	35839
2214	GGCCTGTTGTTAGGCATGCAA	13872	ACAGGAGCCAAACCGGTACT	24856	CAGATTGCCAGGGAAGTGACA	35840
2215	CCAGGGGCCTGAACATCTTT	13873	GACCACCAATTTCAGTAGGGTTTTATG	24857	GAACCAAAGCATCAAGAAGGAAACT	35841
2216	GGTGATAGCAAGGCCAGAGGTTCTT	13874	CAGGAAGGAAGCGATGAACTGA	24858	GGCCTGTTGGGTGATAGCAA	35842
2217	CCAAATGACTTGGAACAGGAGTTTGA	13875	AGCAGGGATTGGCGAAGACA	24859	GGAGCAAAGGGAACCAAATGACT	35843
2218	CCAGCCCTGATTCTGGCATTGA	13876	GGCCAGTTGCTTTCACCAATGT	24860	ACCTGGCTCCAGCCCTGATT	35844
2219	GGGCCTCAAAGCTGCCTTTATC	13877	GTGCTCCCTTTGAAGAACTGGATT	24861	GCAGGCACTGGGCCTCAAA	35845
2220	GCTTCTGGAAGCCTTCGCTAAC	13878	TGTGGAGAAGGGGTCCTCATT	24862	CCTTACTTCTAGTTCCTACTCAGCTTCT	35846
2221	GGTCTGGTGCAGTATCACCACTA	13879	GCTGCCATCAGCTTTTCTGTCGAT	24863	AGCTGAGGTCTGGTGCAGTA	35847
2222	TCAGCAGCCCCTGGTGAT	13880	GGATCAGCATGGAGACAACCAA	24864	GTGACACCAGCAGCTTCCAAAC	35848
2223	TGCTGGTGGATTGGCTCTACT	13881	CAGTGGGCTCACCGTAGCTT	24865	GGGCAGACTATGCTGGTGGATT	35849
2224	AGCATTAGCACTGTGAGGCTTT	13882	CGGATTGTTTTCCAGGTACAAAGTGT	24866	CAGCTGGGCATCTAGGAAGCAT	35850
2225	CCTGGAATCTCAGGATAACAAGGAA	13883	GTTCCTTTTGAGCAGGCTTGTCTAT	24867	CACACAGTCCTCCCTGGAATCT	35851
2226	CACAAGTCCTGCCCTTGTCCAT	13884	CAGATGGGGAGAACTGCTCACA	24868	TGGGCCCCAAGTTCACAAGT	35852
2227	AACTTCCTGGACTGCAAAGTCA	13885	GCAAGGAGCAATGAACAGCTACTA	24869	GCAGAAATGCTTCCACCAACTTC	35853
2228	TGGTCCAATTCAGGGCCACTCT	13886	TTCACAGGGAGGACTGGAGTTA	24870	GGGCATGAAGGCTTGGTCAACT	35854
2229	GCAAATACTGCCAGAACAGCAA	13887	AGACAGTAGCCAGCTTCAAAAACT	24871	TTTATCGGAAAGGGGAGCAAATACT	35855
2230	AGCTGGCCTTGGCACTTGT	13888	CACAACATCACTGCATCTGCATTCTA	24872	CAGCTCCTCTTTCCACTCTGAATC	35856
2231	GGTGTCTTGCAAGCAGGTGTT	13889	AAGCCACCCTGGGAACCACAT	24873	CTGGAAGGTAATGGGAAACTGCAA	35857
2232	CCCAGCACCGGTGAGTGTTT	13890	TGGGGTAGGAGGAGGACACAGTA	24874	GGTGAACGACGGGGATGTTTAC	35858
2233	GCTCTTGCCTACTACAAGGTCATC	13891	GGAAGCCATCAGCATGTGGAA	24875	GCCTCTATGCTCTTGCCTACTAC	35859
2234	ACGCAGCAGTAAGCGTGCAT	13892	CCCCAACCAACCTGCACACA	24876	TCAGTGGCACGCAGCAGTA	35860
2235	CTCCCTACACCAAAACAGAGGATAC	13893	AGCCCCAGTTCACTGTTGATG	24877	CTCACCCTCCCTACACCAAAAC	35861
2236	TGATGAGAGGTGTTGAGTGCATTA	13894	GAATCACCCATGCAGCTGGTA	24878	TCCCAAAATGATGATGAGAGGTGTT	35862
2237	TGGGTGCAAAATCACTGAGTAGT	13895	CCCTATTGTGCCCCAGATTTGA	24879	AGGAAGGAGAAAATTATGGGTGCAA	35863
2238	TGCGCTGGTGTCACTGATGT	13896	CTCCTTGCACAGAAGGGCTCAT	24880	AATCAGGACCGGAGCCCCAGAT	35864
2239	TGTCTGACCTGAAATCACAGCAT	13897	GCGTGCTACATCTCCACTGACA	24881	AAGCCAGAATGTCTGACCTGAAA	35865
2240	GCCATGGATTTAATGGAAAGGTGTGT	13898	GAACATCCTGGGGTGCACAAAG	24882	CCTCCTAGGAATGTCTGCCATGGATTT	35866
2241	CGCACATAGTTGCTGCTGTTG	13899	CCTTCAGGGTCCTGCAGTTCA	24883	GTCTCTGTGACTCGCACATAGTTG	35867
2242	GGCTCCTGGGTTGATTAAATCTGA	13900	ACCATGCAGGGACACACAATG	24884	AGTGGGCTCCTGGGTTGA	35868
2243	CCCAGAAACAATTCCCGTTGGTT	13901	CACAGAAGCGCATCAAGGTTAC	24885	CAACCTTGCACCCCAGAAACA	35869
2244	TCCAGCATGCCCGCTTGT	13902	CCTGCCAGAAGGCGTAAGAA	24886	GGTCTTCTGGCTTTACGTGTTG	35870
2245	TGCCACGCAACCCTTCTTGT	13903	GAGTAAGCCCCATTTAGGCTTTCAA	24887	TGGTTCAGGAATGAGCCACTCT	35871
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2247	TGTGTCCGGGCAGAGACTTG	13905	CCCGGGTGTCTGTCGAGTAAC	24889	GGGGCCAAGAGCTACTGTGT	35873
2248	GGTAGCAAAGCCTCAAAGAATCCAAA	13906	GCAGTGGGGCATTGTGAATC	24890	GGTGGAGGTAGCAAAGCCTCAAA	35874
2249	TGTGGACAGATTGGCCCAAAG	13907	GGGTTCCTGCGTTGGGGTATTT	24891	GCTGGCTAAGCTTTTGTGGACAGAT	35875
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2251	GAGAAGATCCACGGACTCTGTTCA	13909	GCAGCAAGCCAGGAAAGACAA	24893	GCATCTAAATCTTCCTAGCGATTGGAGAAG	35877
2252	AGGTGCTGCTGATTGAGTTCTT	13910	AGCCACCTGCAAGTCAGGAA	24894	CCAAGACAAGGTGCTGCTGAT	35878
2253	TCCAAGACAAGGGAGCCAGAA	13911	TTAGTCTTCTCTCTGCCAGGAAAC	24895	GACCTGAGAAAAGAGTGTTATCCAAGA	35879
2254	ACAGCCCTAAATGAGGACCTGTA	13912	GGTTTGCACCCTGGGAATGA	24896	CTGCATCCAAACAGCCCTAAATG	35880
2255	GGGAACTTCTTTCTCACTCACACT	13913	TGGGGAGAAAGTCAGTATCCAACT	24897	CCCAGCACAATAGGGAACTTCTT	35881
2256	CTCTCTTGGAAGTTCGGTGGATTTG	13914	CAGGACTTCTCACAGCCTTTAATGT	24898	GCACCTTGCAACCCTCTCTT	35882
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2258	TGTCAACAGCAGCACACACT	13916	CTGTCAGAACCAGCAAGCATGT	24900	GCAGGCCCAGAACTTATAGATGTCAAC	35884
2259	GAGCGAGGCTGTCACTAACGAA	13917	TCCCCAGCTCCAGGCAGTA	24901	ACACCACGCGCTGACAGA	35885
2260	CCAGGGCTTTCAGTGCAAATAAC	13918	CATGGGCTGAGCTGAAGATACA	24902	GCTGGCCAGGGCTTTCAGT	35886
2261	GGCCCTGCCAAATTCCTCAGA	13919	GGGAAGTAGAAACCGCCAATCT	24903	TTCCTCCTGGCCCTGCCAAA	35887
2262	TGGGGCCAGCAGAATTACTTG	13920	CGGATTGTGGCTCTCTGTGACT	24904	GGCAGAGGCTGCTATTATAGGTT	35888
2263	ATGACCCGGGAGGGCAAAG	13921	CACCGACTCCTCGCCATTATCT	24905	CCGAGGCATGTGCTGGATGA	35889
2264	GCAAAAGCAGGACTAACACCACAT	13922	TACCTGGTCCAGCCTGACTTC	24906	GGCTGTAGATTGCAAAAGCAGGACTA	35890
2265	TGGCGGATGGGAGGGCATT	13923	CCCGTTGGACTCAGAAGTGAAG	24907	AGGGGACTGCAGAGAAGTCA	35891
2266	GTGACGCAAACATCAGCACTCTTC	13924	CCTGCCCACTCTCAGCAGAT	24908	ACTGGCAGCGCTGTGTGATT	35892
2267	CCTGTGGGGATTTGAGGGTTCT	13925	CAGCTACCCTTTGCCTCTGCAT	24909	TGTCCTGCCCTGTGGGGATTT	35893
2268	GGGATCTTGGGTTAAAATGCAAACA	13926	GACTTCCCCTCAGGTCTTTTCAA	24910	GGAGACTCAAAGGGATCTTGGGTTA	35894
2269	GCCTCCAGAAAAACTTGCCATCA	13927	GCCTGGATACAGGGACACCTATTC	24911	GGGAAATTTTAGACCTGCCTCCAGAA	35895
2270	GGCCACCTGGGAATAGTTTAGTCT	13928	GAAGCTGATGAAACAGTCCTCTCA	24912	GATAAATCAGGCCACCTGGGAAT	35896
2271	GGGCCAGAGAGTTTTGAATAACAAAG	13929	ACTACCCTGAGGCTGATCCAA	24913	CGTATGTTGAGGGCCAGAGAGT	35897
2272	CCCCAGATTCTCAGCTTCATCTCT	13930	GTGGAACCTGGTGGACTCCTT	24914	GCTGCCTTGGTTTTCCCCAGATT	35898

2273	GCTAAAGAAGCTGGGCCGCTAA	13931 GACATTGAGCGGGTCACTTAAAC	24915 GTTGAGGCAGATGGCAGTGT 35899
2274	GCCAAGTGGCAGTGAAAAGAAGA	13932 TGGTTGCTCCAGAAACTACCTAAAT	24916 GGGAAGAAAACGGGCCAAGT 35900
2275	TTGGAGCTTGCTTCCCATTTTTC	13933 AAGACTGGGGACGGGATACTT	24917 GGGTTTGGAGTTAGCTTGACTTTG 35901
2276	CTCGGATCCATCCAGCAATGAGT	13934 GCCGAGACTGCCAGACCTT	24918 AGCAGTCACCCTCGGATCCAT 35902
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2278	CTATTAAGATGGGCTGGCAGTCA	13936 GTGATTCCTATGGGCTTCCCACTT	24920 CTCAGACTCAAGGGGCTCTATTAAG 35904
2279	CCCCTCTTATTATACAGGAGCCTTAC	13937 GCCCACCCTCCCACATTAC	24921 GAAGTTCTTTAGCTGTAACCCCTCTT 35905
2280	TCTCCAATGACCGGGTGACAAG	13938 GGAACACCCTCTGGCACTCT	24922 CTGTGTCCATTCCTTCTCCAATGA 35906
2281	CTTGACTCCCTCTGGATCACATTG	13939 AGTGGAACAGTTGCCTGAGATG	24923 CAGTGACCTCCACTGCTTGACT 35907
2282	CGTGAGATGCGCCAATGCAA	13940 CACGTGTGTAAGTGCAGACTCTTTG	24924 TCTCAACAGGCACGTGAGATG 35908
2283	CCACAAACACATGCCACGAAAAG	13941 CCCAAGGCAATGACAAATGCTTA	24925 GAGTCACAGCTAACCACAAACACA 35909
2284	GCTTCCTTTCACGGCATCAGA	13942 TGAAGGCTCAGGCACAGACA	24926 CCTTCGCGTTGCTTCCTTTC 35910
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2286	CCACGTTTGTGTAGGATGGAATG	13944 TGGCAGCTCTCTCAGCATCA	24928 TGTGATCAGATCCACGTTTGTGTAG 35912
2287	ACCGTTCAGCTTCCCTCCTT	13945 TCTCGCCAAATGCCGTGGAA	24929 AGAATGGCCTGCACCACATC 35913
2288	ACTCTCCCTTCAGACTGGGAAT	13946 GCCATTGGCCAGCATTGACT	24930 TGTGCCATGACTCTCCCTTCA 35914
2289	CAGCCAACCTTGACCTGTGA	13947 GGCTGACTATATGCTTCTGCTGAACT	24931 GCCTGGATGAACCTAGCCAAGA 35915
2290	ACAGCGCACGTGCCATTCA	13948 GGCAGGAGCCCCTCTTCTTG	24932 TGCCTTCGTGCAGGTGACA 35916
2291	GCTACCCAACACCTAGGAGAAATTCA	13949 CAAGGTAACACCCCAAGATGTGA	24933 CCTGGTGCTACCCAACACCTA 35917
2292	GACCACAAGGGTGACAGCAA	13950 CCTAAGCGTCCTAAATGAGGTAAGAATG	24934 AGGAAAGAAATTGCAGACCACAAG 35918
2293	CAATGCCCTCCCGCAGACA	13951 GCCATCTGAAAACTGTGATGGAGTTAG	24935 GCCCTTCCTTCTGCCTGCAAT 35919
2294	GGAGCACAAAATGTGTGACAAAGA	13952 CGGTCTCTGATGTTCTCGGACGTT	24936 GGGGTTTTTGGAGCACAAAATGT 35920
2295	GGGCAGCGATGGTAGTTACCTAAA	13953 ACCATGACACGCCCTTGACT	24937 TGGAGGGCAGCGATGGTA 35921
2296	CAGGGCAACGAAAGTTACCTTCTA	13954 CCTGGCTGGGCATAAGTTTTTGT	24938 GTCCTTCAGGGCAACGAAAGT 35922
2297	TCTCTGGCTTCCAAGGTCTCAA	13955 CTGAAAGGAAGAAGCCACAGTCAA	24939 GGTCAGGGCTTCAGGTATCCATTC 35923
2298	GTGACTGAGCCACAAGTGATGAAG	13956 CCCAGTGCTTGTGGACTCTT	24940 ATCCGTGACGCCCTCTCTGAA 35924
2299	GCCTCACTTGCCTTGTCAGA	13957 GGTGCCTTCTGGCATCAGCAT	24941 GCACAGGGCTGCCTCACTT 35925
2300	GACCTGCTCACTGCAGAACA	13958 CATGGGCCTCCAGGACCTACT	24942 TGGCCTTTGGGAGCAGGTTT 35926
2301	CGTGAAGCCCCTCTACTGACT	13959 CACCTGCCTTCCCCATGAGT	24943 TCTGGTGGGCTGGCGTGAA 35927
2302	CGACCTTCACCTTCACCACTT	13960 CAAGGAGGACCCTGGCTTTTCA	24944 GCCCTGAGAACTGTATACGACCTT 35928
2303	GTCATGAAACTGAATTCCAGCCAAT	13961 CCTCCCGCAACTCTTCTGCTTA	24945 GGCAGTTGAAGTCATGAAACTGAA 35929
2304	GGCTCTCTGTGAGGTAGGATTG	13962 GGATCTCAGGGCTCACCTGTGAAA	24946 AGGCTCTCCCGGCTCTCTGT 35930
2305	CTAGGGCCTGGAGAAGTCCAT	13963 GCACTTCCCCTCACAGTCAGA	24947 GCAGGTCACAAGTCCAGGAAACT 35931
2306	GCTGATCTGTCAGTGTGCGTTAG	13964 GCCGCACAGGATGGGGAAA	24948 GGCCGTGAAAGCTGATCTGT 35932
2307	AGGCCGGGGCATAGCGAGAA	13965 ACCACAGGCTACCTTTCCAATC	24949 GAGCTTGGGAAACTACAAGGTATGA 35933
2308	AGCAGTAAAAGGAGCAGGTTTGA	13966 CACTATGCAGCCAGACCATCA	24950 CCCTCCCAAAAGCAGACAGCAGTA 35934
2309	CAGGTGTGAGTGGAGCGTTAG	13967 CCCCAGGCATTGGTCCTTAATGT	24951 CCGTAGGAAGAACCAGGTGTGA 35935
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2314	CCGTGTGTTTTCCCAAATGCTTTCT	13972 GCCTCCGAGTTGTTTGAGAGT	24956 CGGCTATTAGACCCCAGCAAA 35940
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2316	GGTGAAGGGAAGATCCCATCTGA	13974 TGGCCATATGCCGAGCTAAAA	24958 GACACCAAGGGTGAAGGGAAGA 35942
2317	CTGCTCAAGGCATTTCACAAGTT	13975 CAGAGCCTGAATGATTTCCACACT	24959 GGTATATGCTGAGTAGACAAGGACACT 35943
2318	GAAGGCGGTGTAAGCATGTTAAG	13976 GCCATCAGTGGCTCAGCTT	24960 AGTCCATGGAAGGCGGTGTAAG 35944
2319	TGAGGGATGTGTCTGATGAGCTA	13977 TGCTAACTGACTTACCCAATCCATAG	24961 GGAGTTATCAGTGAGGGATGTGTCT 35945
2320	GGTGGTAGGATGTGCTGTATTGA	13978 TGGGCTCTTGTCTCAGTGTATTCT	24962 GATCCTCACTGGTGGTAGGATGT 35946
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2322	CCCAGCATCTTTGTGAGGAGAA	13980 CTACACTTGTGGAGCTGTCTGAA	24964 ATCTCCTGTCCCAGCATCTTTG 35948
2323	GGCTTGCCGTTTGGAAAACAAC	13981 CACACCCACAGATTCGAGAAGGAT	24965 AATGTACGGCTTGCCGTTTG 35949
2324	ACCTGCGGGAGGTGAACA	13982 AGGCACTGAGACGAGGACAAGT	24966 GGGAGCAGTTGGGACTCAGA 35950
2325	GTCTGCATCCCCTACAATTCACA	13983 GTCCGTCACCTTGGGAGTCA	24967 CACACCTGCTGTCGTCTGAA 35951
2326	CTGCGGGCTGCAGTAAGAA	13984 AAGGCCGGTGGTCTCTTCAA	24968 GCATGGCTCCCATGGTTACA 35952
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2328	GGCTCCCTTGAGTTGTGGGTTAG	13986 ATCCAGGTCAGGCCACAGTT	24970 TGGTTACTGGGCTCCCTTGAGT 35954
2329	TGGCCAGTATGCGAGTGATTT	13987 TCCAGCAACGTGACCAAACA	24971 ACTCCGACGTGGCCAGTAT 35955
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2331	GCCATTGGCGCGTTAATTATCAGT	13989 AGTTGCCCTGAAGCGTGGAT	24973 TGTAGGCCATTGGCGCGTTA 35957
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2333	CAGCTACATCTCAACCGCAAAG	13991 GGTTAGCCAGTCAGAACTTTGTTTAC	24975 CAGGTACTTTGTGTCAGCTACATCTCA 35959
2334	AACCCGACTGACACCTGGAT	13992 CACAGTACCAAAGGCCAGACA	24976 CAAGAAGAAGCAACCCGACTGA 35960
2335	CCCAGGGAAATAGGAGTGAGGAGAA	13993 CTGCCCTGGAAGACTGGAAAGA	24977 TGGGCAGTCCCAGGGAAATA 35961
2336	GTGGCGTTGTTACCTCTCCTT	13994 TGCACCAATGAAGCCACACA	24978 CAGCTTCACAAAGTGGCGTTGTT 35962
2337	GGGTCCTTCCTGTAGGGAGAGTT	13995 GAGGCTGGCCAGATGTTTTCTCA	24979 CCCCGAGGGTCCTTCCTGTA 35963

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2340	GGGAGTATGGGTGGAGCACTTC	13998	TGGATCTGACTTGGAGAGCATTG	24982	CCACCCAGGTCTGGGAGTATG	35966
2341	AGAGCCAGCAAAATATGGGGAAT	13999	ACTCATGGGTACAGAGGATTATAGGAA	24983	GGCAAGGGTAAGAGCCAGCAAA	35967
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2343	GGCTGGAAACTCCACTGCCATT	14001	CTCTGCGGTCCCATTTCTGAACA	24985	TGAGTGCAGGGCTGGAAACT	35969
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2346	CGGACCCAACCTTGTGTCAAC	14004	TTGCTGGGCCCCTCTGTTTG	24988	GGCGGGAATCAGCCTTCATCA	35972
2347	GGGAACTACTCCAACCAGAGTCAA	14005	CCACACCTTCCTTCTCCTTCCAA	24989	CCAATGCTTGGAGCAGGGAACT	35973
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2349	TGCGTGGAGCAAAAGGTTTGA	14007	GGAAGGAGGTCGCTTTCAAAATCA	24991	TGGAGGATGCGTGGAGCAA	35975
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2351	GCCTTATCCTCTACCCTGATCCTA	14009	GGACAGGGGCTCTGAGACACA	24993	CCTGCCACATTGCCTTATCCTCTAC	35977
2352	ACGGCATGGATTGTTACAAGTTTC	14010	GAGAAATGCAAGGACTCCACAAAA	24994	GTCTCTCACGGCATGGATTGT	35978
2353	TGCCGTGGATGACCGTGATGA	14011	GTTGATCCTATAGTGCGAACCTGACA	24995	CCTCAATGAATGGTGCCGTGGAT	35979
2354	GACACGCTCAGAGGATGAGATAC	14012	CGCAATGACAGCTCCATTACTAAC	24996	GCTGCAAAGACACGCTCAGA	35980
2355	CCCTGCCTGGTTCCACTTACAA	14013	CCTTCTCTGCAAGTGTGGTGTCA	24997	AGACATGGTCCCTGCCTGGTT	35981
2356	CAGGGAGGCAATCGGTAAAGAAA	14014	CCCAGTGGGATGATGCTAGATG	24998	GCAAATGTTTCAGGGAGGCAATC	35982
2357	GCATGTCAGCTGGGTCTTTCTA	14015	GAGGCCTGGCAATTTCTGCTT	24999	TTCCAGGTGGCTTAGGGACTTC	35983
2358	GGAACTGGCAACCCTAGCAA	14016	TCCAAAAGCTCTTGATGCTGACA	25000	GCATTGTTTTGCCTGCTAGGAACT	35984
2359	AGTGCTTGTTTAGCTGCTAGAGT	14017	CTCTGTTCTATCTGCCACAAGGTT	25001	GCTTATCTCAAGACCAGTGCTTGTT	35985
2360	GGTTTCCCTGAACTCTGCCTCAT	14018	GACACCAGTTCACCCCAGTTATC	25002	GGGGTCAGCTTGACTCTTGTGGTT	35986
2361	GGTGGCCTCTGACACAGCAAA	14019	GGGCAATGCCTGGAGCTAGT	25003	ACACAGGTGGTGGCCTCTGA	35987
2362	AGCCCGGGCACAGTCTACTAAT	14020	GCAGCAGCTCCTGTCTGAT	25004	GGGCTGCCCTGGATGTGTTA	35988
2363	GGATGAAGGCAGATCCCTGAAAC	14021	GCCCTCGCTGTGGCTTATGA	25005	TGCCCAGACAGCTTGGATGAAG	35989
2364	CCTTGTGTGCATAAAGGGTCTTG	14022	GGTGGACAAGGTGGAAAAGTCT	25006	CAGACAGCCCTTGTGTGCAT	35990
2365	CCATGGCCCTTCCCTTCTTG	14023	AACAGAGGCACCGCCAAGT	25007	TGAGTGCCGGGCTCCAGAA	35991
2366	AGCCCAACTGGGTGGTGCATA	14024	GGGTTCCTCCATCCCCTTCACAT	25008	GGGAGCAGTTCATTGAAAGCCCAACT	35992
2367	GTATGACTCCCCTTAAGGATGAGAAG	14025	TCACCCATCCCACCTATTTACTTG	25009	CCACACTGTGTATGACTCCCCTTA	35993
2368	GGAGAGGCTGTCAGTATGGGATGT	14026	GTTACAGTCTCGGTCACGTCTTG	25010	CCCAAGGAGAGGCTGTCAGT	35994
2369	GACCCAACTGCCAAGACAGA	14027	CACAGTGCACATGAACCCCTTCT	25011	GCCATAGGCTAACCTCACAGACA	35995
2370	GTTTGGCCCTGTACTTCTGTCT	14028	ACCCCACAGCTGGGCTTTAG	25012	CGCCATTTGAGTTTGGCCCTGTA	35996
2371	AACTGCCCCAATGCCAGGATAG	14029	GGGGTGCGCTTGAAGCTTATGT	25013	GGCCTAAGAACTGCCCCAAT	35997
2372	CAGCACATCTGGGACCTGGTA	14030	GCCTTGGAGAATCCTTCCTTAACT	25014	GTGAGTGGCCAGCACATCT	35998
2373	CTGTCTGACCAACTTGCTGTTTG	14031	CCAAGAGCCTGGTGAGTACGTT	25015	GCATTTGGCATCCCTGTCTGA	35999
2374	CTGGGAGGGTAAGGACTGATCT	14032	GAAGTTCTACTCTGTGCTGGTGAA	25016	CTGGAGTATAGAATCTGGGAGGGTAAG	36000
2375	CCCCGTCTGAATGAGAAAGCAT	14033	GAGAACTGCCGGATCAAACAGAAAG	25017	ACCCAGCCCCGTCTGAATG	36001
2376	CCTGAAGGGAGGTTCTGAGTCT	14034	CGGTAGTGCAGGGGATTGTCA	25018	AGGACCCCAGCGTGCTCCAT	36002
2377	TCCCAGTTCAGGGAGGCATTAC	14035	GGAGAACACCAGGGAGAACACATAG	25019	CTATGCTTATAAAACCTGACCCAGTTC	36003
2378	CCCCTCCTCAAAGGGACAAAAC	14036	GAGTAGATCAGTGGCTGTTACCTGAA	25020	TCCAGAGCACCCCTCCTCAAA	36004
2379	CCACTCCAGGGTCCAGAGTCA	14037	AGGCACCAGGAGAGAGTGTATT	25021	AGCAGGGGCCACTACTGT	36005
2380	TCGCCTCCTTCCTGGTCTAC	14038	AAGTCGGAGTTGGCGTCCTT	25022	CGGGTTCCTGGTGCTCATCTT	36006
2381	GCAGAGATGCGACTTCCTTTGTCCTA	14039	ACACCTCCCTGACCCTGCAT	25023	CTAGGAGCAGAGATGCGACTTC	36007
2382	CTGCATTCCAGCCATTTCAAACT	14040	AGAGGGTAAGGGCTATTTGAGGAA	25024	TCCCGGGAACATGCTGCATTC	36008
2383	TGGGTGGTACAGGCAAGAGAT	14041	CAACCAGCTCTAAGCTCTGACA	25025	GCTTTGAGGAGGATGGGTGGTA	36009
2384	CACTGGCAGAAACAAGGAGAGAAGA	14042	TCCTGTTCCATCACTGATTCGTT	25026	GCAGAAGCCACTGGCAGAAAC	36010
2385	GGAAGCTCTCACTCCCCAAAC	14043	GGGTGACAGCAAAGAGGTTGA	25027	CCTAGGACATTAAGGGAACATGGAA	36011
2386	CCAGGCCTCTGTGCTGTTTGT	14044	TGGCAGACAGATGAGGTAGTGT	25028	GCTATGTTGTTCCAGGCCTCTGT	36012
2387	CACGCAGTTGGCAGAGCAT	14045	GGGGTCCTAGGCAGCACTGA	25029	TCCCTGTCCCCACGCAGTT	36013
2388	GGCCTGAAGGAGTTTCCCATCA	14046	TCCCCACCCCATGCAGGTA	25030	GGTCTTCGACTCTCCAAGGAACAGA	36014
2389	CCCATGAATTACTGTGTGGTGTGA	14047	GCAGGGTGGAGAGATACCACTCTT	25031	GCCCAGGCCCATGAATTACTGT	36015
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2391	CTGGAAAGGGAGGAGCCAAAA	14049	AATGAGGCTGGGACAATCAAGAA	25033	TTGGCCAGCGCAACTGGAAA	36017
2392	GAGCTTGCCCAGGCAGATTTCTA	14050	GCTGCATAAAGGGCATTGCTTCA	25034	GTAGGTCTGGGGCAGGAAGA	36018
2393	CACCCACTGTCGCAGCATTC	14051	CCCCAGGAGAGCGAATAATCACAAG	25035	ATGCCCAGAGCACCCACTGT	36019
2394	GCCTTAGGAAAGGGACAGTGTAG	14052	TCCCTGATTCCCCAAGGCTTCT	25036	GCTTATGGAGCAAAGGATCGGTGAT	36020
2395	CAGGATGGCGAGGCAGACA	14053	GCCTGGGAGATGGCTGTTAGTAG	25037	TCTGCCCCTTACCCCAGGAT	36021
2396	CTGTGCCCAAGTGGAGTCATGT	14054	CATGGCCTCAGCACAGTGAA	25038	TGCTTGCTGTGCCCAAGT	36022
2397	CCAGATGAACTGAGAAGGTCAGATT	14055	CCTCATGCCGGATGCTCTTCT	25039	GGACAGGAAGCACAGCAGGTTT	36023
2398	ACATCCCTGTTTGCCCTCTTG	14056	GTGGGCCATGGGTAGTTATGGAA	25040	GGCCCAAAGTGAGCATTTACATC	36024
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2407	ACACCATTGCCTGGGCTCTTAG	14065	GGGAATGTCTGCGATTGGCTAT	25049	TCAGTCTGCCGGACACCATTG	36033
2408	CCACGCAGTGTTGAGGATTAAGTCA	14066	GGTCCCGAATGCTTTCTGTCT	25050	TTGCCCACGCAGTGTTGA	36034
2409	GCTCTTCAAGCCTCAGTGCAA	14067	GGCCGGAAAAGGCTACAGTGA	25051	CCAGATCCTGCCTGCTCTTCAA	36035
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2415	ACAACTCCCACCTCCTGTCTCT	14073	TGGCCGTGCTTGGCTTGTT	25057	ATCCCTCCCAGCCACAACT	36041
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2417	CTAGCCCAGCAAATTTTGGAGTTT	14075	CAGGTGACCCGCTAGGATCTATG	25059	CATGCCATTCTAGCCCAGCAA	36043
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2423	CCTCTCCTCAGATGCCTGTTTCT	14081	ACCATGGGTCCAGTGGGTAAAG	25065	CCAAGGATTTTCTCCTCTCCTCAGAT	36049
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2425	CAAGACCTACTGTCCACTCTGATTC	14083	CCCTTACCTTCCAAGAGCTGTCA	25067	TGGTGATTCTCCAAGACCTACTGT	36051
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2427	GGCCCTGTCAATCATACCAGCTT	14085	GTTTTGCCCCTAGCACCAAGT	25069	CACAGATCTGGCCCTGTCAATC	36053
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2457	TGTGCCTGTGGGTGGAGAAG	14115	GGGGTCAGAAAGCTGAGAGTCTA	25099	TCTTGGAAAGCTGTTGTAGCTTGT	36083
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2462	GGGGTGGTCTCCCCAAACTCA	14120	AGCAGCAGCCCACGGGAAGA	25104	GTGGAGCCTCTCCAAATTGTGTTG	36088
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2465	GCTGTTTATCAGTTTGTGCTGAGTGA	14123	ACTTTGGCCCCATTTCTCCAA	25107	GTGGTAGGACTGCTGTTTATCAGTT	36091
2466	CTGGGCCTCAGCTCTTCCAT	14124	AGGGTGCTGCTTGGGTTGT	25108	GGGAATGGTTGCCAGTACCTT	36092
2467	GACTCCTGGAGCCACTCTTCATTC	14125	CAGTATCATGCGGTCACCTAGTCTT	25109	GGCCTCACTGCTGCCTTCATA	36093

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2470 ACTGGGCTGACCCTTTTGATTTC	14128 TGGCCGCCTTACACCAAAAC	25112 TGTCTGCAGAGGGACTGAAAC 36096
2471 CGAGGGAGATGCGGGTCTA	14129 CAGGCCCGGAACCCTTTCT	25113 CGCACGATCCGAGGGAGAT 36097
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2473 CCAGGGAGGGAGGTCACACA	14131 CCTGGCCGCTGTTACAGACT	25115 AGGGCAGAGATGGGGAAACA 36099
2474 GTCAGCATAGGGCAGTTGCAAAG	14132 CCTAGGAGTGTCCAGCTCTGTTC	25116 GTGTGGGTGTGTGTCAGCATAG 36100
2475 GGAAACCTTAGCTGGGGTCTTTTC	14133 TGACTCGCTGTGTCCTGTGA	25117 GCACTAGGGATCTCTGGAAACCTT 36101
2476 AGAGGTTCCCACTAGAGAGTCATC	14134 AGGCCACAGTGTTGCTCTGTTC	25118 GCCCTAGATGAAGAGGTTCCCACTA 36102
2477 GTTGAGACCGAACACTGGCAAAC	14135 TTGCTCTGAGGCCCTACACT	25119 CAATGTGACTGGGGTGTTGAGA 36103
2478 CCAAGGACATGACTTACCCAGTTT	14136 GTACCTGGCTGGGATATGTGTTTT	25120 GGCAGGAATGCAACCAAGGACATGA 36104
2479 GCTTAGGACCTATCTGGAGTCACAGT	14137 AGGAGAACCCATCCTGCATCT	25121 CACAGACTGGCTTAGGACCTATC 36105
2480 CCAGGAAGTGAGCAGGTTGTAG	14138 GGCTACTGGGGAATTTAATACAGTCT	25122 TCTGGGCCACCAGGAAGTGA 36106
2481 TGTCTGCGTGGCAAGGTTTC	14139 CCTTCAGCAGTGCCTGACAAAC	25123 GAGGTAAGCCAGAGGAAACGAA 36107
2482 GGCCCCAAAGAGGTTGCATTTC	14140 CCAAAGCATAGGCTGTTGTGAGT	25124 AACGTGGAGGGTCAGAGGTTTG 36108
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2485 GCTGCTTCTGAGGCCTTTACTT	14143 GGTTACTGTTGGGGCTCAGGAAA	25127 CTTGGCCTCTAAGGCTGCTT 36111
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2535	GGCTCCTTTACGGTCAGCAACT	14193	ACCCTGCCAGGACACATGAGAT	25177	CTTCACATTAAATTGTGGGCTCCTT	36161
2536	GGATTCTTCCTGTTTGACCGTGTTG	14194	GCCACAGAGCTGAAGGCTAGGATA	25178	GTGGGAGTTGGATTCTTCCTGTT	36162
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2538	ACTGCCTCCCGAGAAGTGA	14196	GGTTTGGACCCTGCTCATATCTTGA	25180	ACACAATCTCTTCTCGATCCACTTT	36164
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2541	GGGAAACAGGCTTATGCAAGACA	14199	CAGGCTTAGCCCCAATTGACT	25183	CGGGTGGGGAAACAGGCTTA	36167
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2544	GCCTTGGTGCTGGTGGAT	14202	GAGCCGGAAGAACTCAGGATGT	25186	CCGTCCCAAACTGCTTGTTG	36170
2545	AGGTACAGGGAGCTGAGAGAAG	14203	GGGATTTGCCCAGGAGTGTCTT	25187	ATCCGGCCTGCAGGTACA	36171
2546	GAAGTCAAGGTGCTTCCAGTGA	14204	CAGCCCTTGGGGTGTAGCTTT	25188	AGGATGGAGCTATCAGTATCATCAGA	36172
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2548	TGGTGCTGAAGGCTGGTTGT	14206	GCCTGCACCCTAAGGACACTTT	25190	GGGTGAATTCTGAATGGTGCTGAA	36174
2549	ACCAGCCAAGGGACCCAGTTTA	14207	GGGTGTCACCTCCATCTACACA	25191	TCGAGTCCGGCTCCCACTCA	36175
2550	CTGGTCCTTGAAAAGGGAATGGTTTG	14208	CCAAGGCCGTCCTTCAATACTTTG	25192	GGCAGGACTGGTCCTTGAAAAG	36176
2551	GGCCAAGCTCCACTCACTCTTC	14209	GGGGAAAGAAAAGCTGGAAAACAAC	25193	CCTCACTCTCACCTCCCAACCTA	36177
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2553	TATGGACTGCCGCTCCTTCA	14211	GGCAGGCAGGAAATAGGAGGTT	25195	TCCCTCCTGCCAGGTCTTTATG	36179
2554	AGGACTTTTGTGCTGGTTTAGACA	14212	AATCTTCCTGGGGTTCATTCTGTT	25196	CCCCTGCAGATTCACAGGACTTT	36180
2555	CAACCGATTCAGGTCCCTTTGT	14213	GTGTCACAGGGTATGGAAGTCACAAC	25197	AGCCCTGCCTTTCTCTTACAAC	36181
2556	CCACTGCGGGGTTAGGTAGTACA	14214	TGACCCTGGAGGGAGCCTTT	25198	TTGTCCCACTGCGGGGTTA	36182
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2561	AGGGGTCTGCTGGCAGGTAT	14219	CCACCTCCCCACTTCCCGATA	25203	TAGTGTGCTGGCTGGGAGGAT	36187
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2563	TGTCTTCCAAATGGCTCACGTT	14221	CGCAGCCCATGCCAATCTTTC	25205	CTGTGCTCATGAATTTGTCTTCCAA	36189
2564	CCAGTTCTGCGCTGGAGTAGT	14222	CGAGACTGAGGCCCACTGTTTT	25206	GGGAGAACCTAGGCTATGGAGTCA	36190
2565	CTGGCTCAAAGACATTGGAAACAAGT	14223	TGGCAAAGCCTGACCTGGAATC	25207	CACTCCATACTGGCTCAAAGACATT	36191
2566	CTGAGGTCTTGCAGCGAAGAAG	14224	GACCGGGATTGAAACCTCCACAA	25208	GGCTGGTGAAGTCTGAGGTCTTG	36192
2567	GGAAAACGGTAGGCATGGTTTACA	14225	CATTTCTCCCAGGAAAGAGGAAGAAC	25209	GATCACAGAACCAGGAAAAGGAAAAC	36193
2568	GGGTGGCCATAAAATCCAGACA	14226	TGGCTGCGCCATCGAAAACA	25210	GCCCTGTGGGTGGCCATAAA	36194
2569	TCCTTGCCCTTGAGGAGCTT	14227	TGTCCCGCTTGCCTTCCTAGA	25211	CCACAAACAAAGCGGGCTAAAATCCTT	36195
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2571	GTGTGTGAGCGCTGGAGATTT	14229	GCTGTTCCCTGCACTTGGAATC	25213	GCGAGACCTGATTACAGGGAGTGT	36197
2572	CCAGACTGGACTAGCCAGGATTC	14230	AAGGCAAGGGGTGAGGAAGTTG	25214	GTCTAGTTGATCTCCAAGACCCTTTC	36198
2573	CTGGGCAGTAGTGACTGTGAGT	14231	ACAGCAGACGGCGAGAAG	25215	AATTGCGCTGGGCAGTAGT	36199
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2576	AGCAGTCTGACATCCCAGTCTTA	14234	GAGGTGATCTGAGCTTGGGCATA	25218	GCCTACATAAGATAAGCAGTCTGACATC	36202
2577	TGCGGATGCGTTCCATGTCA	14235	CAGCGGGCCTGTTACAGAGT	25219	GGACTTCCCTCACCACAGCTT	36203
2578	TGGAGGAAGAAGCAAAGCAAGAT	14236	GGGGACAATCACTGGAGGGTTCT	25220	ACACCAAAGAGATATTGGAGGAAGAAG	36204
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2580	TCATGGCTATCCGCCAGTCATC	14238	TCCCACCAGCTCTTCGTGAT	25222	GCGCTCGTAGGTCATGGCTATC	36206
2581	GCCTTGAAGGATGTGCAGGAGTT	14239	TGTCCTTTCCCCTGCTCTTTAC	25223	GGACATCAGATCTGGGCCTTGAA	36207
2582	CCTCAGCTCACAAAGTTGCTACA	14240	CCAGGCTCCTATTGCTCCTATTG	25224	TGGGACACCTCAGCTCACAA	36208
2583	CCAGGTGGTTGTGGGAAGAGAAG	14241	GAAGCTCTGACCTCCATTTCAGT	25225	CCCTTTTGTTTTTCCAGGTGGTT	36209
2584	CGCCTGCATCATTATGGCTCATT	14242	ACTGGTACAGCCCTGATGAACA	25226	TGGTGAACGCCTGCATCAT	36210
2585	CCTGGGTTCATGTTAATGACAAGAGTTTG	14243	ACCACCCCTCCATGCTTGTCT	25227	GGTCTGTCTGATAGCAAGTTTGTGT	36211
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2587	TGGCCAGCACAGTTCCAAA	14245	GGCCCCAGCCTCAAAGAATGTA	25229	GCGTGGCTGCTGGTTACTGTAT	36213
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2589	GGAAAAGTGCTTGATGAGGCTTGA	14247	GTGCGCCTGACAAGGAAATGACT	25231	CCTGTATTCTGCAGCGTTCTCAA	36215
2590	TTCCAGCAAGGCGGATCACA	14248	CCCGCTGTCAGCCAAGTGTA	25232	GGCTTTCCCACTGTCTTAATTCCAGCAA	36216
2591	GATGGGTATTACCAGGGGAGAAAC	14249	CAGGCTCAGAGGACCTATGTTTG	25233	CATCTGCCCTGAGAGATGGGTAT	36217
2592	GTGGGCAGAATGTCACCATCA	14250	GCCTGCAGCCCTCATACAGT	25234	TGCGACCCTGTGGGCAGAAT	36218
2593	CACAGCGGGTTTGTACCATGT	14251	CCAAGTGGCCAGGGAAAACCTA	25235	CACCTGATCACAGCGGGTTT	36219
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2595	GTCTCGTTGCCCGGAGAGA	14253	GGCCTCAGCCTCTAACTGAGGATA	25237	ACCCAACCCCATCGTCTCGTT	36221
2596	ATCCTGGCACTTGCTACTTATTACA	14254	CCCCGATAGGCTGAAAACACA	25238	TGACAAACTGATCCTGGCACTTG	36222
2597	CTGGTGTCTTGGTTCCCAGAAATGT	14255	GCTGACATGTGGAGTCAACCATT	25239	CCTACCTGCTGGTGTCTTGGTT	36223

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2600	CAGAAGCCATGGTGGAGATTTGA	14258	ACTAATGGTGACATTGGTGGAACA	25242	TGGGCTTCTGGGGCAAAAA	36226
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2603	GCTCAGTGGTTGCTGCAGATGT	14261	TTCCCTGCCCTTCCACCTTTC	25245	GCTGGCTGAAGCTCAGTGGTT	36229
2604	GCTCAAGCCCCATGAATAAGGTT	14262	AGAATTCAACCACCATTGGCAGAT	25246	GGGGTTTCAGCGGACTGTGA	36230
2605	GAGAGACAACTGCATGCTCAGA	14263	GCTGGTCCCTGGAAGGGGATA	25247	AGCCTCTGCCTTGAGAGACAAC	36231
2606	GATGGACTGGTAGTCTGTAACCCAAT	14264	CCATCCCTGCAGGAGAGGAGAT	25248	GCTGGCTGATGGACTGGTAGTCT	36232
2607	ACTGCTCAACCAACATCTGTTCA	14265	TTCCCTCCCCAAAGCCACAA	25249	GGGACACACTAACTGCTCAACCAA	36233
2608	GGGCCATGGCACTAGGGTAA	14266	CTCCTCAGCAGGAGGAGTTTGT	25250	GCAACACACAGCCTGGACATTC	36234
2609	CAAGGGAGGCTGAAGAACGTCAT	14267	AGCACAGGGCTCACAGCTA	25251	GCCATATCTTGTTCAAGGGAGGCTGAA	36235
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2612	TGGTGATGGGGAGCCACTCTT	14270	ACAGAGCTCCTGGAGGCAAGAT	25254	CAGATGGCCCAACTTGGTGATG	36238
2613	GGTGCCTGCTTGTCCCTGAATA	14271	GGCTGCTCAGCTTGCCTGTA	25255	AAGCTGGTGGTGCCTGCTTGT	36239
2614	GATCCTCCCCAAGGGTTAAGAATG	14272	CCCCGGTGGAGACAAGGAA	25256	CGTTTACCAACGGGCGAAGA	36240
2615	GCCACAGCTGTCTGAGGGTTA	14273	CACTGCCAGGGTGGTCAATC	25257	GGAGGCAGGAAGAAGTCCACAT	36241
2616	CCTTTCCATGGACTGTGCAACT	14274	TGGAGACCGTTCTCGGCTAT	25258	CGGTGAGTTTGTCTGCGTTCCTTTC	36242
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2618	GGTGTGTGTGGAAGGACTGTGA	14276	GATGCTACCCTGAGGCTTGAAA	25260	GGTGAGGGGTGTGTGTGGAA	36244
2619	GAGGAAGGAGATATTGGAGGTGTCT	14277	CCAGCCAGCAAGCATCCTGAA	25261	ACCCACCCAGAGGAAGGAGATA	36245
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2623	GAGTTCACCCTTCAGCAAATGAACA	14281	GCCCCTCTGCTCTTCTCACA	25265	GGTGCTTTGAAGACCGATGAGTTC	36249
2624	ACACTGATCTCCACTCCCAGAT	14282	CAATCCTAGCATAGGGTGCTTCA	25266	CTCCTATGTCAGCCACACTGATCT	36250
2625	AGCCTCGAACCTCCGCAAA	14283	CGTGTCGCTGCCAGACAGAT	25267	TGACAGTGGGAGCCTCGAA	36251
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2627	GAAGAAGAGAAGGCAATGTGACCAT	14285	TGTGGCCCTCTCTCTCCAAT	25269	GACAGACGAGGAGAAGTCAAACAT	36253
2628	ACCCCACCCGCTTTCTCCAT	14286	TGAACTGCTGTCCAGGGTCGAT	25270	GCAGGTCAACCTTGGCTCTTAC	36254
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2637	TCTTCTCCCTCACAGCAAATCTTTT	14295	GCAACATGGCTGGTACACATTTC	25279	GCCATTGCCTCCTCCATTGTTC	36263
2638	CCAGGCATCTGATTCAGTGTCAA	14296	GCAGGGTTTGCCTTCTGCTCTA	25280	GTCTGAATGACCAGGCATCTGATT	36264
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2640	ACAGGCCCTTTTGGCTTTGT	14298	TGCTAGACAACCTGGGTAGTAACA	25282	GCGGTAGGATTGGTCTGGGAAT	36266
2641	GGAGGCCATCCAACAGAAGATTG	14299	TGCTCCTCCTCCGAGGTAAAGA	25283	CCTGAGGCATAACCAGTTGATCTTG	36267
2642	AGCAGGCAAGGCCAGAGTAG	14300	CACAGGCAACCTCTGCTCAT	25284	TCTGTCGCTTTGGGCTCTTC	36268
2643	GTGAAACCCCTGTTCTTGGTTGA	14301	CACGGACGCGCATGAAAGAA	25285	CAGACTGTAAAGTGAAACCCCTGTT	36269
2644	AGGAGGGGCACCAACTTACAGA	14302	TGTGTCCTCGGCGGTGTGT	25286	TGGGCCATAAGAGGCTGGTCTA	36270
2645	GGGGCTAAAGTCATCTCACCCTAAAG	14303	GGGAGATCTGGGCTCACGTT	25287	CTCTGTCAATTTGGGGCTAAAGTCAT	36271
2646	CTGCCCCATTGACAGAGGTTCT	14304	GACCTCCAACTTGTGGCAGACA	25288	TGGCTCCTCTGCCCCATTGA	36272
2647	CACCTCCTCACCCGCTATTGTA	14305	GGGCACAAAACCTCTGGATGCAA	25289	TGTGGCAGGGGACACCTTCT	36273
2648	GATGTAGGAGTCATGGGGATCTTG	14306	AGCCGTGGCTGTCCACAT	25290	GTGGTGGCGGAGACACAAAA	36274
2649	GAGCTAGCAGTATAGCACTGGTT	14307	TCCTGGTTCCTCCCACTGCAT	25291	TCTCCTCTTCTTTGAGCTAGCAGTAT	36275
2650	CAAGCGGGTGCAACAGATGA	14308	CAGTTTTGTGGGGCTCTGTCT	25292	TGTGTACCTGGAGGGCACAAG	36276
2651	CACGTGGTCCCTGCAGTCAT	14309	GCCATCCATCTCCTCTTTCCTACA	25293	GCTGGATGGCCACATTGAAC	36277
2652	GCCTGAGGAGAGAATGAAGGGTTCT	14310	TGGGCACCTCAAACTTAACACA	25294	CACCTGGCCTGAGGAGAGAAT	36278
2653	GCAGTGGTGATTGCCTTGGAGTT	14311	TTGGGCCAACTCCCACTCTT	25295	CCACGAGTTAGGGGAGACTTTG	36279
2654	CGGGAACAAGCTTCTTTGGTAAATG	14312	TCCTGTATCCCTGACTTGACTAGAA	25296	CTGGAAAAATGCCCGGGAACAAG	36280
2655	ATACCCTGGGACGAGAGGGACAT	14313	GAGATATGGACGGGAAGCAGAAAC	25297	CAACTCTGGCTGCTCGGTAA	36281
2656	GAAACCTTGCAGGTGTATTGACTCT	14314	TCGACTCATAAATGGCTGTGAAACA	25298	CCCAATAAAATGGCCTCTGAAACCTT	36282
2657	CCACTAGCCTGTAGGGTAAAGTTC	14315	GGGCTTCATTTGCCATACTGAGA	25299	CCTACCAGCCACAATTCCTTCT	36283
2658	CCTTGCCCTCCAAATCTATGTGACT	14316	AGGCCACAAAATGCTTCAAGTTC	25300	GCTAAATTGATTTCCTTGCCCTCCAA	36284
2659	GAGCAAAACCACCAGGGAACAA	14317	GGACATGGAGCAGGTAGTTAGCAA	25301	GCCTCCCTGCTACTTGAGCAAA	36285
2660	GTGTAGACAGAAACCTTGACCCAGAT	14318	GCAGCCAGACCCACTCTGA	25302	GGGGTAGTTGGTGTAGACAGAAACCTT	36286
2661	GCCTGAGGTGGAACTTTGGTCTT	14319	CCCAATGTTTTAGTCTGAACCTGAAG	25303	TCTGACTGCCTGAGGTGGAA	36287
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2664	GGAGAGAACGTGGATTATAGACCGTTT	14322	CCCGGCCCTAAGAGTCACAT	25306	AGGGAGGAGAGAACGTGGATT	36290
2665	CCCATCTAGTCTTCTGCTGGCGATA	14323	GTGTATTTTGGCAATGGCATCCTT	25307	CAAACTGGATTTGACTAGCCCATCT	36291
2666	TCTTGGCTAGGGCGTTAGTAGA	14324	GAGCCCTTCGTGTTGATGAGA	25308	ATGGGACTACCTGGCTATAGTCTT	36292
2667	GTCTGACCCAAGGAAAACGTTCTGA	14325	GCAGCACCGGCAAACTAATACAAT	25309	GGACACAGTCTGACCCAAGGAA	36293
2668	GAGCTCAGGTCTGGACTACTTGAA	14326	CCCCTTGCCTAGAGTCATCAGA	25310	GTCTGGACAGGAGGGCTTCA	36294
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2670	GGGTGGATGATGCTTGGGTAAC	14328	ACCTGGGTTTAGGCTGATGAGAT	25312	TCTGGAGGTAGGGTGGATGATG	36296
2671	GTGAGGCAACCCAGAGAATCATA	14329	CTGGCCTAGGGTCTTAGTGGTT	25313	AGCCAAGCATGGGGTAGTGA	36297
2672	CCCACAATCAGGAAAATTGCTTCA	14330	GGACTTCCCCACCAGCGTTTTT	25314	CCACTTCCCTTCAGTATAAATCCCACAATC	36298
2673	CAGGGAACTGGGCATAAGCCTTT	14331	TCTTCCATGGAGCTCTTTCCAAATC	25315	CCTGAAACAGGCTTTAACAGGGAACT	36299
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2685	GGTTTGTGCAGCTGGTTGACAT	14343	GCTGGATGAGGGAACACCTGATTTT	25327	GAACAGCTTCATTGAGTGGTTTGT	36311
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2815	TGGGCCGAATTCACCACAGT	14473	ACAGTCTGGCCACAGTCTCTT	25457	GCTAAAGGGTCTGGGAGGTTTG	36441
2816	CCAAGCCGATGCCCACTCTA	14474	GGTGGCACAGACTTGGAGATGT	25458	GGCTTCAGGGCCTTAGACACTTG	36442
2817	CGTCTTTTTCCCAGGGTTCGTACT	14475	GGGGAGTTAGCCTGGGACCAAT	25459	CGGAACTTTCTCATCTGACACGTCTTTTTC	36443
2818	CTCAGACAGATCACTGCTTTGAAGA	14476	GGCAGGGATTCCCAGGAGCTAT	25460	GCATCTTCAGGTCTCAGACAGATCACT	36444
2819	CTCATCCCTTTCTCCCCAGCAT	14477	GCTGCCCTCCATGGTCTGT	25461	TGAAGCACATCTCTCATCCCTTTC	36445
2820	CCCGAGGCTTGTCGTTTTAGTT	14478	GCTTGAGAAACCCTGCCCTAGTA	25462	AGAGGTTTCCCGAGGCTTGT	36446
2821	CAAAGCTCTTTGCATTACTGGCTTAC	14479	AGGGGAGCGCTTGGACTATAC	25463	ACCATTGCATGCCTGACTTCAA	36447
2822	ATGGCAGTAGTGCCTTCTCTTC	14480	CCCGGCTGGTAGCTCTCAGAA	25464	CACTGTCAGAGCCGTCATCTTGT	36448
2823	GTGGAAATGAAGAGAGGGTTGACAT	14481	ACCTGCAAGTTACTGGAGCTTT	25465	GGGCAAGCGTGGTAAGTGGAAA	36449
2824	GGGGAGTGACAGAAGGGAATAGGATT	14482	AAGCTGCGGCTCCTTGTGT	25466	GCCACATCTGGGGAGTGACA	36450
2825	CGTGACCTCGGCCTTCACT	14483	CAGAACCTCCTTCTTGGCAATGAT	25467	TGAGCTGGGGCGCTACTAC	36451
2826	GACAAAGACAGCTTGGCAGTGA	14484	CAGCGGCCTCACTCACAGTATT	25468	CTGGGTTTGTCTTACTAAAGCCAATG	36452
2827	CAGTGCTGGGTCTACAAAACACTTTC	14485	CCCTAGAACTGCACCGAGTCAT	25469	GCACACAGTGCTGGGTCTACAA	36453
2828	TGAGGAATTCAAGGCTGAAGATGT	14486	GGGGTACGTCGGTGTCATC	25470	CACAATGTTACAGAGGGTAGAGTCAT	36454
2829	GGCTTGTCACCCCACCAATGAA	14487	CCAGTTTTCCCCACAGAACACGAT	25471	TCCCAATCGAGGGCTTGTCA	36455
2830	GGCAATGGTGAAGCAGGTAAAAC	14488	GGGAAGCCACAGAGAGCAAT	25472	CCCCAGGGGCAATGGTGAA	36456
2831	CCTATCCTGAGACGCAGGTGTGTA	14489	CTGCCTTCCTAGGGCTGACA	25473	CTGTGCAAAGAGGTCTCATCCTATC	36457
2832	CACCCCACACTGTCCATTTCA	14490	TGCAGATGCAACTGTGTCCAA	25474	CCCGAAATCATAGTCACCCCACACT	36458
2833	AACCTTCCTGCAAGGAGACAAA	14491	GGGGTTGCAGCCTAGCTTAACTT	25475	GCCAATGAGCAGCAAGGACAA	36459
2834	GGGTGGGAAGTCTCCTCCTAGA	14492	AGGAGGACCGCAGAATCTTCA	25476	ACAGAGTGGGGTGGGAAGTCT	36460
2835	ACGTTGAGAGCCAACACCAT	14493	CTCACACTGGCGGGTTTTCA	25477	ACTGCGGGTCACGTTGAGA	36461
2836	AATTGTACCAGTGCCTGAAGTGA	14494	GCCGGCCAGTATGCCCTATT	25478	GGCAGACTATTCCTTCCGCAAT	36462
2837	TGGCACAGCCAGGGAACAGAT	14495	CTCTCTCTCCCATTTGCAGAAGT	25479	AGCTCCAATCGCCCATCTTG	36463
2838	TGGCTACGACTGTCCTCCTTCT	14496	GGTGGTTGTCGATGTCAGGAGACT	25480	AGGGGCCGTGTCATTTTTGT	36464
2839	GGCAGACCCATCTTTCACAATGA	14497	GGTTTCTCCACGTAAGTAACAAAGTCT	25481	GGAGAGTGGCAGACCCATCT	36465
2840	CTGGGGACAAGCGCTCCTT	14498	GGGACACAATTCACCCATAACATCT	25482	CCGGAAATGCTTGCCGTCAT	36466
2841	CTGGGGTCTTTGTACTCTGCTATT	14499	GGAATCTGCTTCATTCCTTGCTCTT	25483	CCTGGAAACTGGGGTCTTTGT	36467
2842	GCCTGCTGTGTCAGAGGACAT	14500	ACATCCTGCTCGACCCCAAA	25484	TGTGGACTTGCCTGCTGTGT	36468
2843	CACCAGGTACTCGATCCATCTCT	14501	GCAGCTCCAAAGAGCAGAGGAGATA	25485	CCTTTGGTCACTTTTCACCAGGTA	36469
2844	AGTTCCGAGATGACCCCATGA	14502	TGATTAAGCTGCATCGTGCATTG	25486	TGGTGGCCAAGTTCCGAGAT	36470
2845	TCTTGGGGACCAGTGCATCA	14503	GCCCAAGAACTCCATTGCTAAGTTC	25487	GTCCACGCCACCATTATCTCTTG	36471
2846	AGGGGACAGGGAGAGTGATCT	14504	ACCCAATTCAAGGAGCATTAGCAT	25488	GGGGTGGGCTCCAGATACAGA	36472
2847	CAGAACAGGTGGTAGGGAGAGA	14505	GGGTCCCAATACTGCAACTGA	25489	GGGTGAGAGGAATGCAGAACA	36473
2848	GCTTCCCTCATTCTTGGGAACT	14506	GATGCACCCTCCACTGGAAAA	25490	CGCAAAACTTGATGCTTCCCTCATTC	36474
2849	CTTCCGTGGTCTATGGGTTTATGA	14507	TGTGTTTCTGCACTTTGAGGAGTT	25491	CGATCTCTAAGGATCTTCCGTGGTCTA	36475
2850	CCTGGACTCTTTCAGGTTCTGAAGT	14508	CAGGTGGCAGGAGCAGACAA	25492	CCGTGACACCTGGACTCTTTC	36476
2851	GCAGGCCTTAGGCTTTGCTA	14509	GGAGGAAGGGTTCTGAGCATTC	25493	TGCTCCTCAAACATACCAAGCAT	36477
2852	GAGAGATGCTGCACTGGACTCA	14510	ACTAGGTCTGGGAGCCTCTCCTT	25494	AGAAGCCATGGTGGGGAAGA	36478
2853	CAGCCTCGTGGAAGCAGAAGTA	14511	TACCTGTGGCAGCCCTTCTTCA	25495	GGTCAGCAGCCTCGTGGAA	36479
2854	GGCAATGTTTAGGAGGACTTTCTCA	14512	TCTCCAGCACTGGTCTGTCT	25496	GCGACATGATTTGCAGGCAATG	36480
2855	AAGGACATCTGCGAGGAAAGTT	14513	AGAGACGTGGCACTTTGTTGA	25497	GCGGTGCAGAGCACAGAGA	36481
2856	CCTCAATCAAACGGGGCAACCAA	14514	CAGGCCAGCCTGGTAACCTAAC	25498	GAGCTCTAAACCAAACCCTCAATCAAAC	36482
2857	TCTCCCGCTGACCCGGTGTA	14515	GCCACTTGTCAGGGCATCAA	25499	ACTCACCGGCTCTCCTTTCTCT	36483

2858	TGACCAGTTCCCACCCTCTGT	14516	AGGGGCCTGTTTGGGACAATG	25500	GCCTGGACAGACAACAGCTACT	36484
2859	GGCCACGTGAAGATGACAGA	14517	GACAGCCAGGTGAATGTTCTACACA	25501	ATGTCTGGCTGTGGCTGTTG	36485
2860	GGTTTGTGGGATCAAGACCTTCT	14518	TTGCCCACTCCTGACCTAGT	25502	CCCTGAGCTAGGCCTTGGTTT	36486
2861	TGTCTGTCCCCAAGCCAGACT	14519	GCAGGGACCATACAGACTCCAA	25503	GCCACAGCCAGAAGAAACACA	36487
2862	GCCCATCTAGCCTGTGTTAGGAAGA	14520	CCTCCACTCCCTATGGTGCTTAG	25504	GGCAACAGACACAGCCCATCTA	36488
2863	GCCTGGATTTGGATCGTAACTCTAC	14521	GCTTGGGATTGCACGGATCA	25505	GGAAAGAACTGGAAGCAGGCAACT	36489
2864	AAGGCTGTTGGACAGACACAA	14522	AGCAGGAGGGTGAAGACTGT	25506	GGATAGGCTGGTACCATGAGAAG	36490
2865	TGGCGCCATGACAGAGGGATA	14523	AGGCCACGATGGCCACTA	25507	TGGACAAGCCCTTAACACTTGATG	36491
2866	GCCTGCCACCCACAGAACTT	14524	CCATCCATCCTCCAGGTGTTC	25508	GGCTCCAAGGATTAAGGTCCCAGAA	36492
2867	GGAGCCTGTACTCTGCACTGA	14525	CGTGAACTCTGACTCCTTTTCTGGAT	25509	GGTCAATACTTAGGGAGCCTGTACT	36493
2868	GTGGCGGGAACAGCAATATGA	14526	TCTCACCAGCTTGTCCCTAAATTC	25510	GTTTCCTAAGTGGCGGGAACA	36494
2869	GGCCCTAGACTGGAGCCAAAAAG	14527	AGGGGAAGACGTTTGGGGTAA	25511	TTGCCCTTTGGCCCTAGACT	36495
2870	AGGACTCCATCTGCCTTGTTTC	14528	CCAGGGAGCCTGCACACAT	25512	TGTTCCCACCAGGACTCCATCT	36496
2871	CACCAGCCTCACCCTTTGGATT	14529	GTGGCATAGGCTGTCCTGCAT	25513	ACCAGGGCTGGGAAGTCA	36497
2872	CTCCTGCTGTCCTTTCTGCAA	14530	CTCTCTAGCATGCGGTGGGTAA	25514	GAGCCTAGTGATCGGGGTTTCT	36498
2873	GTTGCCAGACCTCCCGTTAC	14531	GGAAGGCAGTGGACGGTAATTG	25515	GCCTGATGTGTTGCCAGTGTTG	36499
2874	GGAAGTGGTGGGAGGGAGAA	14532	GCCTTCCCCTACTTTACTGTCGAA	25516	CTGGGGAAACTGCATGGGAAGT	36500
2875	ACCAGGAAGTTGGATGGGACTTA	14533	GGAAGGAGCCACGCTTACCAA	25517	GACACAGACTGAAAACCAGGAAGT	36501
2876	CGCAGGCAATTATCTCCTATACTTGA	14534	AGGAGGAATACTCACCCCTGTTC	25518	GTGCGTCTCGCAGGCAATTATC	36502
2877	TCACCTCCTATTCACTCCTCAACA	14535	TGGAATGGAGGACAAATTCCTGAAA	25519	CTGTCTTCACACCTTCACCTCCTA	36503
2878	CAGAGCCTCCATTTCCTTGCAT	14536	TGCCTCTCCGCCAGTGTATG	25520	GCAGGCCACTCTGCTCTCA	36504
2879	TCACCCATGTCACATCCTGTCT	14537	GAGGGCTGGACAAGGTGGAA	25521	TGGGCACCTCACCCATGTCA	36505
2880	CATCAACAAGGTGGTGACCTGAGA	14538	CTGTGGATGTGCTGTGAATGTTGA	25522	GAAGAAAAAGGGCCGTTCTACCAT	36506
2881	CGTGCGGAAGGCAGTTCAAT	14539	GAGTTGCTGGGTCACGGAGTTT	25523	TCACTGGCTGACGACTCCAA	36507
2882	TGTCTCCGGCACAGGTTTG	14540	CCCCGAGAAAACCTTTCAGCACAA	25524	CCTACTGGAGTTTGGCCTGTCT	36508
2883	AGGTCCCAGCCTTGGTTCATC	14541	AGCATCGCCATCCCAACACA	25525	CACGGAGACCCCACCATCA	36509
2884	TGAGTGGTGTTTCAGATCATCACA	14542	ACAGGCTTTGCCCTCCTATCT	25526	CCCTGATGCTCTGAGTGGTGTTT	36510
2885	CACCTTCCCCACTCCAAGTTTTG	14543	TAGCCACCCTTCCTCCACTGCTA	25527	GATAGAGCACATAACCTGAGCAATTC	36511
2886	GGAGCCCTGAATGAACCATGA	14544	TTGGGCCTGTGAACCTGTGT	25528	CACAGAACTGGAGCCCTGAAT	36512
2887	GAAGCCCTGCAGAGTCAAGAGA	14545	GGGTTCCTCCACGCACTTGT	25529	TCACGCTAGGTGGTGGTGAA	36513
2888	GGAAGGGCCTCAACCATCCAAA	14546	GGTGCCTCTGGGCTCAGTTTT	25530	AGGACATGGAAGGGCCTCAA	36514
2889	TGGCCACCATCTCTGCTGTCA	14547	CGTGTCCCCAGAGCCCAAT	25531	CCCATGGCCCAGATGAACAGT	36515
2890	TCCTGGCACCATGACGTATCT	14548	TGGCCATTCGCCCTACTGT	25532	TGGCAGTCCTGGCACCAT	36516
2891	ACAGAGCCAGCAAGCCCCATA	14549	GTGTTAGGTCAAAGGCAGGGTTCT	25533	GAAGCCTTGGCAGACACAGA	36517
2892	CCAGTGCCACCTGGGTATGT	14550	GTGTAGTGGGTTGGCTTAGGCATT	25534	CAGTGTCAGGGTGGGACTCA	36518
2893	GCTTACCAAGGAACGCGTAGA	14551	ACCATCCAGTCCGAGTGCTT	25535	GGAGGGAAGAACCTGCTTGCTTA	36519
2894	CGTGTCTCTGGCTTTGTGACT	14552	GGACGGGCAATGTCCATGT	25536	ATGTCCCTGTGCCGTGTCTCT	36520
2895	CAGCCAGTCACTTCGTTTGTCA	14553	CAGGCCACACACCCCTTTATCT	25537	CCCTTGGACTTCAGCCAGTCA	36521
2896	TCCTTTGCACTTAACGCTCTAGT	14554	CCGGAATCCCTCAGCAAAGTCT	25538	GCCCCTGCTCCTTTGCACTTA	36522
2897	CCACCAAGGGGCCTTGACA	14555	GGCATAGCAGACACTGCCGATT	25539	CCTAGGGTAGCTGCCACCAA	36523
2898	GTTGGGCGGGAAAAGCAAATTC	14556	CCCCTGCATCTATCCATTCCTATTC	25540	GCAAATGAGTGTTGGGCGGGAAA	36524
2899	CAAAAAGACCCCTGACCACTCAAG	14557	ATCTCCTCTGCTGCGTGACAAG	25541	GCTCACACCCTCTTCACCACAA	36525
2900	GTGGTGCTTGTGCGTGTGT	14558	CTGTACATGTGGCTGGAACAGA	25542	ACAGCCCATCTGTGGTGCTT	36526
2901	CAGAGGCCAGATGTAGGGAACTGT	14559	TCCAGGACGTGGCACAAAGT	25543	GGAGGCAGAGGCCAGATGTA	36527
2902	TGCCCAGCTGGTTGGAGACT	14560	CTCTTCCGTCTCCTCCTGCAAAC	25544	TGTCAGCTGAGGGGATGAGT	36528
2903	GTGCCCTGTGCAGACATCACT	14561	TCCAATCGCCTGTCTGTGTTC	25545	CATCGACATTGCCTAAAACCTCTTG	36529
2904	CCAGAGAGTGGGTCCACAAGGTA	14562	CCTCACATTGCCATCCAAGCAT	25546	AGGCTCACGTGCCAGAGAGT	36530
2905	AAACCCCAAGAGGACCTTGAAC	14563	GTCCACGTGGGTTTAAATTCAGCTA	25547	GGCCCAGGGAAGAAGCAACA	36531
2906	CCAAAGAACCGAGCCCTTCACT	14564	CACAGACACAGCCCCAAACACA	25548	CAGACCAGATTACGCCCCAAAG	36532
2907	TGCGCCAGGAAAGAAGTATGTT	14565	TTGGCCAAGGGGACCCAAGAA	25549	GGGCTTTTTGCGCCAGGAAA	36533
2908	CCTCAGTGGGGAGAATATGCCTTT	14566	AGTGTTGGCGTGGAGGTCTCT	25550	CCGACCTCAGTGGGGAGAA	36534
2909	AGCGGAACGCTCACGGAGATA	14567	CAGGCAGGCTGCACACAAA	25551	TCGCTGCCACAGAGGGTGAA	36535
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2911	TGCGGCGTTGGGTGGTTT	14569	TGTGCGTGCACTCCGTGAT	25553	CGCTTAGGCACAGGGATTGA	36537
2912	CAGTCACTGAGAATCAGGCAGTGTA	14570	ACCCTGCCCTGCTGGACTA	25554	GAGGCAAGAGACAAGTCAGTCA	36538
2913	AGTGTGCAGTGCCCGAGAGT	14571	GGGTCCTCTTGCCATTCGTTCA	25555	GAACACATGAGCCACAGACAGT	36539
2914	GGAGACAACGGCCCTCAAACA	14572	TCCACTCCCTGCGCCTCACA	25556	ACGCCCAGGAGGAGACAAC	36540
2915	CCAGGCAGGCTGGAGTTTCA	14573	CTGCGTCCGGGAGTCATGT	25557	AGGAGAGCTGAGGACCAGACA	36541
2916	TGAAGCCCACAGCCATCCAA	14574	TGCGCGATGCTGCAAGATGT	25558	ATCTCCTGGTGGCGCTGAA	36542
2917	CAGGTGATGGCGCTGAGATTC	14575	ACATGGAGGCGAGAGTCAGA	25559	TCCCCAGCCAGCCAGGTGAT	36543
2918	CTGGCCTCTGGGGACAGAA	14576	AGGCCTTCCTGAGGCTTCGAT	25560	CCCATGCCAACAGGACATCT	36544
2919	TCTTGCCTCATCTGCCCTCTGT	14577	GCCCAGTCCGGAAGGGTTCT	25561	GGACCGACTCTTGCCTCATCT	36545
2920	CCCGCAAGTACTCATGGCTCTTC	14578	GGTGCACTTGGAGTCACAGACA	25562	TGGACCCCGCAAGTACTCA	36546
2921	CACACACACTGAGGACGCTTTC	14579	GCCACCACGCCAAGAAAGGAA	25563	CCCCTGACCACACACACTGA	36547
2922	TGTCTTGGCCGATGACACAA	14580	GGCAACCCAGTGCTGAGGAAT	25564	GGCCTGCCCTTTGATCTAGTTG	36548

2923	CCCTGACCGCTGATACAAGCAA	14581	TGCTCGGCGTGTCTGCAA	25565	CGTGCAGTGAAACCTGGAGTGA	36549
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2925	CCCTCACACAAGCCACCTGTTT	14583	AGGGGTCTGGCTGAGCATATC	25567	GCATAACCTCAGTGCCCTCACA	36551
2926	AACCCAGGACTGTGAGAGTGA	14584	GGAGGTCCTTCCTTGCCTCAAT	25568	TGGGGCTTTATTTGGGAAGTGTAAA	36552
2927	TCCACCCCTCTGCGTAACTTTG	14585	GTGGGCCCTTTATTGACCAGGTT	25569	CTCTTCCCAGGTCCCTTCTTTC	36553
2928	ACGCACCAGCTCCCCTTTCA	14586	GCGGCCTCAGGAAACCTT	25570	GCTTTTGCTCTGGCCATGTAAG	36554
2929	GGGGCATAGCCCAGGAATGA	14587	ATGCTCCCCTCCCTGTCCAT	25571	TTTGGGAACCCGGGGCATA	36555
2930	GGAGACGGGATGGTGGAGAAT	14588	GCGAGTCCCATCTGCTCTTC	25572	GGCCAGCTAATTCCAGCAGACT	36556
2931	TCGCTGCCAAGCAGTGGAT	14589	AGGCTCTGGGCTCCTTGT	25573	AGACAGAGCAAGGGGCTGAT	36557
2932	TGGCTCCCAGAAGTGCTGACT	14590	GACTTCAGGGCCTCCAACCATT	25574	GCTCCACAAATGGCTCCCAGAA	36558
2933	CTGTCACGCTTTGTCACGCTTTG	14591	TCCCCTGCACCACTTCTCAGAT	25575	CGCTGTCTGTCACGCTTTGT	36559
2934	TGCCCGCCCATTCCATCAT	14592	TGGGTGCTGGGTGCACAGT	25576	AGGGAGTCAGCGGACGTT	36560
2935	CGAAAGCCACAGGACGTGCTTA	14593	GGAACGGCCCTGCCAAAT	25577	GTGGGTCTGTGTGGAGATGGATAC	36561
2936	GCAAGATCAGCACTTCTAGGACGTT	14594	AGTGAGCGTCCCGTGGTT	25578	TTGGGAGCAGGGCTGGAA	36562
2937	CCCCGAGGGGAATCAGCAT	14595	GTGGCAAGGACAAGCTGTATCT	25579	CCCCTCCAAGGGGTGATTGT	36563
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2940	TACCTCCACCGCAGACAGAA	14598	GGCTTGAGCTTGCAGTCTTG	25582	TGAGCACGTCAGCGTCTCTAC	36566
2941	GGTTTGGCATGGCCTGGAAAGT	14599	GCTCCCTGAAGGGCAGTGAAG	25583	CCCAAGGTGGGGAAGAAGGTTTG	36567
2942	GCCCATTTCCACCCTAACTGTGT	14600	GCTCTTGTGGAGAAGGAGACAGA	25584	GGGTATAAAACTCCCAGAGCCCATTTC	36568
2943	TTGTCTGGGGAGGGGAAGTT	14601	TGCTGGGGCAACCTCGAT	25585	GGAGGGAAGACTCCTCACTTTGTCT	36569
2944	GCCTGGTTCCCCTGACATTCT	14602	TGCACTGCCCTAGAGCTGCAT	25586	TTGAAGCGGGGAGGCCTGGTT	36570
2945	CCACTAGCGTGGGGTACTGAA	14603	ACCCCGCCTCACTCGGATGT	25587	GGTGATTACATCCCCGCCACTA	36571
2946	CCATGACCAGAGTCCACCTTTC	14604	TCCTCTCTCCTCTGTGGAGTTTTA	25588	CTGCCCCAATCTTCATTTCCTTTC	36572
2947	CACTGACATCCGTGTTCCCAAACT	14605	TCGCACGTCCCTGCTACAAG	25589	CCAGGGTCTTGTTTCCACTGACA	36573
2948	TGCCGTGCATGAGTGCAGAT	14606	CACAGTGGCAGTTCCGGATGA	25590	ATTTGGCGTGCCGTGCAT	36574
2949	AAAGGGTGGGACGTCTCGAA	14607	TGACGAATCAGAGAATATGACCTGTAG	25591	TGCCCATTGGCTTCCCCTAA	36575
2950	AAAGGGCCGTGTGGGACACAT	14608	GCCAGGCTCAGACTCATCATCTTG	25592	TGAGGAAGGCCCCAAGGAAA	36576
2951	CATCAGTGCTCCGTGCTTGTTC	14609	TGGATGAGGCCCTGTTCTGT	25593	TGGGCTTCGTGGCATCTTCA	36577
2952	CCAGCCATTCTATCCTCCTCCAA	14610	TCCAGCTTCCTCAAGGCAGTCA	25594	GTTGACTTTGATCACCAGCCATTC	36578
2953	TTTGGACGGTGGAGGTGAGT	14611	CCAGCCAAGAAGCAGCAACA	25595	ATGCCCTTGCGTAGGGTTTG	36579
2954	CACACTCAGTTTCGGGCACAGT	14612	CACGGCCTTGCTTTCCTGAGA	25596	TTAGCAGGAGCCCCACACTCA	36580
2955	GCTTCCCAGCACCTCCTCTAAG	14613	AGGCCCCGGAGGGAAAAGAA	25597	CCACCTATTGCAGGGAGCTGTT	36581
2956	ACCATGGTGGAAACTCCATTCATT	14614	GAGGACTGTGCCAGGGAAGTA	25598	GCTGTGTACCATGGTGGAAACT	36582
2957	TGGATCTGCCCACACCCTTAGA	14615	TGCTGGACAGACGCCCAGTT	25599	GCTGCAGATGAATGTCTCTGGATCT	36583
2958	GCCGAAGGACAGGCACACTT	14616	GTCCTCAGGTCCCAGTGGTAGTA	25600	GCCAGCGTACACATCCCTGAA	36584
2959	GAGACAACCAAACCCTTCCCAAGT	14617	GTGCTTCCCTTTTTGCCCTCTA	25601	CCCATCTCTTCCCTGAGACAAC	36585
2960	TACCTCCGGCCTGACCAATCA	14618	GGAGAGGGGCTTGGGAAGTGA	25602	CAGAGGACATTAAGAAAACTCCCTCTGA	36586
2961	GCAGCTCAAGGGTGTTCGGAAAT	14619	CTGCCAGCACACCTATGTGATA	25603	GTAACTAATCACAGGGCAGCTCAA	36587
2962	TGCCCTGCCCTTGTCAGTCT	14620	GAAGTTCCCCAGTGCTCACCAA	25604	CTGCCTGCCTTCTCTGCTTTTC	36588
2963	TCCCATGTCCCGATCCGACTTC	14621	CCTCACCAGCTCCCAACGTTTT	25605	CTTGTCAGAACTGCTCCCATGT	36589
2964	CTGTTACCATCCACTGGACCAA	14622	TCCACACCGGCCCATCAT	25606	GGGCCTGAGGAACTGTTACCAT	36590
2965	GCCTGTGAGGAGGACTTTTCCAT	14623	TGAAGCACAGGTGGGAAGAAG	25607	ATGTGTCCTTGGGCCTGTGA	36591
2966	GACTCTGTGTGAGAACAGCACTAT	14624	AGAGAATGGAAGAGAAGGGACTGT	25608	TGTGCCAGACTCTGTGTGAGA	36592
2967	GTCATGGTGAGTCAAAGACTACGTTA	14625	AGAGGCTCCACCCGTCTTCTT	25609	GAGGCTTTGTCATGGTGAGTCAA	36593
2968	CGCTGTGAGCCTCCACTGATTT	14626	TGAACAGATGGCTACGAATGTATGA	25610	TGCCTGCCATAGGGGAGCATA	36594
2969	CACACCAGGTTGTCTCTATTTACACTT	14627	ACCCTGGTCAAGAGGGAAGTCA	25611	CCAAACACACCAGGTTGTCTCTA	36595
2970	AGCCTGGTGGTAGAGGGAGAAG	14628	CAGCCTGGCTTGCCCAAAG	25612	AGCTTCTCAGCCTGGTGGTA	36596
2971	GGCAGTATCCAAGGCAGGTGTAG	14629	TGGCAGGTTCTGGGCTTCATC	25613	GAAGGCTGGAGAATGTGGTACA	36597
2972	CAGCCACTACCCTGCAACCAT	14630	AGGGGATGGCCTCTTGAACACA	25614	TGGGGTCTTCTGCAGCCACTA	36598
2973	CCTCATGTACTCCTGGTCTCACA	14631	TGGAGCTGTTTCTCCTAGTGTCT	25615	ACTGCCTCACTCCTCATGTACT	36599
2974	CAGCCCCAACCTCATTAGTCAGTTA	14632	GCCTGTCAGCAGGCAGACTT	25616	AGGGCAGCCCCAACCTCATT	36600
2975	CACAGGCAGGTTAGAGACGCTTTC	14633	CCTTTGGCTGTGTAGGCTTCGAT	25617	CCAAAAACCCACAGGCAGGTTAG	36601
2976	TCAGGCCTCTCTCAGTCGAA	14634	GGTGGGTCTCCAGGTCACTGAA	25618	AGGGTGGGGACGTGTAGTCA	36602
2977	GATCCTCAAAGCCTGTAGGAGAAG	14635	TGAAGACAGCCTCCCACTCTCT	25619	GCGAAACGTGGTGATCCTCAAAG	36603
2978	GTATCCTGGTTTCAGACACTGAACTT	14636	GCCGGGGCCAACTATTTGCTA	25620	GGGCTCCAAAACGTATCCTGGTT	36604
2979	GCCCTCAGAGAGCCACAAGA	14637	GCAGGGAGCAGGGAAGGAAGT	25621	GGAGGTGGGGCAGAATGGAATC	36605
2980	TCGGGACCTATCCGCTGCATT	14638	CTGGGGCAGCTGAGGAATACTT	25622	CCAAGCTGTCTCGGGACCTAT	36606
2981	GCCTGGACTTTTGATTACCCCTTA	14639	CATGAACCTGCAATTTGGCTGAT	25623	CCAGTTCCTATGCCTGGACTTTT	36607
2982	GTTCCCTCTCTCGGCTGACAATG	14640	CCAGCCCAGGTTTGCTGAATGT	25624	CAAGGCCCTGTTCCCTCTCT	36608
2983	GAGCTTCACATTCCATGTGGCTTT	14641	TCAGTCCTGAGCTGGAAATACCTA	25625	GGGGACTGCAGAGATATGAGCTT	36609
2984	TTGAGTCTCCCCATCTCCCTTT	14642	CCCACACCCATCTTGGGTCTTT	25626	AGTGAAGTTGGAAACCAAGGTTGA	36610
2985	GAGCAAGGCTGATGTCTTCACA	14643	AAGGCCCAGCAGGGAGGAA	25627	TGGGCCAGAGCAAGGCTGAT	36611
2986	CGCAATGCTGACACACATCCTTT	14644	GGCATGTTTGTCGTGGGGATGT	25628	TAGAGGCCGCAATGCTGACA	36612
2987	GGAAATTCTGCAAGGAGGGAGACA	14645	TCTCAAACTGAGCATTAGCTCCTT	25629	GTGCTGAAGGCCAAGAGGAAA	36613

2988	CTCGCAAGATAACACACAGAGTAACA	14646	ACCACAGGACTGGATGGGAAAA	25630	CCAGCAGCCCTCGCAAGAT	36614
2989	CTGGAACCAAAGGGCAAAGAAAG	14647	GGAGGAATGATGGGGAAGTCTGT	25631	TCAAGGTGACCTCTGGAACCAA	36615
2990	GCTGTCCACGAAGAGCCACAT	14648	CCCTGTGGCGAGATCCACAT	25632	AGCCAGGGCTGTCCACGAA	36616
2991	CACCGCTAGCCACAGAGGTTT	14649	GGATCCTTGGGGTGTCGTTTCA	25633	GGGCACAAGCAATACTTAGGCAGAA	36617
2992	CCCACCCTGCACATGGGTTT	14650	GCTCCTGTTGTTACTGGGTAGGTT	25634	TTGCGGAGAGGGGCCTTCA	36618
2993	AAGGAGCCCAGTGACCCTGAT	14651	CCTCTCCTCTTTGTGGTCCTTCAAC	25635	GCTGGGAGGGCTGAGTATGATGTA	36619
2994	ACTGGACTGTGATGGTTGCTAAG	14652	CAGTGTGATAGGTTTCCGTGAACT	25636	GGGGTGGATGGGAATATTGGGAAAC	36620
2995	TGCTTGGGTATAAAGGGAGAGAGA	14653	TCAGCCCCTTCACCCAACTAA	25637	GGTGGAGTGGTAGATGTTCTTGCTT	36621
2996	GATAATGTCAAAGCCGGTAGTGATTG	14654	GGTGACCACCCCTGAATAACCAT	25638	GCTGTGCTCAGCCAGATGTGAT	36622
2997	TGAGGGAGCCAGGGACTAAGA	14655	AGCTCTTTCAGGTACAAGAGTGAAA	25639	GGACTCCCTCAAGCAGGAAGTTG	36623
2998	TCACAGCAGGCCTTGGACCAT	14656	AGCAGCAGCCTCTTGCTAGT	25640	GCACAAGTGCTGTTCTCAAAATCACA	36624
2999	GCCACCTACTTGCAGGTTGAA	14657	GCCCCATCAGCGATGTGGAATA	25641	GCTCTGCTTCTTGCCACCTACT	36625
3000	TGGTCACATACACGACTGTGTT	14658	CTCTGAAACCTGGGGAAATGCAA	25642	GAGCGCAATGTCTCCCTGTATG	36626
3001	ACCCACCAAGCCATGTCCTCTT	14659	CTGCCAGTTTTGTTGCCCTTTT	25643	CTGGGATTTCCAACCCACCAA	36627
3002	GGATTGAGGCCAGGCAGACTA	14660	CAGGTCAATGGACGGTTAGGTACA	25644	CTGGTGGCCAATGGGGATAACT	36628
3003	CCCAGGGCTACCTCGGATTTC	14661	CAAAGGCAGAGACTTCCCTGATG	25645	GTCCCTCACTTTTCTAAGCCTCTGT	36629
3004	GCGTGTTAGAAGGTAACGCATACT	14662	ACCTCCTCCTTCCCGATTAAACT	25646	GCGTGGCGTGTTAGAAGGTA	36630
3005	GAGCCGGGAAAGTTTTGTCCAAT	14663	GGAGACCGCAGCTCCAGTTA	25647	TTGCCTGGAGCCGGGAAAGTT	36631
3006	GTGATGTTCTGGCTCCCTCTTTG	14664	GGTCTCGGGAAGCCAACAAT	25648	GCTTCCTCTCTTTCATGTGATGTTCT	36632
3007	GGCGTAGAATGCAGCAGACACT	14665	AGGCCAGCAGCCAGAAGTTT	25649	GAGTTTTGTGTGATAGAGGCGTAGA	36633
3008	CTCAGTGCCCTTCCACATCAGT	14666	CCCCTAGAAACAGACCCACTAATC	25650	GGCTAGTGCCAGGCTCATATCTCA	36634
3009	GTGGATGGCTTCCTAATTCCAAGT	14667	TGACTTCCACGGTACACATCTGA	25651	GATGTTACAAGTGGATGGCTTCCTA	36635
3010	GAGTTCAGAGGGCATGGGTTT	14668	ACCGCTGCAAATAGGCACTAC	25652	TAGCTGCTGGCTGTGAGTTC	36636
3011	TCTGGCTCCAGCACTCACTA	14669	CCTAGGAGAATGGCACTGGCTTA	25653	GGTCTAGGTAGTGTGAGGTTCAGT	36637
3012	CCAGATTGTACCCTCAATTCCTCTCA	14670	GTATGGTAGTCACGCAACACAAAA	25654	TCACGTTCTTTCCTTCCAGATTGT	36638
3013	TCACCTCTCTCTCCTCCACCATTC	14671	ACACGGAGGGCATCGTGTTG	25655	CTGAAAGGGGCACATCCCATCA	36639
3014	CAGGCCACGTCGTCACACT	14672	CCCTGCTCTCCCTGTCCCATA	25656	GCACAAACACCCCACAGGAT	36640
3015	CTCACACATCGCCGGGAAAAAG	14673	GTGCAGGTGACATACCAAGCTGTT	25657	AGGCATTCCGAGGCTCACA	36641
3016	GCCAGCCATAACACCTCACA	14674	GTGTGGCCCCAAGGGTGTT	25658	GCCCAATGGCTGGGATATTAAAAAG	36642
3017	AAAGGCCCTGGGGTGAGGAA	14675	CTGCTCTGCCCACACAAACA	25659	CGGGAGCAGCAAGTGCAAA	36643
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3019	GTCCTACCCAATGACTGACTGATAC	14677	GTGCATGGAAGCTGGGCTTT	25661	GCAGGTGTCCTACCCAATGACT	36645
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3021	AGAGAGGAGCACTGGACACA	14679	ACCTCTTGGTGGAAGTGGACAT	25663	GCTTGCGGCAGCAGAACAA	36647
3022	GCTGCTGGCAGTGCTCTCT	14680	AGGTGAGAGCCAGCTGGAAA	25664	TTGAGGCCTGTTGGGTGCTT	36648
3023	AGCCGTCACTGGTTGTGTCA	14681	GACTTTGAGATCGCTGACTGATTCT	25665	GAAGTTGCAAAAGCCGTCACT	36649
3024	CCCGCCACCACATCTTGCTAAA	14682	GGCAAAACCCTGGCTCTACT	25666	TAGGTGCCCGCCACCACAT	36650
3025	GACAGAGTGAGTACGACATGGAAAC	14683	AGCCCCTGCTCCCAAGGTA	25667	AGGCACTGAGACAGAGTGAGT	36651
3026	GTGCAGCAAGGCTGTGATGAGT	14684	TGAAGCCACTGAAGGTAGCTTTT	25668	TGTTAACCCCGTGCAGCAA	36652
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3029	CTGCTCCCCAGCTTACCTTTTG	14687	CAGTATCCTCACCTTGGTCTAGACTTT	25671	ACGCGCCCAGGTGATGTTG	36655
3030	TGGCCCCTCCTACAACCAAAAC	14688	CCAAATTGACCCACTAGGTGTTGT	25672	CTGCATCTGGCCCCTCCTA	36656
3031	GCTTTGGGCTGGTGACTTCT	14689	TCCACTGGAAGTGGCAGAAAAT	25673	GCCTGGATGGACTTCAAACTTGCTT	36657
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3034	GCCACTTTCCAGCAACGTATC	14692	CATTTTACTGGGGAAGAAATGGAGACT	25676	CTTGGGAGTTGGAGCCACTTTC	36660
3035	CACTTGCTTTTGCATCCAACGATTAC	14693	CCCAATGCCTGGCTCCTAATTC	25677	GGAGGATTTCCACTTGCTTTTGCAT	36661
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3037	GCAGGCACCAACACCTCACAT	14695	GCTGGACAGTGTTGAAAGCCATAG	25679	TGACGCATGCCTGGGAAAAG	36663
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3040	CTCCCCAAATGAAGCTGACTGTAG	14698	GCTGGGATTGCCTTCTTGTTTTCA	25682	AGCCATCCAGGCTCCCCAAAT	36666
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3042	GGAAGGGGCAATGGCACTTAG	14700	GCAGAAACTGTCAGACTCCTACTTC	25684	TGCAGGGGAAGGGGCAAT	36668
3043	AGGGGTTACGGTGTGGTTCA	14701	GTGTTGCAAACCAACCCACAGA	25685	CCACCTTTGCATAGTTCAGTAGGGGTTA	36669
3044	ACCTCCTGCTCACCTGTCAGT	14702	GCCTGGGCTAGACACACACT	25686	CCCATCCTTAGCCTCATCGATAC	36670
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3177 GACTAGAAAATTGCCGTGGGTCTCA	14835 TGGACACACAACACCCAACA	25819 CCCCTGGGCTACGAGTATATGTGA	36803
3178 CAGATCAGCTGGAGGGAGCATT	14836 GGGTAGGCATTGTTCCACACTCT	25820 TGTGGAGCAGCCCAGATCA	36804
3179 ATCTCCAGCACCTGCACTCA	14837 GGTCAAGTCATCAGGTGGAGTCA	25821 GGGCTATGATTAGGAGAGCCTATCT	36805
3180 GTGCTGTTGCAAAGAGTCGAGAA	14838 GGAAAACGTATCACCTTTCCCAGAT	25822 TCCGCAAGCAAGTGCTGTT	36806
3181 GGTTGAAAGATGGAGCCCTCTGA	14839 AGTAGGCTTTGCAGAAGTGAAGT	25823 AGGCCAGGGAGTGGTTGAAAGA	36807
3182 GCTACTGGGGAAGATTGCTTGA	14840 CACGGCTCACTTGTTGACTTTG	25824 GCACCTGTGCCCAGCTACT	36808

3183	GCCAGACGAGTCATCTACCCTTGT	14841	TGCCTTGGATGATGAGTTGAGAAG	25825	CTCTTTCAAGCCAGACGAGTCA	36809
3184	CAGTCACTATTACTGTACCAGGAGTGAT	14842	GCCTGTTTGCGCAGGTCTGA	25826	GCTGATGTGTGCACAGTCACTATT	36810
3185	CACCCAAACATTGGCTTTTCTTCCAA	14843	GACGTCCCTGCATTCCCAATCT	25827	CCACGGATCCTCACCCAAACAT	36811
3186	GAGGCACTGGTTTTGGAAAAACAACA	14844	ACCCACCCAGCGACAGGTT	25828	ACAAAAGGATGAGGCACTGGTT	36812
3187	CATGGTCCCCACCCACAAG	14845	GACTGATGAGTCTGGGTTCACATC	25829	AGGTGCAGGGCTCACAGTAA	36813
3188	GAGGGAGAATACAGTCCATGTTTGT	14846	GGCTGCCAACCACCACTAGA	25830	GCGGCTCTGAGGGAGAATACA	36814
3189	AGGGAGGGAAACTGGTGAGGTA	14847	CACCTCTCAACACTGGTCTTTCT	25831	TTGTTGGAGGAGGGAGGGAAAC	36815
3190	GGCAAAGTGGAGAGATCCAGAACA	14848	AGAGATCTGGGCCAGCCAAAG	25832	GCAGGTTCAGGCAAAGTGGAGAGA	36816
3191	CTGAGAGGAGAAACTGATGCTTCTT	14849	TAGGCACTGCAGCCCTTTCA	25833	GAGAAGCCCACTGTCTCTCTGA	36817
3192	GGTAACCACCAGTGGCCATTCT	14850	TTGCCACGCAAGGCTACTT	25834	GAAGGAAGACTTAAACTGGTTGGTAAC	36818
3193	TCTGAGGGGCTGGGAACTGA	14851	ATGGCTGGCTGCCCAGGTAA	25835	TGTGGTCTCAGCTTGGTCTGA	36819
3194	CCAAGTGGTCCCATTTCCCCTTA	14852	CTCTGCTGGTAGGGGTGATTGAT	25836	ACATACTCTTCCTTCCCTCCAAGT	36820
3195	GGTGTTGCTGGAAAGGTAGGAT	14853	GGTTGTCAAATCCCAAGTCCAGTCT	25837	AGATGAAGTTAGGTGTTGCTGGAA	36821
3196	AGGAGGGTCACCTTTGTGTCT	14854	GCCTTTCAAATCCAGTGGTGACAAAC	25838	CCCACCAATCACCATAGCCAGTA	36822
3197	CCAGAAACCGTGAGTCCTGTCA	14855	GGACCCAGCCTACATCACATTC	25839	GGACATGGAACTGTGACCCAGAAAC	36823
3198	GAACCGCATAGACCAGTCTACCTA	14856	TTGGGGACACTGTGGAGCAA	25840	CCCCACAAAGAACCGCATAGA	36824
3199	TGGCATCAACTCCCTTTCTTCAA	14857	CCTACACAAGGAGGGAAGAGTGA	25841	GCAAACACATGTTGGCATCAACT	36825
3200	TTGTCCCTGCCTGAAGGACTCA	14858	GAATTCCCAGGTTGAGGAAACCTA	25842	CTCACCCCGAGACCCTTGT	36826
3201	GGAGCAATGTCTACAAGGATGAGATG	14859	AAGCCCCAGCCCACTGGAT	25843	GAGGGAAATCAAGGAGCAATGTCT	36827
3202	GGCACACAGTCATGGTAGAATAAACTT	14860	GTCTACAGCACCTTTCCACACA	25844	GTGGATGGCACACAGTCATGGTA	36828
3203	CAGTGACCAAGTGGGAGAGAAG	14861	TCCACCCAAGGTGCCTTCTTA	25845	CATGGCACACAGTGACCAAGT	36829
3204	CACAAATTCCGGGAGATGAACTGA	14862	GCCTTCACAGCCAACACTGA	25846	GCCCGGGAGCTATGACACAAAT	36830
3205	CTGCAGCAGAGGTTTGCAAGT	14863	TCCTGCTAATCTGGCTTTCGTT	25847	GGCAAAACTTTTCCTCAACCCTCTTAG	36831
3206	GACAGGACTTGCACAGAGCATGA	14864	CCCACAGGAACTGTCCATCCAATC	25848	ACACCAACAGGGACAGGACTTG	36832
3207	GATGACAGACACGCAGGAAGA	14865	CCTGTCCCCAGGACATTGAAGT	25849	ATGGTCAACGTGATGACAGACA	36833
3208	ATATCCGCCCCATCTCCTCAGT	14866	CCCAAAGAAAGCCCGTGTGGTT	25850	TGTTTGTAACCCCTCCCTCTCT	36834
3209	CAAACCCAATGCTACACTCCACTT	14867	CCACCACCAGATGGGAAAGGTACT	25851	CCTGTTTGAACAAACCCAATGCTA	36835
3210	GGGCCTGCCTCCAAGAGTTA	14868	ATGTCCCCTAGGCAGGCAGAT	25852	CAGGCCCTGGGGAATACAGA	36836
3211	GTCGGCAGTTCTGGGCATGA	14869	CTCTGCCCCTGGACACTGAA	25853	TGGGAATACGGTCGGCAGTTC	36837
3212	CTCACAACCCAGAAGCCCTTGA	14870	TTTCCCACGGCTGCTCACT	25854	CACCTATGCTTCCCCTCACAAC	36838
3213	GCGCCTTCCTTCCTCCGTTT	14871	CAGTACAGAAAGTCCTGTGGGAACTA	25855	TGTCCCTGCGCCTTCCTT	36839
3214	TGCTGGCCAGACCCTGCAT	14872	CAGGGCTCGTAGCCTTGTTCTA	25856	GCCACAGTTGACTCTGCACTCCTT	36840
3215	GCTTTGCAGACCAGCAAGGAA	14873	AGGGCAGATTGCTGACTTTTTGA	25857	CCAGCCCATTTTGAGATAGAGCTTTG	36841
3216	GGCCCATCTTGGGGTTCAGTT	14874	GCTCCACACTGCCGCAACA	25858	AACCCCTTGACGGCCCATCTT	36842
3217	CCAAGGGCAGGCAGTGTTTTG	14875	ACAGCCACCCTTGGCAAAC	25859	CCAAAGGGTCGCTACACCAA	36843
3218	CCATCGTGGAATTTTTGTCCAATCT	14876	ACAACATTGACGGCTTTGGTATG	25860	CCAGATGGAAAAGCCATCGTGGAA	36844
3219	GGAGAACTGGACGGTTGTGAAG	14877	GCCATGGTCCCTCCCAAGTTAC	25861	ACGAGGCCCAGCTGAACAA	36845
3220	CCTGTTGAAGCCACTAGACCAA	14878	AGACTGACCAGCCCGGAAAA	25862	CCTCAGCTTCCCCAGATACCTGTT	36846
3221	GTGTGATCTGGTGGCTTCACA	14879	CCACTGCACCTGGCCTGTTTTT	25863	CCAGGACAGGCATTGAGTGTGA	36847
3222	AGTTCCCTGGGTGGAGGAACAA	14880	GCCTCTGGTTCCCCTTTCTGT	25864	TCCCTCAGATGCCTGCTGTTAC	36848
3223	GTCCCAAAGCAGCCATAGACA	14881	GGAACACAGCCATGCCCATT	25865	CCTGCTCGACTCTGCTATTGT	36849
3224	GGTGCAGTTGAATGGTGGCCTTA	14882	GCTGCCCAGCGTTCAATATC	25866	AGAAATCAATGGGGTGCAGTTGA	36850
3225	CCTCCTGCCACCACGTGAA	14883	CGGAAGGCATAGGGTAAGCACTGA	25867	GGCACTTCCCCATCACTCTTTCT	36851
3226	GTGAGCAAGAGACCCTGACTGA	14884	CAGGGATCAAGCTGTGGCTTAG	25868	GGGTGGCCTGAGCTAAGAGT	36852
3227	CACTGCACCATGCTCTTCATGT	14885	AGGGAGGCAGTAGTAGGTTTGA	25869	AGTCACCCCACTGCACCAT	36853
3228	GATGGCCGGAAGCAGAAGT	14886	AAGGGCTTTCCCCGCTTTTG	25870	GGCAGAAGGGGAAGCAAACA	36854
3229	TCAACCTCCCGGGCTAACATGA	14887	TCCCCGCTACCCAGGAGTCTAA	25871	TGGGTTGCTGCAGACTCAAC	36855
3230	CATGGAATCACTCAGTGTCATAGTCA	14888	GGTAATTTCTGGGCTGACAAGGTT	25872	TTGACTGCTACTCCCATGGAATC	36856
3231	TGGACACACAGGTGGAAGATG	14889	CCACAGGGCATTGCAGGTTTAC	25873	CTGAGAGCTTAAACTGGACACACA	36857
3232	ACTCAGTGGGTTCTGGATGTGA	14890	CCCCTGCCCAGTTTCCAAAT	25874	AGGAACTTCCCTCTCCTTTACTCA	36858
3233	CCACCTAGAACCCACGACAAA	14891	GCAACTTTGACTCACCTCACTGACAA	25875	TGGGACACCTAGACCACCTAGA	36859
3234	CTCACAAAGGGCTACAGGGCATT	14892	CGATGAGGACACTTCCTCTTTCTGA	25876	ACCAGGTCCCAGACCTCACA	36860
3235	GGATAGGCAACTCTCAGGGCAAAT	14893	GGGTCCTGCAGACAAACCTT	25877	GCACACAAAATGAGGATAGGCAACT	36861
3236	CTGGCCGATGGGGTTCATC	14894	CTCCCAACTGAGATTTGCGATATAATCTTC	25878	CACTTCCCAGTTACCCCTCAGTTC	36862
3237	CTGGGCTCAAACAATACTTCTACCTTAG	14895	CACCTGTGGTCCCAGCAACT	25879	CCTCAACCTTCTGGGCTCAAAC	36863
3238	GAGGGACTAGCACAGCAATACAAAG	14896	CCACCTCCAGGCCTAAGAAATC	25880	AGAGGTAGGCACAGAGGGACTA	36864
3239	ACACCCAACAGCCCCTGATAGA	14897	ACTTCCTCCCTGGTCCTGATGT	25881	GGCCTTAGGGACACCCAACA	36865
3240	CCCTGATCTTGCCTAACCCTTCAGA	14898	CACCCATGCTCCTTCCATCAGA	25882	CACACATGCCCTGATCTTGCCTAA	36866
3241	GCCAAGATCCACTGTTAGACCAAGA	14899	GCTGAAAGCTGTGGTTTGGTTTTG	25883	CACATTAGGCCAAGATCCACTGT	36867
3242	GGAAAGCTCTCTGAGCTGAAAATG	14900	AGCCCCAGTTCCCCACAACTAA	25884	GTTGATCATCATGCAGGTGGGAAA	36868
3243	ATGTGATGGCTTTGGACCAGAT	14901	TGCTGTTGGAACTGGAAAGAGTT	25885	GCCCTTTTCAGCTGTAATGTGATG	36869
3244	GGAGACAGCTAGGGTGTGGACAT	14902	CCATCCCCGCTCCAACTCA	25886	GGACGTGAAGAGAAGCGGTTT	36870
3245	GTCCACCTGACTTGCATTCTTTCTCT	14903	CCCTCTGGAAGCTTGTGTCTAGTT	25887	CCCACATGTCCACCTGACTTGCAT	36871
3246	CCCTCCCAACTCTGAAGTTTGT	14904	TGTATCTGCGGCTGAATTGAGAA	25888	AACCAGACCCTCCCAACTCT	36872
3247	GCTGTGTCAACCCTCAAAGTTCAGA	14905	GGTTTGGTCTGTGGCTGCATAG	25889	AGGCGAAGAGCTGTGTCAAC	36873

3248	TGACTACAGCTCACTGCTCTTG	14906	CAGTTAAAGTCCGAGGAGGTGATG	25890	TGGGCTGACCACTGACTACA	36874
3249	GTCATCGGAAGTTAAGCCGCATCT	14907	GCAACTGAGCATCGGAGACT	25891	CCGAAGTCGTGTCATCGGAAGT	36875
3250	GCAATGTAGGAGTGGATGGCTTT	14908	CCTTCAGCAACTGTAGCTCCTCTAC	25892	GCTGGATTAGAGTAGGGGCAATG	36876
3251	GGGTTTCAACAACTCAAGTGCAA	14909	CGGTTGCCTCTCTCTTTACTGAT	25893	TCCGTCTCAGGAGGGTTTCA	36877
3252	CTGGAAAATCACCTAATTCAAGCCCAAATC	14910	GGTGCGAGGAGGGGTTTGT	25894	CTGCCTCCTTCGATCTTCTGGAAA	36878
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3254	CTGGCAAGGGTTGGAGACACAT	14912	GCTTGCCAGCCAACCTGTTA	25896	CAGGTAGTTTGTCGAGAGCAAGTCA	36880
3255	TGGGGACTTGTGGTGCCTTTTC	14913	CCAGTTGGTCCATGGGCAGTTA	25897	ACAGACCAGAGTGGGGACTTGT	36881
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3257	GCATAGCTGGTCACACACTCT	14915	GGAGGAGAGAAGCCTTGGGATTTG	25899	GAGTCTCTTGAGGCACTTAGCAT	36883
3258	GGCTCCATCCTCAGGCTTCA	14916	CCAGGAAGCTGCAATATGGAAGTGA	25900	GGACGTTCAAACCCTGATGAAAG	36884
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3261	CAGGCTATGACATCAAGGCTTTCT	14919	AACACCATCGTCCGTCATGATT	25903	CAGCTAAATCCAACAGGCTATGACA	36887
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3263	GAGGACCAGGGAGTGGAATGAAGT	14921	TCCGACCCTTCTCCCACATT	25905	GCCATCACTGTATCCTCTCCATCACT	36889
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3267	CGCGTGCCTTACATTGGTGTCA	14925	CCGCTTGGCTCTCATTCTCT	25909	GCGAAACGCGTGCCTTACA	36893
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3271	GAGGACTGACAGGTTCCTGCTT	14929	AGGCCTGGGTTGCCTTGTAAC	25913	CTTGACTTGCTGAGGACTGACA	36897
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3274	CCTCAAACCTCCCTGTGGCTAA	14932	GGTCATGAACATGGAAGGAACAAGATT	25916	TGCTGATCCACATACCTCCTCAA	36900
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3305	TGGTTTAGCAAGCAGACAGCAT	14963	TGCCCTAGAGCAAAGGGTATATTTC	25947	CAGCTGCAAGGTGCATGGTT	36931
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3309	GGTTCTGAGGTTCTATCCAAATGTCTTC	14967	GCCACTAGCCTCCATGTCAAG	25951	CCTCTCTGGGCATTATGGTTCTGA	36935
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3311	TCAGGTGTAGCGGGCAGTTAGT	14969	AGGACAGGAGGCAGGTTCAAGT	25953	CCGTGGCTGTCTGAGATACACT	36937
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3339	CCTCGAGGAACTTCGCCTTT	14997 CGGTTCTGAAACAGGGAGTCTT	25981 AGCCGCTCCCTTCCTTTTC 36965
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3341	TCCGCTGCACTCAAAACGAA	14999 TGTCAGCAGAAAATGTACAGTGCTA	25983 CCAGAGTCAGCAGTAGGAAGGAGTTC 36967
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3343	GCACCCAAAATGGACATCCAACA	15001 GCCTCTGGCCAGCCTTCAAAAT	25985 GACTCGGTTGCACCCAAAATG 36969
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3345	GCACTAACACGTAGCCTGAGTAATG	15003 TGAGCCTGGTCCTCAGACAAGT	25987 TCCTAGGATCTGCACTAACACGTA 36971
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3352	GGGACATTTGACCAGAGACAGAA	15010 GCATAGCATCCTGCTCCAGTT	25994 AGAGAGGCAGAGGGACATTTGA 36978
3353	TCTCCACCTACCCCTTTCTGTAG	15011 GGACTAGGGAGCCATGTGGAAGT	25995 CCATTCGCAGTAATTCTCCACCTA 36979
3354	TCTGGCAGGGCTGGAGTAGA	15012 TCCAGTCTCATTTTGCACTGTTCT	25996 GCATTGAGAAACTGAAGCACATCT 36980
3355	CCCAGCAGCAATGTGGAACA	15013 GCTTCCCTGGTTGGTAATACTCTGT	25997 CAGCTTAATTCTCCCAGCAGCAA 36981
3356	TCTTTCCGCTCTCCCTTCTCT	15014 GTAGCGGTTGCTGCTTGATG	25998 CCAGGCAAGTGACCCTGTGTTA 36982
3357	CCAAGACACGGAAACTCCTCAT	15015 CCAACGTAGGTCTGGACTGATG	25999 GTGCAATCGTGCTTTCCAAGACA 36983
3358	GGATCACTTGACCACAGGAGGTT	15016 TGATGCGAACATGGCTCACT	26000 GGTGTGGTGGGAGGATCACTTG 36984
3359	GATCTAGCTCCTGCCTACTTCATGT	15017 CTGGGGCAGAGCAAGTGGAAAA	26001 TCCTCTAAGGAGTGGCATGATCTA 36985
3360	CTGTGTCCTACTGAACGTTGTACTGA	15018 GGCTCCTAACCACTAAGAACACA	26002 TGCAAGTCTGTGTCCTACTGAAC 36986
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3364	CCTGGGACATAGGGAGCACAT	15022 CCCAAGGAACACAGACCCTAGT	26006 CGCTAATGAATTGCCTGGGACATA 36990
3365	CGGTTGAGAGGTTATATCTTAGGCCTGTTA	15023 GCCCGGCTGAAAGCAACCAT	26007 CCCTACAGAACGGTTGAGAGGTT 36991
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3369	GCAGTTTAGCCTCTGGGTTGAAG	15027 CACGCTCCAGCAACAAGAAG	26011 GTCACTGGGAACGAGTGGTTCAA 36995
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3371	CCCATGAAGGTTCTTGGCTTTG	15029 CACCACTGACTCACCCTTGAAT	26013 GTGGAGCTTAACCCATGAAGGTT 36997
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3377	CGATACAGCTGTTGTCTCCAGAA	15035 CTGATCCATAGGCCGAAGTGACAA	26019 GGGGATCAATAAGCCATCGATACA 37003

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3380	TGGAAAGGCTGGCTGTCTATG	15038	CCCACTTTTGACCCTGGCCTTA	26022	TTCTTCTGCAGACACAAATGGAAAG	37006
3381	CAGTCCATCTTCTGTGCTGCTA	15039	GGCCTGAGCAGGTGTTTTAGTA	26023	TCCCTCGCCAGTCCATCTTCT	37007
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3393	GTCTCCATCAACCTCACTTGTTTTAC	15051	CCGTGCCCGGCATATTATTTTAGT	26035	CGGCTATCTCTGTCTCCATCAAC	37019
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3395	GTGTTTTGCTGTTCTCCAGCAT	15053	GGTTGCTTCTGCACCTGGAT	26037	GGAGCAGGGTGTTTTGCTGTT	37021
3396	GTGGGAGAAAAGTAGCAGTGGAT	15054	GCCTTTAGTTTGGGCCAGAAGAACT	26038	GGGCTTGGGTGTGGGAGAAAAGTA	37022
3397	CAGAGCAGGTTTGATGATCCCGTTT	15055	CGGCCTTGGCTCCTGTAGTTT	26039	AGAAGAGCAGAGCAGGTTTGATG	37023
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3399	TGGCTGCCTTCTCCCTTCAT	15057	CACACACTGAGACTGAGGAAAGA	26041	CTGAGGCTTTAACTCTTTGACTCGAATATG	37025
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3407	GCAGACTCAAGGTCACACTATTTCTCTT	15065	GGCAGAGGAGGCAGGGAGTTA	26049	AATATGCAGACTCAAGGTCACACT	37033
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3531	GCCCCATAGATCCAAAGCATAGTGTAA	15189	TGGGCTATGGCCTTCAGTTTG	26173	GGGTCTGCCCCATAGATCCAAAG	37157
3532	GGTTGGGTTGAGAAGTGCCTCAGA	15190	TGCCCAGTTCCCAGCACACT	26174	CTGCTTTAAGAGGTTGGGTTGAGA	37158
3533	CCCCGAAGGATCTCGTTAAAACA	15191	GCTGGACCCAGACCCTCTGA	26175	CTCTCACTGTAAAATGCCTGTGAATC	37159
3534	CCAACTGGCATTCCTGCCTCTA	15192	CGAAGGTGTCTGGGCTGCAA	26176	CTTCATTACCTCCCAACTGGCATT	37160
3535	GGCGATCATCTTTAACTACTCCTTCT	15193	TGACGATGAGTGGTGGTAGGTT	26177	GACAGGCACTTGGCGATCAT	37161
3536	CAGAATGGGTGGGGCAAGACA	15194	CAAGCACTTCCTCACCTGGAT	26178	GGACTTCCCCATACCAGGATCT	37162
3537	AGGGAGCCTCAAGTGCATCA	15195	TGGGCCAAAGGCCCCAAGA	26179	CCAATGATGGCGCTCAGCTTTTT	37163
3538	AAGCTCTTTCCACTTCAGGATCTTT	15196	AACATTTCAGCCAGAGGGAACA	26180	CAGGCCAAGCTCTTTCCACTTC	37164
3539	TCCCCAGAAGTGGTAACAGGTA	15197	CCAGCCTGGGAGGCTACAGA	26181	GGGTCTGTAAGATTTTCCCCAGAAGT	37165
3540	AGCTAGCAGGGCTGATGCAA	15198	CGCGAGGAGTACAAAATAGGGGAGTT	26182	CTGAGCTAGGATTCAAGGCAGCTA	37166
3541	GTCTCACCTTTTCCACCCTTGAATG	15199	GGCAAATGGAAGGGGTCACA	26183	ACCTGGACTGAGTCTCACCTT	37167
3542	CAGACCACAGAGCCCGAAGT	15200	CCAAGGTGTAGCTAACTTTTCCTGTAG	26184	AAGACCACCCAGACCACAGA	37168
3543	TGCAGTAGTTGAGGTGAATGGTTT	15201	TGACATCCTCCTGAGTTGCTGCTA	26185	GTGGCAGGTTGAATGCAGTAGTTG	37169
3544	GAGGAAAAGCAGCCAGACATACA	15202	ACGGAGGTGGGAGCAGGTT	26186	CACAGCTGCTGCTTTGAGGAA	37170
3545	GTGGAAGATGGACTGGCCTTGT	15203	CACATCCAACGAGCTGCAGTAGA	26187	GGGGTGTGTAGAACCTGTGGAA	37171
3546	GAACCCAATCTGTCTACCTTGTCA	15204	TCACTCCGCCACTAGGAGTTG	26188	GGCCTCTAAGAACCCAATCTGTCT	37172
3547	ACAAGGCGCCCAAGTGATCT	15205	CCGTGGGAAGGGATTGTCATC	26189	CCATCAGAGAGTGGAGGTCACAAG	37173
3548	CGTTCAGTTGCCATCTGCTGTTC	15206	CCTGGCAATGGCAAGGAAGA	26190	ACCCAGGAACCGTTCAGTTG	37174
3549	CACCGAGCCATCCCTGAGTTC	15207	GCATCCACAGAATCCGTTCACT	26191	GAGGATAATGCCTCATCCCTTATTTCA	37175
3550	GGGTGCCCACTCAGGGTTATTC	15208	ACAGAGCAGGAGCTGGAGCAT	26192	GGTAGAAGGGTGCCCACTCA	37176
3551	ATGTGGCCCAGAACCCACAA	15209	CCAGCTCAGCCATTCACGCATA	26193	CTGTGGAGGCTTTGGAGCAA	37177
3552	CGATCCCACTTTGAGGTGAACTTGA	15210	CCTTTCCCTCTCCCCTTCTCA	26194	CCTGGCCGATCCCACTTTGA	37178
3553	CCTGGTTGTCTCTGTCCCAAGTCA	15211	CCTGACAGACACCTCCCATACCTT	26195	TGGGCCTATCCTGGTTGTCTCT	37179
3554	CCCTTGCAGGGATCCAGATG	15212	GTGCCACCACCCTCATCTAAAC	26196	GAAGTGGCAAGGACAGTTAGAAGT	37180
3555	GGCTACCTCCAACCATCATCTTG	15213	GGTATCAGCTAGGAGGCTGTGA	26197	GTGACAGTCTTTGGCTACCTCCAA	37181
3556	ACATAGGTGGTAGCCAGGAAGT	15214	GGCACTGGGACTCGTCAAAAC	26198	GACCTAGTGCCTTCAGTGAACA	37182
3557	AAAGAAGTCTGACCATGCCTTGA	15215	GCGGTTCTGCAGCATGACAA	26199	GGGAAGTGGATCAGGTTTCCAT	37183
3558	CCCAGGCCAGCTTGTTCTCT	15216	TGGCCCAGCCGCAGTCTAA	26200	GGAACAGCAGATCAGAGAAGGTT	37184
3559	GTGTAAGGGTGAGTCTTTCAGCAA	15217	TCCAGTGAACGACTCTGTGGTA	26201	AGTGCTTGGCCCTACAGTTG	37185
3560	CCAAAACAGGCAGCCACAAGA	15218	CAGCTAATGTGGGAGGAATTTCTAGACT	26202	CCTGCATCTTTGAGGGTAGCCAAA	37186
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3562	ATTCCTTCCCTCCTGGGGTCTA	15220	TGGGATGGGGCCGTAGCAA	26204	GAGCAGGGATCTCAGGCTCTTT	37188
3563	GAGCCCAAGTTTCTTCACTTGGAAA	15221	ACCCTGAGAGGGTGCTACAATG	26205	GCCTCACTGAGCCCAAGTTTCT	37189
3564	GGGCCATCAGTGAGCTTTCTTC	15222	GCCTCTCTCGGGCTCTTGACT	26206	AGTGCCAGGGCCATCAGT	37190
3565	GGATCTCCACTGTTGCCTAGAATG	15223	AGAGCACATCCCACCTGAAGA	26207	AGCCCTGGATCTCCACTGTTG	37191
3566	GGTGGTTCTGTGGGTTTCTGT	15224	AGAGCCTGCTCTGTTCTCTGT	26208	ATTGCTATCACATAGGTGGTTCTGT	37192
3567	GGGGTACTTTCAGCTCTCTCTAGT	15225	TAGGGCCCTTGGACCTGCAT	26209	CTCTCAGTTAGTGATTAAGGGGTACTTTC	37193
3568	AGCAGCAGCGGAAGCACAT	15226	AATGCCGAGCTGGACTGGTT	26210	AGTACCTGGGGACTTGACTGAA	37194
3569	AGGAACACAGAGGAGGAGCAA	15227	ACCTGGATGTCCTGAAACTCAAC	26211	AGGTTGCCAGGCCCTGAGT	37195
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3571	GGACTGCAGAAAATGTTACAGGTCTT	15229	GTTCGTTTGCGGAAATGCAAAA	26213	GAAGGAATTTAGAGGGACTGCAGAA	37197
3572	CTTCTCATTGGAATCTTGGCTCCTT	15230	CAGGGCTGGAGAGACAAACTT	26214	CACAGTCTTCCATCTCTCACTTCTCA	37198

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3575	GCCTACGAGGTACAGCTCAGGATA	15233 TGCACAGGCGTGTCACATAC	26217 GCTTCCCATTGCCTACGAGGTACA 37201
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3580	ACGGTCCATCGCTTGCATCT	15238 GGGAGAGGCGTGAAGGAATTGA	26222 CTTTGTGTCTACCACGGTCCAT 37206
3581	AGAAGCATCTGAAGTGCCTTGTT	15239 AGATGGATCACAGGAGGCGTAA	26223 TCATGTGTACCAGAAGCATCTGAAG 37207
3582	CGGGCCAGGGAAAGAAAAGATG	15240 CCCAGGAGTGGGAGGGAATTTT	26224 TTACTCAGCGGGCCAGGGAAA 37208
3583	TCCACAGGGCCTGGTACAT	15241 TCCACCCATTCAACAAGGGTTT	26225 GAGGGTGGCACCATGTTTGT 37209
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3585	CCACAGGTGGTTCAGATAGACTCTACA	15243 GTGATCTGGGGCCTTCACTTC	26227 GGGATCCACAGGTGGTTCAGAT 37211
3586	TGGAGGAATTCCACTGCTTCATT	15244 CCTGACCTGTGCGAAGAGATTAATTG	26228 TCCTATAAATCCTGGGATGGAGGAA 37212
3587	TGGGAAGGCAGAGGCAGTTCT	15245 CCACTTTGTTAGGCCTGGGATCCTT	26229 GCCCACACAAGGTCAGCATT 37213
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3589	CCTGGATAGGTAGCAGACAAGTACTAA	15247 GTCCCTGTCTGGCGTGTTTT	26231 GGAGCTCTCATCCTGGATAGGTA 37215
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3592	CCAAAGCTGTTCCGGTTCTTG	15250 GGAGTAAGAATGGACAGCAAGAAGAT	26234 AGGCAGTGTCCCAAAGCTGTTC 37218
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3594	TGAGGTGGGCCAAGTTCAGA	15252 GGTGGAAGGGACGCTTGAGA	26236 TGGTCCTTGCTGCCTTCTTC 37220
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3597	GTTGCCTCAAAGCCACACTTG	15255 CCTAGGACAGAACCTCTCCCATA	26239 CAATTGGACTTGTGTTGCCTCAAA 37223
3598	CCCTGAGGTTCATTGAGCACTTT	15256 TCTGGCCCGGCATACTCAA	26240 CCGCCTTTCCCTGAGGTTCATT 37224
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3605	CACAAATAAGCACAGCGGGGAAT	15263 CAGAAAGGCAGCAATTTGGACAT	26247 GATCAACCTGCATTGTCCTGTCA 37231
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3607	CCAAAACAGCTGGCTACCCTATTC	15265 CCAGCCTCCCTGAGCCCTAAT	26249 GGGAATTGTCTCTTGACTCCAAAAC 37233
3608	ACCCCAAGTAGCCACCATTG	15266 GGCCTTGGGTCCAAAGCAAAGA	26250 TGGCTGCCCACCCCAAGTA 37234
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3636	CTTGAACTGGTCCCATTGTCTAGT	15294 CTTGAGGACGGCAAATGCTGTAT	26278 AGGGCCCTGTCTCTTGAACT 37262
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3640	TCTGGGTTTCAACTACAGCCAAA	15298	ATCTTCCTGGTGTTTCACTCCTTT	26282	CTTTCTGGATGTCTGGGTTTCAACT	37266
3641	GTGTCTGATGGAACCTGCCCAATC	15299	CAATCAACCTAGTTCAGGAGTACAGT	26283	CAGGAGTGGGTGTCTGATGGAA	37267
3642	CACATCCGTGTAAGTTAGCTGCTTT	15300	TTCTGGCCTCACAAGCAAAGT	26284	GGAATATAGGGCACATCCGTGTAAG	37268
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3644	CACCATGCATAACCACAGGGCAATA	15302	TGGGGCCAGCATTTGGTGTT	26286	GATAGCGTGCACCATGCATAAC	37270
3645	CCACACAGGCCCCATTACTTCCTA	15303	GTACCTTTATCCCCAGTGGTCTTCA	26287	CACTCATTGCCCTCCACTGT	37271
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3651	TCCCACCTACCTGCTGTTCTTG	15309	CATGCCAGAACCATTCTCGTCTT	26293	GCCTGAGGTCACATCCCACCTA	37277
3652	GGTTTGACCCCTCTTGCTTGTTTG	15310	GGCCAGCAACTGGGTCTCTA	26294	CCAGAGCCCTTCAGCAAGAAA	37278
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3655	CAAGCTGGCCCTGTGACTGA	15313	CATACCTAAGCAATTTGGCTGCAA	26297	TGTGCACTTCCTGGCTCATTT	37281
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3658	GGCTGACGGCAAGACATGAAG	15316	TCATGGTTCCTTCTGGGGAAATG	26300	GCTTGAAGAAGCTCTTGGTGATGATG	37284
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3669	GACCCAGGGTGCCTGGTAAA	15327	GCCTCACGTCTTTTAGCTCTGT	26311	GCTGGGACGTACCTAAGTGGATGA	37295
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3690	ACCTCTAGACACACTGCCTATCT	15348	AAGCACAGTGGTCATGGTTGA	26332	GCTGAAATGAAGTGACTCTACCTCTA	37316
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3692	CGGCTGCTGTCCTGCTTTG	15350	GCACCAGAGAATTAATCAGATCGTTTG	26334	TCCAATCAATAGGTTGCCAGTGT	37318
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3694	TGAAAGGTTGAAGCTGCACAGA	15352	GTGGCGGTCACTGCTTAACT	26336	GTGGGAGTACAGTGAAAGGTTGAAG	37320
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3701	CGCCCTGGAAACTCACAGCTT	15359	CCTAGAGATTACGGGCAGTCCAAA	26343	CATTAACATTAGCGCCCTGGAAAC	37327
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3704	AGAAAGGGCAAGGTGGTTTTGT	15362 AGGCCCTCAGCGAACCTT	26346 GGTCTGGGAGAATTAGGAGTAGTAGA 37330
3705	CTGTTGATACCTTGAGGTGCTGGAA	15363 TGCTCCAACCCCTGATCAAC	26347 GCTGTTTCGCAGCCATCACTGTT 37331
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3707	CAGCGGCAAAACACCCTCTT	15365 GGCCTGGGAAAAGGAGCAAGAT	26349 CAGCCGTCAGCGGCAAAA 37333
3708	GGCCTCGAAGGCATCACCTA	15366 GTCGTCCTCTATGTGGAGAATGAGA	26350 TGCCTTGCTGGGCCTCGAA 37334
3709	GGGAGAAGCTATGAGCTGAGTGT	15367 ACCCAGGATGAGTGCAGAGA	26351 ACCCCTTTCTAGGGAGAAGCTATG 37335
3710	TGTCCCACACGCTGCATATC	15368 CCCCTCAAACAGACCACACATA	26352 TCCATTCTTTCCCCACTGATTTGA 37336
3711	TCTGGCAACTACAGTGATGTGAAA	15369 AGGGAGCCCTTTGGCTTCTACA	26353 CACAGACAGGATCTGGCAACTAC 37337
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3713	TCCACCTTCCTGTCCGAAGT	15371 CTTCCTGGGGCAGCTAGTGT	26355 GCCCCTTACACCACTTTCCACCTT 37339
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3715	TGCTGGGCAACCAAGCTCAT	15373 TAGCCTTCCCGAGCTGAAGA	26357 GCGATACGTAGGCGGAGCTT 37341
3716	ACGCCTCACCCTACAAGCCATA	15374 GGGACGGAAGTGGCCTGAGA	26358 TGGGGTGCTTCTCTCTGGAT 37342
3717	CAGCCTGTTTCAGGGTGACA	15375 CTCCCTGAGGCTGGCCTTTC	26359 GAGAGGAAACTGGTGACAATACAGAT 37343
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3719	CGCCAGTCAAAACAGCCGTTAG	15377 AACGAGGAAGGCCGGTGATT	26361 TCCGGCTACGCCAGTCAAAA 37345
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3721	CTGTAGTCCAGGCTGTGGATGA	15379 TCCGCAGGTACTTCATAGAGGAT	26363 TTGCCCTGGTCGCTGTAGT 37347
3722	ATGGGTCCATGGGAGGGGACTT	15380 CCACCTGATGCATTCCCACTCA	26364 AAGGGGACTTGGCCCACAGAT 37348
3723	GCTTCTCGCCAAGGTCATCT	15381 TGAGGGATCTTGCACAGTAACTTC	26365 CCTGTGACCACCCTGCTTCT 37349
3724	CTTCTGAGGGTGTGGAAGCAAA	15382 AGGTGCTAGGCACTCCTCACA	26366 TGTGCCGGCTCTCCTCTTCT 37350
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3728	GGAGAACTGGGGATGCTGCTTT	15386 GGTGGGAGTAATGGCAGTCTGA	26370 GTGCAGAATAGGCAAGGAGAACT 37354
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3764	TTGGAGGCAAGTGGCACAGA	15422 AGACCCCATTCAGGCACTCT	26406 TCTTGCCCTGGTCCCACAGT 37390
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3766	AGAGAGGGAGCCCTGATCTGA	15424 GCTGGAGTGAGTGAGGCAGACT	26408 ATCGGTTTCCTGGGCTGTTG 37392
3767	CCCCAGACATGGTAGAGGAGATAC	15425 GGAATCCAAACAGGCACAGTGA	26409 TCCACTTGTGACCCCAGACA 37393

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3894 CCAAGGACTCCTGCAATCCTAGTCA	15552 AGGGCCTGTGAGGGTTGA	26536 TCTCACCAAGGACTCCTGCAA 37520
3895 CACTAAGACTTGCCTCCCCATCAT	15553 GGTGGAGTCAGTGGAGCCAAA	26537 GCCTCAAGCTTTCTGTAAAACACT 37521
3896 CCTTCCTCACAAGTGCTTTCTGAAT	15554 CGGCCAAAGAGGTTTCGTTCT	26538 GTGTTCCACCAGCTAGGCACAT 37522
3897 TCCTCAACCCTTTGCACACAT	15555 GTGGCCCTTGCTGACCATTA	26539 GCTCAACCAGCTCACTCCTCAAC 37523

3898	GTGGCAGTCTTAGCTTCCAGTT	15556	CTTCCACCAAGAGATACAACAGTGA	26540	TGGCCGTGTGGCAGTCTTA	37524
3899	CAGCAAAGCAGCAGGTGACAGA	15557	ATTCTGGGCTGGGTCCCAAA	26541	GGAATCAGGATGCCATGGGATGAA	37525
3900	AGTGGCTGTCAAGCCCAACT	15558	CTTTTCCCCTTCCCATCATCTGATTC	26542	AACTGACCAAAGTGGCTGTCA	37526
3901	GCCTAACACAAAACAGGTCCATTT	15559	CACATGGTAGCAGGGATTGCAAGA	26543	TCCCCAACCCACCATTCAGTTG	37527
3902	GGCTCTAAGACCATGCTACCTCATTC	15560	GGGGTGTCCACCTCGCTTATG	26544	GCTAAGTTCATCACAAGTCTTGGCTCTA	37528
3903	TGGGAACCAGCACATGGATTG	15561	CTGGCAGCAAGAATCCACCTT	26545	CGATTATGGCTCTACCGGAGATAATTG	37529
3904	TTCTGTCGCTGTGCGTCCTT	15562	CCATCCCACTTGACCCCAATCA	26546	CTTGCCTGACATTTAGCCTTCTGT	37530
3905	CCACGGGTGAGTCATCCTCATATC	15563	ACGTGCCAAGCACCCAACAA	26547	GCGTGTGAGCTATTTAAGAGCAGAT	37531
3906	CCAGCCATTGTGTTGAATCCTTT	15564	CAGAACCGGAGGCAACAGT	26548	CCTTAGCTTCCAGCCATTGTGT	37532
3907	GGGTGGTACTGTGCGTCCAA	15565	AGGCACAACTACCTACTCACAAAG	26549	GGACAAGTGAAATAATCAAGGGGTGGTACT	37533
3908	GGGGCTCATTTAAATACTCCCTCTGT	15566	CCAGAGCAAACTCAGGTGACAAC	26550	CCCTCTGGCTAGGGGCTCATTTA	37534
3909	TGCTATTTGGGTGTCAGTCCTTT	15567	GGGATTACAGGCTACTTTTCTAGTCTT	26551	CTCCAGCGACCCTTGCTATTTG	37535
3910	CAGAGCCCAAGGGCATTGAACA	15568	GTAACCACTCCCTGGCTACTGT	26552	GCACTGCCACCTGCTGATT	37536
3911	TCTCTACCCTGACAGTGCAACA	15569	CTCTGAGCTCTTGCGTTTCTGA	26553	GGGAAAATGTTTTGACCAAGGAGTTCTCT	37537
3912	AAGGTTCCATCTGTGGAAGAGTTT	15570	TGTGCTCTCAGCACTTTTTGTGA	26554	TCAGGAAGCAACTAAAAGGTTCCAT	37538
3913	GGCTGCCAGCAGGTTGTCT	15571	ACCTCCAGGCTTGTGCTACT	26555	AGGCGCCGAGAGCAAGCTA	37539
3914	TCCTCGGGAGAGTGACCTGTAA	15572	ACGGTGGAGGCTAAACAAAACAT	26556	CTGCAGGCCTCCATCTTGTT	37540
3915	GGAACTGCAGACAAGAGCTAAAC	15573	GGTCTGACCACCCTACTGAAGA	26557	GGCTGATCTTATTGCAGTGGAACT	37541
3916	CACATGCATCCTGTCCTAACTGT	15574	GACAATGTCCCCTGACTGGAATTT	26558	CCCTCTGTGGAAGAATGTGTGA	37542
3917	CAGACCTGTTTCCTCATCAATCTCT	15575	CCTCAGGGAGCTTGTAGTCTCT	26559	CAGCAGATCCTACAGACCTGTTTC	37543
3918	GGGTTCTTCTAATGCTGGAGACA	15576	GGGGTTAAGCTTTCAGGAGCTAAG	26560	GTCTTATCGTCTTCACAGGGTTCTT	37544
3919	TGTCCTATAGCAGACTCCCAAGT	15577	GGGCTTCAGCTGGAAGTTGTTG	26561	CTGACCCTGAGCCTGCTTTTTG	37545
3920	GCAGCCCTTGCCGAGAATAGA	15578	GCCCCAAATTTGCAATGCAGTAG	26562	TGCCAGCCTTCCACAAACTTC	37546
3921	CACTCCGATGCCCTGGTATTCA	15579	CTCTACGGAAATATGGGCCTGATG	26563	GTTTGTCCTCCTTTATCACCACACT	37547
3922	GTCCTGTGAGGCATTGTGGAAGT	15580	GGAAGCCAAGCAGTGACAGT	26564	AGTGGGCCTTGTCCTGTGA	37548
3923	ACTGCATGAAGGCTCTGTGATT	15581	GTGTGATCCAGAGCAAGTCTTCTA	26565	GGAGCCTGAACCACTGCATGA	37549
3924	GCCAGGACTCTAAAGGGAGCTTGT	15582	CTGCATCCCACGTTGGCTTT	26566	AAGGGAGGGCCAGGACTCTAAA	37550
3925	TCCCACACCTCAGCCTTGGAA	15583	CCACAGAACCAGAGGGACTTGAAC	26567	CCCCTTCCTGGGTACCACCTAAT	37551
3926	ACTGGCCAGGCTCCCATTC	15584	CCAAGCAGGGATTAGAAAAGTCTGA	26568	GGTCACAGCTTTCCCCATCATA	37552
3927	TGCCCTTCTGCCTACTGAAAATC	15585	AGGCTAGACCTAGGCTTTGGAT	26569	CCCTTGAAATACCTAATTTCTTGCCCTTCT	37553
3928	ACCCCTCCACAACCTCAAGTCA	15586	GCCCAGGGAAATCCTCTCTGT	26570	ATGCCCCACCCCTCCACAA	37554
3929	CACCTTAGCAGCCCACTTTCT	15587	CATGTCAAGGGCCTGTCAGT	26571	CCCTGCTGGGCACTATATCACCTT	37555
3930	CAGCAGGCTCCAGTTCAATCT	15588	CCCACAAGCCAAGCCAACAA	26572	GGAGGTGCCTTTGCCAAACA	37556
3931	GGAAGGGATGCAGAGCACAGATAC	15589	CGATTTTTGCCTCCTGGAACGAA	26573	GGACTTTGGGCCGTAAGGAA	37557
3932	CACCTACCCAGTGGTAGTAGTGACA	15590	GAGTTGGGTGTGGGGTGATGTA	26574	GGCTTAAAGAGTGACCAAGCACCTA	37558
3933	GCCCCTACTGATAGTGTGCGTAAAG	15591	GAAAGACCAGCTGTTCCATCCAA	26575	GGAATGTTGTCTTAAATAAGCCCCTACTGA	37559
3934	AATTCTGCCGGATCCGACTTT	15592	AGAGTGTGGGATAATGGTGGAAGA	26576	GAATTTCTGCTTACTGGGCCAATATC	37560
3935	TTCTCTCTGCATGGGTTGCAT	15593	CAGGTTCTCACATTGGGACTGACT	26577	CACTCCACCGTGCTTTTCTCTCT	37561
3936	GTGGGAGTTCTGTCCCAGAGA	15594	ACAGGAGTGTTCCAGCTAACATC	26578	AGGGTGGCAGCCTTCTTCT	37562
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3939	AGACGGCCTCTGGGGCATT	15597	GTCCACTCACAGGTGGAGATGAGT	26581	CTTTTTGGGCTTACCATCACTACAGA	37565
3940	GGTGGGCAATTGTGGTGCAT	15598	TGCCATCCGCTTTGTTTCCTA	26582	CTTCTTCTCACTGGTGGGCAAT	37566
3941	GGAAATCATCCTGCCACCAGTA	15599	CAGGCAGGTGGAGGAACTCA	26583	CTGTGGAAATAAAGGTTGGGATGAAAG	37567
3942	TCGGTGGCCTTGGGAAAC	15600	TTCGCCAGCCACGAGGAAAT	26584	CCTCAGGACTGGAGTTTACACGAAGT	37568
3943	CCCCAGGCAATTCAATTCATTCATCA	15601	GCCCCAGCTTGGACAGATG	26585	CGAGGACCCCAGGCAATTCAA	37569
3944	AGCTTGGCTGGGTGGGTTTT	15602	GAAGGAGGCCCTAGGGAGGATTT	26586	CCGATAGGAGGGAGGGAGCTT	37570
3945	AGCTCCAGGTCTCAGTGCAT	15603	TCACTCAGACTGGGGTGCAA	26587	GAGGCAGGACGATTTCTTGACCTA	37571
3946	AGGTGGCCTGGGAACCTCTT	15604	GGTCCGCTGACCTCTCAATCTT	26588	AGGCCGAGCCAGATCCTATTG	37572
3947	CCAGTTTCGCCCTGCTGAAGA	15605	AGGAACTGGAAGCCGCTCTCT	26589	ACCCACCCAGGCCAGTTTC	37573
3948	GAGTTTTCGAGTTCCAAGTTCCAAT	15606	GCACAGTGAGCCACTCTTCT	26590	GCAAGGCTAGAGTTTTCGAGTTC	37574
3949	CCAGTTCCCGGACATAGACACT	15607	GGGTGGAGGGGTGTCTAAAATG	26591	TAAGCCAGGGCGCCCAAA	37575
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3952	GCTCGCTTTTCCTTCCGCCATT	15610	CCGCTGTTGCCTTCGGTGAT	26594	TCTCGCGGCTCGCTTTTC	37578
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3955	GAACGGAGGCAGTGAGATCAAG	15613	AGGCGGGTGTCTGTGGAAGAT	26597	CCCTGTGAGGGCAGGATGAAC	37581
3956	CCCCACCACAGCATGAAGATGA	15614	TGCCAGCTCAGTATGTGGAAATG	26598	GAGGAACAAGGGCAAGGTGATG	37582
3957	GGGGCCACAGGTGCTAATGAAA	15615	GTGCATCTAATGACCAGGAGAAGTCAA	26599	CTGGTCACACTAAGGCACCAT	37583
3958	GGTCACAAAACACGCGGGAAA	15616	TCCCCGCAAATTCTTTCCTCTT	26600	GGGAGGGGATTTAAGGTCACAAAAC	37584
3959	GCTGAGGTGACCTTCTCCAGATTTTC	15617	ACTGGGGCTCCAGAATGTCA	26601	GGTGTGACTGTGTGTTTACAGGTT	37585
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3961	GCTGTAGAAGGGAAGTTAGCACAA	15619	AGCCCCTTACGAGAAGGGCTTAG	26603	GAGACAGAATCCAGAGCTGTAGAAG	37587
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3963	CCGTGGAATGTGGCGATCTGA	15621 ACCCGAACAAGCTGGCATCA	26605 ATGTTTAGAACCCCGTGGAATGT 37589
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3965	GGGACATACCACTGAGTGCAAA	15623 CCGCGAGAAAGAGAAGAGTCA	26607 GCCCACTGAAGAAATTGGGACATAC 37591
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3972	TGCTCTGTCATCAGGCTGAATC	15630 GAGCCCAAAAGTCTGGCACTAC	26614 GCAGGGTGTTGCTCTGTCATCA 37598
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3987	GGGAAAGCAAACCTACACACAAAC	15645 ACTTCCCTCAGCCCTACCTCTA	26629 GGGCTCCTAGGGAAAGCAAACCTA 37613
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4021	CGCACAATGGGAGGAAACAAAA	15679 CCTTCGGCCTTGGCTCTAC	26663 GGTAATAAGACTGACCATCGCACAA 37647
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4030	GCCGCTGCTGACAGCTACA	15688	GCTAGAGAAGAGCTCCCATTGTTC	26672	AGGCATCGGTCTGAAGGACTTG	37656
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4038	GGTCTGGCTCACAGTGAATCTT	15696	ACGTGCAGAAGGCAGCAA	26680	GGATGGACTAATATAAGGTCTGGCTCACA	37664
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4042	CAGCTGGGGTCTTTCATTCACA	15700	CAGAGTGTGTTGGGGAAAGTTTGGTA	26684	CATTTTGCTACAGCTGGGGTCTT	37668
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4045	TGGCAAAGTGAAGGGTGACATC	15703	GATGAAGGGCCCTGATTTTCCAT	26687	CCAGCAAAAATTGAGAGCTGGCAAAG	37671
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4047	AAACTTGCCAAGACATCCTCCTT	15705	GACCTAGCAACTCTTGAGGAAACA	26689	CTACACTGCAGAAGTAGCTTAAACTTG	37673
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4055	CAGAACATATATCCCTGCTCTCTCTTT	15713	CCCTGCCCCTCCTCTCATGT	26697	CAGAGAGTGGGGTAGTATACTCAGA	37681
4056	TGAGCAGATGGAGCTGTTTGT	15714	AAAAAGCTGTGGCTCTCTTCTCT	26698	GGATGAGGCTGACACTGCAT	37682
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4069	CAGTGGCCTAAGGTGGAGGTAA	15727	GTAACGTTTGCCTGGTCCATCT	26711	AATGCAGGCAGTGGCCTAAG	37695
4070	AGGGCGCCCAGAGATGAAAAG	15728	TGCCCTCCACCTGGCTTTC	26712	TGCGCCAGCTATGGACACA	37696
4071	ATCTTGGACCACAAACCTCAAGT	15729	AGAGTGGAATTACCAGGGAGTCT	26713	GGTGTCATCTTAAATCTTGGACCACAA	37697
4072	GTTGTCCTTCTCCGGTTGCATTC	15730	GCAGGGTCCGGCCCTAATGA	26714	AAAATCAGGCCTGTTGTCCTTCT	37698
4073	GAGCCCAAGGGCACCAAGT	15731	GCCTTCCTCTACCAGGGTTCTACT	26715	ACACCCGGTTACGCAGAGA	37699
4074	GCAATCGACTGAGGTACTTGGGTAAG	15732	CTGTGGTCGGATTCGTGGAA	26716	CACAAGTAAGAGCTGCTGAGCAAT	37700
4075	CGAGGAGGTTACTCTGACCACACT	15733	AGTGGCCAGGGAGGAAGAACTA	26717	AGGGACCGAGGAGGTTACTCT	37701
4076	CTAAGACCCAGGACGGAGTCAGA	15734	CGTGACCTCCACCCCTAGT	26718	GCTGCAGCCAGAGCCTAAGA	37702
4077	GAACCTGAAAAACAGGGTGTGGAT	15735	TGTTGGGTCCAGCCAGTCTCA	26719	GGCTATGGGTTGATCAGAACCTGAA	37703
4078	AAGGGGTGCGTGAGCAAGT	15736	CCTGCTGTGTGCAGTGGTTGAA	26720	AGCAAGGGGTGTGTGAGCAA	37704
4079	ACAGGCTTTCTACAACAGGTTCA	15737	TGTGAAAATCCCTGTTGCTCTTCA	26721	CCTTCAGCACAGGCTTTCTACA	37705
4080	GGGGAGCAGTGTGGACTTG	15738	GAGAAGAGAGGTGATAGGGGACAT	26722	AGGGACAGCACAGATGGATGA	37706
4081	GAGGAAGAATCTATCTGTAGGCACAA	15739	GCGCTTGCCCATAGAAAGTGA	26723	TGCGGTATGACATGAGGAAGAATC	37707
4082	GCCCAAGAACAGGCCACTCTATG	15740	TGGACGACCTTTTGCCATTTTTG	26724	GAGTGCTTTCAGCCCAAGAACA	37708
4083	CAGAGCCACACGTCTTGATTTGA	15741	AGGCAGCAGAGAGGTCCAAA	26725	CGGGAGCCTTCCCTAAGAAGGTTTT	37709
4084	GGCCACCAAGGCTGTGAAAC	15742	GGGCTTGAACCTCAGCTCTATCATC	26726	AGTACGGAGAAGGCCACCAA	37710
4085	AATGGTGCCGTCACCTGAGA	15743	CTGACCAGAGAGATTGGGCCTTCT	26727	AGCTAGCCCCACTTGCATTG	37711
4086	GTTCGGAGGTTTGCCAGCTTTG	15744	ACTGTTCAGCTAACGGTGTCAAG	26728	TGATCAGAATGAGTTCGGAGGTTTG	37712
4087	CTGGGTTCAGGAGCAACTAAGAAAC	15745	GAAAGCCTGAGAAGGTGCCAAT	26729	ACCTAGGAAGGGGTCAGGTTCT	37713
4088	TCTGTGTTCCCCTCCGTCTTT	15746	CGACGGGCAGCTGTCAAGA	26730	CGCCTTCATGGTGATCTGTGT	37714
4089	AGCCACCCTCTCCCAAGACAAA	15747	TGCCTCCTTTTCTCAGGGTCTT	26731	ACTTGTTCAGGGTCAGAGGAGTAG	37715
4090	CACAGCTCTCATCACCTACGAA	15748	GCCTTGAATGTCAGGCGAAATAAC	26732	AAGCCTTCCACAGCTCTCATC	37716
4091	AGGGAGCTGCAGGTTCATGT	15749	AAGAATGCCCTGCACTGTGT	26733	AGCAGCTACGAGGCCCTTCA	37717
4092	CCTGCGAATAACACGTTTCTGTAG	15750	GGGAGAGGTATGCCAGGCTCTAA	26734	CTGTCGCACATTTCCTGCGAAT	37718

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4095 ACCAATCCCGAGGTGCCTAAG	15753 AGGCAGCCCCTGCTCACTT	26737 GGCAGTGGAAGCCTTTGATACCAATC 37721
4096 CACACTTTATGGAAATGCAGAGACTGA	15754 CACGGACGTGCATGACACTA	26738 CCCTGGAACCTTCCAAGTTCACACT 37722
4097 AGTGCCTGCTTTGCATCTACATA	15755 CAGGAGCAGGGGTGTATTGAAAG	26739 CCCAGCCATTTAGTGCCTGCTT 37723
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4099 AAGCACTGGATTTCACTGAGACA	15757 AGGGCTGTCTCCTCTCCTGTTT	26741 TCAAAGAGGCAAAGCACTGGAT 37725
4100 GGCAGAGAAGCCACATCCAACT	15758 CTGCCCCACCATATCTGAGAAG	26742 GCAAGGCTACCTAGGCAGAGAAG 37726
4101 GCCGAGGAAAAGCTGACAGT	15759 TCTTTACCGCATGGTTCTAACACAA	26743 ACGCACATGCCGAGGAAA 37727
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4159	CCTCTGTTGGTGAGGTGCTAAG	15817	TTGTATAACACGCTGCAGGAAAAG	26801	AGTCCTGTGTTAGCCTCTGTTG	37785
4160	GCTGACAGTGGCTCCTGAGA	15818	CTAGTGGCTTGCTCAATTCCTATTTG	26802	GGAACTGGATGTGGCTGACAGT	37786
4161	TGGCCTTCCATGCTCAAAGAAC	15819	GCTGGCTTCTCCAGTAACAATGA	26803	GACATGAGCTTGGCCTTCCAT	37787
4162	ACACGTGCATGCGACACTCA	15820	TCCTCACAAGAAATGGACAATGGAT	26804	TGAGAGTCGTACAGCAGCAAAT	37788
4163	CAGTTTCAGTCCTCTGCTGGACTT	15821	CCTGGCCGTGCCTGATATTTCT	26805	CCTGGGCCCCAAAGACAGTTT	37789
4164	TGGTCATATCGCTTCTCCTCTTTG	15822	AGGAGAGACACAGAGGGACATTT	26806	CACATCAGTGCAATCCCTGCTTTC	37790
4165	ACAGGCACCGAGAGGATGAA	15823	GTTTCTCTGGCCATTTAGGACTCA	26807	TGCCCTCGAAATCCTGTGAGA	37791
4166	CACCAAGCCGATGAAATTGATCTTG	15824	GACCTTTCTTCCTTTCTCTCACCAA	26808	GGCAAGTTTCACCAAGCCGATGA	37792
4167	GACCTGCAAGATGCCCAGTAAG	15825	TAGGGTCCTGTCCCCTCTCT	26809	TCTGTCCAGGAGACCTGCAA	37793
4168	CCCAGCACCCTTGCCTGATTTA	15826	CCCAAAGCAGCTAATGAGCATTGCATA	26810	GAGAATCAGTGGGAGCTGCAT	37794
4169	GCCTCCTTCTGTTGACTATTCTGT	15827	TGTGTGTTACCAATGCCAAACAATC	26811	GGGTCACCATTTTGCCTCCTTCT	37795
4170	GCTTGGGCAGGCATTTCTCTT	15828	TCTCAAGAAAACCTGTGGCAAGT	26812	AGGTAATCACTTGGCAGTAATGCTT	37796
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4173	GAGAGCTGCAAGTCATCCTCATTC	15831	CTGCTGACCTGACTGTGAGTGAA	26815	GCCATGTGTATTTAGTGCTGGTCTT	37799
4174	AGGGAAATCCAGCCTTCTCCTT	15832	CAGGTCCACCAACAAATCATCATC	26816	ACCACCCAGGCAAGGGAAAT	37800
4175	TGGCTTATGCTCTGAGGGTCTA	15833	GCAGACATGTGGGTGACAGA	26817	TGTGGTCCCACTCTGGCTTATG	37801
4176	GGGGTGCCGTAGTGATCAGTAGAT	15834	GCTGCGGGCAGCATTTTAAG	26818	CCAAGGGGTGCCGTAGTGA	37802
4177	AGAGCCTGGCACTGGGTATT	15835	CACCCTGGAGCCTCAATCCAT	26819	TCTGCGGGCAGCTGTTAGA	37803
4178	GTGAGCTTGGCTCAAATCCATGA	15836	AGCCTTGCAGGCCCTACT	26820	TGGGCAGACAGGTGAGCTT	37804
4179	CAGTTTCCTCTGGCACCTTTGTTC	15837	ACAAGTGATTCTCATGGCCACTT	26821	GACAGCCATTCATCCCCAGTTTC	37805
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4181	CACATCACCGGACGCCTTT	15839	GGGGAAGCAGCTTTCACTCTCT	26823	GCTCAGAGGTCACGGTCACA	37807
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4184	CCACAGGCCCTCATAAGCCTCTA	15842	ACTGCATTGTGCGTGACCAT	26826	TCCTTGCCACAGGCCCTCAT	37810
4185	CCAGGGCAAAACAGAGGTCCAT	15843	TCCTGAGCGTGGTGGAGATG	26827	GCTGTAGGAGCCAGGGCAAAA	37811
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4187	TGCTGCCCACCTGGCATTT	15845	GGAGCAGCAGCCATACTCCAA	26829	AGGTGAGAGGAGCAGGTGTT	37813
4188	TGCGCACATGACTCCTCTAGT	15846	CTCATGACTGGTTGTTTCTCCTTGAA	26830	GATTAACAACACTCTTGCGCACAT	37814
4189	GCAGATGGAATCAGCATCTCTCGAT	15847	TCCTCCTGCTCCTGGGTCAT	26831	GAAGGAGCTGCAGATGGAATCA	37815
4190	GCTGGGCTATGTGGCTTCAGAT	15848	TGTGGTCTCTGAGCCTGCTGTT	26832	AGGAGTCATGCTGGGCTATGT	37816
4191	TGTGCTTGGCTTAGGCTAGAGT	15849	CATGTGGCTCTAGAGGGAGGAA	26833	GGCTGTGGCTTGGGAGGAGTATAA	37817
4192	AGGCAGGGACTGGAGGTAGAAAG	15850	GGAGGCTGCCAGAAGTCAGAAC	26834	GCTGCTGCCTTGGACAGAAA	37818
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4194	TGGTGCGGTCACCCGTGTT	15852	ACTTCCCCTGTAGGCACTGTCA	26836	GCCAATGAGGCAATATGGCCAATGA	37820
4195	GGCCCTATCCCTTGAGAGTTGT	15853	TAGCGCGCGCGACAAACTA	26837	GCAGGGCTGATGGCCCTAT	37821
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4197	CCTCAGAACGATGAGGGAATGAAC	15855	GACCTCCAGGGTGCATGTCAAA	26839	GTACTTTCTGCCTCAGAACGATGA	37823
4198	GGCCACAAGAATCCCAATCTCA	15856	TGCTGCCAGCCACTTTGTGT	26840	GGCGGAGATAGAGCTATGAACTGA	37824
4199	AAGTTCGAGGCTCACTACTTTTCT	15857	CTGCCTCCACTGTGTCTTCTGT	26841	GGTGGGTAGGTTCTAAGCACACCTAAG	37825
4200	GGGGATCTGGGGAGTATCTGGTAT	15858	TCTCATGGTTGGGTAATGCAAAGA	26842	CCAAAGCCATGATCTGGGGATCT	37826
4201	GGGTAAGGCAGAGATGTGAACTGT	15859	GTGTTGGGGCAGACCTTCAGTA	26843	GAGGTCAGGGTAAGGCAGAGAT	37827
4202	CCGCTTCCTTGCCTGGGAAT	15860	CTCCACCTGCCACACTCTCT	26844	GCCTTGGGCACGTCACTTC	37828
4203	CCCATTGAGGAGGGCTTTTTGA	15861	GGCTCAGGGCCCAAGGATCT	26845	GGTTCTCTTCCCAACTATCCCATTG	37829
4204	GACAGGACAGCAGTGTCAATGGTT	15862	GCCTTTATTCCCTGCCCCAATC	26846	GACGTGCAAAATCACTGAGAGACA	37830
4205	GCTCTTTTGGTACCCGGAACTCTAC	15863	CTCTGATTCTGCCCCTGGAACA	26847	GCAGGAAAGCAGCTCTTTTGGTA	37831
4206	CTCACAGCCTGACAGCTGAA	15864	GCCTGCACTCTCCCACAACTATG	26848	ACACAGGCCTGATATTTTCTCACA	37832
4207	GAGCAGGTCAGTCTCCAGATCCTT	15865	CTCCCACCAGCATCCAAACACA	26849	ACAGGTGGAGCAGGTCAGTCT	37833
4208	GGCTTGTCCTGAATGCCAGGTA	15866	CAGCAAGCAGGCTCAGGTACT	26850	GTTGGGTTGGCTTGTCCTGAA	37834
4209	CCACCCTGAGCTCTTCCTGTT	15867	GCATCAGCATCACTAACGAGGTCTTC	26851	GGCTCTTAGAAGTGGCCTGTGTT	37835
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4213	GGAAGCATGGTGAACTGCACATT	15871	CCGTGTCTTGGCAGAAGCTCTA	26855	GCTGGAGGAAGGAAGCATGGTGAA	37839
4214	CTGGAGGTCTCTCCACTGCTTT	15872	GGCACGAGCCTCGGAAGAT	26856	CCTATAAGCTTGCTGGAGGTCTCT	37840
4215	TTGGGTAATGCTCGCTATGTCTAAT	15873	GATGGCGCTGAATGGAGTCT	26857	CCTCCACCCTTGTATCGTGAGT	37841
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4217	CCCAGGGAATACAGCCAGCAT	15875	GGAATATACAGACCCTTCCTTCAACT	26859	AACTTACGGGCCCAGGGAATAC	37843
4218	CATATGCCCTTATGCCTGGATTCT	15876	TCCCTGGCCTCCAGTGATTTG	26860	GCAGGATGGTGTTAATTCAGGGAGAT	37844
4219	GCTGAAGCCAAGGGAAGAGGAGAA	15877	CCCAGAGGTGCCTGTGACAT	26861	AGGTTTCTAGGTGGGGCTGAAG	37845
4220	AGCCAGTAGCTGCCCTGAGAA	15878	TCCCGCTGCGTGTTAGTTTC	26862	ACCTCTCCTCCACAGCCAGTA	37846
4221	CCAGAACCTGGCCATCTCTTCCAT	15879	GCAGGAAGCCACACAGAAGTT	26863	GGAGTAGAGCAGGAGTTGGGAATAG	37847
4222	CCCGATAAGCGTCTGATGGAAAC	15880	TCCGTTGTGTGCCAATGCTA	26864	TCCCAGAGCTGACCCGATAA	37848

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4224	TCCAGGCTTCATGGCACTCCTA	15882	CACAGCTCCAGGGGCATCATA	26866	TCCTCACCTCCAGGCTTCAT	37850
4225	CAGAGCCCCGTTACTCTTTCTTGTAG	15883	AGTGTACAGGAGTGGCTTCAGT	26867	GAAGAATCTCAGAGCCCCGTTAC	37851
4226	GGGGAAGGGAGATTGGTAGAGA	15884	GCCTCTTCGGGTCCCTGTTCT	26868	GGCTTAATTGGAGGGGAAGGGAGAT	37852
4227	CCGAGCCTTTGTTTCACTCTGTTC	15885	CTGAACAAGGCGCTGAAGGAAGA	26869	CAATCAGTCCCACGGTCCATTC	37853
4228	GGGTTTGTCTTACCTTCAGCTTCT	15886	AGTGCAGAGAATACCCGAGACA	26870	GCCTGTATTTGAAAGGGTTTGTCTTAC	37854
4229	TCAGCCAGCAATGTTAAAAGACAAG	15887	GGGGCTGACTTATTTGGTGGTT	26871	TGAGGCTGGCTTCCAAATTTCA	37855
4230	GGTATCGTTCTAAGCAGGGAAAAGA	15888	GCCCAGGTGAAGCAGAACTTTG	26872	AAGTGAAGGCATAAGGTATCGTTCT	37856
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4232	AGGACAGCAGGTCAGGAACCTT	15890	GGGAAGCCCCACTTTAGGAGTT	26874	GGGAAGCTTGGGCTTTGCTA	37858
4233	CCAGGTGATCTGCCAACCTATTCT	15891	CCTTTTCCTCCCCAGGACTTC	26875	GCCAAATGCAAAAGCCAGGTGAT	37859
4234	GACTTCTGTTACATGCCCCTCACTT	15892	AGGCCCATGTGGCTGATGTTAG	26876	CGGCCTCATCCAGACTTCTGTTAC	37860
4235	GGCAGGAAACCTGTAGATGGGAGTA	15893	GTCCCCTGTCCATAGGAGAGACT	26877	CACACTGAGCTATAAGGCAGGAAAC	37861
4236	GCTGGGGAAGAGTCTGTAGACTTAG	15894	TGTGATCTACCAGCAGCCATTATT	26878	TGCCAGCTGGGGAAGAGT	37862
4237	GCATAATGAAGCCATTCCCAACCTT	15895	GCACCTGCGAAACGTGGAA	26879	GTGGATGGGATCTGTCTGAGCAT	37863
4238	CCAGGACCACAGTTGGAATCCTAT	15896	TGTTACAGGCAGAAGTGCAAGT	26880	TGACCCACCAGGACCACAGTT	37864
4239	AGGAGTGGCACCTCCCAAGA	15897	TGGCACAGCCCACCCGAAA	26881	TAGCAGGGGCCACAGCTTAG	37865
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4242	AGGGTCGGGCTGCTGATGTTA	15900	AGCCTCAGAGCCGCATGAAG	26884	ATAGGGCTGCCTCCCAGAGA	37868
4243	GGGAATTGCCCTGAGCAAATACTAAC	15901	GCCTGGCCTGCACTGAGTT	26885	CTGACTACTGTCAAAGAGTTAGGGAAT	37869
4244	GCCAGCTCAAAGGCCATACA	15902	GGCTATAACACCCTGCTGACAT	26886	GAGTCACCTGTAGCCAGCTCAA	37870
4245	GGAGTTTCTTGGCAAAGGAGCTATG	15903	CAAACAGGGCACGTCCCAAAG	26887	CCAGTTGAAGCTCTCTGATGGTT	37871
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4247	ACCGAGGTTTAAAGAAGGCAAGT	15905	CATGAAGTGCTACTACGACTCTTAACT	26889	CCCAGGCCTTACCGAGGTTTA	37873
4248	AAGGCAGGAATGTTGCAGCTA	15906	GTGGACAACCCGTCTCAGATTC	26890	GCCTGTTAATACTCCAGTGAGGAGAAG	37874
4249	GCCGAGAAATGCGGCCAAGA	15907	CGCCGGACTGTCCACTGAT	26891	CACAACCTGAGTGCCGAGAA	37875
4250	AGGAGGGGTACAGATGCCCAAA	15908	CCTCCTGCTGCACATCTTTCT	26892	TTCCTCCCAGGAGGGGTACA	37876
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4252	ACACTGTCCTTGACTCCTTTGTT	15910	TTGAGGATGCGTGGGATGTAG	26894	GGCCAAAAGGTGAAACACTGTCCTTGA	37878
4253	GGTGACTAAGCAGCAATTCCCTTTAC	15911	TGGGCCTCGTCAGGCCTTTAT	26895	CTTGCCAAGCAAATTGGTGACT	37879
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4255	GTTGCCAGCACAGTCAAGCTTTT	15913	ACAGCGGAGAAGGTGGTGAA	26897	GCATGCTGCCCTACTTGTCA	37881
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4257	GCCCAGAACCCACTGAAATTATTC	15915	AGGCTAGGCAGGCTTAGGATTG	26899	GGACAACTAGGGACAGCCTCTCT	37883
4258	GAGGGGTTGATGAGAGCAAAGT	15916	CTCGCAGAGCAGAACCTCAT	26900	TCAGCAGTGTGAGGGGTTGATG	37884
4259	GCCGGCATTAAAAGTCCATATCTAAGT	15917	AGCTCAGGGAGGCGGAAGTATT	26901	TGAGTCACGTGCCGGCATTA	37885
4260	CTGTGCAAACATCAAGGGATTAGAAG	15918	CCCACTAGGCCACCCTCCAT	26902	CAGTGTAGACTGCTTTCTGTGCAA	37886
4261	CAGCAAGCTCAGCTGGGGAAAA	15919	GACACAGATCTCTGGTAATTCAGAGCAA	26903	TGTGTTCTCAGTTGTCTGGGTAAG	37887
4262	CATGTCTCTGGGAAGTTGCATGT	15920	CCAACAGCAGGTAGGCAAATG	26904	CCAGGTACCTCCATGCCATAGACA	37888
4263	CTCTGCAAACCCAGGCAGGTAT	15921	GTACCACTCAGACCAGGAAAACA	26905	GGGGAACACTCAGGTCTTGTGAGA	37889
4264	AGGAGGTTGGTGAGGCAGACT	15922	CTCCCCACCTTCGTCTCTGA	26906	GAGGATAAATGGAAGAGGAGGAGGTT	37890
4265	TCTGCCCTTCGCATAGACCTT	15923	AGATAGCAATGCTGCAACGTAAGA	26907	GTGACTCATTCCAGGTGTAGAGGAT	37891
4266	CACCTTGATTGGCAGGTTGACT	15924	GTGCAAGTTGCTGTTGTCTCACT	26908	AAAAGTAGGGCAGTCACCTTGATT	37892
4267	CAGTGCGTTAGGCCCTGAGA	15925	TCAGGAACCGTGACTCTTTTTCA	26909	ACGTGCAAGCAGTGCGTTAG	37893
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4369	TGAGGGTGTCCAGGCAGTTTG	16027	GTCACCGACACCCTGTGGAT	27011	TGAGCCACTGTGCCTGGATA	37995
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4407	GTGGACCTGGATGACCTGTCTTG	16065	AAGCCCTGCCCACCCACTGA	27049	CGAGACCCGATCCCTGAAGT	38033
4408	CGCCATGTTTCAGGCCACCTT	16066	GCTGCCTTGGGAATAGACGTTCA	27050	ATAGCCGAGCCGCCATGTTT	38034
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4419	GTCTACACACACGCCTCAATAGT	16077	TTTCCATTTCTCCCTCGCTTATTCT	27061	GCACTAAGAAGTTGCTTTGCTGTCTAC	38045
4420	GGGAGAAGGCGCCTCTATTTCTGA	16078	AACTGTTCCTGCAGCCCTTT	27062	CCCAGCCATTCCCTCCAACAT	38046
4421	TCCCTCAAAACCCACGTATTTGT	16079	TCCCAGCACAATGCCTGTCT	27063	GCATATCCCTTGACTGTTCCCTCAAA	38047
4422	CACAGCCCTGCTTTAGGAAGA	16080	GCCTTCAGTATGTCTTGCGTCTTG	27064	TGCCAGTAGGGAGTCACTGAGA	38048
4423	TTTGAGCCCAGGAGGTCGAGTCT	16081	GGAGGGCAGTGGCACAATCATA	27065	CCAAGGCAGCAGGATCATTTG	38049
4424	TCCCTACACACTCCCATGAAGA	16082	AAGGGTGGCACCGAAGTCAGA	27066	CTTTCCGGTCTCCCTACACACT	38050
4425	ACCCAGACTGGCCTCCAATTC	16083	GGCCAAGGTGGAAGGATTGCTTA	27067	CATAGATGGGGCATCACTGTGT	38051
4426	GTGGCCTCGAGGAAGCATAA	16084	AGGTGAAGCTGAGCCACTTG	27068	CCAGTAGACCCACCATGTCTGT	38052
4427	GTGCATGAGTTTCACATGTCTCTCTT	16085	CTACCAAGTCTGCTGTCCCAAA	27069	GCAGAGAGTGCATGAGTTTCACA	38053
4428	TGTGGGGCTGTAGGACACAA	16086	CCCACGCTGATTAATGCTCCTTTG	27070	GTGCGTGAGAATAGATGCTGACAT	38054
4429	GGGGTCAACTCCTATCACCTTTC	16087	GAGAGGCTTTGAGGCATTGAGA	27071	GGTGGTGGGGTCAACTCCTA	38055
4430	GTGGCAGGGGTCTTTGTACTTC	16088	GGCAGTTGGGGAGGAAAGGTAT	27072	TAGCCTGACTGTGGGAGTGT	38056
4431	GGCAGAGACGCTACAGTAACGAA	16089	GCAAGGCTGGACCAGGAATGT	27073	AGGCCCTGTTACTGGCAGAGA	38057
4432	GAGACCCTTTCCTTCTGCTTGA	16090	AGACAAAGTCCTCCCCACTCT	27074	GCTGCACAGCTCGTAGCTCTAA	38058
4433	GAACAGCACAAGGTGGGACAAAC	16091	ACACAGGCAGCGTTTGCTT	27075	TGCCTTCCACAGGGAGGAGAA	38059
4434	CTGTTTGTAAGGTCTCTGGGTATGA	16092	ATTAAGCCCCATGGGTGGTATG	27076	CCAGGACTAAGGGAGTTGTCTGT	38060
4435	CACCATCAGAGCTGGGGTCTTT	16093	CAAAGTCGGCCCGTCTCACT	27077	GCACCAGGCCACCATCAGA	38061
4436	GGATGTGAAAGCTGGAGCACTGT	16094	CCTCATCCAGAGATTGCAAGGTT	27078	GGTGCACAGCAGAGGATGTGA	38062
4437	GACATCTGTGTGCTTGTTAAGGGAATGA	16095	CTACCTGCCTGCACTGTAGATG	27079	GGGTGGACATCTGTGTGCTTGT	38063
4438	TCCAAGTTCAGTGCCAGTTCTATC	16096	AGCCTGCTGATGCAGGAGTTG	27080	GGGATTCAAGTCCACATCTTTTGATTC	38064
4439	TGCCTTGGCCCTTGCCTTTT	16097	GCGAGACTGATTACAGACTGAGCTA	27081	ACCAAGATCCACTCCAGCAAAA	38065
4440	CAAAGGGAAGACACGAACTCCTT	16098	GCGCTGAAGTGTTGCATTCTGT	27082	CACAGAGCCCAAAGGGAAGACA	38066
4441	CGATGTGGCGGTATACAGTGTTG	16099	TGGCCGAGGCTGTGAAGGTA	27083	TGCAGTCGATGTGGCGGTAT	38067
4442	CGCTTCACTGGCCAGAACTTG	16100	CCAGCTCCCCTTGTGGTTAAA	27084	TCAGTTTTGCTCACGCTTCACT	38068
4443	GCATTTGTTCATTGTGCGTGTGT	16101	GGCATGCCTTTCCACCTAGT	27085	GCTCTATGTGCTAGTGTGTGCAT	38069
4444	AGGGATAGAATTGCTGACCAGATAGA	16102	AGCCTCCACTCCCCTCCTTCT	27086	GCACAGTCAGTTGTCAGGGATAG	38070
4445	CGAGCCGGACAGCAGTTCATTA	16103	GCTCCCCGAGGCTGTGAGTAA	27087	GCAACACAACACCTACAGGAGACA	38071
4446	CGTGAACTGCCCTCCTCAGA	16104	GGGTCCACAGTGTCCGTGTTT	27088	AGAGGAGGCTCCAGCGTGAA	38072
4447	GCAGCTGAGGAGTTCAGGGTTCAA	16105	AGCCCCAGCTCTCCCATGT	27089	TCTCTGGGCAGCTGAGGAGTT	38073
4448	CAGGCTTGGAAACTCAGGGACTT	16106	TCGACTGCTCTGTGCCCATCT	27090	CAGCCACAGGCTTGGAAACT	38074
4449	CCCACATGGGAGACCGCTAA	16107	GGTGAAGGCAAAGTCCTCCTT	27091	GGTTCCTGGTGAGGGAAGATGT	38075
4450	GCTGTGGTTTTATGCTCCCCTAAAC	16108	ACAGCCCTGCTGCTGCTT	27092	CACCCCTGACTTAGCTGTGGTT	38076
4451	ACCGCCTGGTTCCTGATTCT	16109	CACACACAGTCCTAGGCCATAC	27093	TTGCTCACTACCGCCTGGTT	38077
4452	CAGGGGAGGCCTAGAGTTAAGTTC	16110	TCTGGGTCGCGTAACTCCAT	27094	TGCATAGACAGGGGAGGCCTAGA	38078
4453	GGGGCTCTCCCTCTCGATGTA	16111	GCTGGCGGAGCAGTTCGTA	27095	CGTCCACGAAGCCCCAGTA	38079
4454	CGGGCCACCTGGAGAAAGAATAG	16112	AGGCAGCCTGGCCTTACA	27096	TGGCCCCACTACTTCCTGAAC	38080
4455	ATGGGTGGCTTCGGCTCAA	16113	AGGCTTGGCAAGGCCATCA	27097	GAGTGTGGCAGTCGGTACTAAG	38081
4456	GCCAAGTTTTTCAGAGAGCAGCAGAT	16114	AGTCGTGGGCTGTGTGCAT	27098	TCTGATGGCCAAGTTTTTCAGAGA	38082
4457	CCCTCGCTCACCTTCTCTTC	16115	TGCCTGTTATGCACAAGCACTA	27099	CTCCAGGAAGCATCTGTTGACT	38083
4458	TGACACTGACCGGTGTGGAT	16116	GGGGCAGCTGGAGACTGGAT	27100	TGGGGACTTGGTGTCCACTGA	38084
4459	CCTATCCCATTCCTCACAGCCATT	16117	AGGGAATGGGGAATATTGAATGCTT	27101	TCTGGATGACGAAGCCCCTATC	38085
4460	TGGCTGGAAGCTAATTTTGGTTTG	16118	GGGTGGGGCTCAGTGTTGA	27102	CAAGCAGCTCTTGGCTGGAA	38086
4461	GAGGCCACCTACGGTATTCAAAC	16119	GCAGAGCAGGGTGAACAGT	27103	CATGTTACCCAGAGGCCACCTA	38087
4462	GACTCCAGAACAGAAGGGTCTTG	16120	TGCCCAGGCTTCTTGGTGTT	27104	GGAGGGGACAATTGCTGTGTGA	38088
4463	AGAAGCCTCCCCTCCCTCTGT	16121	AGGAGGATGGGGACGAAGGAT	27105	CCTTCCCTAAAGTCCCAGGAGAGAAG	38089
4464	GTGGCTAGAGCTTTGAGCCTATG	16122	GTTGCTGACAGGTCAGTGTCT	27106	TGTGTCCAGTGGCTAGAGCTT	38090
4465	CCAGCTGGCCTGACACTCAAA	16123	CCCTAGGCCACTGCAGCAA	27107	CGGATGTGCAGGTGGAATCT	38091
4466	GTCCTGGCTGGCTAAAGGTTGT	16124	GACAAGCCTGGGGAAACTGT	27108	CCCACAGAGCTCGCCAGAAA	38092
4467	GGGCTTCCCAAACACACAATC	16125	ACGCAGCACCCAGAGGTAAT	27109	AGGTGACTAGGGCTTCCCAAA	38093
4468	ACAACAGATTACAGGAGCACAGTTA	16126	TGTGGGCCACCTCTTTTTGAA	27110	CAGGTTTGGACTTGAGTCAACAGA	38094
4469	GGTGGATGAATGCCCTGTCAAC	16127	GCCTTTTGTGCAGTGGTCAGCAT	27111	TGGCTGAGGAGGTGGATGAATG	38095
4470	TGTGGGCCCCTGGAGCTACT	16128	TTAGGCCCCACTGCAGATCA	27112	CCGAAGGCCTCTGGGCTTTA	38096
4471	GCGTCCGTGTCACTAATTTTCTTG	16129	GGGGAACCACCCAAGGTTCTTT	27113	ACATGTGCGTCCGTGTCACT	38097
4472	TGCCCCTCCTGAAACCACATTC	16130	AGTGGCACTTCCACCTGTGA	27114	TCCAGTTGCCCCTCCTGAA	38098
4473	CATGGCTGGAATGAGTCAAGTGCTA	16131	AGACCTCAGAACGGAGTTGTTC	27115	AGGGATATCATGGCTGGAATGAGT	38099
4474	TGACTCCTGGGAAGCTAGACTTAAA	16132	AGACGGCCAGAAGTGACTGT	27116	AGGCTGGCACAGGTATGACT	38100
4475	TCTGATTATGAACCCAAGGCTGTT	16133	CACGAGCACCAGGCATCA	27117	GCGAGGCTGGGCAGTATCTGATTA	38101
4476	CTCCCTGTCTGCATCTTTCCTTAT	16134	GGGCAAAGCTCTTGAGACCAGAA	27118	GTTGCATCTTCATCTCCCTGTCT	38102
4477	TGAGTGACCATCCACTGACAAG	16135	AGTGAACGCTGTGGGTTTGTT	27119	TGGTGGATGCAGCTGGTATG	38103
4478	GAAGTCTCCATGTAGCCACTGAAT	16136	CGAGAGCCTGGGGTAGTTTTG	27120	CCACAGAAACACTTCTCGCTGAA	38104
4479	GCTCTGTGCTTTGGGAGACTGT	16137	AGTGTTCCGCTTGGCTGGTA	27121	GATATGCTGAGCTCTGTGCTTTG	38105
4480	GCCAGGAAGCATCCATCAGACA	16138	GAGAGAACGGACAACTTTTCCCAACA	27122	GACTCGATCTAGCCAGGAAGCAT	38106
4481	GCCACTGGAAAACCTGCATGAATGA	16139	GCAGCCTCCACAGAGAACAT	27123	TGCTGTGTGCCACTGGAAA	38107
4482	GCGAGGACTAGCTTGCCTCTTT	16140	CCTGCAGCCAAGTGCTTACTCT	27124	TCAAGAGGAAAGGCGAGGACTA	38108

4483	ACTGCTGTGTTGCTAGCACTT	16141	TTGAGCAGCTACCATATTCACACA	27125	GGCAAGAACCCTGTGTGTTTCTTC	38109
4484	ACAAGGCATCGCCACACCAT	16142	CACATGCACACACACTGGATAGA	27126	AGGCTTGACTTGGGGAGGACAA	38110
4485	GCCTACATTCCTCCATCAGCTT	16143	ATTTTGCCCCAGAAGCTACAGT	27127	GCTCTTCTAGCTTAGGCCTACATTC	38111
4486	ACAATCCACCCCGGTCTTCT	16144	GCTCAGACCCTTGTGCTTCTCT	27128	CGCAAACAGCAGCTCTGAGAAA	38112
4487	GTAGCAACTCTGCAATAGTCTTTCCTT	16145	GAGACTGCATTGGCGTACTGA	27129	GGTACAAGGGGTGTAACCATAACAGT	38113
4488	CCTTTCACTGCTTGCTGTGGGATA	16146	GCCACCTTTCCTCTGCAAGTT	27130	GCCATCATTTCCTTTCACTGCTT	38114
4489	CGGCCTGCAGAATCCACACTATT	16147	TGGCAGCAAGAGGACTGACA	27131	CAGAAATAGAATGTGAACCCAGGTAGT	38115
4490	GGCCTTAGTCATTGCGCCAAAC	16148	GGATAGGACAGTAGCCATGGAGTT	27132	GGACAACTAGCCTGGCCTTAGTCA	38116
4491	TGTGTCGACCCTTTCCTCAAAC	16149	GCTTTTTGTAGGAGTAAGAAGGGAGAA	27133	CTGGTCCTGGAATGTTCTGTGA	38117
4492	TGCAACTGGGAAGGCGAAAG	16150	GGACACCCGAGACCTGTGCTA	27134	CCTGGAAAACCAGGTGAACCGATCT	38118
4493	GGCAATGGCACTACTGGTACA	16151	CCACCAGCTGCTTGGCATA	27135	CAGCCCAGCACACTGACAAT	38119
4494	GCCACAAGCTGTGAGCTGTCT	16152	CCTGAGCCTGGCCTGAGATG	27136	GCAGTGGTCCTGAGCCACAA	38120
4495	TGGCGGGGATGGGGATCTCA	16153	GGTCCACGTCCGTGCCTTTATT	27137	TGCCTGCTACCTCCTTTCAGT	38121
4496	GGGACCCTTCTCAACTCTACTCTCT	16154	GGGAAAAGCACGTTTGTTCAGT	27138	GAACTCTGGGACCCTTCTCAAC	38122
4497	GCTCCTGGGAAGACATATGCAA	16155	GCTTCTCTTTCCTCTCCGAACA	27139	GAGAGAAGAGCTCCTGGGAAGA	38123
4498	TTCTGGCTCCACTACTGTGTTAC	16156	AGGCTCAGGGAGGTTAGGATAC	27140	GGGCTCAAATTCTGGCTCCACTA	38124
4499	GGGAGACACTGAAAAGCAAGACT	16157	CCATGTCCCTGGCACTCTGTTT	27141	GTCACTGTAGTGGGAGACACTGAAA	38125
4500	ACAGGAAACCCAGAGAGTTGTAATAG	16158	ACTTTGGGATTCTGGAGGACAGA	27142	TGGGCCTGGCAGAACAGGAAA	38126
4501	GTGCCCTGTGTCACAAAAGCAA	16159	AAAATGCCTCTGCTTCTAGGATGA	27143	TGGCATGTGCCCTGTGTCA	38127
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4503	TGCCCCAGATCTGTCCAATGA	16161	GCTACTTGGATAGGCCTTGCTTTG	27145	GGGAATAACCAACCCCATGGAGCAT	38129
4504	TTGAGCTGAGAGACAAACGCTTA	16162	ACCGTGCCCAGCCTTGTT	27146	GCAGGGGTGGGTTGTTTTGA	38130
4505	CCCAGAGCCACACGCTCAAA	16163	TGTGCAGCTGGCGTGAAT	27147	GCTCCACGATGCGCTCCTT	38131
4506	TGGTTGCCTTCCCAAATTCGTA	16164	AGGCCCCACTTCTTGGGATT	27148	CCAGTTAGGTACTCCTGTGGTCTGA	38132
4507	TGCCTTTTCGTGCAAAGCTAAC	16165	AAACACACTGGAAGCCTGAAGA	27149	GCTGAAGCCCTAGGTTTGCCTTT	38133
4508	GTGAAGGGAAGGGGCAACTGT	16166	ACGCGGCAGGTGCCTAGGAT	27150	GGCTAGCAAACACCTACAGTTGTGA	38134
4509	CTGCAATCACAGAACCAGTCCTAGA	16167	GGAGTCCACCTCACCGACTTAC	27151	CCCACGGTTCTGCAATCACA	38135
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4514	GCAGGACATTGCCAGGGCTTT	16172	TTTCCAGAAATTCAGGCCACTTTG	27156	CGGAGGCATGATTGCAGGACATTG	38140
4515	GTGGGTAAGCTGTGCTGTGTTG	16173	CCTCGGGTTCCAGAGTCTGTTG	27157	AGCAGACAGAGCAGTGGGTAAG	38141
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4517	GGAGTGTTGGCACCTGTTCA	16175	AGGCACCGCATACCCTGAAGT	27159	TGGTGAGGGGTGGAGTGTTG	38143
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4521	GGATTTCCTCCGAGATGCATGTGA	16179	AGGCGCAGCCCTTCCTCAA	27163	CCCGCATTTACGCGTGGATTTC	38147
4522	AACTGGCATGGTGAGCTGTATT	16180	GCTTTGAAGAGCGAGGTCACA	27164	GTGCAGAGTTCCCAGTGCTAT	38148
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4524	CAGGCAGTGACAACTCAGTCT	16182	GCTTTTTGGGGCATGCAGTT	27166	TACCAGGACAGGCAGTGACA	38150
4525	CCTGGCCTTAGAGGCAGCAT	16183	GCAATGCCAGAGAGGCAAGA	27167	CCAGTTCCAAGCCTGGCCTTA	38151
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4534	GGGCTAGTGTCCATAGGCTCAA	16192	CAACTGTGCCAGGGGTCCTAAA	27176	AGACCATCCAGGGGCTAGTGT	38160
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4537	GGTCTTGTCAGGAGTTTGAGCCTAT	16195	CCACACTGTGTTGGCCAATGT	27179	CTCTGTATCTGGTCTTGTCAGGAGTT	38163
4538	CTTAAAACCCTTTGCCTTCTTGCTA	16196	CGCCACTGGAACCAGAACT	27180	GCTCCCGTGGTCATCTGTGTTT	38164
4539	AGTGGGAAAGACCTCGGTGACA	16197	TGGCTTGGGTGTGGGTTCAA	27181	TACCCCTGGCGGCTGCTTGTA	38165
4540	GGAGGTCAATGTCAGGGGTCAGA	16198	CAGGTGCTCCAGGCTGTGAT	27182	GGTCAGGGTAGGAGGTCAATGTCA	38166
4541	CCCACAGGACAGGGACTATGT	16199	GTGCTTTTGGACCACTCCCTTTG	27183	CCATGTTCCCCTTTGGACTAGAGTT	38167
4542	GACCCCAGAAAGAGCCAAGTCT	16200	AAGGTCCAGGGAGTGGACACA	27184	CCTCCGTGACCCCAGAAAGA	38168
4543	GAGAGGTTTTGATCTCTCGGAGTTG	16201	GCGACGGGAGGAGTTGAGTTG	27185	CCCACCAGATTTCTTCCTTCCTGAGA	38169
4544	CAATTAAGAGGGCTCCTGGAAACT	16202	GCAAAGGGAAGTCCAGAGGAAAC	27186	CAGCTTGTCGCTGGAGCAAT	38170
4545	CAACAGCAGGGTTCACACAAC	16203	CTAGGACAATCTCACCCCACAAG	27187	CCCAGCCCCAGGTTTACAACA	38171
4546	ATTGGCATGGAAGTCTATGGAACA	16204	CCATGCAGCAAGGGCCTAT	27188	GCTGGAGTTTCAATTGGCATGGAAGT	38172
4547	GGCAGCAGTGATAAGAACGGATTG	16205	CAGCAGCCTCTGGTCTCTTC	27189	GGCTTTGGCAGCAGTGATAAG	38173

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4549	CAGGCAATGTGGTTTTGGCTTA	16207	AGCCACTGCACTGGGCTTA	27191	GACCAGCCTACATTTATCAGGCAATG	38175
4550	GCCCCTCCTTCATTCACTCAACT	16208	CCTGGCCCAGAGTAGGTGTTTA	27192	CCTGTCAGCCCCTCCTTCAT	38176
4551	GATTGGGACTGTGCCTCTCAAAG	16209	TGGGAGAGGCATGTGGAATGA	27193	GGGCCAGGCAGATTCAGAGATTG	38177
4552	CATCATCTCTTGCCTGGGTTATTGTA	16210	GGATGCAGGGACATCAGCTAAG	27194	GACCAAGTTGCCATCATCTCTTG	38178
4553	GCACTAGAAGTGACCTTGACTGT	16211	TCCCCTACCCCAACCCTAATTAAA	27195	ACCCTCCAGGCACTAGAAGTGA	38179
4554	GGCCTCTTTAGCAGCCCTTATC	16212	AATGGGCACAGCCACAGGTT	27196	ACCCGACAGCTCAGGTCAAT	38180
4555	GCCCTGCCTCTAAACAACCACATA	16213	GCCCAGCCAGGCTAAATGACTT	27197	TGCGCCCTGCCTCTAAAC	38181
4556	CCCACTGTGCTCAGCTCTGTTT	16214	CCTGAAGCAGGTTTGGAAGACT	27198	CCTCTCTGTAATGCCCCACTGT	38182
4557	CTTGCCAGTTCACCTGACTGTAT	16215	TGGGGCCACCTACCTGGATA	27199	GGTTGCCTCTTGCCAGTTCA	38183
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4559	ACTGTCGGTCAGGATCATTTTGAA	16217	GGTGTGGGGAGACCTCTATGTT	27201	TCAACCACTGTCGGTCAGGAT	38185
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4567	GACTGTGGGACTCTGTGCAA	16225	GAGTTGAGTTTCGAGTGCAAAACTTG	27209	GCCAAGCATGGAAACCATTTGACT	38193
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4569	CCAGCATACTTGTGACCTGAGAGTTC	16227	TGGCTGACCAGGGGCATCTTT	27211	CCCCAGACCAGCATACTTGTGA	38195
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4571	CCTGTGCAAGTTCCCAGAGAGA	16229	TGGGTTCGGGGCCCAACAA	27213	GAGGGAAAGTGACCTGTGCAA	38197
4572	CAGAGCTTGTACTTGGACCGATGT	16230	GCATTTCTGTGGTGGGATAGTGT	27214	GTCTGACTGCAGAGCTTGTACTTG	38198
4573	GTTGATTTTGGTGGGCTTCAGTT	16231	GTCTGCCTCACTAGTGGCCTAT	27215	CACTTCCCAAGCTAAAGGGGTTGA	38199
4574	GCATCTGCAACTGTGTGATCTTG	16232	GGGAAACAAGCACAGACTCCAA	27216	AGCCAGCATCTGCAACTGT	38200
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4577	CCTGCCCTTTGCTGATGAAAC	16235	ACCCTGGGCAAGACACTCT	27219	TTGAGACCAACCCTGCCCTTTG	38203
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4579	CCTCAGAGCATTTGAGGAACAGAA	16237	ACCGCCCCAGGAAGCTTAG	27221	GGAACACACAGCCTCAGAGCAT	38205
4580	AACGTCATCTCCACCAATGAACT	16238	GCTGCAGAGGCTCAAAGCTAGA	27222	CTCCAAAGAACACTGCCCAGTAA	38206
4581	GGTTGTCAGCACTCTGGACAAAA	16239	AGGAAACCAGATCTGTGCACTTG	27223	GCTTCCTGTAATGGGTCTAAGGTT	38207
4582	CTGGGGTGTATTTTAGCTTCAGAGT	16240	TGGGTGAGGCTACTGTGAGT	27224	GCAAGGAAGTAATTAGATCACTGGGGTGTA	38208
4583	GCCCCTCAGGTTGTGTTTCT	16241	GTCAAAGGGGAAGCATGCAAAG	27225	GTGGGTTGTTGTAAAGCCACGAT	38209
4584	CTCTTCTGAACTCCACACTCACA	16242	TCCAAGAAGAGGTGTGCAAAATGA	27226	CGAACCCACACTTCTCTTCTGA	38210
4585	GGGAGAGGCGTATTCTGTGATG	16243	CAGTACCTGGCCCATAAAAGGTAGT	27227	GAATCAGAATGGGAGAGGCGTAT	38211
4586	AGTAAGCCAGATGCCCAATTTGT	16244	TTCAGCTGTTTCCACCACAGT	27228	CGGCCTTAGCCATGGGAAATCA	38212
4587	CCTTGTCCAGGCCATGTGT	16245	AGGAGAGAGGCGGAAGGAACAA	27229	GTGGGGAGACCTGCTATCTTCTAC	38213
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4589	GGGATTCAGGCTCCTTTCATCTTG	16247	TGGAGGTCAGGTACTGAAGACA	27231	CCTCTTTGGTGACTCAGGGATTC	38215
4590	GGGGAGGCAGACCTGGTTA	16248	ACTGGACTCCTCTCCCCACATT	27232	GGCTTCAAGAGCTTCCCTGTTG	38216
4591	GCTGAACGGTTCCTTATCTTTCATC	16249	GCCCAAGAGGCAGGCATCAA	27233	GACCTTGCTGAACGGTTCCTT	38217
4592	GAGAGTTTGGCGCAGAGGAGTAAG	16250	GCAGCTGTTTCAGCGAATAGTTCT	27234	TGGAAACGGGTGTGAGAGTTTG	38218
4593	TCCTATCCCTCCCCTACCACTT	16251	ACTTGTGATGACAAAGGGAAGGAA	27235	AGGCAGAATTGACGTTCATCCTAT	38219
4594	CGTGATCTGACTCTGGAATGACCTT	16252	ACAGACGGTGGCGTCATCAA	27236	GTGGTTCACAGCGTGATCTGACT	38220
4595	GGTACCTCCCAGATGTCCATTTC	16253	CTGGGTGTGTAGTGCAGTCAGTTC	27237	GCAGAGCGAGGTCACCTTTGA	38221
4596	GTTATGGTGGGAAGAGTGGAGTATG	16254	TCCCTCCAGAGCTTCACAGAA	27238	TCAGTGGGCTGGATGGTAGT	38222
4597	CTGGCTCAAGCTCCCTTGTGAA	16255	TGAGGCTCAGACAGATGATGTTG	27239	AGGCCAAATCCTGGCTCAAG	38223
4598	GCAAGATGTGCCCACCACACAA	16256	AGGGACCCGTTTCAGTTCATC	27240	TGTTGCTGTCTGGCAAGATGT	38224
4599	GGCAGGGAGGTACCATCTTCTTG	16257	CCCCTTTGATGCCACAGTTATTTC	27241	TGGCTTGGGGCAGGGAGGTA	38225
4600	CCTCCACTACTGGTCCTACCAA	16258	CCAGACTATGCCACAGGGCTAATG	27242	CTAATTGTGTTCCATACCTCCACTACT	38226
4601	GAGGAGAGGACACCAGACTTGTTG	16259	TTGCCTTTGCCCGCACATC	27243	AGTGCAGAGAGCAAATTTCAGGAT	38227
4602	GGCCATTGCACTTGCTACTTC	16260	AGGACCACGGGCTGCAGGTA	27244	AAGGCCTGACTTGGCCATTG	38228
4603	GGACACCCTCCACTGGTAACACTAAG	16261	TGTGCCCAGGGTTGCACATA	27245	TCTTTGTTCAAAACGCCCAGAAC	38229
4604	CAGCCCCTGGCTTTATGTTCACT	16262	CCTTGCTGATCCAGGCAAAATG	27246	CACAATCTCAGCCCCTGGCTTT	38230
4605	GCTCATTCCTGGCATCCCTTGT	16263	CCTTCACTCCTGGGTGAGGAAT	27247	CCCAAGGTGCCAAGCTCATT	38231
4606	CTCAGAGGGCCTGATCTCAATGT	16264	GTAGATTAGCATGAGTGGGAACTGA	27248	TGATGACAGAGGAGTAAAGGTCTCT	38232
4607	GTGGGATAGATCCCTGCAGATTC	16265	AGTTCCTCCAGGAAGGTGCTA	27249	GGTGCATGCAAAGAGTGGGATAG	38233
4608	TCTTCTCGAAAGCATGCCTAATCT	16266	ATGCCGGTGGGGTTTATGAAT	27250	CTCAGCCCTTCCACCTTCTTCT	38234
4609	CCCGAATCCCACCTTTGAGGGTTTA	16267	CGGAGCACCACCAGGATTGTT	27251	CCCATCCCGAATCCCACCTTT	38235
4610	TAGCAGGGGACTGGGCATTCAA	16268	CTTGCCATGTGCCAAGTTCTGA	27252	TGGGAGTACCTGGATCCCCTTAG	38236
4611	GGGATAGAGGTCAGACACAGCAGTT	16269	TCTCGCCCACTGTCACCATGA	27253	GGCACACGATGAAAGGGATAGA	38237
4612	TTGGGCGGTTTGCTGTGT	16270	CACCAGGCCCTTGTACTCAGAT	27254	AGGACCACAGCAGCTTCGATTC	38238

4613	GCAGGAACCATACCAACTGCTCTT	16271	CACATTGGGACCCAGGACTAAG	27255	GTCCAGAAGGCAGGAACCATAC	38239
4614	GCCCTAGCTCCATCTGTCCAAT	16272	GTACAGCCCCAAACATGACTCT	27256	TCAGGGTGCCCTAGCTCCAT	38240
4615	GCAGCCTTTGCCTTTTGCTAAC	16273	GCGTAGTCCAGGCCCTAGAGA	27257	CCCAACCACAGCAGCCTTTG	38241
4616	GATGACACTGGGGTCGTGTGATAG	16274	CCCCAGCCTAAGGTTTGTCCTT	27258	AGGTTCCCTGCCTTAGGGATGA	38242
4617	CTCCCCTGCATCTAGGTGTTTAC	16275	CCCTACCCACACCACACAAAA	27259	AATCCTCCCTCCCCTGCATCTA	38243
4618	GTGCAAGACAACCACTGGTGAGA	16276	CCCTCCGCAAGGGGAAATGAAA	27260	CCATCCTATATGTCAGGTGCAAGACAAC	38244
4619	CCTGTTGCCTCTGGAAACTATCT	16277	CCAGATGAGCTCCCAGGACTTTG	27261	GGAGGTAGCAGCATTTACCTGTT	38245
4620	TCCCCAGCTCCAGGGAAGAA	16278	TGGTGGACAGGAGTGGGAAGTT	27262	TCTCCAGCCGAGGGTCTCA	38246
4621	TTCTGGGAGTGAGACCGTTTG	16279	GTTTTCCTCCACCCCTTTTCTCTTC	27263	GGGAGTGTAATTCAGGGAGTGTAATTCT	38247
4622	CCGGCTCTAAATCCTGGCATTTTC	16280	CCTGCCTCTCGCCATACTCATC	27264	TGGTCAGCTCCGGCTCTAAATC	38248
4623	CCCTGCTAGGAACAGTCTGATGAA	16281	CGCACACCCTATTGGGAAGGAA	27265	TACCGTCCTCCCTGCTAGGAACA	38249
4624	ATCTGGGCCTCCTAAGGTCTTC	16282	CCTCTGCTAGTCTCCTGAATGTTG	27266	CCACTGCAGTTTACAGTGTGAAGCAT	38250
4625	ATTGGCAGCACGAACCCAGACA	16283	GCCGCAGTGGCTGATCCAT	27267	GCCTGGGGAATGCTACTGATTG	38251
4626	AGAACAGGCCCTAGAGCTGGAA	16284	AGTTGAGAAGCAGGGACTTGAATC	27268	GGACAAGGACTGTGTAGAGAGAAC	38252
4627	GTCCAGCCCTTCACTAGATGTTAG	16285	TGATTTTCCCAGGGTCTGTGTCT	27269	TGTGGTGTCCAGCCCTTCACT	38253
4628	TTGTTGGAGGGCGAGCAGTT	16286	CAGCCACTCACTGACCTTGACA	27270	GTGTGTCCGAGCACATTGTTG	38254
4629	GGAGTTTCTCATTTTCTTCCAGGCAAT	16287	AAAGCCGGCCACCCCAACA	27271	GGGGCACGTGGAGTTTCTCATT	38255
4630	GACCTTGGCAAAGTGACCTCATC	16288	AGCTCAGCCACTCCAGACAAG	27272	TGTAGTGATGGTGTTTGTGACCTT	38256
4631	GCCTTCTGGAGTGGACAGGAA	16289	AATCACCATGTTACCACCACCAT	27273	GGAGGGCACAAAACTGCCTTCT	38257
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4633	GGGATGGAAGGCTGGGGAATG	16291	TCAGAGATTGGATTTCGGTCGTT	27275	AACTGGAGGTGGGGATGGAA	38259
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4635	TGCTCCCTCTGGGAAGCAT	16293	ACAGGGCGGTGCTAGCTTGA	27277	TCCCTCTGGCCCTGTGGTT	38261
4636	ACAAAGTGGAGAATGGGCTGAAC	16294	CCACCCAGTGCCTGAAAGTAAAG	27278	GCTGCATCTTCACGCCTACA	38262
4637	TGCCCGCAGCAGCTTCAT	16295	CCCATGGCTACCCTCACTCT	27279	GCCCAGTTTTAGGGACCAAAGACCAT	38263
4638	CCTTCTTCCTGCTACGACCTTCT	16296	TGTTGAGATGAAGCCAGCAACA	27280	GAGTTGGTCTCCAGAGAATACTTCCTT	38264
4639	GCCCACACTCTTAACCTCTGCACTA	16297	AAGGATGGCAGGACCAGTGT	27281	ACCTCCAGAGCCCACACTCT	38265
4640	CGTTGTGGGACAAGGTCTAAACA	16298	CAATGTAAGCCAGTGACAAACTGT	27282	GGGAGACTTCGTTGTGGGACAAG	38266
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4642	GGTTGAGCACTGTTCCACACA	16300	GGGACAGGCTGCGACACTATG	27284	GGGAACAGGTGTCCTTTCCAGGTT	38268
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4644	CTGTGTCCCAATTTCCCATTTCAGTTC	16302	AAGCCACCGAGGCCTAACTCT	27286	CCTCCTTATGCCACTGTGTCCCAAT	38270
4645	GTCCTCTGTAGCTCAGACACTGTT	16303	GGCCAACTCCCTCTCCCTACA	27287	TCCGAGGCAGGCAACTCTACA	38271
4646	GGTGGTTTGCTAAAGACCACCTGTA	16304	AGGCCCAGGCAAGAAGGAAA	27288	GACTCCATAATAAAAGTGGGTGGTTTGCTA	38272
4647	TGCCCACACCTACTGCCATAC	16305	AGCCCACGATCTGCAGGAA	27289	GAGGAGCTGCCCACACCTA	38273
4648	CTCTGTCTCCCAGTGGTCAGTCT	16306	GGTGCCACCCCAGAAATCACA	27290	AGCTCCCGCTGGCTCTGTCT	38274
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4650	CCCCAGGCAATTGGGACAAACT	16308	TCCTGCACACCTCCTGGACAA	27292	TGTCTCCCTGTTCCCAGTGTCA	38276
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4652	ACACACATCTGTCTGTGTGATGA	16310	TCCCTGCATGCCCGAATTT	27294	GGGACAGATGGGATGAAAACACA	38278
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4657	GTCGTTTGGAAACTGGCTTCTGA	16315	AGAGAATAAACGCATGCGGAAGA	27299	AGTACAATGGATCTGTCGTTTGGAA	38283
4658	CAGAAAGAGAGAGGTTTGTCCTCTTG	16316	TGGAGTTCCTCCACGCATTG	27300	GCCTCCACTCCAGAAAGAGAGA	38284
4659	CAACACCACCTCTCCATACCTTGA	16317	CGTGGCCCAACCACAGAGATT	27301	CCACCACCTCTGGGCAACA	38285
4660	TGCAGCAAGTGCGTGCATTC	16318	CTTCCTGTCCACCAGCTTCTTC	27302	TCTCTGGGGCAGCTGAAGTT	38286
4661	GCTGGGCCATAGCTCTATGTTTTC	16319	ACCGCGCCTTGCTGTATCAT	27303	CCCAAAGTGCTGGGCCATAG	38287
4662	GTTCCACCTCTCCCTGCAAAGT	16320	CAGAATTCCCGGACAGCACAGA	27304	CCTTTTGAGAACCAGCTCAAATGTT	38288
4663	GACTTTGGGAACACAGAGAAATGGTT	16321	CATTCCTCAACCTCTCAGACCAA	27305	AGAGAGGACTTTGGGAACACAGA	38289
4664	GGAAGCGGGTGAATAGACTGAAG	16322	GAGTTAGAGATCAAGTGGGGAGATTAC	27306	TCTGTCTGGAAGCGGGTGAA	38290
4665	GCCCTGCTGTAGTGCCTTAGTT	16323	AAGATGTGACGGTTGGTACAGTT	27307	CACATCAAAGCCCTGCTGTAGT	38291
4666	TTCAGGCCCGGCTATCTGT	16324	CCAAATTCCAACCTGACCCTGGTA	27308	CTGGGGCAAAAGACTGATTTTCTTCA	38292
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4668	CCCCAGCGCAACCTGGTAAT	16326	TCCTCTAAGGTGAAATCGCTCAAA	27310	GGCAGACGCCACTTTCTACTCA	38294
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4685	GCTTTGGGTGGGTCATTTCCTT	16343	TGCAAATACCCCATAGCCAGATG	27327	CCACCTATGTGCCAGGAAGCTTTG	38311
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16462 TGGCTTCTCTCCCCACTGTGAT
16463 GCCTCTAAGTCCATTCTTTGGATTTG
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27392 AAGGGGCAAGGTGCTGAAG
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4808	TCCCCTTGCAAGACCGATTG	16466	GTTTGTCTCCTCTCGCCTTCA	27450	AGCAGAAGGCTACCGTCCAA	38434
4809	GTTCTACTCGGCTTCTGGAAAGAT	16467	CCCTGACCTGTCCTGGGTCAT	27451	GGAAGTAGTGGTGCCAGCATTG	38435
4810	CACTCCCGGGTTTTGTGTGAAT	16468	GGGCGTGCACCCGAATGAA	27452	CACACCAGCATCTCTCCACAT	38436
4811	ACTGCCCACCCCTCAAAAC	16469	CTCTGGGAGCAGTGAAGGTTCT	27453	CGAACAGGAGCCTCTTCTCACA	38437
4812	GCTAGACCCTCCCTCCCAATGT	16470	AGGAATGGCCTGGGAGCTTTTG	27454	GTCCTTTTCAATGGCGTTGCTAGA	38438
4813	CAGAACCCCAGCGCGATCT	16471	TCCGTCACCACAGTGGGGTTT	27455	CGACAACAGACCCCTCTCCTTCT	38439
4814	AGCGGAGCCTGGGCAATGT	16472	AGTGCAGCGCAGCCCTCTA	27456	CAGAGGCCTGAAATCGAGGATGA	38440
4815	GCCGGTGCCATTCGTATCCATT	16473	TGTCAGGGCCTTGTGGGATTCT	27457	AGGCAAGCCGGTGCCATT	38441
4816	CGAAGTCCCAATGGCAACTTTCA	16474	CGACGAAACCAAGAGCAAAGTGAAC	27458	AGGCACCGAAGTCCCAATG	38442
4817	GGTCGCGGATTGTGCTGTCTA	16475	GCAGCAGCCCCTGGAATGTA	27459	AGGAGGCAGGTCGCGGATT	38443
4818	ACGGGGCCAACGAGGGGAAT	16476	AAGTGCCCCAGCAAGCTGTCA	27460	GTGGAGATCGAGGCTTGCTTCT	38444
4819	CCAGGCCCAAACCCACTACAAA	16477	TCCCAGTGGTCTCTTGGCTTGA	27461	CACTGTGTTTCCAGGCCCAAAC	38445
4820	GGAGTTTTGCACAGGGACTTCT	16478	TCACAGGGTCACACGGAGTCA	27462	GGGGAAAGCAGCGAAATGGAGTT	38446
4821	CAGTGGGAAAGCCACGGACAT	16479	GGTCGCATGTCACCCTGTGT	27463	GCATGGAGACGAGTCAGTGGGAAA	38447
4822	GGCATAGGGGACCAGGAAAGTCT	16480	GGTACCAACCGATGTCTCTTTGCAT	27464	TCTGGTGCGAGCTTTGCTT	38448
4823	GGGTTTGACTCATGGGAGTGTGT	16481	GTCACATGCCCACCCACAATAC	27465	GCACTGAGCGTGTAGGGTTTGA	38449
4824	CCCCTTCCTGGATTTGCTTCT	16482	TCGTTCCCGACTGTGGTGAGT	27466	CCAAAATACTCCCCTCCCACGTT	38450
4825	GAGAGAACTGTAGTGGCGATTTTCT	16483	GCTGCTGGGAAGCTCTGTTTG	27467	GCAAACCTCAGTAGAGAGAACTGTAG	38451
4826	TGCCAAGGTGTCCTGTCTGTTC	16484	TGGAGCTGCTGCCCTGTTAG	27468	GCCAAGAAAAGACATTGGTCACTT	38452
4827	GCGTTGAAACTGTGCTCTCAACT	16485	CCAACCAGCCTGATGGTCTCTT	27469	GGCCTCTTCAGCGTTGAAACTGT	38453
4828	GCCCACATGTGTTACTGGTGGAT	16486	CATATGGATTCGCTGCCGGTAA	27470	CTGATCATGGCCCACATGTGTTA	38454
4829	GCAGGCAGCCGTGAGCATA	16487	GCATCAAACCAAACCAGACACA	27471	ATAGCAGCACCAGGCCACAT	38455
4830	CTCTGCCTCACCCATTCTTGT	16488	GAGGCAGGGCCAGATAAATTCT	27472	GTGCGCAGTTTGTGATTCTGA	38456
4831	AGTTGAAGGCGCTGGAGAAG	16489	AGGCTGTCCAAGAGTCCAAGA	27473	CGTGTTGGGCTCAAAATGCTGTTC	38457
4832	ACGCACACACCGGCATACA	16490	GCACAGGCCTGGAGAGCAA	27474	ACACCTGCCCACGCACACA	38458
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4835	AGGCTGCCCTTAAGCACGTT	16493	CGGCATCCAATCTGCTCTTAGT	27477	GGTACAGCCAACCAAATTAGACAAG	38461
4836	CAGGTCAACGTCTCATCTTCGTT	16494	ATGAAGAAGAGCGGGAAAAGGAT	27478	TGTGGTTCCAAGTCAATCACATCT	38462
4837	TCTGCCAAGGGGTCACCAT	16495	CCCAAGGTCGTCGCTACTTGT	27479	CCGGAGTGGAATCATCCCTAGTTC	38463
4838	GCATCAGTGCCCATTCACACA	16496	TGGGCAGCGCTCTGCTTCT	27480	ATGGTGGGCTCTGCATCAGT	38464
4839	CCGAGTTCCAGCCATTTGTAAAGTTC	16497	TCCCCTCCCAGCTACAGAAACT	27481	CTCTTACCGAGTTCCAGCCATT	38465
4840	GCCCTTCAGGCTTCTGTGAGTA	16498	CCTAGAAGTACGTACCCACCAACA	27482	GGCTGTAGGGAATGAAGGAACA	38466
4841	GCTACACTTCCAGCTAGCACTTG	16499	TGGAGGAGAAGGTGGCTGTTTG	27483	ACTGTGATGCAGGCTACACTTC	38467
4842	GGCTCACATGCTTTCCAGTGA	16500	GGGAACGGTAGTGAGGATGAAG	27484	GGCCAGTAGCATTGGCTCACAT	38468
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4844	CAATCCTCCCACCTCCAGAGTAG	16502	CACAGTGGCAGGCACCTGTAAT	27486	ACTCAAGACTGGACTCAAGCAATC	38470
4845	AGCCATGCCCACCCACTGA	16503	GCAGGGAGAAGCTCCCCTAGA	27487	GCTCAGACTTGGGCAGAACA	38471
4846	ACCCCACAATTTCCACTCATTCT	16504	ACTGAGGGAATGAGGACTGGAT	27488	CAGCTCAACAAAATTGACCCCACAAT	38472
4847	CGGCCCTTGTTACATATCCAGTT	16505	GGCAACGCAGGCAAAGTGT	27489	CACACTCATCGGCCCTTGTTAC	38473
4848	CAGGCCAGGGAGAGCTTTGT	16506	GGCTCCCCTCTCAGAGCAAAA	27490	GCAGAGAGGAGGCGAGTGA	38474
4849	CCCAGAGCATAGAAGCCGATCA	16507	GGACTGGAGTCTGTTGGGAGAGA	27491	CGGCAAATGTAAACCCAGAGCAT	38475
4850	CCACCTTTACCCAGGAGAAACTCA	16508	GACGGCTCCGTCTGTTGAGAAA	27492	TCCTCTCACTGTTCTTCCACCTT	38476
4851	TGCTCTGATACACGAAAAGCATGT	16509	GCACCTTTGCCTTAAGCATCATTG	27493	CCCTGGAAGGTAATTTACATGCTCTGATAC	38477
4852	CCAGAAACAGCAGGGGAATTGT	16510	AGGGATAACATGTCTCGTTCAACTT	27494	CCATGAGGCATAGCATTTACCAGAAAC	38478
4853	ATGCCCCAGGGAAGACCATTC	16511	CAGCGTGCACTGTCTGAGTCT	27495	GCGACTTTGAGCCCATCCTTCT	38479
4854	GCGAAGCACCAAAGGGTGATGT	16512	AGCTGTGGGTTTCCGTGTGT	27496	AGCACTGGCGAAGCACCAAA	38480
4855	AGGCTTGGCCTTGCTTTCA	16513	GAGGGAAGCCCAGCATTATGAAG	27497	GTGTGCTTCAGCATGCCAGTTAG	38481
4856	CCGGGCGTGTTTGACTTACA	16514	CCTCTCCTGTCTTCGTAGTGAGA	27498	ATGCCTTTTCCGGGCGTGTT	38482
4857	CCAAAGGTGTAAGTCGCAGTCT	16515	GCTTGAGCTGGGTTATTGTGTTC	27499	TGAAACTGGAGAACCAAAGGTGTAA	38483
4858	CTGGAAGGTCTGTGGGGTCTGTA	16516	TCTTCCTTGGGCGTGGGAATG	27500	CTGAGTCACCTGGAAGGTCTGT	38484
4859	GCTCTGTGCTTCCTCATCTGCTT	16517	GGCTGAGCCTCACAGTAACAA	27501	GTAAGTGGGGCTCTGTGCTT	38485
4860	CTTCATCAGGGCCAGAATCCTT	16518	CAGGGACACAAAACCCCGATCA	27502	GCAGGTTCAGTGACTCTTCATCA	38486
4861	TCCATCTCCCACCACCTGTT	16519	TGGGCCTAGGAAGGTTCAGT	27503	TGAGTCCTAATCTCAGCTCCATCT	38487
4862	CGCGGTAACTCCACAATCACA	16520	CGCGATGACATGGAGGTGAA	27504	CTTATCTCACCACGCGGTAACT	38488
4863	GCAGTTGGCAAGACAGCAAGA	16521	ACATCCTCAATCACAGACTGCTT	27505	GGCAGCAATTGGTGAGAGACAAG	38489
4864	CACTGGCTTGGAACATGAGGCTTA	16522	TCTCCACAGGGTCCTGCTCAA	27506	CCTGTTGACACTGGCTTGGAACA	38490
4865	ACAGTCCTCACTCCACGTCACA	16523	CAGACTCTCTGCACCTCAGTGT	27507	GGGCAGCACAGTCCTCACT	38491
4866	GGCCATGGGATCTCTGTGATAACTA	16524	GCTGCTTCCACATTACGTTTGAGA	27508	GGTTTGTAGGCCATGGGATCTCT	38492
4867	GTTAGCCTCTGACTATTCCTTACATCA	16525	CTGGTCCAGCCTGGGAGTTG	27509	CCGTGCCTGTGTGCATGTTA	38493
4868	TGGTGCAATGCCTCCTTAAGTT	16526	TGCGGTGGACAAGGGAAGT	27510	GCTCACTCCCAGTTGGTGCAAT	38494
4869	AAACGCTGCCTCCCTGCTCAA	16527	CCGGCCTGGAGGGATATTCTTG	27511	GACCCAAAGAAGCCAAGGTCAGA	38495
4870	GTTCATTCTAGGAGGCAGTGGAA	16528	TCCTGCAGCCAAGCTCACA	27512	CTCACCTACTCTCAAGGTAACTGTTC	38496
4871	ATCCCACAGGTGGGGAGCTT	16529	GCCTCCAGGACTTTGAACCAA	27513	CCACCTGTATGGAGCGGCAATA	38497
4872	ACAGGAGTGCCTGGCTAATGA	16530	GATGGCAAGATCCTGTCTCTACAA	27514	GTCTCCCAAGTAACGGAGACTACA	38498

4873	TGGCCACACCACCCTAGCTT	16531	ACACACAGGGAGAGGCCGTTT	27515	TCGGTGCCCCTTGGCTTGT	38499
4874	GCTCAAATCCACCCAGAGGTCACA	16532	TGAGCGCAGACCTTCCTGAT	27516	GAGGTAAAGCCAGGAGCTCAAATC	38500
4875	CAAGGAGAAGACGGCCTGCAA	16533	GCGTCCCTCGGCTTGTAA	27517	GTGAAGTAAAGGGGCAAGGAGAAGA	38501
4876	ACGTGCACTGAATACCGGAAAG	16534	TCCCGCAGGTGAAGCCTCTA	27518	CCGTCCAACGTGCACTGAAT	38502
4877	AACCCACGAACAGCCTCACT	16535	GGCCTGAAGCCCGGGATATT	27519	AGCTCCGTCCTAACCCACGAA	38503
4878	CGTCTGCTGCGCTGTTAGAAAC	16536	ACGCGATGTCCTGGAAAACA	27520	CTGCGGTGTAGATAACTGAGTGAAG	38504
4879	TGTGACTGGAGGTGGCATCACT	16537	CAGTCACCTGAGTGAAAGCTAAGT	27521	GTGCAGAAGCACCTTGGTTGT	38505
4880	CACGTGTGTTCTACCTGATGCTGAA	16538	CCCAATCCCGCAGCTCTCA	27522	GTGTTGTGTGCACGTGTGTTC	38506
4881	GGAGAGAGGGTCTCACCTTGT	16539	GCCAACAGCAGAGAGAGAACAGA	27523	TTGCGTTCAGGGAGAGGAGAGA	38507
4882	TTTCACCGCCGCACAGAGAA	16540	TCTGCCTGTCCCCAGTCTTG	27524	CCGCCTTCACCTCTCTTTGAGT	38508
4883	TCAACGCCCCGGGCCAATTC	16541	ATGCCTGGGTCTCTTCCTGAGA	27525	GGAGCTTTGAAAACTTCCGACACTCT	38509
4884	CCTTGAAGTCCATTTTCACCAGTATG	16542	CCCTGTGGGCTCCTGGTACA	27526	CCACTTGCTGGAGTTCCTTGA	38510
4885	ACACCCAATGAGCTTGGTTTGA	16543	CCAAACAGCTGGCCAGTGAAG	27527	GGGTGGCATGAACTTTACACCCAATG	38511
4886	ATCCAGCCTTCTGGGCCATTC	16544	TGGGTTCCCATGTGGTGTGA	27528	CCTGTTTGCAGATCCAGCCTTCT	38512
4887	CCTCCTCCCCTCTTGCACAGTTA	16545	AGGAGTGGCCAGCAGTGAAA	27529	CCCTTGGATTCTGATGCCATCTCGTT	38513
4888	CTCTCTAGTTCAAGCTCCTTGCTTGT	16546	GAGGATGAGTGCCTGACTGTGT	27530	CTGGGATGTTAATTCCCTCTCTAGTTCAAG	38514
4889	CATGCTGGGGACAGACTCTCA	16547	CCGAACCCACTTGCCCATTG	27531	ACAGCGCCCTCTGCTATCCAT	38515
4890	CCAGAAATGTGGCTTAGAGGAAGGAA	16548	GTGCGCTCGGAAGGTTGAATAG	27532	GGCGCAACCAGAAATGTGGCTTA	38516
4891	GCTGGGGTGGGATTGCTTGA	16549	CACGGCTCACTGCAGCTT	27533	ACATGGTGGTCCCAGCTACTCA	38517
4892	CATTAGCAGCGGACAGGACCAA	16550	GGCTCTTCAGAGTGATGGGACAT	27534	GGGAGACAGCCAGAGCAACTCATT	38518
4893	GGAGACCGGTGTTTTCTTCTCTTG	16551	TTGTGGAGCCCCAGTCTCTGAA	27535	TCCCTGGAGACCGGTGTTTT	38519
4894	CAAAGGCACTGACAGGTGTGT	16552	GTCTTACTTTCTCCGTCCCAGTAG	27536	GATCACACCCAAAGGCACTGA	38520
4895	TCCATTCCCCTACTCCCAGCAT	16553	GTGGTGACAGAAAGCAGCACATC	27537	CGTGCCCATTTGCAATCCATTC	38521
4896	GGGAGCAAGAGTGGATCGTTCT	16554	TCCTCCGAGCCACTGCTCAT	27538	GAAGAAGAGATTCTGGGGAGCAA	38522
4897	GTGAGTCTAGGCTTCCCAGCAA	16555	GCCACAAGCCCGACCTTT	27539	TGGGTTGGATTTGGGTGAGTCT	38523
4898	CGCGTTCACAAATCGCTCTTC	16556	GCCACCCTACGTCCTGTGT	27540	GCTGCTTCGCGTTCACAAA	38524
4899	CCCTTGTTTCTTTCCTCAGGGCTAA	16557	GGAGTCTGTTGAAAAGGCTGGTT	27541	CCTCCCTCGTTAACTTCCCTTGTTTC	38525
4900	AACGCCCAGCTGAGGTTTGA	16558	GTCCCCAGGGAAAAGCCATTGT	27542	GCTGCAAGGGCTTCCTGAAA	38526
4901	CTTTTCCCGCGTGCTGTGAT	16559	AGGGGAGATGCCTGTGATGA	27543	TGAGGGAGGCCCTGTCCTTTT	38527
4902	GTTATGTGGGAAGACCCCAACA	16560	AGGCACCAGTGAGCTGATGT	27544	CCCTGATTCTCAAGACCTCTTTGTTG	38528
4903	GCCTGGGAAACTTGTGAATGGAA	16561	AGAGGTGGGCAGCACTGA	27545	CCAGGGGCCTGGGAAACTTG	38529
4904	CCGCTTGAAAAGCCACCTGCTA	16562	CCCGGCTGAGAGCAGACAA	27546	CAGCGATGGAGCTAGTCTGTGA	38530
4905	CTTCACCTGGAACCCAGAAACA	16563	CAGCTCTCACCTGCGTTTTG	27547	GGAGGTGTAACCAACTTCACCTGGAA	38531
4906	GGCACCCAGCAACTGGATCTT	16564	ACAGCCGCCTTCCCAAAACA	27548	CGCATATAACAGGCACCCAGCAA	38532
4907	GCCAACACAGGCAGGATTGA	16565	CGGTCACTAGGCACCATCTAATTC	27549	TGGGCCGTCTTTCCCTGAGA	38533
4908	ATCTTCCTTGCCCTGGAGTCTA	16566	TGGCACGGCCACGAACATC	27550	CCACGTGTCTTCGTCCATCTTC	38534
4909	TGCGGATGCGGTTTCCTTCT	16567	GGAGTAAACCACCTGCCTCTCT	27551	GGGGACCTTTGCTCCGTTTG	38535
4910	GGAAGTTGGGGAAACAGGAGGATA	16568	CCAACCCCAACAGAACAAGACCAA	27552	CCCAGAAGGAAGTTGGGGAAAC	38536
4911	GCGAGGTTAGCCAGGGTTAG	16569	ACAAACAGCCTTCCGTCTCTTC	27553	AGGCTGTAGACGGCGAGGTTA	38537
4912	CCACTTGGCAGTGAGGGTATTTC	16570	AACCACCTGGCCCTCTGTAG	27554	ACACGCCCCAGTCTCCACTT	38538
4913	TGCCAGCCCCAGTCCTGTGA	16571	GGGAGAGTGCCCTGTTTGTTGT	27555	GCCCTGCAGAACTCCCAACT	38539
4914	CACTGCAGCTTGAGCCAACA	16572	GCGTTTGCACTGGGAGATGAAG	27556	ACCACCCTCTCACTGCAGCTT	38540
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4918	AGCGGCGCAGTGAGTACA	16576	TTGCGGGGTGTGCGGAGAT	27560	AAGTGGCAGCGCCAGTGGAA	38544
4919	CACACTGGCTCCAGGACACT	16577	CGGGAGCTGCTGCTTGTTG	27561	AGCCACACAGGACAGGACTTG	38545
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4922	GCTGTGGGTCTTGCTCTGCTTT	16580	GCTTGCTGGTTGGATGTCAGT	27564	AGGTCTTGCTGTGGGTCTTG	38548
4923	CCCCACAGCAAGGACATAGGAA	16581	TGCTCTGTGGGTGTCACATTC	27565	AGTGCCTGGCATCCTGCTA	38549
4924	GTTCCCCAACAAGGGGCATCT	16582	GCCCAGAGCTCCATTTCACTGT	27566	TGAGGCCTGGGGTATTTGAAGA	38550
4925	AGCTGGGTGTGCCTTTGATG	16583	TGTTCATGGCCAAAGGTGTGA	27567	GCCCACAGAAGGTTGTTTTCGTT	38551
4926	GCCTTTCTCGTGACCCTTGT	16584	CGGCCAGGAGAACCAGAATCTT	27568	GCTTGGCCAATGACAGCCTTTCT	38552
4927	GTGGAGTTGGCGGTGGATGTA	16585	GTGCGTGTGTGGTGTTCTCT	27569	ACCAGCCTCAATGTGGAGTTG	38553
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4930	CCCCAGGCGTTTCTAAACAATCA	16588	CCTACTGCTTGCTGCTAGTGT	27572	GAAAACAACCCCAGGCGTTTC	38556
4931	AATTTCCAGGGTGCGTGGTT	16589	CAACGGTAAGACTGACCAGACA	27573	GCTTCTCTGGGTCTCAAGACATAAT	38557
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4936	GCACCACTTGCTTGCTCAGT	16594	CCCAGCACTTCCCTAAGCAGTA	27578	GTGCGCTTATTGCACCACTTG	38562
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5119	TGACCGGCCCATTGGATGA	16777	GTGCGGGGCCTCTGAACATA	27761	AGGCTGCAGGTCCTTTCTGA	38745
5120	GCATCCCCACTTGGAAGAAATCAAAG	16778	GCTCCAAGACCAGCCATACCTA	27762	CCCAGCATCCCCACTTGGAA	38746
5121	CTCACCTCACTCTGTCAATGGTAAAC	16779	CTTTGCTGCTAGGTTGGGATCA	27763	CGAGCTCACCTCACTCTGTCA	38747
5122	ACTGTCCCCAACCCAGTTCA	16780	AGGGACAGCTGCCCAGGTT	27764	TGCCTCCCACCTCTACCCTATT	38748
5123	ACGCAGGTGATGACCACAGA	16781	GCGTGCACAGCTGAGAATCCTT	27765	TCATGATAGGTAGATACGCAGGTGAT	38749
5124	AACCCTAGGGGCGGTGTGT	16782	TCGGTCCGATGGTCCCTTAGA	27766	GGACCGCTTCGGTGTGGAA	38750
5125	AGGACCCCAAAGCCCATGAAGA	16783	CCCAGGTCTGAGCCACTTACTCT	27767	TGGGCAGCAGGACCCCAAA	38751
5126	GGGTACCTGATGGCCTCTCTTTG	16784	GCCCCATGCCTATAACCTGCTA	27768	AGTGAAGCCCGGGTACCTGAT	38752
5127	CCTGTGCGTGGGATAGATAAGCAA	16785	CTATGAAGGCAGGGACTTCTGTT	27769	AACAGCCTGTGCGTGGGATA	38753
5128	TTGCTGCCCTGGGACTCA	16786	GTGGTGGAGGGAAGAGAAACTTG	27770	TCTGCCTCTTCCTGCTGGTT	38754
5129	AGAGCAGGAACACTGGAAAACTT	16787	TCCCTTGGCCAACTCCCTTCT	27771	GTCACAGTTAAAGAGCAGGAACACT	38755
5130	CAGGGGAGAGGGAGACAGTCAT	16788	CCCCTCTCTGACCATCCATATCT	27772	AGAGGCAGTGGCAGTCAAAC	38756
5131	GCAACCCCACACTTTCTCTGACT	16789	CCTTGGGCTGAGTGTGGTTAAG	27773	GCACACCCAAGGTCAGCAA	38757
5132	GCAGAGGGAGAAACAACCAGAAA	16790	AAAGATTGGCTGAGGCTTCTGT	27774	GAGTAGGATTAGGCAGAGGGAGAAA	38758

5133 CTGAATCATTGGAGGGCTGTGGTT	16791 ACGTTTTCCTCCGTGCCAAA	27775 GGAAGGGTTTGGCTCTGAATCATTG 38759
5134 GCTGGGCAGCAGGTTGTAGTAA	16792 CAGAGCACATGACCCAAAGACT	27776 GCATATGAGGGGCCAGGGTTTC 38760
5135 CCCAGAGGAGCGGTAGTCAAAA	16793 TCATCATGGTGGGTCTCTGAGTA	27777 CCCTGTATGGCAGGGAGAAAGA 38761
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5137 CAGTGCAAAGGCCTGGAACA	16795 GCACAAAGGTTCATGGACAGGAAAGA	27779 CATGTGAATTGTGGTCAGTGCAAA 38763
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5139 CAGGGTGTTTGTTATCCTCTCTGACT	16797 TAGGGGACGGGGACAGTTGTAG	27781 ACCCTCACAGGGTGTTTGTTATC 38765
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5141 GACTGGGACTCGCAAGCAAACT	16799 TGAGGCCAAAGAGGAAATGATTCT	27783 GCATGGGGCAGGAGTGTAAAGA 38767
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5143 GCACTTTTGGAACCCAGCCTTT	16801 TGGATGCACACATGCAAGAAAGA	27785 GCCTCCAGCAACAATGAGCACTT 38769
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5169 GCCGCTGACATGCTTCCAAA	16827 CAGGCCTGTGGTGGGTTTTTGT	27811 CCACAGTATCGCCGCTGACAT 38795
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5174 ACCCCTCATTCTGCGCACTT	16832 TTTCTATAGACTTTCGCCAGTGGAA	27816 CAGGGAAATCCTATAACTACCCCTCATTC 38800
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5176 CAAGCAACACTCCCACCTTTG	16834 GACTCATGCCTGTATCCTCAGCTA	27818 GGTCTCAAACTCCTGGGTACAAG 38802
5177 AGGGCCGACCGTGGTTTTC	16835 CAACTTGTGGGTGCGGGTAT	27819 GCATGCCCATTGAGTCTGCTACT 38803
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5200	TGGAGGGAGCGGCTGATTG	16858	AGCCATCATGCCCAGTGCAA	27842	AGCTCAGACCATTGCCTCTTG	38826
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5202	AGCCCATAGCCCTGTGGTTACT	16860	GGCCTGAATTCACACAGGCATT	27844	ACACAGGTGCAAGCCCATAG	38828
5203	GCTGTGCCTGTTGTCAGCAATG	16861	CAGTGGCCCACCAACCTCAT	27845	ACCAAGGCTGTGCCTGTTG	38829
5204	CATGAAGTGGCCACCCTGTTTG	16862	CCAAAAGGCATGGGGTAGGGATT	27846	CACCGGCTCTGCCAAAACAT	38830
5205	GCCCTCAGCTTCCTGAGCTA	16863	TCCCCTGGCTGCTCTGAGAAAT	27847	TGTCTCGGCCCTCAGCTT	38831
5206	CTCTGCTTGCTCTCTGTGCTT	16864	ATGCGCTTGGTCCCTTCCTT	27848	CCAACCTCATAGCCTGACACTTTC	38832
5207	TGGTGAAGAGGTCCCCTAAATTCT	16865	TGGGCCATCCACAGCTTTC	27849	CCTGCTTCTTTGCCCAGATTTTTG	38833
5208	TGGCTGTCCTCCAAATTGGTTATC	16866	GGCCGGAAGGAGACCAGACTA	27850	CCAATGAATGGCTGTCCTCCAA	38834
5209	ACTGGAGGCAGCACCTGGATT	16867	TTTTGCCCCAGCTGGACTCT	27851	GGTTGGGTGACTTTCCCAGACT	38835
5210	CTGGAGAGCTTTTCTCTACTGGAGCTA	16868	TGGCTGGCATCAGCCAGATTTC	27852	CAGCTTCTGGAGAGCTTTTCTCT	38836
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5213	GCTCCTTGCACTCCCATCTT	16871	CACAGTGTTGATGGCATTGTCTCT	27855	TGATGCCTGCACAGCTCCTT	38839
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5215	GACCAACCCAACTCTCCCCATT	16873	TGAGCCAGCCCAGAGTTCAGT	27857	CCAGTGACCCAGGGACCAA	38841
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5217	GATGCAGGAGTGCCTGTGGAAT	16875	TGCCCTGCTCAGCCTCATT	27859	TGGGCAAGGGCATGTGGTAA	38843
5218	ACGGTGGCTGCTTCTCCTTT	16876	GCAACAGACACCAGCACATC	27860	GGCCAGGAAGCTTGAGGAAGAT	38844
5219	AGGCTCGGGGACACTCAA	16877	GGACTAAAAGAGAGATCCAGGCTAAC	27861	CTGCAAAGCACTTTTGGGAACAT	38845
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5221	TGCCCACCTCCCCGCTTTCT	16879	TGCCGTCAGCCCTGGAAAAA	27863	CACTTCAAAGCCCTCGTCCAT	38847
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5223	TCGTTGACCCTGCCATGAGT	16881	GAGGAGGGCCAGAGCAATATC	27865	CCTCTTGATCCTTTGGACTCGTTGA	38849
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5240	CAGTGTCCCAGGCCCTTGTAG	16898	TCCTCTTGCTGCAGTGTGATT	27882	AGAGAGAGCTGAGGGGTACAGT	38866
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5242	GGCTTACGGGTAAGAACAGGACAAA	16900	CTGTGTTAGCAGGGATCAGGTCTA	27884	GGAAGAGTTGTGGGAGGCTTAC	38868
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5246	CCAGATAACCCTCACAATAGGTTTCT	16904	GGAGGAGGCTGGTGTTCATAAG	27888	TGGTGACCAGATAACCCTCACA	38872
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5250	CCAAAGTGGTTTGGGGCATTTC	16908	GTCCCAGTGGCATGAGAAAGTTG	27892	GGGGTCAGAATCAAGTTCCAAAGT	38876
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5255	AAGCCCTGGATGTGGAGTCA	16913	ACCCCTCCCCTCCTGAAATCT	27897	TCCCATGAACACAGTATGGTAGAAG	38881
5256	GAGCCCCACACTGGACACT	16914	CACCTGATCCGGCCAGCTT	27898	CCTTGGGTCTCGCAGACTTC	38882
5257	GCAGGTAGCAATACAAATCCAGTTCA	16915	TTCTGCACCCTACCCTGTCT	27899	GCTTGTCTACATTGCAGGTAGCAATACAA	38883
5258	GGCCATTATGTGCTGGGGATTG	16916	CTCCCACCCCACACACATTTCA	27900	TGGCCTGCCACACAATGACT	38884
5259	TTCAGCCGGTGGTCCTGACA	16917	AGCGGGAAGCTCCTACTCAA	27901	TGTGCAGAAAGGAGACGCAAA	38885
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5262	CTGGCCAAATCCCACCCAAA	16920	AGGACAACCAGAGGTCACTCTT	27904	GCGGTAAAGAAGCTGGCCAAATC	38888

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5265	CCTGGGAAAGACGCTACCTTGT	16923	GGATGAACAGCAGTCTCAGTGGTT	27907	CTCAAACCAGCCTGGGAAAGA	38891
5266	ATGGGCTTGTGGTCCTCTCT	16924	GAGGCTGCTCTCCCAGTCA	27908	TCAGGGAAGGGATGGGCTTGT	38892
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5273	CTCCAGGAAAGGGCTCTGGTAAG	16931	AGCCAACAGCCTACCCAAAC	27915	CCTGAGAACATGGCTCCAGGAAAG	38899
5274	GGCTCAAACAATCCTTGTGTCTCA	16932	ACACACCTGTGGTCCAAGTATC	27916	GCTCACTGCAGTCTCAACTTTTG	38900
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5276	CCTTTATGTCTGCAGGTGTCAATC	16934	AGGCCATCAGCCTGGGAAGA	27918	CCTGTGAAATAACAGCTGACAATCCTT	38902
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5279	GGAACCACTACACCAAAGTTGTCCTA	16937	ACTGCAGCGCCTGTAAGTTG	27921	GGGATCGGCCTACTGAAGGAA	38905
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5283	GACCATCACACCAGATTGCTTTGT	16941	GCACTGGACGACTGCTGGTTT	27925	TCAGGGCTACCTGACCATCACA	38909
5284	GCCCAGAGAAGCCTTTTAGCATTTACA	16942	GGCTCCTGGCTTTTGGCTTCT	27926	CCTCTCTCCTAACAGCCCAGAGA	38910
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5288	CAGCTTTTGCCAGGAGCTCAA	16946	CCCATCCACAGGTCACTGAGGTA	27930	ACTGGAAGGAGGGAACAGCTT	38914
5289	GCTGTAAGGACTGTGCCATGAGA	16947	GGAGGGCCACTGTTTACCGAAT	27931	AGCCCTGCTGTAAGGACTGT	38915
5290	GAAGACAGCCCCAAGAAGCATTT	16948	ACACTGCCTAGCACATGAAAGA	27932	AATGCAGAAAGTCATGGTGTGAAG	38916
5291	CTGCTCTTCCCCATCATTCACT	16949	CTGGTCAGGCAAGGAGAAAGT	27933	CACCAATTGCCACTACTGCTCTTC	38917
5292	CCTGCTGCAAACCTGTGTGAGA	16950	GCCAAGTGGAAACAGGATATGAGA	27934	GCTTGGGTTATGCCTGCTGCAA	38918
5293	TTTTGGGAGGCGGGTTCTGT	16951	CCCTGCAGCTCACACAGCAAT	27935	CTGCACTCAGGACTTGGAGTTTTG	38919
5294	ACTCTGGACAGCAGCACAAG	16952	GGCATACACCCACACCATCCAA	27936	GGCTCTAAAACGCAGGGCTGTT	38920
5295	CAGGGAGCCAGAATCTCTGAAG	16953	CCCATACCAACTCCATCTCCTATG	27937	GACTAACAACAGGGAGCCAGAA	38921
5296	GCTGAACGGAAGGACAGACA	16954	GCGAACACCCGTCCTTCAT	27938	ACAAGGCCCCTCCTGCTGAA	38922
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5300	GGCACCCCAGAAAAGTCTAGTT	16958	CGCGCACACATGCACACA	27942	GGGAGTGGCACCCCAGAAA	38926
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5476 GACTTCCAACCTGGACTTAGACAT	17134 TCCACATGCCTCAAAAAGACACA	28118 CCCTCAGCTCACAAGAACCTGACT	39102
5477 CTCGCAAAATCCTCAGATCCTTTGT	17135 GGATGTGTTTGTGAGCCGAACAAG	28119 AGGACCCCTTTCCCTAGAGTTC	39103
5478 GCAGACCATGGGGCTCTACTTAC	17136 ACCCCTCGGAGGACGTGTCA	28120 AGGACACCAGGAGCAGACCAT	39104
5479 CTCCCACCACATAAGCAAAGACT	17137 CCCAACACTGTCACCCATCAT	28121 TGTGGAACCTCCCACCACAT	39105
5480 CACTTGCAGCCGACTTTGGTA	17138 GAGGACAAGGGAATGCACGTA	28122 CACAGGCTGGCAGTTAAAACAGA	39106
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5482 GCAAAGGTCTTCATGGGCAAGTCT	17140 GCTTGGCAGTAGGACAGACCAAA	28124 CCCAGGCTCTAGCAAAGGTCTTC	39108
5483 GACCAGAGCTGGCAAATTCTACA	17141 AACTGGCCTCACTCTGGAAAG	28125 GGTTACAACAGCTTGTAGGTCCTTGA	39109
5484 GCCTGGAATGTGATCCATCAATGTT	17142 GTTCCCTTTCTCCTCTACCTAGCAT	28126 AAAAAGAGGGCCTGGAATGTGA	39110
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5486 GGCTACTCCTCCCAGTCTGTTGT	17144 AGGAAGGGGATGAGCAGGAA	28128 CCTCTGTGGAGGATAGAATGGCTACT	39112
5487 CCACCCTGTTGGATTTACTTTCTTCA	17145 TGAGGACTTCCCCTACCACTCT	28129 GCCTTCCACCCTGTTGGATTT	39113
5488 GGAATCATGTGGGGTGGAATCT	17146 TCAGGTCCACCAGTGTGAAAC	28130 CCTGCAAACTGCAACAGGGAATC	39114
5489 AGTGAGGGCCTTACACAGTGA	17147 GGGATGTCGAGGTGCTAGGTT	28131 CTGGATGCTAATGTGCAGGAGTGA	39115
5490 GAGGAGAGACAGCAGTGTTTGTAA	17148 GGGACCATCCCTGACTCTTACATT	28132 GGGAAGAGACCTAGAGGAGAGACA	39116
5491 GCTGCTGTGATTGGGGAGTTG	17149 ACCCGGCCATCTTTGGTACA	28133 GGGATTCACTGGCTGCTGTGAT	39117
5492 CTCCCCTGGTACTCTGGAATTGA	17150 GCTATAGAGCCGTCTGCATCCAA	28134 GGCCTTACTCCCCTGGTACT	39118
5493 GTGCAGGTAGAAGAACTGATTTCTCA	17151 CCATGGTGCCAGAAGGTCTTGA	28135 GCCAAATTCTGTGCAGGTAGAAG	39119
5494 CCGCTGTGTTGCTTGCTTTCT	17152 GGCTGGACACGTTCAAGCTTAC	28136 TCACTTCCACCGCTGTGTTG	39120
5495 ACTGTCAAACGGCTGTGCTT	17153 AGGTAGATGGCAAAATCTGTGGAA	28137 CCATCAGTGTAGTTCCTCTAACTGTCAAAC	39121
5496 AGTCAAGCAGGAGGACTTGGAT	17154 GATGGAGCCTGAGGCATTAAACA	28138 GGCCAGAAGACTGCAAAAGTCA	39122
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5521 AGGCTGGTGGCTGGAATCATCT	17179 GCACCAACTAGCTGACCTGTGA	28163 GGCATCAGCAGGGGTGATTTGA	39147
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5544	TGCACCTAGCCCAGTGTGTT	17202 GTAATCCCAGCAGATCACACTTTCTT	28186 AGTTCCGGGCTTTGCACCTA 39170
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5604	GCCACCTACCCACCCACTCA	17262	GAGGCATAATCCCAAATCTCTCTGT	28246	AGGCAGTGAGAAGCCACCTA	39230
5605	GGCAATGCGAATGAGTTCCTACA	17263	GTGTTATGTGTCAAGCCTTCTGTTC	28247	GGAACAAACTGTGGCAATGCGAATG	39231
5606	CACCACCAAAAGGCTGATAAAGTGAA	17264	GTCTGTGAGAGAAACATTCCGTGAATCAAT	28248	GCTATGGAACAAAAAGACACCACCAA	39232
5607	CCTGAGGCTCCATGCTTTTAATAACT	17265	CCTGGTTCACATGCTGTGGTGTA	28249	TCCATCCTGAGGCTCCATGCTT	39233
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5629	CTGCCTGCTATTTCCCAACTGT	17287	CCCGGCCACAATGGCATCT	28271	CCGCTGGAACTGCCTGCTATTT	39255
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5652	CAAGGCTTCCTTTTCAGTCCTAGT	17310	AGGATAGAGAGGGGCCAAATGT	28294	CCCTCACCAATTCAAGGCTTCCTTT	39278

5653	CCGTCTCACTGCTGTCTTCTGT	17311	AGGGAAGGACTCCCAGGGAAAA	28295	GGCCAAGATGCCGTCTCACT	39279
5654	AAGCCGAACGAAATAGGAAGGAA	17312	GAAAGTGCGGATGGAATGGAATTG	28296	CATCTTCTCAAAGCCGAACGAAA	39280
5655	GGGCAGCAGCAAAGAAACTTG	17313	CTGTTGAGATGAAAAGGGAGTGGAT	28297	AAGCGAAGGGCAGCAGCAA	39281
5656	GCCTAATGAGATTTAAGGCAGGGACTCA	17314	CTTTGGGCAGACCTAGAGCAA	28298	CCCCTTCATCCTCATGCCTAATG	39282
5657	GGGGATATCCTGTGTGTGGTTGA	17315	GGAAGCCACAGACCCTCAAAT	28299	CCAAGCCATGGAAGTTTGGGGATA	39283
5658	GTGGGATAACACCTTTCCCCAAA	17316	GCTAGAACATCATACATCGAGGCATAC	28300	GGATTTGGACTCTCTTAAAAGTGGGATAAC	39284
5659	GCCCATGGATGGCAGCAAA	17317	GCCTGAGCTGCATTCAGACATAGT	28301	AGGACAGTGGCCCATGGAT	39285
5660	GCAAGATTCATGCCGCAGACA	17318	GCCTGGGGAATTATAAGGAAGGGATT	28302	CCACAGCCTGGCAAGATTCA	39286
5661	GTCGTTGCTGTCTCTGCTTTAGT	17319	ACTAACCCTCCCCTCCAGTGT	28303	GGTCCCTTGTCTGGTGTCGTT	39287
5662	GCAGTGATTCTCACACCCTACTGT	17320	CGCAAGTCTGCATTCCAACAA	28304	CACAGGGAGCAGTGATTCTCACA	39288
5663	GGAAACGTAGGAAGTTCTCTAGTGTGA	17321	CTGGGAATCTAGTTAGAGAGGCTGTA	28305	TTGGTGAGAGCTGGGAGGAA	39289
5664	CCCATCACTGCCTCATGTGTTC	17322	CGACTTCACCATCTTCCTTCGTCTCT	28306	GGAAAGCAGTGCCCATCACT	39290
5665	GCCATTCCAATTTATGGGCAGTCA	17323	CCATTCAGAGCTCTTGACTCAATGATAC	28307	CTGAGTCTTGTTGGGAGCCATT	39291
5666	AGGAGCTGGCATAAAGTTGAACA	17324	GAGCAGAGAAGACACCTCATATAGCTT	28308	CTTCTCTGAAAAGGAGCTGGCATA	39292
5667	GCGAGGGAGGATGAGAAATAGGTTGT	17325	TAGTGGCGAGGAGGCGAGTT	28309	GGTGTTGATAGCGAGGGAGGATGA	39293
5668	GGTCAGCCTGTGTTCAGGATCT	17326	GAGTCTGAAGCCCACAACTATTTTCA	28310	AAGGTGGGTCAGCCTGTGT	39294
5669	GTGGGTGCCAGACATGTAGATCAAG	17327	GCAAGCAGCTGGCAGTTGAA	28311	CCCTGTGGGTGCCAGACAT	39295
5670	GCAAACTTCAGGGGAAGATCACT	17328	TGCCTGGTTACCTCCCATTTAC	28312	CAGGCACCAAGAGCAAACTTC	39296
5671	CTGGTACTTACCCTGTGCCAGAAG	17329	GCACAGACCAACCTTGGTAGTAAC	28313	GGGTACAGGTGACAATTAGCCTGGTA	39297
5672	CTGTAACACCTGGGAAACCTGATGTAT	17330	TCAGAGGGTGAAGAGGAAGTTTTC	28314	AGAAAGTGGCAAATGGCTGTAAC	39298
5673	AGTGCTTTCTCAGTATCTCCCAAAAG	17331	GTGATGGGCTGATGGTACCTCTT	28315	CTCCTAGTCCAGTGCTTTCTCAGT	39299
5674	CCCCATTTGTAAGCCGGGAACA	17332	TGATCCCAGGACAAACACGATT	28316	GGCCGTAAATAAACCTCCCCATTTG	39300
5675	GAAGTCAGAACGCTGCTTGTTC	17333	CCACACCACGGTCTTGTCTTAG	28317	TGCAGACTCAGTATGAAGTCAGAAC	39301
5676	ACCTGTCTTTGAGCTGTGCAT	17334	TGAGGCACTCCCGCCTTTGA	28318	ACCCTGGGACAAACCTGTCT	39302
5677	GTCTGCTTTGAACCGAGTCTTTG	17335	CTGAGAGGACATTTGATGGGTCTAA	28319	CCCTTACACAGTGTCTGCTTTGA	39303
5678	GGCTCAAACAATCCCACTGCCTTAC	17336	GTGGCGGTGAATGCCTGTAATC	28320	CCTTCACCTGTAGGGCTCAAAC	39304
5679	GCTTGAATCTCCCACTGACATCAA	17337	CAAGTATCTGGACAAGCTGATAAGGAA	28321	CATGGGCTTTTGTGGCTTGAATC	39305
5680	CTCCCCGCATGTGCATGACTAA	17338	GCCAGTGGATTCTGCGTCAA	28322	AGATGTGTCTCCCCGCATGT	39306
5681	CACACTGACCCTTGCTTCTTCA	17339	GCATTCCAGGATGGGAAAACCATA	28323	CACCAGGCTTCAAACACACTGA	39307
5682	GTCACCATGTAATTTCCCAATGGTT	17340	GTCTTTCCAAAGTTTCATCCACACATC	28324	GTGGCCCTAAAGTCACCATGT	39308
5683	GCAGCACAGGCTTATTTCTTGACA	17341	CCCCACATACTCTCTGTGTTATTGAGATG	28325	GGGAATATGGCAGCACAGGCTTA	39309
5684	GAGTGGATGAACCCAGCAAGTTC	17342	CCAGTGTGACATGACTAGGATGTTG	28326	TTTAGTAGTAGCTCAGAGTGGATGAAC	39310
5685	GAAAAGCTCTCACTTTCCCTGATCT	17343	TCTGACACTGAGTTGGGAAGGAA	28327	ACAGGCTCTGCTCCACTAGA	39311
5686	GCTGATGCCTGATCTTGAGCCCTAA	17344	CTGGTTCTGCTAACATTGGTGTCAAC	28328	GCAAGGCTGATGCCTGATCT	39312
5687	GTTGTGTGATGCTCACAGACTGA	17345	GGGGCAAGGGCTGCAAAAGTAA	28329	TCACATGGGCATGTTGTGTGAT	39313
5688	TGGGGAAAGAACCACCCAAAAG	17346	GGTGCCTACCCTATGTGATTTCTGA	28330	TACCCGAGGCTGGGGAAAGAA	39314
5689	CCTTTATGGGCACCAACTGATTTC	17347	ACTGCCCTGTGACACTGGAA	28331	GGGCTGGTCTAGATGCTTCCTT	39315
5690	CTGAGGTTCTATGCCTAAAGAAACCAA	17348	GAGCTAAAGTCTGAGGAGGTTCTGTTTTTA	28332	GTGACTTCCTGAGGTTCTATGCCTAA	39316
5691	GCTACCAAAATGGGGCTAGGAAGA	17349	TGGAATGGTGTAGCCCAGTGA	28333	GGCCAGCGTAGAGCTACCAA	39317
5692	GCAACTGTGAACTCAGGAGCAAGT	17350	GACCTGCTCAATGCTTCTGGTAAG	28334	TGTCTGGTATGCAACTGTGAACT	39318
5693	GGGGATTGCTGATTGGTTGGATTG	17351	GGGGACAGCTTTGATTCCCTATG	28335	TGGGCTAGGGAATGGGGATTG	39319
5694	CAGCCAGCACTAAACGTAGAGAAAG	17352	CACAGCGTAGGCAATGATTCTTC	28336	GCAGAAAAGAGTAAACAGCCAGCACTA	39320
5695	GGAAAGAGAAATAATGGGGAAGCATGTGAT	17353	GCCTGGTGGAACTGGCTATTGT	28337	TCAGTGTGGACATGGAAAGAGAAA	39321
5696	AGGGAAGCGTACTGAGTGCTAT	17354	CTGTATCCCACCAGAGTTGGTTAG	28338	GGCTTCAAGAAAGGGAAGCGTACT	39322
5697	TGGAAACAGAGGCAGCTAAATCA	17355	TCTGCCCCTTCTAGCTCTGCTT	28339	CTCCAGCTCCTGCTTTGGAA	39323
5698	CCTCTCTCCCCTCGGCTCTA	17356	CCCCAGCTCTTCTCTTCCTAGAT	28340	CCTTTCCCTTGAGTCCCTCTCT	39324
5699	GCTCGTAGTGTGAACTTGGGAACT	17357	CCACTTAGTCCTTTCAGCAGCCAAAT	28341	CACCACTGGCTCGTAGTGTGAA	39325
5700	CAGGCTTGGACTAGAAGTACACCAT	17358	GCAGTTGGCAAGCTGGAGACT	28342	CTGGACTTCAGGCTTGGACTAGA	39326
5701	GCAGGTTGCTTGGGTTGACA	17359	GAAAGAGACACACAACAAGGCTATG	28343	TGGAACAAGGCAGGTTGCTT	39327
5702	CTGAGTTTTCAAAGGCTACACGTTACTTC	17360	GGGTGGAGTGAAGCCAATTCCAA	28344	GCAACCGGTCGAATATGCTCTGA	39328
5703	GCTGTCCCCATACTAGTGACTCAT	17361	AGTGGGTGTGCAGTTTGCTT	28345	GGACAGAAGAGCTGTCCCCATACT	39329
5704	AGCTACGTGGACCCCACTGTT	17362	GGTGGTAGAGCAGAGTCCAGTGT	28346	GGCTGTGCTTGTCTTCAGCTA	39330
5705	GCCTTTCTCGGCCTTTGGAATC	17363	CTTCAAAAGTTATGCTGTGGTGGTAGA	28347	CACAATGTTCCGAGTTGCCTTTC	39331
5706	CCATGGACTAAAGGGTTATAGGGTTCTA	17364	GCTGTCTTTGTGGTCTAGATTGCTTT	28348	CCCACCACCATGGACTAAAGGGTTA	39332
5707	CTGGTAACCTACTAAGGCTTGTTTTG	17365	TTTCCTCTGGCCCTGACCAT	28349	CCCATGTATCTCTTCTGGTAACCTACTAAG	39333
5708	CCCTGCACACCCTCTCAGT	17366	GGGAAACAAAGTCAATCCTCCATTTC	28350	GAGTTCCCCTGCCGACTTG	39334
5709	TCCCTTAAGTCCCGGAGCAT	17367	GGGGAGTTAATCAGATTTGCTTTGAGA	28351	GGCCCTCAGAGATCTGTATTCCCTTA	39335
5710	TTGCTACTGGGCTCTGGTGAGA	17368	GTGGTGAGACTTGGAAAGGGGTTT	28352	TGGGCGAGCACTTGCTACT	39336
5711	TGATGTCCAGACAGAGTGTTCAGA	17369	GTGGTTAATGGAAAACAGGGCATAG	28353	GGGGACATGTTTGATGTCCAGACAGA	39337
5712	CCCTGTGTTTTTGGAAGACACCATGT	17370	CACCTGGATATTTCAGAAGCCCTTGA	28354	GCTTTTACTCCCTGTGTTTTTGGAA	39338
5713	GGCCTGAGTTCTAGCTTTGGTGTTG	17371	CCTAAGTCACTCCACAGGACACA	28355	GGGTCTAGACAGGCCTGAGTTCTA	39339
5714	GGTGGGATGGAGGATTCTCTTTGT	17372	CTGAGCTAGGTAGCCTGCTCTAAA	28356	AGAAAGAGGTGGGATGGAGGAT	39340
5715	GTGTCCCAGAACGAGCACTT	17373	GGACTACTTGCGTTTTGATTGCTA	28357	GAGTGTCAGTTGTGTCCCAGAA	39341
5716	CGGGACCAGTAGTCTTGTTTTAATCACATA	17374	GAAGTCACGCATTGAATCAGAGTAG	28358	GGCGGGACCAGTAGTCTTGTTT	39342
5717	GCTCTCCCTAGACATGGCGACTA	17375	CTCTTTGAGCTTGGAGTCTCTAATACA	28359	GTGCACATGCTCTCCCTAGACA	39343

5718	GGGGATGTAGGGTGGAAAGAAAC	17376	GTGTTCAGCCTCTGCCTATTTTTC	28360	CCGTAAACCAAGTGGGGATGTAG	39344
5719	CTGTGCTGGTAAGAGAATTAGCTGGAAT	17377	GGGCCTTTTTACATGTTCCAGAAG	28361	CCTCAGTCTGTGCTGGTAAGAGA	39345
5720	GTTGCCTCCTTTGACCCTGAA	17378	GTGAGTCATGCCAAGATGAGATTG	28362	CTCAACATGGAATTAAGTTGCCTCCTT	39346
5721	TCTGGGCCATTCGGTAACATC	17379	CAGTACACGGAAGCTGATGTAAGA	28363	ACTCTTATCTAGGATTCTGGGCCATT	39347
5722	GCATAATTGGGCCTAGTGGGTTATG	17380	GAGAACAGCACCAAGCCTCTCA	28364	TTGGTCCCCGTTGTTGCAT	39348
5723	CCTGAGGAAAGAATCCCTGAGCTTT	17381	GGACTGAGACAGGGGTTTTAACTGA	28365	GACTGGCACTCCTGAGGAAAGA	39349
5724	GAGGGAGATGACCTCCACTGA	17382	AGACACACGGGGCCTTTG	28366	CCTGGGGAGGAGGGAGATGA	39350
5725	GCAGTTTTGCACCAACCTAATGCTT	17383	GGAGCATTTTGACGTGGGTGATT	28367	CAGAACAGCAGTTTTGCACCAA	39351
5726	CTGGCTCTGCAGTTTGGAGAAAAG	17384	TGGTTTGGGTTCTGAATGACACA	28368	GGATGCACTGGCTCTGCAGTTT	39352
5727	CCAGATGGGCTGACACAGTCAT	17385	CTGTCTTAGGAATAAGGCGAGGTTGAAG	28369	CTCTTGGATTCACGGCCAGATG	39353
5728	CACTGTGGAGTTTTTCCTGGTTGTAA	17386	GTGCCACTACAGCTGGAAAGGAT	28370	GGACATGATTGGTTTCACTGTGGAGTT	39354
5729	TGGGAAGTCCTGGCATGTAAC	17387	CACCAAGGCCAATCCCTTCA	28371	ACCTATCCTTGACGTGGGAAGT	39355
5730	GCAGCGACAATGAAACCCAGTTC	17388	GTGGGTGAGCTACTTTTCTCAGGAT	28372	CCTGCAGCAGCGACAATGAA	39356
5731	TGCAGTGTTGATGTCACATGCTAT	17389	CCCATTGTAGACCATTTGCTCTCT	28373	CCTAAAGGGAAAAATGCAGTGTTGA	39357
5732	CAGGGTTCTGCAATGTACCACAA	17390	GGCCCTGCCATTCAAAATCACCTT	28374	GGCCTAAGTCAGGGTTCTGCAAT	39358
5733	TCCACTTCCACTGGCTGACA	17391	CCATACCATGCAGGCTTACTCAAG	28375	AGTGCTCACCCCTTCCACTT	39359
5734	CACTGCATTCCCACTCATATGCTTCT	17392	CACCATGGCTACTGACCCATT	28376	CATAACTACACTGCATTCCCACTCA	39360
5735	TGTGGGGAAATTTGCCTATACACT	17393	TTGGGGCTCGGAAGGAACAT	28377	GGAACTCAGCTTTTCATTGTGGGGAAA	39361
5736	GGGTAAACAAGGCTAAGAAGCAAAG	17394	GGGAGTGTGACTGTAGAGAATGTTAG	28378	AAACCTGCTGACAGGGTAAACA	39362
5737	GGAAACACCTCAACCCGCATT	17395	GGCAGAGCAGGAGACTAGGAACA	28379	CCCGTGCTTCTAACAGATTGGAAA	39363
5738	CATCCTCAGACTGGGACTTCTGT	17396	TGGCTGAGGGGTCAGTTTTATG	28380	CCAAGCACCAAGGACATCCTCAGA	39364
5739	TGGTGCTTAAGAGTAGGCTGATTC	17397	CTGTGAACACAGGAAGTCCAAGA	28381	TTGGGTTGAGATCCAGCAGATG	39365
5740	TGGTTCTGAGCAAATTGTGGTACT	17398	CAACGCCAGACCTTTCTTATTTGT	28382	GGTGCTTACATGGTTCTGAGCAA	39366
5741	GCTAGAGTGCTAAGCTTTCTGTCA	17399	GTTCTTAGGATCCTGTCCTGCTTTAC	28383	CTGGGATAAGAGCTAGAGTGCTAAG	39367
5742	GGCATTACCAGCTACTGAGATTTTAGA	17400	CTGCCCTGGTAAATATCCAACTGA	28384	GACTTAAGAAGGCATTACCAGCTACTGA	39368
5743	CCACACCAAGAATGGTTATCAGTGTGAA	17401	GTGGGACTAGAGAACATATCTGTGAGA	28385	CTGGCCACACCAAGAATGGTT	39369
5744	AACCCAGCATATGAGTCGAATGAA	17402	TGTTTTGCCTGACATCTTGAGTGA	28386	CCAGCTGCCAGAGGTTTCACAT	39370
5745	AGGGGATGACCAACCCTGAT	17403	TCTTGTTGCTCTTGAAGGCATTG	28387	GGTTGGAATATGAATGTGGAGGGGATGA	39371
5746	GGCTGTTCTTCAACAAGAAGTGAATG	17404	GTTGCTTTGCATTCCAAGCCTTTC	28388	ATACCAAAGGCTGTTCTTCAACAAG	39372
5747	CCAGGATTAGAAAGTTCAACCCAAGT	17405	GGAGGGAAAATGCACTGAGACA	28389	TCCCACTCACTTTCCAGGATTAGA	39373
5748	CCGCCAAGTCAGTTCAGACAGA	17406	CCGATGGGATGTGGAGGTGAT	28390	ATGCATCCCGCCAAGTCAGT	39374
5749	GCTCCTCTCTTTGTGAGGCAAA	17407	TCTGGTCTTAGGCTGGAGATTAGT	28391	TGCCATTTTTGCTCCTCTCTTTG	39375
5750	CGTGTCTGCATTCTCCCTTCA	17408	GGTGAGTGCCATGTTGTTTACTGT	28392	CACTGCATCCACTCTCGTGTCT	39376
5751	CCCACATGCAACCCAGTTCTTC	17409	TGTGCCAGCCTTCTGTATCTTG	28393	TCCTTGAAGCCCACATGCAA	39377
5752	GCCTTTCTGACAAACTCCCAGATA	17410	GATCCTTCCCACTCGAGGAAAC	28394	TGAGGACCGAGATTTTGCCTTT	39378
5753	AGAGATACCGATACTCTGTCCATCAA	17411	AGGATCCCAATCCTAGCTGTCA	28395	GCATTAGTACTGCAGAGATACCGATAC	39379
5754	TCTGTGTTGCTCACTACTGCTT	17412	TGGTTGGGAGACACACGGAGAT	28396	ATGGTCTGGCTGCCTGTTCT	39380
5755	CTCTTCAGCTCAACCCTCCCTTTT	17413	TGGGCAGAGTGCTTGGAAAG	28397	AGGCCCAGCTTAGCTCTTCA	39381
5756	CCAAAGTGAACCTAAAGCCTTACTCCAA	17414	ACCAACCAGTAAAGTCCAGTAACCTA	28398	TCTGGGACCAAAGTGAACCTAAAG	39382
5757	GGCCCTTCCTTTAGAGCTTTTCA	17415	AGCTCCCCAGGCAATTCAGGTA	28399	GGTTCAGAAAATGGCCCTTCCTTTAG	39383
5758	GGCTGCAGCATAACTGGCTTTTG	17416	GTGCCCTTTTCCCTGTCCAGAA	28400	TGCACAGGCTGCAGCATAAC	39384
5759	GGCTGAGGTCATTAAGAGCTAGTGT	17417	AGGCCTCCAGGACAGGGAAA	28401	CCACCCAGGCTGAGGTCATTAAG	39385
5760	CGGAGATAGGAGCACACTCTTG	17418	GGTGCAAATAGGGTGTGATTAGAAGGAA	28402	CGGGACCTTGATTTCGGAGATAG	39386
5761	CCCTCTCTTCTTCATCAGCACTCTTT	17419	CAGAAGCTTGATGACTTCCCAAGAAC	28403	GGGAGTAGACACATACCCTCTCTTC	39387
5762	CAGAGAACTCTGAAGCTGGCTAGT	17420	GAGAAACCAAAACCCAGGTCTGT	28404	CCTCTGGATTAGGTAATGAGGCAGAGAA	39388
5763	TCCAGGTCTTCTCAAGGCATCTA	17421	GTGCCCTAACTTGGTGCTTCTCT	28405	GCTAAGCATGTCAGAATTCCAGGTCTT	39389
5764	GCCAAGTCGGTGGCGAGAA	17422	GGAGGTGTGTCTGGCTTGCAT	28406	AGCCGTCATCCAGCCAAGT	39390
5765	GTGTGTGGGCTTCGCTACAGAAA	17423	CCAACCGGACACCTCTCATACA	28407	CCCAGTTTCCAGGGGTGTGT	39391
5766	CCTCTGTTTGCGCAGAGATCACA	17424	CTCCTGGGGTCCCTTTTTCTCT	28408	GACTAAGTGTGTTGCCTCTGTTTG	39392
5767	GACAGGAGTGTTAAAGGTTCCAAATAG	17425	GGCCTTTATTTGTTCCTGGTCAGT	28409	GGCATAGGTTTGACAGGAGTGTTA	39393
5768	CACTGGCTCTCTGAGACTCTTAAATC	17426	GACTTAAGTTTAAAACCCAGGCTGTTC	28410	GAGAATTATACATCACTGGCTCTCTGA	39394
5769	GTGGCTCTCAAACCAGAGACCAA	17427	CCAGTGGAGATGCCTGCAAATCA	28411	AGCATGCATGTGGCTCTCAAA	39395
5770	CTGCCTACTCCCCACATCTTTAC	17428	GGAGCCCTATGGGCCATCAA	28412	ACAAGCCCCATATCTGCCTACT	39396
5771	GCTTGTTCCACTCCCGTCACA	17429	GGGCTGGAACCAATGGCTAGT	28413	GCCTTCCCATCTGTGAGCTTGTTC	39397
5772	CATTGCAAAGAGTTCCAGTGAACA	17430	GGAAACCACTTGGAATCTGGTAGT	28414	CTGCAGTCAGACATTGCAAAGAGTT	39398
5773	GGCATGGGACTTCACTCTGACA	17431	GGGAGCAGAAAGAGGAGATCTTAG	28415	GGGTGAGGGCATGGGACTTC	39399
5774	GCTCTTCCAAAACATTGCCACATATC	17432	CCTGGTTTTCAGGGAGGGAAGA	28416	GGTCCCACATGGCTCTTCCAAA	39400
5775	GGACTGCAGGATACCTGTTGCTT	17433	TCGCTGAGGGCTGTCTCT	28417	GGAGCTAAAGGGACTGCAGGATAC	39401
5776	CCACTAGAAATTGGATTGACCCAACA	17434	TGGCGGGGAAGACTAACGTTTC	28418	TGCCAACAACCACTAGAAATTGGAT	39402
5777	TCGATGCCAACTCTATCCATGAAA	17435	GCTTCCTACCTTCAGTAGTATCAGTAGT	28419	CTGATATCCAAATCGATGCCAACTCT	39403
5778	GGACTGGCTACAAAGACTGCCTAAT	17436	TGGGGATCACAAGGACGAAAG	28420	GCGTGATTAGGACTGGCTACA	39404
5779	GAGATGGATGGATTCTACCCCTAAAGTAAG	17437	AGCCCTCACCCCATTTCTGGAT	28421	GGAGGAGAGGAGAGATGGATGGATTC	39405
5780	GCCTCTAGACTCACTGGTCCAA	17438	CCCACTGCTAACACTGACATAGAA	28422	TTCCCCAAGATGCCTCTAGACT	39406
5781	CCGGACTTGATGTCAGATTCAAGCTA	17439	GCAAGCTCAGAGGGCTTATTCAAG	28423	TGGATTCAGCCGGACTTGATG	39407
5782	GACTTCGCACAAGTTTTCCTTCAT	17440	GGAAGGAAATGATAGAGAGAGGCATTG	28424	CTCTCAGTGACTTCGCACAAGTT	39408

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5784	GCCAGTGGGAACAATCTGGAGAA	17442	GCTTTCTATCAGGCTCCCAGTT	28426	AGTAGAGAGCCAGTGGGAACA	39410
5785	GGCTGGAGCATGTTCCTTCAATCT	17443	GGCTGGGCCTCTACCTTTTGAA	28427	ACGTGTTGGCTGGAGCATGT	39411
5786	CACCCAACTTTTGTTTGGAGAGCATT	17444	GCTCGGGTTAAAGTTTCACTGAATATG	28428	GGCAACATTTTCCAACACCCAACT	39412
5787	CTGTCCACCGTACAGGATCGTT	17445	CGGTCCTATCCATTAGAAGGGAGTCT	28429	ACTCGGGCGTGGAACTGT	39413
5788	GTGCTAGTGAGAGTGCTGGTTT	17446	GTGCTGAAAAGTCTGTGCCTAAG	28430	AGGGGTACCCATTCTGCATGT	39414
5789	CCTGGCCCAAGGTGAGAATGTAG	17447	GGTGTGCCCTGCTTGGATACT	28431	TCTCCAGCTGGGTGTCTCTATC	39415
5790	CTGGGGCTTTAGTCTTTTGCCATA	17448	CTGCATGCATACTTTAGGCACATATC	28432	AATTCCCTCATCTGGGGCTTTAG	39416
5791	CCCAAACACCTACAGAAAAAGAATCGAACT	17449	AGGTAGGTTGAGGCCAGATTGT	28433	GAGGTCTCCCAAACACCTACAGA	39417
5792	AGCTGCTAGAGTGCCGATGT	17450	CCCATTCAGCCACACGCATTAC	28434	AGCACTCAATAAACTCAGCTGCTA	39418
5793	ACCATACCCCAGCAAAAGAAACA	17451	CAAGGTGGTTTAATCTTCACTGGATGT	28435	CAGTTAGGACCACAATCAAACCATAC	39419
5794	CTCGGCCCACTTAATTCTAGATGTTC	17452	TGGTAGGCATAGCGGCAGTT	28436	GTCCTCTCGGCCCACTTAATTC	39420
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5796	TGGGGATTCCACAAACTTTGGTT	17454	TGCCTTCATCCTCCCTGTCTCT	28438	ACCAGATGGCATTGGGGATTC	39422
5797	ACACCATCTCCTGGAAGTAACAGT	17455	GGGGCATGTTGCCTCAGTTTC	28439	CCCCTGCTCCAAACACACCAT	39423
5798	GCCCTTTGCTGGCTGACACA	17456	CCATGATCTCCAAAAGACCTTGCTT	28440	TTTGTGGTTGCCGCCCTTTG	39424
5799	CTGCTTTTGTGGATTCAGGGTGACT	17457	TGAATGCCATTACCTCTCTGTGTT	28441	CAAGGATAACTTTCCACTCTGCTTTTG	39425
5800	GCCATCTGCCATATCTCAGAGAGTCAA	17458	GGACAGCAATTTCTGGGTTAAAGGAT	28442	CGGAGAGCCATCTGCCATATCT	39426
5801	AAGGGAATTCCTAGATGCCAAACTT	17459	GCTTCTGTACCAGGAGTGGCTTAC	28443	CGCCTCTTAAGCCAAGGGAAT	39427
5802	CGCCGAGTGATCTTAAAAGCAGACAA	17460	TTCCCCTCCAATCTCAAGGTTTAC	28444	CAAATCCCGCCGAGTGATCTTA	39428
5803	AGAATCCTGGGGTATGTTAGTGATTG	17461	GCAAGTAAAGGGCTCAGTGGCTAT	28445	TTTCCTAGATAGAATCCTGGGGTATGT	39429
5804	CGAGGCTAACCAAGGTCAGATAATA	17462	AGATCTAGGCCCTCTGGTTTCA	28446	AAGACACAACGAGGCTAACCAA	39430
5805	CCAGCTTGCATCAAGAGGACTGA	17463	TCCCTGAGCTTCCCCTCCAA	28447	GTGAAGCATTTCCAGCTTGCATCA	39431
5806	CCTGATGATGGCAGAGTCTGAAG	17464	CCCCAGATTTGTACCACAGAAATCA	28448	TTGACAGCGGCTTCCTGATG	39432
5807	CCATCCTCTACTTTAGGGTGACTTTAACA	17465	CCTGTAGGAGAAAAGCAGTCAGT	28449	GGATCAGGTTTGCCATCCTCTACT	39433
5808	GAACTGCTCTCTGGCTTCCTT	17466	CAGCTGAATGCATGAGGGTGACA	28450	GCTGCTTTTCAGAACTGCTCTCT	39434
5809	GAAGCAGTTCTGGTCTCAAGATGATA	17467	CTCTTTGTGAGCCAAGCTGCTAAA	28451	AGGGCACTCCAGAAGCAGTT	39435
5810	CGGCGAAGCACTAAAAATGCACTA	17468	CAGCAGGAGAAATTCTTGCCTAACA	28452	GAGCATCGGCGAAGCACTAA	39436
5811	CCTTAGCTTAGATATTACGCTCCAGCATT	17469	GGTTATTGGCAATTGCTGTGGAT	28453	TCCCAGCTGCTCCTTAGCTT	39437
5812	CACAGAGTCCTGAAAAGCAGGAA	17470	CGGACTAATACACTCAGACAGACTTC	28454	TTGGCCAATGGAATCACAGAGT	39438
5813	GAGTCCAAGCAGATCATGTGTGT	17471	CCTGTTCCCTCTTGCAGTGCTT	28455	GGACAGCAAAGAGTCCAAGCAGAT	39439
5814	GCGAGAACGACCATTCAAAATCA	17472	CCCTGTAAGCACTCATCACACGTT	28456	TTCCCAGCGAGAACGACCAT	39440
5815	GCCTATGTCACTTCTCTCCCTTGAAC	17473	CCCCAATACACAGACTCTCCATGTCA	28457	CAGACTTCAATTGCCTATGTCACTTCTCT	39441
5816	GGCCAGAAATGGTCTTGCAGCTT	17474	AGCTAAGCAGAGCTGGGGATTC	28458	TGGGACTGTGAGGCCAGAAATG	39442
5817	GCATTAGAGTTGCTACCGCTTTC	17475	CTGCTTACAAATTACTGGGCTAGTTGATCT	28459	TCTAAGTCCTGTCTTGCATTAGAGTTG	39443
5818	GGCAATAGGGCACTCACAAAC	17476	GCCTCCTGTAAGGATGTTGGAA	28460	ACCGGGACACCCATAGGCAATA	39444
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5820	CAGTGTGCTGGTTCTGGTTGAGT	17478	CAAGTGAAGACTTGGTTGCTGACA	28462	GTGGTGTCTACAGTGTGCTGGTT	39446
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5988 GGGGTGAGAAGGGAGTATGTAGTAG	17646 AATCACCTGTGGCACTTGTTTAAG	28630 TGAATCCAAATCTTGGGGTGAGAAG 39614
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28813 CACCCTCTTACCAGCAGCAACA
28814 ACTACATCCTTCAGTGGCAACATAC

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6174	TGGCAAGAGCTGACATTTACCAA	17832	CTCATCCATTGATTCCGTTCAGTAAAG	28816	GATGGAGAGAGTGATTGGCAAGA	39800
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6176	ACCAGGAACCTTCTTGGGCATA	17834	CTGCTCAGATGCCATAAATGTTTGAGA	28818	CCTGGACCTTTCTTAATACCAGGAACCTT	39802
6177	CACCATGCAGGGGTTTATTACTGA	17835	CTGCAGCATGGCTTTCCATTTC	28819	CTCATTGTCCACAGATTGATCACCAT	39803
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6179	CCTTGCTTGAAATTTCCCGTATAGATG	17837	AAGGGCCGATCCGGAAACTT	28821	GGGTGTCATTTTCCTTGCTTGAA	39805
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6181	GGGCTGGAAGAGGCTGCAAA	17839	AAGCTGGTGGTGGTCACTGT	28823	AGAACCCATGGGCTGGAAGA	39807
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6185	GGTGCCACTGACTCCAAGGAAA	17843	AGTAAGTAGAGGAGTAATGGAGTGTGA	28827	TTCCCAAGGTGCCACTGACT	39811
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6187	GATGGTGCCTTGTTGCTGAATC	17845	CCACTTGTGAGGACACAGCATTC	28829	CTGTCTCGAAGATGGTGCCTTGT	39813
6188	CTCTCTGCTTGTGAGTGGTGACA	17846	GAGAACCAGAGCCTGTGTCTTCATAG	28830	CCTCAGTGTGCTCTCTGCTTGT	39814
6189	CCCCACACCAAGTATTTTTGCTCTT	17847	TGGACTGGGAAAAGAAGACACTAGA	28831	CCTCAAACTACCCCACACCAAGT	39815
6190	GGATAGGTCAGTTAATTGGGATTCAGA	17848	GGAGGACAACTTGTTGTTTGTGGAAT	28832	GAGCTGGACTTGGATAGGTCAGTT	39816
6191	TTGCAGGAGCCCCTTCCAAT	17849	GGGAGCCTTGTGGACCAGAAAT	28833	AGCTAGTTTGCTCCTACGTCTTG	39817
6192	GGGTGCCAGTTCTGCTGCAT	17850	GGCCTAGAGTTGCAACCTTACGAAT	28834	GTGGCTTGGGTGCCAGTT	39818
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6203	TGGAGAAGGGCCAACATCAGT	17861	CGCATGGAGATTGTGCTGCTAAC	28845	CTGACATGTGGCTGGCATTCT	39829
6204	GCAGGTTTTTGGTTGCCTTGA	17862	AAACCCACTCCAACAGCCTTT	28846	GGCACAGCAATATGCAGGTTTT	39830
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6223	GAGGTCAGTTGGACCCTTCAGA	17881	ATCAGTGGGTCATTTTGTGGTACT	28865	GCCAGGTATGTGAAGAGGTCAGT	39849
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6225	GAAATGTCCTTTCCTTTCTGCACTT	17883	GGCCAATAACTCAGCTTTGCAAGAAG	28867	GTCTCACTTACAGTGGAAATGTCCTT	39851
6226	GGATTCCTGGGCTACTCCATTTTTC	17884	CACATTTCAACAGGTGCCTCTTATCCTT	28868	TCCAAAGGATTCCTGGGCTACT	39852
6227	GCCCTGCCACTCAGCTTTCTATC	17885	GCCTCTTTATCTAGAGTCAGCCAGATG	28869	ACTGGAAGCCCTGCCACTCA	39853
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6233	GCTCTCTGGGAAGACAGAGTTC	17891	GTCCTCTCCACATGAAGTAGTAAATAGTTG	28875	CGTAATAATGCCCTGATGCTCTCT	39859
6234	GGGTGCTGTATTGGAATCCTTAGT	17892	GCCAACAGCAGATCTAATGGGATGTA	28876	GTGGGAAAGAAGGGTGCTGTATTG	39860
6235	GGGGTTGAAGAGAAAGGTTAGGACAAG	17893	GTCACATATAGGCTGCCCATTCTCTAA	28877	GTTTAGTAGCTTGGGGTTGAAGAGA	39861
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6237	GCAAATGTGACCCTAGGCAGAAAC	17895	CCCCTTCTTAGCTTGATCATCTGCTTT	28879	ACCCCAGTGCCTGCAAATGT	39863

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6250	GCTGCTGATGAAAGAATCACATCATCT	17908	GGGGTAAGAGCTCCTCTGCAT	28892	TCCGTCCTTTGCTGCTGATG	39876
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17984 TGAAGGCCCCAGCCACATTAC
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6370	GCATTGGGTGCACGTTGAGA	18028	TCCTTTGCAACTCTGAGCTTTGT	29012	GCACTGTGCGAGGCATTG	39996
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6378	GGCTATGGCTTCACTCCATCTTTC	18036	GACTGTTTGTTCAGCCTAAGTCCTAGA	29020	GCAGGTTGGCTATGGCTTCACT	40004
6379	GTTGAAGTTCCCTGCATTTCACA	18037	GGCTGACCTCTCTCCTGTGTT	29021	GTTTGGGACAAGCTGAGTTGAAG	40005
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6385	TGTTTGTCATCAGTCTGGAATGCTT	18043	TCACCCATTATCTTGGACCCTTTTC	29027	GGGGAGGGTGTTTGTCATCAGT	40011
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6387	CTGAACCAACTAAAAACAGGTGGTGTTG	18045	GGGATATGAATTGCCTTCTTCCCACATA	29029	AGCAGCCTGAACCAACTAAAAAC	40013
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6389	CCCTGCTTTTCCTAAGGGCAGATTT	18047	GGAGAAGCAGTTTCCAAGACATGA	29031	AGTGGGATCCCTGCTTTTCCTA	40015
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6411	GGGAAGTTCAAATTGCTGAGCTTGTAG	18069	CCCAGCCACCTCCTAATGGACTA	29053	CCCCAAAGCTAAGCATGGGAAGT	40037
6412	CCAACCAGCTATGCTACCATTTCTAC	18070	GTGGAAAGTCCTCAAGCCCTCTTAG	29054	CCACTGCTTAGACCAACCAGCTATG	40038
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6422	CCTGGAATGGTTTTATCTGACAGGTT	18080	CGCCCAGCCAGACTTATTCTCA	29064	CTCCAACTTCATCCTGGAATGGTT	40048
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6504	CCACACTCTCAAGATCCTGTTGGTTA	18162	GAGGCTCTGCAGAATGTTCCTAA	29146	AGGCTGTCACCACACTCTCAAG	40130
6505	GTGTCAGACATGGCGGCAAAT	18163	GCGGCTGCACAATTAGAGTAGAGA	29147	GTTCCCCAGGAGGTGTCAGA	40131
6506	GTGAGTGCCTCTATCTGGAAAGGAT	18164	CTCAAAGGCCCAGGTCTATCTGATG	29148	TCACTGGAGTGAGTGCCTCTAT	40132
6507	GTTGGATGCCTAATGATTTCCCTTCA	18165	TCCTCTCCTCAAACAAGGGATATTTTG	29149	GCCAAAGCAGCACTGTAAGTTG	40133
6508	GGTGGGATGCTTTACGCAGAGA	18166	GGGAGACTGCATGTATTTCTGTCTGT	29150	GGAGCCAGGTGGGATGCTTT	40134
6509	GGGGTTCTGTTTTCTCCCATTCTTC	18167	GTGATGGGCCTGAATCCAGTATTATG	29151	CCATGACCTGTGGGGTTCTGTT	40135
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6511	GAAGAACACCATTGGATCACAGAAC	18169	CCTGGGCTTGTGGCCATATT	29153	GGAGAAACAGAGAAGAACACCATTG	40137
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6513	CTCCATCCTCTCTCAGAACCTCAGTT	18171	CTCATCTGGTCCAAGCTTCTGT	29155	CTGGGACTCCATCCTCTCTCA	40139
6514	GAGACGCTCTCCCATATCCAGTCTA	18172	GGCATGTCACACACTCCTACAT	29156	CACGGAGACGCTCTCCCATA	40140
6515	CCGTGGCACAGAAAGGAGATGT	18173	GGTTCAAATCCTGGCTCCACTATC	29157	AGAGAAAACCGTGGCACAGAA	40141
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6517	GGAGAGTAGGGATTTTTACCTTTTGCATTC	18175	TCCGTTTTCAGGGCTGGGATA	29159	AGCACAAGGAGAGTAGGGATTTTTAC	40143
6518	GTTTCACGAACCTAAATGTGGTGAT	18176	GGAGGCGAGAGTCCTTTCCTTTTG	29160	GTTTCCAGGAGTTTCACGAACCTAA	40144
6519	CCCAGCTCCATGGCAAACAT	18177	CCCTCCTGGTGTCTTCCAGAAA	29161	AAGACTGTGGCCCAGCTCCAT	40145
6520	AGGGCCAGGTAGCTTACTGAAG	18178	GACTCTGCTTTCACGGATGGGATT	29162	AAGTGGTGGAAGGGCCAGGTA	40146
6521	CCGCAGTGTCCACCTCAGT	18179	AGGCTGAGGACTAGGCTCTGAT	29163	AACCTCACAACCCGCAGTGT	40147
6522	ACAGGTACAGCCATACTCAAAAAGTAG	18180	TCCCTGGTACACCTTTACACAAATC	29164	ACAGAGAACAGGTACAGCCATACT	40148
6523	GCCACTGGGAGATGTGTCATAAAC	18181	ACAGCTAGGCCCTGGGAAGAT	29165	TCAGCTAGCCACTGGGAGATGT	40149
6524	AAGAGAGCTTGGGTAGACAGTTTC	18182	GGAGGAGGTGGTGGACACAAAT	29166	CGTGTGCTAATGAGGAAAAGAGAGCTT	40150
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6530	GAGGACTATATGTGGGAGTGCTTGA	18188	GGGAACCCATTTCCGTGTGTTG	29172	TGATCTGATCTTGGATGTGAGGACTA	40156
6531	GTTGCCTCTTTAATGCTCTGCAAAT	18189	GCACTCAGAGGAGGGGTGTGTTAT	29173	GTCCCTAACACCATGTTGCCTCTT	40157
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6534	CAGATCCCTCTGCTGGTACAGA	18192	AGCACTGGTCTGGAAGTGAGA	29176	TGGGGTAGTGAAGGATTGATTCAGA	40160
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6556	GCACCTACCAGTTGTCAGAAACA	18214	GCCATTGCCTTTTGCCTCAGTA	29198	CCACTAGATAACAATAGCACCTACCAGTTG	40182
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6563	GGGGCTGTTAGGTGGCACAA	18221 GGGTCTGCATGATATGCTGGTA	29205 CACCTCTATGTGGGGCTGTTAG 40189
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6568	GGGTAGAGGGACTGGTCTACCAAA	18226 ACTGACCCCAGGAGTTTCATCT	29210 AGAGGCAGGGTTTGGGGTAGA 40194
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6672	GTCATCCCTCATCAAGGGCAGAA	18330	AGGATGAAGGCAGAGAGGACAT	29314	CAACCCTGGTTGCTCTGTCA	40298
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6675	CCAAAGTGTCACCAGCAGAGACAT	18333	CCTTCCCATATTGGGTCAGTGTCTGTA	29317	ACCTCCAGGATGCAAGCCAAAG	40301
6676	CTGGGGATGAATCTGCTTTACCAT	18334	CCAGCATCCCTACCTTCGAGAAAC	29318	GGGGACATCTGGGGATGAATCT	40302
6677	CAGCGATAACTTCCTTCCACTCAA	18335	GCAAGACAAAGTCCACCCCACTTT	29319	AGGCTCCAACAGCGATAACTTC	40303
6678	GGACCAGCCTCTACTTGCTACTGT	18336	CCCCATGTTTGCAGTGGAGTTC	29320	TCCCAGGACCAGCCTCTACT	40304
6679	TGTAACCTAGATGACCATGCGTTT	18337	CGAGGAAGCTCTCCATCTCATTAGT	29321	GCCCTGATTGCTGTAACCTAGATG	40305
6680	GCCAGCTTTTTGCTATTTCCTTCCAT	18338	GCAAGCATCTGTACTTCCTCCAT	29322	GACTTCAGAGCCAGCTTTTTGCTA	40306
6681	AGGGGTTAGGCGGAAAAAGTATG	18339	CCTGTTTCTGCATCCTTGGTCAT	29323	CCATGATAGGAGTTTTGATAGGGGTTAG	40307
6682	GGAGAGACAATCAACTATGTCTCTTGTGA	18340	GGACCAAGGTTCAGCAGCCTAT	29324	GGACTAATAGGATGGAGAGACAATCAACT	40308
6683	CCTCTTTTCCCTACTTCCCACTTC	18341	GGCTTGGGAGCATTTGCAGTT	29325	TCATGTCATCCCTCTTTTCCCTACT	40309
6684	CACTTGGCCATAAAGCAATTCTCAAC	18342	CTGTGATTCGTATGACTTTGGGCTTT	29326	TCAACCACACACTTGGCCATAA	40310
6685	GACGGCAGCAGAGGATTTTGA	18343	GAGCTGCTCAGGCTGCTTTA	29327	TCCCGGTATTCGTCGCTAGA	40311
6686	GAATGCTGCCAAAGGGAACTGA	18344	CCAGCTTTGATTCCACACTGATTT	29328	GCTCAGGTGAATGCTGCCAAA	40312
6687	GCAGAGGAAGTTAGAGGTGGGATATT	18345	GAAGCAGCTGAGGTTCTACCAA	29329	GCTGGGCTTGCTGTGAAAAAG	40313
6688	GTGGACTTTCAGCGCCAACT	18346	AAGCAAACACCGTATCACTCTGA	29330	GACTGGGTGTGTGGACTTTCA	40314
6689	CTCCTAACTCACGTCCGGGTAT	18347	AGGAGCCGGTCCTCACTGTAG	29331	TGCCAAGGTCATCCTCCTAACT	40315
6690	CCAAGGGGACATTTAACGCTGTGA	18348	GTGGATCCCCTCTTCCTGAGTCAT	29332	ACATTCTGCCAAGGGGACATTTA	40316
6691	GACTTTAGAACTCCTGCTACCAACATT	18349	GACCTGATTGTCCTGGTGGAGGTA	29333	GGCTAACAGGAAACCTCTAAGACTTTA	40317
6692	GGGAGTGGTAAAAGGCTTGGACTTC	18350	GGCCCAATCAGATGCTTTGGTCAT	29334	GCAGGGCTGGGAGTGGTAAAA	40318

6693	CAAGGTGTCAGTCTGCCTTGT	18351	CACCCTTCTACCTTGCCATTAGTAT	29335	GGAGAATGTCCTCACAAGGTGTCAGT	40319
6694	TGCCTGCAGAAGGGGATGTGA	18352	CCAAACAGTGTCGGGTGTTGA	29336	TGGGGAACACTGCCTGCAGAA	40320
6695	AGGGGAGGAACATGAGGCTGAA	18353	GCCAAACGTGCTGAGTCTTG	29337	CCCAGGGTAAGGGGAGGAACAT	40321
6696	GGTGGCTTCAGGAATAGGGGACAA	18354	GGAAGGGGAGGAAAAAGGGAAA	29338	CAGTGGGTGGCTTCAGGAAT	40322
6697	GGTTCTAGGATCTGTCTGGGAGATCAA	18355	AGATACGAGGCTCGACATTTTACTAAG	29339	GCCCTGTGGTTCTAGGATCTGTCT	40323
6698	ACTGCTCCTAAGATAGGCGATTTC	18356	CAGCTGGACGCTTGATTAGTCT	29340	GACCTGATAGCAAAACTGCTCCTA	40324
6699	CACCAAGCATACCTGAGTCATACCTA	18357	CAGGCAGGGCGTGATATGT	29341	CCCATACGTGCACCAAGCATAC	40325
6700	AGCTTCCATCCGTCCCATCTAT	18358	CCCTGCATCGGTGGCAAAAT	29342	CAGGGAAATCCCAGCTTCCAT	40326
6701	CGATAGCTACCCTTGCTTTTTCCTCTT	18359	GGCCAGCTTTGAGTGTTACAGAAC	29343	GAAACCGATAGCTACCCTTGCTT	40327
6702	CCTGCTCTAGGCTGCAAGTGTT	18360	CCAGCAAACACACCAAGGAAGT	29344	ACCCTCGGCTCCTGCTCTA	40328
6703	CTGGACTCCTGCTGATAGAGTCTTG	18361	CAGGGATCAATAAGGCACTTGGAGTA	29345	CCCCTGGACTCCTGCTGAT	40329
6704	GCCCTTGATCCTCCTAGGCTATTG	18362	GAAGAAACAGAGAGCAACTGAGACTT	29346	CCCTGCAAATACTGCCCTTGA	40330
6705	AGTGGCTGAGAATGAGACCTCTA	18363	TGCCCTTAAACCATGGCAGATG	29347	GGGTTGCCAGTGGCTGAGAAT	40331
6706	CCTGTTGGTCCAAATCCTTACTGA	18364	GAGCAGTTTGGAAGAAGTCCTATCAT	29348	TGCAGTGAGGGTTCCTGTTG	40332
6707	GACTGTGGTTTCAACACCTTATACCTA	18365	GTGAAAGATTTACCCCTTATGGTCCTA	29349	TGCCTGCTTGACTGTGGTTTC	40333
6708	CCTTCTGGCTAAATCTACCTGCTCTTC	18366	GGCTGAGGCAGTCAGAGTGT	29350	CAGGTCTCTCCTTCTGGCTAAATC	40334
6709	CCATGTGAGGTAGATAGGATTTGCTCTAGT	18367	GGGACCTCTAGGTTTCAGCTTCT	29351	CTGCATCATAACCATGTGAGGTAGA	40335
6710	CTCACAGGCCAGGGATTTCAGA	18368	AGTGGGAACTTCAGAGCTAGACTT	29352	CGGCAGCCTTCATTCTCACA	40336
6711	GAGCTTGAGATCCTTTCTGAAGAAGTT	18369	CGAATTCCTTGTCCTTGTAGCCTTTC	29353	CAGCTTGAGCTTGAGATCCTTTCT	40337
6712	TGTATTTGGGTGCATGAGTTGGTT	18370	CTGAGATAGGTCATCTCTTGAAGACAGAGT	29354	GGGGAAACTGCACCTTGTATTTG	40338
6713	GGGTCCCTAATTTTGGTGATGACT	18371	GCTAGCAGAGCTCAGGAAAGTTG	29355	TGCTGCTGATGGGTCCCTAA	40339
6714	GCAGCCATTGTCTGTAGGACTTGAT	18372	ACACATGACTGTAGCCAATTCCTT	29356	ACTTCAAAGCAGCCATTGTCTGTA	40340
6715	GCAAGCTCATTTGCAAGGTAGAGAAC	18373	TGCTCTTAGAAACCAAACCAACTCT	29357	CTGTTTCATGGTTGCAAGCTCATTTG	40341
6716	CCTTCTTGAGTCTGATCACTCGTT	18374	AGGAGACATGGGACAGGTATGTT	29358	GGGGTCTCCTTCTTGAGTCTGA	40342
6717	GGGATTTCCCACACTTCTCCTCTT	18375	CCAGGGCTACACAATAGAGGTTTCT	29359	CCTGGAGGGATTTCCCACACT	40343
6718	CCACCTCGAAGCTTTAGTACCCTAAG	18376	GGAAGGAAGAGAGAAGCCTCAGTATC	29360	GTACTCATCCACCTCGAAGCTTTA	40344
6719	GACCATCCACAGACTTAAAGAGGAA	18377	GCTGAGGACGGCTGCTTGATA	29361	GCCTGTGATGGCTTTCTGTTGA	40345
6720	GCTCCAGCCTAGATACAACACTTG	18378	CACATGACCCGTTCTGGGTAAC	29362	GCAGTTCAGTGCTCCAGCCTAGAT	40346
6721	GACAGCCAGCTAACACTGTGGAA	18379	GACCCTTGAAGGAATGTAGGCAGTA	29363	GCCTCAACAGACAGCCAGCTAA	40347
6722	CCACGAAACAGGACCATGACA	18380	GCATACATGCTTCACTGCACTTC	29364	AATGGTTGAGTCCATCCACGAA	40348
6723	ACCCAGACACGGCTTGACTCT	18381	CCCCTTTGTCATTGAGGAAAGAGTTT	29365	ACTGAATTCCACCACCCAGACA	40349
6724	CCACCGTCTATGTCAACGCCATT	18382	GAAGGTCACTCTGGCTTCATTGT	29366	GACATGAGGACCACCGTCTATG	40350
6725	TGGAGGCAGAGGTTTAGAGAGAA	18383	CCTGGGTTAATTCCCTTCCCACTCT	29367	TGGAGAAATGGAGGCAGAGGTT	40351
6726	CTGTCACCTCTTTCGTGGTACAGT	18384	CACGTTCATCATAGCTTGTCAACACT	29368	GGAACTGGATGCTGTCACCTCTTTC	40352
6727	GAGGCGAGATAGAAAAGTAGTTGGTGAA	18385	GGTTCCTTCCCATGGCTACACT	29369	GCTCACACTACTGAGGCGAGATAG	40353
6728	GTGGTACTGAAACCCCAGTTTAGCTT	18386	GAGGAGTTCCCAGTAATGCAACTAT	29370	CTGCTGGTGGATGTGGTACTGA	40354
6729	TGCCTTAGTGCTGGCCTCTCT	18387	CCACTTAGAGTGTTGCTTTTAACTCCCATT	29371	GGGAAGGGCTTATCTGCCTTAG	40355
6730	ACCAAAGCCGTGTATTCGTTTATTC	18388	GTTTACACCCTCAGTCTACACAAGAA	29372	AACCCAACCAAAGCCGTGTA	40356
6731	GGACCAGGTTTCTAGCAGTGAGA	18389	GATTTCACGGCAGAGGTGTGT	29373	CCCTGTCTGTAGGACCAGGTTTCT	40357
6732	CACACAGCCATGGACATTCAGA	18390	ACTAGCTGGAATGGTGGTAAAGAATAC	29374	GGACAGCAAAATACGCCAATATCACACA	40358
6733	GATGTAATGCTCAAGAGGCTGAGA	18391	TCTTCTGACAGCAGTGCTACTCTA	29375	ACCCCAGTAAAGATGTAATGCTCAAG	40359
6734	CACCAGAAGCAGTTTGTCTTCAGT	18392	CCCTGAGTTAGTTCAGTGAAGGTGTATTG	29376	TGGTAAAGATCACCAGAAGCAGTT	40360
6735	GCAGTCACACCAGGCTCAAATC	18393	CAGGTTACTACTGCTGGTGTGCTT	29377	AAGGCCTGGGCAGTCACA	40361
6736	CTGGCTGCAAATATGTGCCTGTTAC	18394	CTGGATGAATGGCTGTACACACAGA	29378	TGGCAGCTGGCTGCAAAT	40362
6737	CCCAAAGTCATCAATTCTTCCTGAGA	18395	ACTGCTGTTTGGGGATAAGCAT	29379	GGGCTCCTCCCAAAGTCATCAA	40363
6738	CCCTACCTGCATCTCCTCAAAC	18396	GCAAAACATGGGTCTAAGCCTGTA	29380	GCATTTTACTAACCCCTACCTGCAT	40364
6739	AGAGTGGCAGCAGTGGGAATAG	18397	GGCTTTCCAAACACGTCTGGAGTT	29381	GGCCCCGTTGGTGATATGATGAGA	40365
6740	CGGTTTTACCCTGAAAAGAAACCAAAC	18398	CTCCCTGCCTGTGCCTCTATTT	29382	GGCCTAGAATAACGGTTTTACCCTGAA	40366
6741	ATCTGAGAAGTAGCACTAGGGAGAA	18399	CCAATTTAGTCTGTCAAGGTCCCTTTC	29383	GTGAGTCACTGGCCAATCTGA	40367
6742	GCCTTGACAAACAATGTGAGCAAT	18400	GGGATGGGGAAGAAGGGCTTACTA	29384	CACGAGAAAGCCTTGACAAACAA	40368
6743	GTTTGCCTCTTGGGCTCATTG	18401	TGGACCTGGAGGCAGTAACTTG	29385	GGCCTGACAATGTTTGCCTCTT	40369
6744	CAGAGGGTCTGGTAAACTCTGGTA	18402	TGAGGCTGAACCCATCAGAAGA	29386	GCAAGTTGTTCAGAGGGTCTGGTA	40370
6745	CCTGTCTTTGAATGGCTCTCCTCAGA	18403	TCCAGACCTCGTGCTGCTT	29387	CACCGGGATCATTGTCCTGTCT	40371
6746	GTGGGAGAATAGTGGTTCTGCCTAT	18404	CGTTCTTCAAAAAGCAGACTGGACCAA	29388	GAGAAGGAATGACTGTGGGAGAATAG	40372
6747	CACCCATGCTAGGGACTCTTGA	18405	GGTTTTCACCAATGCCCCTTCA	29389	TGTGTCTCCTCACCCATGCTA	40373
6748	CAGCAGAAACAGTGGGATGTAGT	18406	CTCGCTTAAGATCTCACTTTGAGTAAC	29390	GGTGTGGCAGCAGAAACAGT	40374
6749	ACCTGTGCATCTGCCTCTGTTG	18407	GTCTGTGGGTGATGAGGACCAT	29391	GCTGTCAACACCTGTGCATCT	40375
6750	GCCTGCTTACATCCCACTCAAAAG	18408	GGGAGCTGGGATCTGAGTAAGACT	29392	TGAAACTTCACAGCCTGCTTACAT	40376
6751	TCTGAGTCTCTCCTTTCAGGACAA	18409	GGCATTTCTAGACCCACCTTGTATC	29393	GTGGATGATGTCATTTCTGAGTCTCT	40377
6752	CCCTTCACTCATTCCTGGGGTTAG	18410	CGCTCCCAGGAACCAAGGTATT	29394	GGGCCTCTCCCTTCACTCATT	40378
6753	GCATGGCAAAGACCACTTCCTT	18411	GTGGTAGGAGTAAGGAATTTGCAGAAAAC	29395	GGCTTACACTGCATGGCAAAG	40379
6754	GGCAGGGTGTAGGGAGAAAAG	18412	TCTAGCTCATCCCCAATGTTCTTTC	29396	TGGGCTTGGCAGGGTGTAG	40380
6755	CTGGCTGTTGATGAGGAAAAGGGTAT	18413	CCCGGGATTTGTTCCCTAAACA	29397	AGCTGGCTGGCTGTTGATGA	40381
6756	CACAGGGTGTCAGACTTTAGGGAGTA	18414	GCCTGGGGTAATGAATTCTGCTGAT	29398	ACCCACAGGGTGTCAGACTT	40382
6757	GTGAAAGGAGGTGCTGTTAGTAATTG	18415	GCCTCAGACCCTGGCACTCA	29399	TCGGGGCCCTAGATAGTGAAAG	40383

6758	GTGCCATATTGACCATCTTCCTTTTAC	18416	CCCAACCTTAACCAAAGGAAGCAA	29400	GCTGTCCCAGTGCCATATTGACCAT	40384
6759	CCCACCTTCAGGGTGTGTGATG	18417	AGCCCCTCACCGGAAGTCTTATC	29401	TGGAAACCCCACCCACCTTCA	40385
6760	GGTGGTGGAAATGTTCCGATGA	18418	CCTAGGTGGAGTCTTCATTCTCTCA	29402	CCAAAGGAGTAATGGTGGTGGAA	40386
6761	GTCCTGGACCATGGGCAGAT	18419	GTCTGTGAATCTCACACTCCTCACA	29403	TGGCCAGCTCAGGGTTGA	40387
6762	CTGTTTCACTTGCACTGTGTGT	18420	CAGTTAGGGCTGCTTGTCTTAGTCT	29404	TTGTGAGGACAGCAGTCTGTTT	40388
6763	CCGTAGACTGTCCAGAGAGATGACA	18421	GACTCTGTCACAATCAAGAGAAGGAATG	29405	TGGGGCTTTCCGTAGACTGT	40389
6764	CCAAAGCACGAAACGCCTTGT	18422	GGCTCCCCAACTATCATGTATGTCAAATG	29406	AGCAAGGGCCAAAGCACGAA	40390
6765	TCCATGCTACAAGGGCAGAGT	18423	GACTTTGCAAGCCATACAGCTTCT	29407	CAGCCACACTTACTTCCATGCTACA	40391
6766	GGACACAGAATGAACCACTGACTGA	18424	GGGCTCACACAAAGCTGACTTC	29408	GGGGAGGGAGGACACAGAA	40392
6767	GACAGCTTAGACAGAAACCTCTTTTG	18425	CCCCTGGAGCTGCTAATTTGA	29409	CACCTGGAAATTGACAGCTTAGACAGA	40393
6768	GCCGAAGATCTCTGCATTTCACA	18426	GGAAAGGAGAGAAATTGAAGGCATTTG	29410	GGAAGAGAGCACCTGGAGTTTC	40394
6769	TGCCGTGGAACGGTCTGTTT	18427	TCAACTGAGATTCAGAGCCCTAAGA	29411	TAAGTCCTCCTGCCGTGGAA	40395
6770	GCAGAGACAGAAGGCAGGGTAAGAAA	18428	GCAGGAGATCCCCTTTACCCAGTT	29412	AGGGTGCTGGCAGAGACAGA	40396
6771	GGGATGCAACTATCTCCTTCCCATT	18429	GCCAAGTTCCAGACACCATCACA	29413	CACTCTTTCTTGGGATGCAACTATC	40397
6772	GTGTGTGGATGAAAACTCTGGTCTCTTA	18430	ACATCATGGATAGGCTAGAGGTTATGA	29414	TGGGTGGAAGTGTGTGGATGA	40398
6773	GCATCTGGCATGCACTCTTCACTTAG	18431	ACAAGGCAGGAGGAGCATTAC	29415	AAGGGGCAGGACAGCATCT	40399
6774	CTCCAGACCTCTTGAATTGACTGA	18432	GCTGCTCCAAGCACAGACCTA	29416	GGAAGGAAGCATCTCCAGACCTCTT	40400
6775	GTTCCAGTAGAGCTGGATACATACAA	18433	CCTCTCCACCCTTCTGTACTCT	29417	CCATGGAGGATTCATGTTCCAGTAGA	40401
6776	CTGGGCCATTTACCATATGTCAGGAA	18434	TGGACAAATTCCAAGTGCTCACA	29418	CCTGCTTAACCCTGGGCCATTTAC	40402
6777	CCATGTGGCAGGTGAGGATGT	18435	TGGAGCAGGATTCCGGTAGGTT	29419	AAGGTCAACTTTCACCTCCATGT	40403
6778	AGGAAGAGCGTATGTAGAGAGCAT	18436	CCCAGTTCATTTCCTCGTGTTCT	29420	GTGAGGAAGAAGGAAGAGCGTATGT	40404
6779	CCCAGAATTCCCCTGCTGAAGA	18437	GCATGGGTACTGACCCGTTTCT	29421	GGAAGGGAAGCCTTTCCCAGAAT	40405
6780	GCTGTGGTTTCAGAGTCTCACCCTAA	18438	CCCTGGAACTCGAGAGATGCTT	29422	GGCTGGCTGTGGTTTCAGAGT	40406
6781	CACTCCAATGCCTCCTGTGGTT	18439	CCTTGGATCCTGGAACTGTGCTT	29423	TGCTCACCCTCTCACTCCAATG	40407
6782	CCCTGTGAGACTTCATCCCCTTCA	18440	GAGGAAAGGATTACATTCCGGAGAA	29424	CCCAACCCCTGTGAGACTTCA	40408
6783	GGGGAAGTTTTTGAAGGCAAAACAA	18441	GCCAAAGATAACGGATTCAAGGCAACT	29425	AGGTTCAGGCAAGGGGAAGT	40409
6784	AGGGGATTAAGAGGCAAAGGAATG	18442	CAGGGACTGGGCAGAAAACA	29426	TTCTGTCAGCAGAAGGGGATTAAG	40410
6785	CTGGGTTCAAGTTTTGGCTCTTC	18443	CCCAGACAGCTGCGGCTAATAA	29427	CCCAAGTCTTTTCCTTCTGGGTTCAAG	40411
6786	AGTGCCTTCGTAAGCGATTCA	18444	GGGTCTTCATTTTCTGCCGTACCTATAAAC	29428	GTATTTCAGTTCCTAGTGCCTTCGTA	40412
6787	CTGTAGACTTTGGCTTATTCGACCTT	18445	CAGCACAGAGTTTTATCCTCCTCTAC	29429	GCTCCATCTCTGTAGACTTTGGCTTA	40413
6788	GCTGGTCCTCTGGTTTTGTAACCCTATT	18446	CCCCTTACTCACCTGGCCAAAG	29430	CTTTGCTGGTCCTCTGGTTTTG	40414
6789	CCATGGTCAGCATGATATCCTCAA	18447	TGCACCAGTTCCTCCGTGAGA	29431	CGGGGCAGTGTACAGGAGTA	40415
6790	AGGCTCGCCCTCTCCTCTT	18448	GGAGCCACTGGTCAGAAGACA	29432	TCCTCTGCCCTCCCTGCTA	40416
6791	GCTCTCTAGAAGCGCTTTCCCAAA	18449	GAAAGCAGACCCTGAGACCAA	29433	TCCCTGCACTGCTGCTCTCTA	40417
6792	CTTTCAATTCCAGAGCTTGTCCCATA	18450	ACAGAGATCATAGGTGGCATCCTT	29434	AAGCAAGAGTGGCTCTTTCAATTC	40418
6793	CGAGAACTACGGTTTCAAAGGAAAGAAG	18451	GCTTCCACCTCCACATTCCTGACT	29435	CCACTCTCGAGAACTACGGTTTC	40419
6794	CGGCAAATGCAGCACAAAATC	18452	GAATAAAATGCATCCACGCTCACT	29436	TGCCCAGATACTCGGCAAATG	40420
6795	CCTGGTTCAAACGGGAGCGTAT	18453	AATACGCCCCAGCCCTTGTCA	29437	GGTTGGCTGAAAAACCTGGTTCAA	40421
6796	GGATGCTGTCGATGTCACTCT	18454	CTGTGTACCAAGTGGATGGATGA	29438	TGGCTTAGGGCCCAGAGGAT	40422
6797	CTCCACCATAGGGAGTGACTTTG	18455	GGGCAAATTTAGCCACCCATGAAAG	29439	GCAAAAGCTGTCAACTCCACCATAG	40423
6798	TGTTCCCACCTCGCCACAAA	18456	GCGTGAATGCGTGGGAAACA	29440	TGCCACTGATGGCCACTGTTC	40424
6799	GGAGTCTTTTTCAAAGCGTGGGTAAC	18457	ACACAGCCACATACCACACATC	29441	CCCATCTCCACTTTTGGAGTCTT	40425
6800	GCAGTAAGCACCAGTGAATATCTGATT	18458	GGGAGTCACCAGCAGAGTCATA	29442	GCTGCAGTAAGCACCAGTGA	40426
6801	CCAGAGATACTTGAGATGGTCACACT	18459	TTGCAGGATGACAGACCAAAGTTA	29443	ACGAGAACATCCAGAGATACTTGAGA	40427
6802	TTGCCAGAGTGCTGTCATGT	18460	CCGAGCCTATGCTGAGGTGTAAG	29444	GGGCCTTGATTTGCCAGAGT	40428
6803	AGGATTGGCATAGGAACAAGTGAT	18461	CAGAATCCTATATGCTAGGCTCATAACCAA	29445	TTGCCGGAATGAGGAGGATTG	40429
6804	CCGTCTTTGTGCCACCTGTTG	18462	GGGAATTCTCCAACCCCATTTCT	29446	CTCTAGCCTTTCAGTCCGTCTTTG	40430
6805	GGCACAGATTAACTGCGTCTGACT	18463	TGGCCATAGAGGCTGAGAGCTT	29447	GACACACAGATAATGAAGGCACAGA	40431
6806	GCGTATCTTCTGCATACACTAGCTT	18464	TCCCAGTGCCCACTTCACT	29448	CCACGTGGCGTATCTTCTGCAT	40432
6807	AAGGCGATGGGAACCTCAGA	18465	GGCTGATTATCGGCAGAATTTGGTT	29449	CCTCAGCTGTCAGCCAGCAA	40433
6808	GCCAGAACCTGGTCTGAACTCCAA	18466	CCACACTAGGAATGTGGGACACAA	29450	GCACTAACGTAGTAGGCCAGAAC	40434
6809	GGGGAAACCATGAAGGCTTGAA	18467	TCATATTTCTCCATCCCAGCTGTTAC	29451	ACACTGATCCTTATTGATGGGGAAAC	40435
6810	GGCCACCATTGGCAGTATCATC	18468	GCTAGATGATGATGTCCTCTACCAAA	29452	ACAGCACGGCCACCATTG	40436
6811	CGTGGGTGAAGCGCAGAGTA	18469	CATCCAGCGTCACATTCTGTCT	29453	AAGCATGGCCCGTGGGTGAA	40437
6812	TCAGGGCATAGGGCCTAGAAG	18470	GGTGTGGGGCAAGACTTGTTTC	29454	TGGCCTGGCTTCAGGGCATA	40438
6813	GCCCACAGAGACCATCCATCT	18471	GCTCTGGGCTGATTGAGTTGCATTC	29455	ACAAAAGAATGGAGGCCCACAGA	40439
6814	GTGGGATTCAGCCATTCCTGCTA	18472	CCATCACCCGAAGCCTCACTTT	29456	GAGCTAAGTGAAGTATGAGTGGGATTCA	40440
6815	CCTCAGACAGAGCTGGGTTTAAATTCT	18473	GCCTTAGGCCATGTACAATGTGA	29457	GGCTTAGAGCACTCCTCAGACAGA	40441
6816	GGGGACAGCAGCGTTCCTT	18474	GGCTGCACAGACCACTCAATTATC	29458	GCCCCTTGGCCACATCTTAGT	40442
6817	TGCTACTGGGTGCCAGGAA	18475	CTGTATTATCCATCACATGAGGGTCTTG	29459	ACCATATGCCGGGTGCTACT	40443
6818	CCTGCCACATGTTCTATCTGTGTCT	18476	CTCACCATACGGTTGTACTCACA	29460	TTTTCCTTACTGATCCTGCCACAT	40444
6819	CAGTGATTGTCCTAAGGCCAGGTT	18477	CATGGAGGATTTCAAAGCAAGGAAGT	29461	GGCCAGCACAGTGATTGTCCTAA	40445
6820	GGGATTCTCTAGGAGGAGATAACTTTCTGA	18478	GCTGTCACAGACTGCATGGAA	29462	TTGAATGGGATTCTCTAGGAGGAGAT	40446
6821	GGAGCAGGTTGGAGGTTAGCAT	18479	CACCATCTTGGTCATCTCTTTCTTTC	29463	GCTCAATTCGGAGGAGCAGGTT	40447
6822	GCTGTGGAATGCAGTTGAATGACTTTCT	18480	GGTGAGTACCCTAGCAACCAGAAG	29464	TGGCTGTGGAATGCAGTTGA	40448

6823	TGCTGAACGTCACACAGTGAT	18481	ATGGCCCCTGGAACTGGAAA	29465	TGGGGAGTGAAGATGCTGAAC	40449
6824	GCAATCTTGGCTAATGAGACCATTT	18482	CATGGCTTTCTGCAGACCATTTTT	29466	GCCCATGGCAATCTTGGCTAATG	40450
6825	GCCCTTCTATTCTACACTGACGTAAA	18483	GTGATATGTGTGCAGGTTTCTCTGTTT	29467	CAGCTCTGAATTTGGCCCTTCTATTCT	40451
6826	GGAGAAACAACCACCATGGGAATGA	18484	TGAATGCTGCAAGAGGAAATGGAA	29468	GGAGCAAACACGTGGAGAAACAAC	40452
6827	CACAGTGCTGACTTCCTTTCTACT	18485	GACGCCATGTGCTGTAGATTACT	29469	GGCTTGATCACAGTGCTGACTTC	40453
6828	GGGCCACCTGGATGTAGACTTTTG	18486	CCCACAGACTGATTCAGAGTCGTTAC	29470	TCAGGGCCACCTGGATGT	40454
6829	GGGACAAATCTTCCTACCCCATTC	18487	GAGGCCGAGCAGCATTTGAT	29471	CGAAGTTATGGCTGGGACAAATC	40455
6830	AGTACTGGGATTTGCTACAGAGAAG	18488	TCAGTGGTGCAGCATGCTTAG	29472	TGCAGCACAAAGTACTGGGATTT	40456
6831	GGGGAGGTATCAGAGCCTTTTTCTGA	18489	GGGTGGCACAGATATGAGGAAACA	29473	GGGGTCCTGGGGAGGTATCA	40457
6832	CCATGAAACTCAATGACACTGTTTCTGTCA	18490	GCACAACCATGTACCTTTGGAAATC	29474	GCCCTCCATGAAACTCAATGACACT	40458
6833	CTGATCTTGTACTGCTAGCCCTTATAG	18491	TGCCTCATACAGACCCGACAT	29475	GCCCTTTGAAAACTGATCTTGTACTGCTA	40459
6834	AATGGCAATGACCCAACATGAAG	18492	ACATTTGCCAACCCCTGCTT	29476	GGCTGCTTCCCTAGAAAGACAACA	40460
6835	GGAGGAAAGCAGGGATGCATGT	18493	GAGGCAGTGCCAAATATGAACTGATAC	29477	TGGACACCCGTCAAGGAGGAAA	40461
6836	CTGTGCCTCAAGGAGACAGTGA	18494	GAGATCCCAAATGCCCTCCAACA	29478	ACGCTGCTGTGCCTCAAG	40462
6837	CTGCTGTCAACAGGCAGTGAAA	18495	GCTTCTTGAGGCTAGGCTTGGAACT	29479	TGGCCAACAGACTGCTGTCAAC	40463
6838	ACTTCGGGAGCCACAGTCCAA	18496	GTGTGTTGCATCTCCAGCTAATG	29480	GGACAGCAAAGGCTGCTTGACT	40464
6839	CCACCTTGTTTTAGATTTTGGCTCTGA	18497	CAGCCTCCAAGTTGAACCTAGT	29481	GTGGGATGCCCACCTTGTTT	40465
6840	CAGACAGGCACCTTTCAAACTCTTCT	18498	AGGGCCACCTCTCTGGGTTTTA	29482	CCCCATCAGACAGGCACCTT	40466
6841	TGCTGATACACTGACTCGGTACT	18499	GCATTATGGGGTTGTGGGTTGT	29483	GCAAGACACCTGAGATGCTGATAC	40467
6842	GGGAGCCAGTCATTAATCGAAATGT	18500	CCCGAGGAAGCATTTGCTGTGA	29484	ACAGCATGGGAGCCAGTCAT	40468
6843	GTGCCTTACCTTTGCCAGTTCTGT	18501	GGCATGGTTCTTCCAACTCCAAGT	29485	CCTGCAGACAGTGCCTTACCTT	40469
6844	CTGAGACTGGATTGGGATAAGGAGATG	18502	GCCTGGCCACCTATCATTGT	29486	GGCATGGTAGACTGAGACTGGATTG	40470
6845	CCCCAGCTTCATCTCCTTGGAA	18503	TGTGGCAGTGGAAATCCCAAA	29487	CCTTCTGCCCCAGCTTCATC	40471
6846	GCATGATAGGAAGTGTGCGTGATAG	18504	GCCAGCACCCCACAAATGTTAC	29488	GCGTGATAGGAAGCGTGCAT	40472
6847	CACCTCCTGGTTAGAGGCATTC	18505	TGCAGTTCCATTTCCAATAGTCACA	29489	AGAGAGCTTTCACCTCCTGGTTA	40473
6848	ACACTGAGGACAGGAACTACTGA	18506	CCCCAAACTGGATGTTTTTCCCTCAT	29490	GCTTCCGAGAGGTTACTGATACACTGA	40474
6849	GTGAGTGTGGTTAGTACCTCCTAATCTT	18507	CCCACCCATGTCTGTTCTCTATTT	29491	CCAATTGATCCAGTGAGTGTGGTTAG	40475
6850	GTCTGGCCATTCCAGGGAGATT	18508	TGAGGGAACTCCCCTGACCTTT	29492	CCCACCATGTCTGGCCATT	40476
6851	CAGCCTTGTTGCTGGAGGGAAT	18509	CCACCTGTTCAGGGCCACTA	29493	AGGTTCACGCAGCCTTGTTG	40477
6852	CGAAATTAACAGAGTTCTCTCTGGTTCCAA	18510	CTTCAACTCTACATTTCTGAGGGTCAT	29494	GGGCTCCAAAGTCGCGAAA	40478
6853	CTGGAGCAGGAAGGCTTGTTAC	18511	CCCCTACACACCCATACAGACA	29495	ATCCAATGAAACTGGAGCAGGAA	40479
6854	GTGTTCCTGGACATGTGTTTGATACCTA	18512	GCAATTTGCTTAAGGACAGGGATTGTATC	29496	CGGTGTTCCTGGACATGTGTT	40480
6855	ATGGCCCAGCCTCTGAGTGA	18513	CTCCCTCAAAATTCCCTGGTTCT	29497	GTGGGCAACACCGAGAGATG	40481
6856	GCACCACGAGCTTGGACTTTCA	18514	GCCTCAGCCTCCCAAACAA	29498	CCCACAGCACCACGAGCTT	40482
6857	GCGATGCTTCTGGATCTGCAA	18515	TCCCTGGTGTGGATCTTATTTTCATC	29499	CAACCGCTTGCGATGCTTCT	40483
6858	CCAGCCTCTGAAGGAATTGGTCTATG	18516	GCTTCCCTGGAGACCACAGT	29500	CCTTTTCCAGCCTCTGAAGGAA	40484
6859	TCCCCTGATGGAGAGTTGCAT	18517	GGGTCTTCGACTTAGCATTTAGTCT	29501	TCCACAGGAAACCTCCCCTGAT	40485
6860	GCAGTGGAAGATAGGCAAGAGGAA	18518	GCTAGCTCCTGTTTCCATCAGCAT	29502	CTTTAGCAGCAGCAGTGGAAGA	40486
6861	CCAACAGGATTGTCGGTGAATTG	18519	GCTTGGTCCAAAACCTCATTGATTTAC	29503	GAAGGAAGAGCCAACAGGATTGT	40487
6862	CCACCCTGAATTTTCTAGGTGTGAAC	18520	ACACGGTTGTACATGCGTAAGA	29504	AATACCCTTCCACCCTGAATTTTCT	40488
6863	GCTCACTACGAACCTGAGACCTA	18521	GTGTGGAGGAAGGGGAAGAGTTC	29505	CGGAGATACCAGCTCACTACGAA	40489
6864	GACCAGGAACACATCTTGAGAGTTAAG	18522	CCCCTTGCTTTCATGGTAACACTT	29506	GAGAGATGACCAGGAACACATCTTG	40490
6865	CCTGCAACTAGCAAATTCGGCTTTT	18523	GTGGCTCCTTTTCACATAGTTGTTT	29507	GGATGCCCTGCAACTAGCAA	40491
6866	TGAGATTCACCACTCTTCCTGAAC	18524	GGACATGGGAGCAGATTTTACACT	29508	CCCCAAACCCTCTCTGAGATTC	40492
6867	GGTGCAACAGACTGAGAGCAATCTA	18525	GCACACATGGGTTCTCAGTGAT	29509	TGCAGGGTGCAACAGACTGA	40493
6868	GAAGCAGTGGGTGAGATATAGGATGAT	18526	CCCCTAAAGCCATTTCGGTTCTTC	29510	CTGTGAAGCAGTGGGTGAGAT	40494
6869	AGTGAGGCTCAGTGCCATCTTG	18527	ACATGGATAGAAAGGTCTGGCTTAATG	29511	CATGTTTGGTGTGCAAAGGATGA	40495
6870	GGCCTTTCCAACCCGATTGAGTA	18528	CAGTTTGGCCCATGCTGATG	29512	AGTTGTGTTGGGCCTTTCCAA	40496
6871	GGTCCCATGTAGGGAAGTCTCTT	18529	CAGAACCCCTCCTGAATAATGGAAGT	29513	TGACCTGTCTGGTCCCATGTAG	40497
6872	ACGCAGACAGGGACCAGAT	18530	ACCAGGCCACTCCCTTGTGT	29514	GGAGGTCAGAGACGCAGACA	40498
6873	CAAGTTTCAAGGAACAGGCACTAAG	18531	GGCTTTCCTGGATGGACAAACAGAT	29515	GGATGCACAAGTTTCAAGGAACA	40499
6874	CAATGCTGGGATCTTTCCTCCAA	18532	AGAATCCTGTCTATTTACAGGGGTAGA	29516	CACACTTCCTTCAATGCTGGGATCT	40500
6875	CCGTTCGGTCAAAGGACATGCAA	18533	CAGTTGGCCCAGTCAACCAA	29517	GGCAAATAGACAACCGTTCGGTCAA	40501
6876	GCTACTCTGGGTGGTTCTAACTGTTG	18534	CTGGCAGTGATCTCTCTTTTCTCT	29518	TCAGGCTACTCTGGGTGGTT	40502
6877	CTGCCAATAGCTAGGGGAGCAT	18535	CCCACAAGACACTACCCACTCCAT	29519	GTGTTTGTCCTTCTGTCTGCCAATAG	40503
6878	CCAGGTTAGCATACAGAAGGCCAAT	18536	CTCAGGAACTTTGGAGCATAGATCA	29520	GGCATCCAAGAAGTTTCCCAGGTT	40504
6879	CTCCCTAGTGCTTGGTGCTACT	18537	CATGACAGTTAAGGGGAGAAGGAAAA	29521	CTCCAACCTCTCCCTAGTGCTT	40505
6880	CGGATTTGGAGTAGTGGGCAATG	18538	GGCATGTTTACTCCACCAACAACA	29522	CCCACTTGGTTCGGATTTGGAGTAG	40506
6881	CCCATCGGGAGAATCACACACT	18539	GCTTGAGAACTGAGGTGCCAAA	29523	GTGTTGAACCCCATCGGGAGAA	40507
6882	GGCTCTGGAAGAGAGTAGGCAAAT	18540	GCCATCTGCTGCTGTTAGCAT	29524	AGCGCTAGGCTCTGGAAGA	40508
6883	CCGTATCTCCTCCAGTCCATTACTGT	18541	GACCTTAGGCAAAGAGGTAACACAAAC	29525	TCAGTTGGACTCACACCGTATCT	40509
6884	ACACCTTTGCTGATCTCTGCTATAC	18542	GAGAGCTGAGTTTAATGAGAGTTGAGGTT	29526	GGGCACATTCCATCAACACCTTTG	40510
6885	CTGTTCCTGCAGATTACTTGCTACTGT	18543	TGGAGATACAGTGGTTGTGTTTGA	29527	CAGTGTTCTCTGTTCCTGCAGATT	40511
6886	CCTGTGGTTCAAGCCTCCAAT	18544	GCTTCTAAAGGATACGGTGAAAGTGTAG	29528	CTCATCCTAATACTGACCTGTGGTTCA	40512
6887	AGATCAGGTTCTAAGGGTCATACCAA	18545	ACAGTGGTAGAGATGCAGAAAGATG	29529	CCCATAGCCAGTCAAAGATCAGGTT	40513

6888	GGAGAGAGGCTAACTGACATATACTGGTAA	18546	GAGGTTCAAATAACCAAGTGGAGAAG	29530	CTGAGGAGAGAGGCTAACTGACA	40514
6889	GCCCAGTGTCTGATGTGTAGAAAG	18547	CCCAGATTCAGTCCTGTAGTGTAAC	29531	GGGAAAAGCCCAGTGTCTGATG	40515
6890	GAACAAGGGATGTCTCCTCTTCAA	18548	GGGTGAGACAAACATAGGTTCTTTACTTG	29532	TGGCATTTGCAAGGGGAACAA	40516
6891	GCAAGGACACAAGCGAAGAGACT	18549	TCGGGACCAGCCATCACT	29533	AGGGAGGAGGCAAGGACACAA	40517
6892	GCACCATGGGTTTGCCTCTACT	18550	GCACTGTTCCAATGTCCAGGTT	29534	TGTGGGGCACCATGGGTTT	40518
6893	AGCCTCCTATGATTCCCAGAACT	18551	CTGGGAGTAGGCATTAACCCATTG	29535	CCACCCACAACAAGCCTCCTATG	40519
6894	GCAGAGGTTCAGAGGGAAAGATGTTC	18552	TCCAGCCCCTACAGAATTCTATTTTTC	29536	AAGGAAGGGGCAGAGGTTCA	40520
6895	GTCATCATGTGACAACCTGTGACT	18553	GCTCAAGAGCCACACGAATCT	29537	CCATTGAGCCCAGGTCATCATGT	40521
6896	GGCACGAGTAAGTGTGTCTGA	18554	GGGCTGTCACCTCTTCTGTGA	29538	GGGGTGAAAGGCACGAGTAA	40522
6897	GGCCTGCATGGGTCACTATT	18555	ACGCTTCATTCCCTCAGTCTCA	29539	GCAGTGACATGGCCTGCAT	40523
6898	CCAGTCTGTCCTTGAAAACAACACA	18556	GTCTGGTTGCGCAGTGAACA	29540	CCGAGACTCATCCAGTCTGTCCTT	40524
6899	GCATCCGCCTTAGATAAACAAAGAA	18557	GCCCTGCTACCCCTTTCCTATT	29541	AGCTCTTCTGCATCCGCCTTAG	40525
6900	CACATTGACTTCTTTCCATGCCCTAT	18558	GCGATGCCAGGAACATCTCT	29542	CCTACTCGTTAACTCACATTGACTTCT	40526
6901	CCCTGAAGTGAGTCAGGAGCTGTA	18559	TGGCCACACAGCCTACCTT	29543	ACCTGCTGTGTACCCCTGAA	40527
6902	TGCCATCCCATTGAGGAGTGA	18560	CTACCTAAACCTCTAATCACCTAAGACTTG	29544	AGGCCACTTGCCATCCCATT	40528
6903	CCATCTTTTCTAGAGTGGGAATTGTCT	18561	CCAGAGCCATGAGAGGAAAAGGATT	29545	CGGCATCACTACCATCTTTTCTAGAGT	40529
6904	CCTGGCCTCTGATTCCATCCAAAC	18562	GGGTGATGTGAGATTCCCTTAACTCT	29546	GCCACCCTGGCCTCTGATTC	40530
6905	GGGTTTTGCTTTGTGGTGGTGTTG	18563	GCACTCTCAAGGCGTAAGACCTTTT	29547	GTGGCAGAAAAGATGGGTTTTGCTT	40531
6906	AGGCACCCATTCCTGTCACTTG	18564	ACATAAGCCCATGCCAGGATAATAAG	29548	GCCCTTTCCAGGCACCCATT	40532
6907	CGAATGGGTATCGATGTCCTTGA	18565	CCAACCAGCCAGTCCAAAAAG	29549	CCTCCCAAGTCAACGAATGGGTATC	40533
6908	TGACTGCGTTGAGCTTAGAAGTTA	18566	GTCAGGCTGCGTCATTGCTA	29550	GTGACAAGTTCAAATGACTGCGTTGA	40534
6909	GTCCAAGTCCAGCCTTCACA	18567	CAGAAGAACGAGGAAGGAACTGT	29551	TCCTGAGCCTGTGTCCAAGT	40535
6910	GCTGGGAACCTTGGCCTGTATATC	18568	GGAGGAGCTGGTAAGAACTAGTCA	29552	AGGCCACGCTGGGAACCTT	40536
6911	GCTTCCTGTTTCCACGTAGGTTTC	18569	CCACCAGACAACTTTCTCCTCTT	29553	CTTCGTCCTTGCTTCCTGTTTC	40537
6912	GGCTGGAAAGGATGGTGCTAT	18570	GAGCAAAGAATGTGCTATAAGGGAGAT	29554	GCTGTCACCTTTGGCTGGAAAG	40538
6913	GAGTGGCTAACTGGTCCTAAATTCAAA	18571	GCATGTGGGTTTTCCAGCAAAT	29555	CAAGACAGATCCTGAGTGGCTAAC	40539
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6915	CCCTCTGGCAGTGTGTTAGCAGAT	18573	ACAGGTTTCTTTCAAGCAGGATTTC	29557	TGGGTCCCCTCTGGCAGTGT	40541
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6917	CCAGAGTTCCCTCTTTGGGAATCAGA	18575	TCGCTGGATTTCTCCAGGATTAC	29559	TCCCCTCCAGAGTTCCCTCTTTG	40543
6918	CAGGACTTGTGTCTGTGGCTAAAC	18576	GCCCAGATGGAGACACAGCTT	29560	GAGCTGAAAGGCACAGGACTTG	40544
6919	GCTTCTCGTGACTCATTCATGTTT	18577	GTCACAGCCTGCTTGCCTAT	29561	AGGAAATCAATTGCTTCTCGTGACT	40545
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6922	GGTGATGGCTGATGAGTGGTT	18580	GCCTCCTGTGTGAAGGTCGAAA	29564	CAGTTCAAGCCCAGAGGTGATG	40548
6923	GTCACTTGCTCACCCCAATGA	18581	CCTGCCCCAAACTCCAAAGA	29565	TGGCCCTGAGCTTGTCACTTG	40549
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6925	CCTTCAGGTACTGAGGGTGTCTCT	18583	GGAGGACAAAAGGAGCTCTATTCAA	29567	GCTGCCTGCCTTCAGGTACT	40551
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7016 GACCTCACCATCATCCATGTGTTC	18674 GCCCTCAAAGACCTCACAGATT	29658 TCTCGCTCTGACCTCACCAT 40642
7017 TGCTCCCCTGAGATGGAGACT	18675 GCTCCTGACCTCACAACAACTTTCT	29659 TGGTGACGGGAGTTGGAATG 40643

7018	AGCCATCTACCACTGTTCTTTCAAT	18676	GGCACAAACCTGAGAACTTGAAC	29660	CCATTTCAGCCATCTACCACTGT	40644
7019	GGGCAAATTCCCCGACATCAGA	18677	CGCTAGCCAGGTTTCCGTTTAG	29661	CCAACCTCAGAGGGGCAAATTC	40645
7020	GGTATCCGAATGGAGGACCAAGTAAT	18678	GCGAAGGACGTCAGCGATGTAA	29662	GTTCCCTGGAGGTATCCGAATG	40646
7021	GGCCCGCCTCAAAAATCTACCAA	18679	GATTGTGGTATAAGGTGGGTTTAGTTG	29663	CCTTGGCCCGCCTCAAAA	40647
7022	TGGCCAATAGAATGTGTGAGGAAT	18680	CTCTCAAAGGCTTTTACCTGGGAGTA	29664	GGCATGGCTGTGACTTGCTTTG	40648
7023	GGGCGGAAAAGATACACCCCATAA	18681	CCAACCTAGCCCCACTAATTTCT	29665	CACACTGAAAATAAAAGGGCGGAAA	40649
7024	CAGCATCGCAGTCCTGTTATTTC	18682	CAGAAAGTTCACCAGAGCACAATC	29666	CGGAATGAGGATGGCAGTTACA	40650
7025	CACGCTTAGATTTCAGTGAGCAAGA	18683	GGGAGTATGCCCCTTTGCTGTT	29667	GCCTGTGCCATCACGCTTAGA	40651
7026	CACTGGTTTCTAGGACCATGTTAAAAG	18684	GTCAGCAAGAACCCCTTCAAGT	29668	ACAAGGAAGCTGTCACTGGTTT	40652
7027	GTGAAGATCCACAGCTCGGAATAC	18685	GCTGTGGTGATCGTGGTTGT	29669	AGGAGCACGGCTGTGAAGAT	40653
7028	GCTAGGTGGTTACTTTAGGTTGGATAG	18686	TCTTTCCCCATCTAGAATGCTCTTTTC	29670	GCTAGAGGATCGCTAGGTGGTTAC	40654
7029	AGGTAACGCACTGACTTTTGAGT	18687	CAGGCTGCTCTTGGGAAGTT	29671	GCTCCGGGCTTCAGTTTTAGGTA	40655
7030	AACTCGCCAGGGCTGTTGAT	18688	CTCCCTAGAGAATCCTAAGACCAACCAA	29672	AGGGTTACGGGCAGAGAACT	40656
7031	CCTCCTCAGATGACCAGAGAAATC	18689	GCATGGCTGAAGCTGAATCTTG	29673	CCCTCTCCACCCTCCTCAGA	40657
7032	CCTCAGTCAGGTTTCCAGAAGGTACT	18690	GCCCACAGTGGTTCAGTCTTAG	29674	GTGTGAATGCCCTCAGTCAGGTT	40658
7033	GCAACCGGTGCCACCATAGA	18691	GCCGGAGAGCAACGCACAT	29675	ACACAGTGCTGGCTGCAA	40659
7034	CCCCTAACTCGATTGCCAACAGAA	18692	GCAGGCTAAGATTACTCGTCTAAACA	29676	GAAGCTCTTACCCCTAACTCGATTG	40660
7035	CCTGGGAGGTAAGCCCATTGTAAG	18693	GAACTTGAATCTGAAGGGACTATGGAT	29677	TGATGGGCTTCCTGGGAGGTAA	40661
7036	CCAGACCTCAGAAATGTCCTGTTC	18694	GCAGGCTGTGAGACCATGAAAAC	29678	TGGTTGACTGCCAGACCTCAGA	40662
7037	GGAGACTGGCTTTGGCAACACA	18695	CAAGGAGACAGATCCTTGGGAAAG	29679	TCTTGGCAGACAGGGAGACT	40663
7038	TGCCACACAACCTGGTGCTT	18696	GGCAGGAACTCAGGACTCAAGA	29680	GCAGCTTGCTGCCACACAA	40664
7039	GGATCTTGCGTCCAGGCCTTT	18697	GCCACCCTCCACTACACTCCAT	29681	TGAGGGCCTGCTAGGATCTTG	40665
7040	TGGGTTTAGGCGGCATCCTT	18698	CCAAGAGGAACTTTTTGGCACTGA	29682	GCAGTCGCCAACTTCTGGGTTTAG	40666
7041	GGCCTGCATCAAACAGGGAAT	18699	GCCCACCTTTGGCCTTCTCT	29683	GTCAAAGCTGGCCTGCATCA	40667
7042	GCTGGATGGTATGTAGCCAACT	18700	GGGGAGAGGCACTGAATACCTT	29684	ACTGCTGCCCTCTGCTGGAT	40668
7043	CCCCAAAGTCTGTGCTTTTCCTACT	18701	GCTTACAGTAGATGCGGGAAGA	29685	CCTCTCCAACCCCAAAGTCTGT	40669
7044	CCTCTGGAGCATTGCAGATCA	18702	AGCTGAGCATCGGTAAATTCCAA	29686	TGGTCTTGCCCTCTGGAGCAT	40670
7045	GGATTCCAGCAACTTTCAGCAGGTA	18703	CCATGGCTGACCATGAACTCTAC	29687	GGCTTAGAAGGATTCCAGCAACT	40671
7046	CGCTTGTCCCAGGATGGACTTAG	18704	CACCAGATGGCTGATTGTCTCTTC	29688	GCAAGGAGCACATCGCTTGT	40672
7047	AGGGCATGTCCACTGCATTC	18705	GCATGCCTGGGAGGTAAAGAATTG	29689	TGTGAGTGCAGAGGGCATGT	40673
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7058	TCGCCCCTTGAGTGAGAGAAGA	18716	CAGCTGTTGCCCAAGCTTTCATC	29700	TTACCACCTCGCCCCTTGAGT	40684
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7071	CGTCTCAGTGACTTTGGCAGTGAT	18729	AGCCGGCAGGAGGAGGAAGA	29713	TGAACCCCGTCTCAGTGACTT	40697
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7078	CCTTTATTCCTGAAGGATCTGGGTTGT	18736	CCATGCCCTGGGATTAAGGTCAA	29720	AGCTACCCAAACCTTTATTCCTGAAG	40704
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7151	CTTGCACAGTGTAGAATCTGAGGAT	18809	CCAGAGCACTCCTCTCCCAAAAAG	29793	GAAGAGTGAGCTTCTTGCACAGT	40777
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7160	CACTTTGGGCTCCTCCTTCCTTT	18818	CTGTAACTCCCATCGTAGCCTTCT	29802	AGATTGCCACCTGGACACTTTG	40786
7161	TGGGGATGACTACTTTTGCTATTGT	18819	GGGGAGAAGAAAGGGAGGACAAA	29803	GGGATGGTGATGGGGATGACTA	40787
7162	GGGAAGGATTCCAGTGGTCGAT	18820	GTGATGCACAAAAACAGGCACAA	29804	GGGAGAGCAACAAGAGGGAAGGAT	40788
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7164	CTGGGGTAGTATGTTTGCCCTTCTA	18822	ACTTCATCCCTCGAGTTGTGTATC	29806	GGTGACCTAGCTGGGGTAGTATGT	40790
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7167	GGTGGTGCCTTATTGCTGCAT	18825	CTGCATTGTGAGGACACAGCATTC	29809	CTCTACTTCCAAGGTGGTGCCTTA	40793
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7170	GGTGGTACTGGCCCTGAACA	18828	GGAGATGAGACTGAAAATAGGTAGGTT	29812	TTCCACAGCAGGGTGGTACT	40796
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7172	GATGAAGATGGCACTGGATGTTTC	18830	TCGGTAGCTGTGGGAGGTAGA	29814	CGTGCCTCCTGCAAGATGAAGA	40798
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7174	CAGCAAAGCTGCAATTTGTCTATCT	18832	GAAATGGAGCCATTAGGTAAGGTGTT	29816	GGTGACACTGCCAGTAACAGCAA	40800
7175	CCTTGTATTCATTCCATACCTGCTCTTTG	18833	GCTGGCATAGGTAGCCAGGAAAC	29817	GCCCTCCCCTTGTATTCATTCCAT	40801
7176	CCCAGACCCACCTTACAGGTTCT	18834	AGGGGACAGGTGAGCCAGAAAT	29818	TGAGCCCAGACCCACCTT	40802
7177	TCTTGAATCAGACAAAGAGCACCAT	18835	TTGCTGGGCTGTGGGCACTTC	29819	CTGGAACTGCTGGTTAATCTTGAATC	40803
7178	CATGGACTCAAGACTCCACACA	18836	GGGGATCCACATGGTGAATGT	29820	TCTCCAGCGTCATGGACTCAAG	40804
7179	GGTCAGAGCAGGCATTGTTGT	18837	TCCACCAGTCAGCCAAGACTCA	29821	GGTAGAAAGAAACAATTCCAGGTCAGA	40805
7180	TGGGTTTGAGGCACAGTTAGATG	18838	TCCCAACTGCTTGTTGAGGTT	29822	GGGTTTTCAGGGGTGGGTTTGA	40806
7181	TGTGGACCAGAGCCTCATGTA	18839	GGAAGCCATGACTAGGCTACAGT	29823	GCAGATGGAGGTTACCAAACTTGT	40807
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7183	TCTCAGCTCTTAGGCAGAAATCTTAC	18841	CCAATGCCAGGGACACTTCT	29825	CCCTCTTACCCCATTCTCAGCTCTT	40809
7184	TCTGCCCTTACCTCCTTCTTCT	18842	CTAGCTGTTCCTCCAAAGAGTAAGT	29826	TCCCCTTCCTCTCTGCCCTTA	40810
7185	ACCGAGTTAGAGCCTTGACTTTC	18843	GGTGATTGTATGGGAAGCGGAATCT	29827	AACATCTGCCGACCGAGTTAG	40811
7186	GTCCCAGGTTAATGAGTCTAGAGGAA	18844	CACAATTGCCTCTTTGGCAGTTC	29828	CCCTTAAAGTCCCAGGTTAATGAGT	40812
7187	CCCCTGATGATCCACACCCATT	18845	CCACCTTGCCCAGTGAAGAAGT	29829	ACCTGGATGCCCCTGATGAT	40813
7188	CCATTGAATGTAGAACATGCCTCTCT	18846	TGCAACCTGGTAGCTCAGAGT	29830	GCCTTTGCATTGTTTTGGCCATTG	40814
7189	GCCAGCACTTCTGGATGTTACATTG	18847	GGTAGATCAGCCTTCACTGTGTGT	29831	AGTTTTGCCAGCACTTCTGGAT	40815
7190	AGGTTTCAGGCCATCCCTCTCT	18848	GCACCATGGCAGGTCCAGAA	29832	GGAGTTTGAGAGCCAGGAGGTTTC	40816
7191	GAGACTTTCGCAAGGTTTTCAGCAAT	18849	GGTGGTGCAAACTGGTGCTA	29833	CTGATCAAAGGGCAAGGAGACT	40817
7192	GGAAGGGCTGTTTACAGAACAAGTACA	18850	CCTGCCTTTGCCATGTCCAGAT	29834	GCTAAGGTGGGAAGGGCTGTTT	40818
7193	GGTGCCATAGAATTACTCCAAGGGTCTT	18851	GTGTTTCTCACAATGTTCGGTGCTT	29835	TGGGGTAAACGGTGCCATAGA	40819
7194	GCAAGAGTCCATGATAGGCATGT	18852	CCCAGTGCAAGGAAAGGGTAATG	29836	GCCTAGAATTGCAAGAGTCCATGA	40820
7195	GCCTACACTATTTCATCCCATGGTAAAC	18853	ACCTTGCACATAGTTGCATGTTG	29837	GGGATGGCAGCTTATGCCTACA	40821
7196	ACTTTGTGATGTGTTCCTACCTCTT	18854	GAGGAAGCAAGGGAGCTGTACT	29838	CCCTAGGAGAGAAGACACTTTGTGA	40822
7197	GACAGAAACCTGTTTGAACTCATGGTA	18855	GCTAGGTTACTGTATGCCCTTGAAC	29839	CCACCATAGACAGAAACCTGTTTGA	40823
7198	GCTCTAGAGTATGACGGGGACACA	18856	GACCTTTTCACTAACACTGGTGAGACT	29840	CCATGCAACCCGAGGCTCTA	40824
7199	GAGTGCTCAAAAGCTATTCCCAGTT	18857	GCCTTTCCAGCTCTCTGTTCT	29841	CCTAGGCACCTGAGTGCTCAAA	40825
7200	CCTTGGCCTGAATCCTCCTACA	18858	TGTGCAGTTAGAAGGGAGACAAG	29842	GATTTGCCTCCTTGGCCTGAA	40826
7201	GCAGCAGCCTGTGCTTTCAT	18859	CATCCTGTTTTCTCCCTCCATTCA	29843	GCTCATGGCTGATTCACTCACT	40827
7202	ATGTGAGGTCACACTTCCTTTGAT	18860	TGGTGAGGTATAGTGGAAGTTCAGT	29844	CCCGTCCCAGGTGTCATGT	40828
7203	GCCTGCTTTAAGACTTTTTGCAGTTC	18861	GGTATCTGGAGAATGACTTGTTCCAA	29845	GGCTGAAAAGGTGCCTGCTT	40829
7204	AGGTGCAAAGGTAGAGGAACAAC	18862	GCTTCGGTAATGCTTCAGGTGTGA	29846	TTGGTAAGAAGAAGGTGCAAAGGTA	40830
7205	CCAGGACTCTCTCGGTTGAGTT	18863	GCGCCAGGGACAATTTCAGA	29847	GGACTGTAAGCCAGGACTCTCT	40831
7206	CTTGTCTTCTCAGGTTGCTTGTTT	18864	GCCTGTCAGTCACATGCTTCT	29848	CCCCTGCCCCTAAAACTTGTCTTC	40832
7207	GTGACTGTAGCATTCCCAGTTCTCT	18865	GGACAATGATGGAGAAATCCCTGTA	29849	GCTGCCAACATCTGTGACTGTAG	40833
7208	CCCTGATCAAATCATACCTGAAGTCAA	18866	GGCCCAAATAACAGGAAGTGCCATAAT	29850	GGAGAGAGAACCCTGATCAAATCAT	40834
7209	GGGACTTTGGGAACACACAGCAT	18867	TTGGGCTCGTGGTCTGCTTCT	29851	ACGAACGCACACGGGACTTT	40835
7210	CCAACACTAAAAGCAACCGTTTGAA	18868	GGCTAGTGACAGAAGTGTTCCTATAATG	29852	CTGGTGCTTCCAACACTAAAAGCAA	40836
7211	GGGGCTGTATTGCAAAAGATGCTACT	18869	GAGGGTTCAAACTGACAGCAGGATT	29853	AGAATAAAATCTGCAAGGGGCTGTA	40837
7212	GGATGGGAAAGAAATTGGTCTGGAAAGA	18870	GGGCTCCAAAAGCAACACACT	29854	CGAAACACACAAGGATGGGAAAGAA	40838

7213	CTCCAAAGGAACCTTCCCAGTAAA	18871	TCCACAGGCTAAGGGTAGAAGAA	29855	GTGGAGCTCATCTCTCTCCAAAG	40839
7214	CCTTCAGCCTTGTCACGCTTCT	18872	CTGAGCATCAAGCTTGTCCTTCTTC	29856	GTCTTTACCACCTTCAGCCTTGT	40840
7215	ACACTATGTGCTTTTGCCACAGA	18873	CCAGGCTGGGACCTAATTTCAGCTA	29857	GGGCCCAGACACTATGTGCTT	40841
7216	TGGACTGTGCAGGTGTCTGA	18874	GAGAGACCTGAGAAACCCCGAATC	29858	GGATCAGGCTGCTGGACTGT	40842
7217	AGTCCCCATTATTGTGCTCGTT	18875	AGCTGAGAGCAGTCTCTGGAT	29859	GGTTGTAGAACTACAGTCCCCATT	40843
7218	CAGGGTCTCCACTCTGTAACTTTT	18876	GGTTGTAGTCATGATAACTGTGTGCAT	29860	GGGTTAATTCAGGGTCTCCACTCT	40844
7219	GTGGGCTAGAATTTTGGCTCTGACA	18877	AGGAGCAAGGCGCAAGCAAT	29861	GGGGTCAACAAATGTGGGCTAGA	40845
7220	ACAGTAGTGTGAAACCAGGGAAAT	18878	CTCAGAGAATAAGAGGTGGCTTTCA	29862	CGAGAAACGGATCATTAAACAGTAGTGTGA	40846
7221	CCTCCGATTGCTTTTTGTGTTCCTA	18879	CTCTGACCTCAGACATGCAGGATT	29863	CCTAAAATACCTTCCTCCGATTGCTT	40847
7222	CTGGCTTCAGAACTTTCTCCTCTAT	18880	GGCAGACAGGATATTTTAACGCTTGT	29864	CGCTGGTTTCTGGCTTCAGAACT	40848
7223	CAGGCAGGGAGCTTATTCAGATCTAC	18881	GCAGAACCCCTACAAAGAAGGTA	29865	AGACTTCAGGCAGGGAGCTTA	40849
7224	CCCTCAATAAACTGGGAGACCCATTG	18882	GCTAATGACTGGACAGAGATTTCCTTAG	29866	TGCACACACAGCCCCTCAAT	40850
7225	GAAGGCTGAGGAGCTCTGATTTTA	18883	GCTTAGCGTGGTTTAGGCGAATG	29867	AGGGTCCTCCACCCTCTGA	40851
7226	GTAGGTGCAAACAAACTGGGTTTT	18884	TCCATGGGGAGTGAAGTGATCTT	29868	GGCAGAGAGTGAAAGGGAAGTAG	40852
7227	TCACTCCCAAGCTCCGTTCT	18885	GATGAAGTGGGGATGGTTGCAATAAA	29869	CAACTTCACCAGGGGTTTATCACT	40853
7228	GCCTGGGTCCAAAGGTAGAAGA	18886	CCCAGTCTCTCTGTCTGTTTTCAAG	29870	AGTCCAAGCCTGGGTCCAAA	40854
7229	CTGAGGTGAATTTGTAAAGGGTCATTG	18887	GGTCTGAAGGCCGTATCAGTTCT	29871	CAGACCTTCATGCTGAGGTGAATTTG	40855
7230	GGGGCTATAAAAGTGATTACAGCTCCTA	18888	CCAGCAAGTTTGCACAAGACATTTCA	29872	CCCATCCAAGGGGCTATAAAAGTGA	40856
7231	CCCAGCAGATGGGATGGAAACT	18889	CCCTCTCTAGCTCCTTCACACA	29873	AGTAGGGTGGTTCCCAGCAGAT	40857
7232	CAATAGCATGGTGGAGTGCACATA	18890	GAGTGAACCTTGCCTTACCTTTCT	29874	GTGCTCATGCCAGCAGCAATAG	40858
7233	GCATATCCTGCCAACTGTGTCA	18891	GAGGGCGAAGGGGAAGTTTTGT	29875	AGAGGAACAGAGCAGTTTTGCAT	40859
7234	TGCCAACAAGGTGAAATAGCAGAT	18892	CAGAGTGCCATGCATTGTGGTA	29876	GCCCATGCCAACAAGGTGAA	40860
7235	GCAGAAGGGAGAGTTTTGAGAGACT	18893	GTCCACCTCCCATGGGTCATAA	29877	GGGGATGAGCAGAAGGGAGAGTTT	40861
7236	GGCTAAGCTGTTGCCAGGTTCT	18894	CCTGAAAGGGTTAATCAGCTTCCAT	29878	TTTCTTTCAGTGGCTAAGCTGTTG	40862
7237	ACCTGCATTTCCGTGGTGAA	18895	GGACTAGCAGATGGAACAAACACA	29879	GGTTGTGGCTGTAACCTGCATT	40863
7238	CAAGAAAGAGGAGCCGGAATGATTT	18896	GGTGATTATGGGCAGCCATACATTG	29880	CCTGGATATCGAGCAACAAGAAAGA	40864
7239	GCATGGAATCTCAATATGGCTTGGAA	18897	AGAATGTCACAGAAGAGGGAGAAAC	29881	GAAGATGGCAGCATGGAATCTCA	40865
7240	GCGGGGCTGTACCACCTAGA	18898	CTGTTTCCCTCAACCAAGACAGAACA	29882	ACAAGTATGGCGGGGCTGTA	40866
7241	AGCCCCAGAGATGTTAGGATGT	18899	TCCAGGTCCCTGTTGGAGATGT	29883	TCCGCCAATGGTGAGAGAAAG	40867
7242	GGTAAACAGGAGGCAGAAACTGT	18900	CCACTGCCCCATTTTTCACATC	29884	CCAAATCCTGGGATCCTGTGAGGTA	40868
7243	GCAGGCCAGAGTAAGAGCTTCA	18901	GCTTGTCAACAAAGCCGGAACA	29885	TCACGGGCAGGCCAGAGTA	40869
7244	CCCTCTGTGGAATGAGGGTAGA	18902	TGTAACAAGGGCCATGAAGACAAG	29886	AGCAGCACCCTCTGTGGAA	40870
7245	GACAGTGACCTGCTCCATTTTATTAAG	18903	CCCTCCACCACTGGCATGTAAA	29887	ACTAAGACAGTGACCTGCTCCAT	40871
7246	CCCACCAATGAGTCACAAGCATCA	18904	GCCACTGGCTCTATCCCCTAGA	29888	CCAAACCATATCAATGACCCACCAA	40872
7247	CCCTAACATGCCAGTTTCTACCATCT	18905	CAAATGTCAGACCCTGGAGAACA	29889	AGCTTCCCTAACATGCCAGTTT	40873
7248	ACCCAGATGTTCCCAGACAGA	18906	GTCACTAACTACAGGTGAGCAGCAA	29890	TGATGTCAAGTAACTACCCAGATGTTC	40874
7249	CTGCCCAGGTTTTGCAGGAAGA	18907	GCATGCCTGCAGGAGAGATT	29891	CCTGAGCCTGCCCAGGTTTT	40875
7250	GCTGGATTTTGGGAGAGACAGGTT	18908	CAAGCTCTGCTATTAGAGGAATCTGAA	29892	GGAGGAAGGTGGCTGGATTTTG	40876
7251	TGTCCCAGAGAACCCGATCTGA	18909	CTCTGGGCAGGGAATCTCTGAAAG	29893	TTGCCCCAGGTGCTCTGT	40877
7252	GCTCCATCCCTTTCTAGGGCAACA	18910	GCACGGTAAGAAAATGAGGCTTAG	29894	TGTCCCAGCTCCATCCCTTTCT	40878
7253	GGACTGGACACAGGAGCATCTT	18911	CCTTGGATTTCAAAGCCTTCTGATCTT	29895	AAGCTGCTGGGACTGGACACA	40879
7254	GGGTTCTTTCCCTTGGCTGATT	18912	AGGACACAGGCTAAAATCACAACA	29896	CCTGCCCCAGGGGTTCTTTC	40880
7255	CCTCTGAGGCAATACCGTGGAATC	18913	GCTTGAGCGCTGAGTGCTTCATT	29897	GCCATAAATGGGTCCTCTGAGGCAATA	40881
7256	GCTGCTACATGTCTCAGATGAGTTTA	18914	CCCATGTGTCTGGAAACCAATGACATAA	29898	GTATGGCTAGCTGCTACATGTCT	40882
7257	GGACTACAGCCACCAAGTGATGATG	18915	GGACCACACAAATCCTGTGACT	29899	TTGCTTGGGGCAGGAGGACTA	40883
7258	CAGGCTGGTAGCTGTCTTTGGTA	18916	AACTAGAGCTTCCCAGGAGAAAAG	29900	TGGGTGGGCACAGGCTGGTA	40884
7259	GCACTGTCTTACTGCAAGGAAAAT	18917	AGCCTAGTCTCCCAACTGCAA	29901	GGCTGACATAGGGCACTGTCTTAC	40885
7260	GTGCTTCAGCTGTTTCCGTCTCA	18918	TGCCTGGAGGGGAGGAGTATC	29902	ACTGTGTGTGCTTCAGCTGTT	40886
7261	GGGTACGGTAAATGATGATGTGCTT	18919	GGCCACCAAGTCCACTGAGAAA	29903	CGCAAATCAGTTAATGGGTACGGTAA	40887
7262	AGAGCATCCCTAATCATGGTACAGA	18920	CAGGTGTTCAGCAAATTCCTCTTTG	29904	TGCTGGCAGAGCATCCCTAA	40888
7263	GTGCAAGCCAGACATGATGAAACA	18921	CCTGGCGTCGATATCACGGTTT	29905	CCTCAGTGCAAGCCAGACAT	40889
7264	CGACCGTCCACATGTTCCTTTC	18922	AGAGGCTCTGTAGAGTTCAGACTT	29906	TCTGGGCGACCGTCCACAT	40890
7265	ACCTCCAGCCTTTAGCACTTAC	18923	AGGGTGAGGTACCATCTTTCCAT	29907	CTGCTTTCTCATACCTCCAGCCTTT	40891
7266	GTGGAAACTTCAGTGGGAGAGTAT	18924	CCTCATGGCCAGTTTTTCTCCTT	29908	GGGATTGACAGGTGGAAACTTCA	40892
7267	TGGAACCCTGAGCCACCTGTAT	18925	GCAAGGCGCCTCCGTAAGA	29909	CTGCAAGAAGGGCTGTGGAA	40893
7268	GCTGCTGGAAGAGCTACAACCTT	18926	GAGATACTCCAAAGCTCTTTCCTTCTAC	29910	AAGACCCAGGCTGCTGGAAGA	40894
7269	GTCCCATCACACCACAAAGGTT	18927	AGAGCAGCGTGGAGAAGTGA	29911	GCTGTGGGAAATAGTCCCATCACA	40895
7270	GGGGCATCATCCAGTTTTTCTGTA	18928	AGAGAAACCTGTTGTGCTCTTACTT	29912	GGCTAAAGTTGAAGGGGCATCATC	40896
7271	GTTTGGACTGTCTTCCCCTGTTG	18929	GGAATGCTCCCACTGAATGATGAAC	29913	GGACCAGTCCTTTTTGTTTGGACTGT	40897
7272	GGGAAAGAGGTGCTTCTTCTGA	18930	GCCATCCAAGCAGTCTTGACA	29914	GCTGTGGTTGAGGAAGCACTA	40898
7273	GACCCACCAAAAACTAGCACTGT	18931	GTGGTGTGTTCCGCTACCTAGT	29915	TGCCATTGAATTGACCCACCAA	40899
7274	GCTGAGATTTTGCCCAGTACGTT	18932	GACTACAGGATTCGGAGAGGGAAAC	29916	ACCCCGCCTTATGCTGAGAT	40900
7275	CCTGTGACACTTCTCCTGAAACA	18933	AGAATCAAGGTGGCTTCAGACTTC	29917	CCCTGTACTTACCTGTGACACTTC	40901
7276	GTCATTGCTGAGAGACTCTGTGT	18934	CCAGTGCCTGGCTAAGTGTCAA	29918	CATCTCCCCTAGTCATTGCTGAGA	40902
7277	CTGGAAGGTGTGCAAAATAGTCTCT	18935	TCCTTCCCTCCACGGGATTCT	29919	TGGGGCAGCAATGTCTGGAA	40903

7278	GGTCCTCACTTCACATGGGTGTT	18936	CCCCACAGGTCCCTAAGATGAT	29920	ACCTGGGAGGTCCTCACTTCA	40904
7279	GGGATGGTGAGTGGTGGAAGTTAC	18937	GGGCCTGTCTGCAATCTCTTTG	29921	TGGCTGTGGGATGGTGAGT	40905
7280	ATCGCCCAGAAGGTGATAAATAAGT	18938	CCCTGTCACCAGCAGGTTCAAA	29922	AATCCCACCATCGCCCAGAA	40906
7281	CCTTGGAAAACACTTTGTGGCAATAC	18939	CTCTGAGGACAGACACGGTAAG	29923	AGCAGGTAACCTTGGAAAACACT	40907
7282	GCTGTCCTTTATAACAGCGTGGAAA	18940	CCTGACAGCCTACGTGCAAAAG	29924	GGGACCTAGACAAGTGCTGTCCTT	40908
7283	GCCTGTTTCAGGAGTTGCATTC	18941	GCTGAGAAACCTCGACCTTCTATG	29925	GCGTTTCCCACTTGCCTGTT	40909
7284	GCTCCGCAGTGATGTGGAAGAT	18942	GGTCTATGCATGCTAGGCAACT	29926	GGATAGACGCTCCGCAGTGAT	40910
7285	CCAGACCCTGTTGCAGCAATATGTTTA	18943	GATGCCAATTGAACACGGAAGTCT	29927	GTCATATACCTTTATACCAGACCCTGTTG	40911
7286	GCCTGGAAGAAGCCTGAATTGTTTC	18944	CTGAGAGCAGTTACCTTCCTCTTT	29928	AAAGATTTGCTTGCCTGGAAGAAG	40912
7287	TGTGGGCAGAATGTGAGAAGATG	18945	GACCTCGCCACTTTCTTGGAT	29929	CCAGGGAATCTGTGGGCAGAATG	40913
7288	GGCAGATACACACACTGCAGACA	18946	CAGTGTCCATCTGTGAACTTCTATCT	29930	ACTGCGGGCAGATACACACA	40914
7289	CACAGGTGCAAACCATTAATCACAGA	18947	TTGCTTGGTGCGCCCATCT	29931	AGGTCACAGGTGCAAACCATT	40915
7290	CTGAACTCCTCACAGTCTCAGATACA	18948	GTTAGAGCTGGAACAGACTTTAGAGA	29932	CCTGAGCTGTTTCTGAACTCCTCACA	40916
7291	CCTTGCATGCATACTCACCTCTA	18949	CAGCATCGACTGTGATGGTTTAC	29933	GTTCAGGGGTTTCTACCTTGCAT	40917
7292	GTGATCCTCCCAAGTGGCTATTACTAC	18950	GTCATTTCCTTAGGTGGGTGCAA	29934	GGCTCAAGTGATCCTCCCAAGT	40918
7293	GAGAAACCTTTTGCTAGGCATAAGTAG	18951	CACGTGACCCAGTGGAAAAAC	29935	TCACATGCCACCTCCTCTGA	40919
7294	GCTTCCTCCGACTATCGGAATC	18952	AAGCACGGGGCTGAGAAGAT	29936	CCTCGCTATATCAGGGAGTAGCTT	40920
7295	CACCAGGGTTCCATAAACACTGAGAT	18953	TGTGACAAGAAACATGGGTACAGT	29937	TGTGACCACCAGGGTTCCAT	40921
7296	CCACCTGAGGAATGAGTAGCTGGAA	18954	GGGAAGAGCTAGGATTTTCCCCATCT	29938	GGTTGACTGCCACCTGAGGAAT	40922
7297	GCTCTGCTATCCTTGGGTTCTTTT	18955	GGGCAGCCACCTTGTGAAC	29939	TCCATCTCTGGGCTCTGCTATC	40923
7298	GTGAGTTTCCTGTGACCTAACCATGT	18956	ACGTAGGAGCTCCACAACCTA	29940	TGTGGAATGTGAGTTTCCTGTGA	40924
7299	GCCACATGCACACTGAGCTTTTC	18957	TCTCCCAGTTCAGACCAAGTTGA	29941	CCTCCTGCCACATGCACACT	40925
7300	CTTGCCTTGGTTAGGCTTGCTT	18958	CAGTCGGAGTAAGTTTCCTCAACGAT	29942	GCTGTGTTTCCTTGCCTTGGTT	40926
7301	GTCCCAAGAGGGTATGTGGCTAGA	18959	CAAACCAGTCCTTCCAAGCCTTA	29943	GGGAGGAATTTTCCCTGTCCCAAGA	40927
7302	CCCGGTCTTATTGACTGGACTTC	18960	GTGGCTCAACACGGTAGATGTTTG	29944	AGAGCCCATATCCCGGTCTT	40928
7303	TCAACCTAGTTGGGTAGTGTTTTCA	18961	CCCCATTCACATCTCTGCTCTTTTC	29945	CAGAGCCAAACTGTATCAACCTAGT	40929
7304	GGGTGGTCTCTTCAAACTGCAA	18962	CCGTGTGTTGTGAGCATGAAGGTT	29946	TGTAGCCAGGGTGGTCTCTT	40930
7305	CGAGGGCCAAATGGGCAGAAT	18963	CAGGTTACTCACCCACAGCAT	29947	CCAAAATGCCGAGGGCCAAA	40931
7306	TTGCCTCAGAGGAGCACACA	18964	GTGACCACCCACATCATCACA	29948	GGAAGGTCAATGTTGCCTCAGA	40932
7307	GCTCCATCCAAAAGGCATGGAAGA	18965	CAGTAGAAGGTACAGTCCCCAAAAG	29949	GGGTTACCCTTGCTCCATCCAA	40933
7308	GCTGCTGCATAAGCTGCAAGGTA	18966	GGAGTGACTGCTTCTGCTTGAGA	29950	CATGGCTGAGCTGCTGCATA	40934
7309	AGCCTCCCTTCATCTCCTCTGT	18967	GGAGAGAAGTAGGAGTGGGACAT	29951	GGCTCTCAGCCTCCCTTCA	40935
7310	GCCACACCATCTTAACTGGCATCA	18968	GCTGTCAAAAGTGGATTTCACCAT	29952	CACTGGATGATAGCCACACCATCT	40936
7311	GCTCAAAGAGCTTCCAGAGAGT	18969	TCCCTGGACACCACGATCTCA	29953	CCCAAGCTACAGGGCTCAAAGA	40937
7312	GCGGTCCACAAAATGAATGGCTTT	18970	CCAAGCCCTCGTTCTGAAAGT	29954	GTCCTGCGGTCCACAAAATG	40938
7313	CACAGAGGCCTCCAATGCTGTA	18971	GGGTTGGTTAGTCTTGGGGAACA	29955	TTCTCACCTCCCACCACAGA	40939
7314	GGTAGGAACTTTGGAGTCACCCTTTTC	18972	CACAGAAGAGAGGGTATGAATGTGA	29956	GCAGTGAGTAGAGCAAGGTAGGAACT	40940
7315	GAACAAGTGTGGAAGAGTGTGGTA	18973	CTTTGCCTTTGCTAATGTCCATGT	29957	GCAAGTGTGAACAAGTGTGGAA	40941
7316	GAGGACAACACAGTGAACCAGAGA	18974	TGGACAGTGCAGCTCTACACATA	29958	GCAAGGTGAGGACAACACAGT	40942
7317	CCTCTGAGTACCTGTGGGAGTGAA	18975	CACACATCCCGCGAGGGGAATC	29959	GAAAGGTGTCTCTTTACCTCTGAGT	40943
7318	CCAGACCTTGTGGTAGGTACAGTTG	18976	GCATTGAACAGGCTCTTCCCTAGA	29960	GTGGCATTCCAGACCTTGTGGTA	40944
7319	GTCGGCTATGGCAGTATGCTAAC	18977	GTTGCTGTGAACACCTGCATGAAA	29961	GGTGTGCACTTGTCGGCTATG	40945
7320	GATGCTAAGGTTGTTCGGCTAGAATTT	18978	GGCTGAAAATATTTCCTCCATGGTGTT	29962	CTGTGAATGTGATGCTAAGGTTGTTC	40946
7321	GGATTCCGTCTATCATCCAGTGAATA	18979	GCATCTCCACACCTCCACTGA	29963	CAGGCCTTCTGGATTCCGTCTA	40947
7322	AGGCTGGGGTAACCCTTGGAAT	18980	GGCCATCATAGACCATTCATGCTA	29964	TGCACGTAGGCTGGGGTAAC	40948
7323	AGACCCCATATTTCTCCCTATTGAAC	18981	GCAGATGGACGTGATGGGTAGA	29965	CCCGAGCCTTAGAAAGACCCCATA	40949
7324	GGTCAGGACCAAGAGTGGACAT	18982	CCCCACAGCATTCCCAGTCTAC	29966	ACAGAGCTTAAGGTCAGGACCAA	40950
7325	TGCAAGTGTCCATACAGTCTTGAT	18983	GGACAGCCCTCTGTTGTTTCTAA	29967	CCTTAGTACTTAAGATGCAAGTGTCCATAC	40951
7326	AGTGTTAACTCACAAGGGCTCATAC	18984	GCAGAAGTGGGAGAAGTCTGTGTT	29968	TTCCTGCCAGTGTTAACTCACAA	40952
7327	GTCTCATGCAGAGTTGGTGACT	18985	CTGTCAACAGGGCTTTCAAGCTA	29969	CTCTGTCAGGACTCCAGTCTCAT	40953
7328	GGGGCAAGTGGAATGGAAGTCA	18986	CCCCAACACCCATGTATTTGTAGTGA	29970	TGGGAGGGGCAAGTGGAA	40954
7329	GGTGCAGATTAGCAGAACATTCTAAAG	18987	CGCCAGTCCACATCAGAACACATT	29971	CCGCTTGAGGAAAGGTGCAGATTAG	40955
7330	GAAACTGAAGCCCGCTACTCA	18988	CCCTAGCTAAAGGACACTCCTTATCA	29972	CCATGGGTTGGCAGGAAACTGA	40956
7331	GAGCCTCATGTCTTTGAAGCCTTTC	18989	CAAGGAAGGTCAAGTCTTCCTCAGT	29973	GAAGGGTAGAGCCTCATGTCTTTG	40957
7332	GACAGGTTTTGTTCTGGACAATAGGAA	18990	GGCTGGGCAGAGCCAGATAATA	29974	GAGAACAGGTTGATGACAGGTTTTG	40958
7333	CCACTCCCAACACACTTTTCTTCAT	18991	AGGTTCTGGCAAGTGGAAATAAAAG	29975	TGCCCCACTCCCAACACACT	40959
7334	GACCTAAGCTCTTCCTGACTTTGACT	18992	GCCAGGGTCGGTAACTGATGTATG	29976	CGCCAGTCAGACCTAAGCTCTTC	40960
7335	CGCATCCATAAAGAGGGATCCAGAGT	18993	GGCCCCATGCTGAACAGAAAA	29977	GGCTCCCCGCATCCATAAAGA	40961
7336	TGTTGGTTGGTAGAGCTTATTGCTA	18994	AGGACCAGCTTTAGTGTCTGATG	29978	CCCTGGAAACATAGATTGTTGGTTGGTA	40962
7337	TCAGGGCCTCAAAATTCCCTAGA	18995	GCTGGTCAAAAGCATCTTGCAAAACA	29979	GTCCAGGATTCAGGGCCTCAAAA	40963
7338	CTCTTAGCTATATCCCCTCCCCTCTT	18996	GAGGAAATTAGTCTGGCTGAGGTT	29980	CCAGGTTGCCACACATTCTCT	40964
7339	GACAGACAAAACTCCTGCGAGATAC	18997	GCTGTAAGAGCCTGGATACGAGTT	29981	GGGCTTTCCCTCAGACAGACAA	40965
7340	GAGGCTCCTCATGTGCTTGAA	18998	CATGTTCCTACATGTCTCAGAGCAA	29982	GGAAAGTCAGCACAGGGGAAGA	40966
7341	GCAATAAGCCAGGTAGGATTGTTCA	18999	CCCAGACCAAAGTCTTAACATTCCCTTTA	29983	GCACATGTTGCAATAAGCCAGGTA	40967
7342	CCATTTCTGGTAGGTCCTGAGCAA	19000	CTGCAAAGGGGACCTTCCAAGT	29984	GCACCCTGTCTTTTCTCACCATT	40968

7343	GGGCAGCAACTTACACTCTTAATC	19001	GCCCAGGTGGATAGTCAAGACA	29985	GGGATGGGCAGCAACTTACA	40969
7344	GGTGTGACAAGCACACCTTCA	19002	GACTGAAAAAGTTTGGAAAGCATGGCTCTA	29986	CCTGCAGAACTGGTGTGACAAG	40970
7345	GGAGGAAACCTTTGGTAGGGAAA	19003	GGGCATCTGTGTATATAGTTCCCAAGTCTA	29987	GAAAAGTCCAGGAGGAAACCTTTG	40971
7346	GTCACCTTTTGTCCACTTGATTGATG	19004	TCTCACGGGAATCACCATCATTTT	29988	GCTGGTGAAGGACGTCACCTTT	40972
7347	CCACCTCTGTAAACATGCAAGGAA	19005	GGGCTGAAGCAAAGACCATGT	29989	GTAAAATCCTGCCACCTCTGTAAAC	40973
7348	CTCAGTCACATGTAGTTGCTGTTG	19006	CCTGGCTTAGCAAGAATTTGTTTGT	29990	GGTGTAGAGTAGGTGCTCAGTCACA	40974
7349	TGTCTCTGCCTGACCCACAATC	19007	TGGGTGGGAGTGCCTGTAAAC	29991	TGGCCTCAGAGCTCTTGTCTCT	40975
7350	GGCACTTGGCAACATTCCACTT	19008	ACTAATGATGTGACCCACCCAATC	29992	TCCTCCCTTTTGGGCACTTG	40976
7351	TCAAGACATCTGTGCCCGATAC	19009	GACCCCGTTGCCTACTAACAGA	29993	GGGCTCACCTAACCTGGTATCAAGA	40977
7352	GTGAGGAAGACTACTGCCAGAAA	19010	CTGTCCACCACTACCTCGTATCTCA	29994	CCACTTGGTGCAGTGAGGAAGA	40978
7353	CTGGGAGTCTTCAGACCTGTCTTTG	19011	CAGTTACGCTCACTGGATGGAT	29995	TTCGCAGGGTCTGGGAGTCTT	40979
7354	CACTGTGCCTTTGCTGTGAAAC	19012	AAAAATGAGGCTCTGGGAAGTCA	29996	CCGAGTCCAACTACACTGTGCCTTT	40980
7355	GGCTAGTAGGTTTTGCTGGTTCAGA	19013	CCACCCTTTCTGAACTTAGGCATGT	29997	GGTTGTGAATCAGAAGGCTAGTAGGTT	40981
7356	GGAGTAGGACTCCAGCTCAGGTA	19014	CATAGGAATTGCCGGAGACATGA	29998	CCAGGCAAGTGGGAGGAGTAG	40982
7357	GTGGAGGCTAAAACCTGGCTGTT	19015	GCATTTGTGGCTCTAGGGTAAAAC	29999	GTGTTAGAACTCAGTGGAGGCTAAA	40983
7358	CTCAGGTCCACAGTGGCATTCT	19016	TCCCTGGCAATTCCCCTTCA	30000	ACCCCTTGCTGTCAGTGAAC	40984
7359	CGGTTCTTCCCAGAGCCCATAG	19017	GGCCACAGGTTTCTCTGCTTCA	30001	GGATGAGCTTGGATGCGGTTCT	40985
7360	TGTCTCGCTTCTCTCTGGATGT	19018	GAGTGGGAGCATGGATGGTGAT	30002	CAGTGGTTAGATTGTCTCGCTTCT	40986
7361	GTGAGAACAGGTGTCTAGGAGAGCTA	19019	GGAAATCCTGCTACATTTCGCTAGTTA	30003	TGAGGAAAGTGAGAACAGGTGTCTA	40987
7362	GCTCACTTTGCCCACGGTTTG	19020	GCAGGGACTTAATGAGGGCAATTT	30004	CGCGAGACAGATCTATGCTCACTTTG	40988
7363	GCTGGGATTGAACCTAGCTCTGAGT	19021	ACTCCTAGTCACTTGCACAGTCT	30005	AGGAATAATGGGGCTGGGATTG	40989
7364	GGCAGCCTTTCTTCTCCTTGACTT	19022	GACTAGTGTGTCTGCTTTGCGTTT	30006	CCATCTGGCAGCCTTTCTTCT	40990
7365	CCTTCACTTCCAAGGGTATGCTAAG	19023	GTTTTAGCCAGAGGCTCTATATCCAT	30007	GTTTCTCCAGCCTTCACTTCCAA	40991
7366	GATGGGGTTTTTATGGTTGGGCTTT	19024	CTGCTACCTACAGCCAGACTTCA	30008	CTGGCCAGGATGGGGTTTTT	40992
7367	ACATACAGCCTTGCACATTTCTGA	19025	GCTGTTATTTTAGCTCTTGCTGGTT	30009	GGGCGGCCAGAGAAAACATAC	40993
7368	GGGGAGAACAGATGCCAACA	19026	GGTGAAGCTTGTGTTAAGGGTTCAA	30010	GAGAAGAAGGAATGAGGGGAGAACA	40994
7369	CCTGGTCAAGGATCTTATCCCAGAAGT	19027	AGGGGCCTCCTGAGGAAATACT	30011	TTCACCCCTGGTCAAGGATCT	40995
7370	GGGCCTGAAGATACAGGCTTCCTT	19028	GGAACACCCGAGCCATTCTGA	30012	TGCCAAAGAGGGCCTGAAGA	40996
7371	CGAGCTGATCTGAGTACAATCACAGA	19029	GCTACCTGGGCTGTGGTATCTTC	30013	GCCTAGACGAGCTGATCTGAGTA	40997
7372	GGCCAGATTGGACAAGGACTGT	19030	TCTCCATGACTTCCCTCTGTTATTTC	30014	TTCCAGCATGGGCCAGATTG	40998
7373	GCTTGCCTCTATCCTCCTCTTC	19031	CCTCTGCTTTGACTTGCTTCAAAT	30015	CCCTGTCACAGCTTGCCTCTATC	40999
7374	GCCATGAAAGCCAGCATCTGT	19032	GCTTCTCTACCACATAGTGTAATGGTT	30016	ACCAGGATTTGAAGATATGCCATGA	41000
7375	AGTGTGCTTCCCACATCAAAGAT	19033	AGGAACCTGGCCCCTTCTCT	30017	GCCAGAATCCTGGTAGTGTGCTT	41001
7376	GGACAGACACTGATTAAGGGCAACT	19034	TGAAGCCCCAGTCCTACTTGA	30018	AGGTATTTTGGGACAGACACTGATT	41002
7377	CGCAAACATTCACAGCTTAACACA	19035	AAGCTGAGAAAAACAGCCATCTTC	30019	GAGGGTCACCAGAATCGCAAAC	41003
7378	CAGGTATCACTAGATTGTTCTCCCAAT	19036	GGCGTACACTATGGGAAGGTAAGTTG	30020	GAACCACTTCAGGTATCACTAGATTGT	41004
7379	ACTTGGCCAGCTCTCTCACTTC	19037	CAGGGTGGACCTCTCTGAGATATT	30021	TTTCCCTGGAGCATATCCACTTG	41005
7380	GCTGTCCCAAATGATTCCTGCTT	19038	GCTGCTGCTAATTCACCCTGTA	30022	GGCAAAACATATGCTGTCCCAAATG	41006
7381	GGATCAGCGGAGTTTTGGTCAT	19039	CTCATGAAAATTGTGCCGATTGCTAAG	30023	GCAGGTGGAGGAGTACTAGGATCA	41007
7382	GGTTTCTTGGTTTCAAGGGAAGGAATG	19040	CAGTGGTCAGGGACAGCTCTA	30024	GCCTGCTGCTAAGGTTTCTTGGTT	41008
7383	CGGCTTGTGAAAAACACAAGGAA	19041	GGCCTGGCCACTTTAGTACTCT	30025	GATGCTGTCTACGGCTTGTGA	41009
7384	GGATGTGCTGCATCCCAAACA	19042	CCTGAGATGAGGATGGGAGCAT	30026	AGGGAGCAGGCCATGGATGT	41010
7385	GGCATGTACAAAACAAGCCCTTGAA	19043	GCAGACTGACCCTGTGACTTTC	30027	AGGCTGGACAAGGGGCATGTA	41011
7386	CCCAAGAGTTTCAGGTAAGAGTTTGT	19044	TGAAGAAAGCATCTGGCATGTGAA	30028	GGCAGTATCAGAAACCCAAGAGT	41012
7387	GCCAGTTCTGAAAACTTCCACTAACA	19045	GGGGAGAGTGAGTCTAGGTTCTGT	30029	GGGAAGTTTTTCAAAAGGCCAGTTC	41013
7388	GCGTGGTAAGTACGATGGGAAGT	19046	GCGATAGACACTTTTATCCAGCTTTACACA	30030	TGCCAAGGGCGTGGTAAGTA	41014
7389	CAGCTTCATTCAGTACATTCCCTTTG	19047	GGGGTTGAGCTGGTTTGATATGT	30031	GCCCATGACAGCTTCATTCAGT	41015
7390	CAAACTGCAGGGGAAATTATGATCTGA	19048	GCTGTGTCTAGGCTTTTTCGTGATA	30032	GTAGCTCAAACTGCAGGGGAAA	41016
7391	TGACTCCTGCCTGGGTCAAA	19049	AGACGGACTCCCGGCGTTT	30033	AACCAAGCCCCGCCTGACT	41017
7392	AACAGTGGAGAGTGTGCTAAAGTT	19050	CAGTTCACCTGTCTGCTGAGGAA	30034	GCCCAGCAAAGGTTGATAACAGT	41018
7393	TCCCTGGAGTTGTCCTCCAA	19051	CCAGAAGTCTCAGAAAAGGAACCATAC	30035	AGGCTACATGCTCCCTGGAGTT	41019
7394	AGCCAGACTTGGGCAGAAGA	19052	CCACAGTGGGCAGCTATTCTCCAT	30036	CCCGGACTCAGCCAGACTTG	41020
7395	GGCGGAATCAGCATCTAGGATAG	19053	TCCATAAGGTGGTCCCTTGATTAAC	30037	GGGTGGGCGGAATCAGCAT	41021
7396	GCAGGAAGTTAAAACAAACTGAGCACTACA	19054	CAGCCAACTCGTAGGTTCAAACAATG	30038	CTGTCAATGTCTACAGCAGGAAGT	41022
7397	CACACCCAAATTATCCATTCGCTGAT	19055	CCTCCTGAAGCTCAGAATACACCAA	30039	ACCCCAGCCATCACACCCAAA	41023
7398	GTCCTCACACCTACTGTTCCTTAAGTTG	19056	TTCGGAAGCTGTGCATGATGA	30040	GTCTGTCCTCACACCTACTGTTC	41024
7399	AAGATGTTTTCCCTGGCCTTCTT	19057	CCCCTCAGTAATTTTACCTGGGATTAG	30041	CAGTCTGCTGGCTTCAAGATGTTT	41025
7400	GAGAGGTTCCTTCTCGGTCTTAC	19058	CGGGGCGCAGGCTAGAAAA	30042	GCTGGGTGGTAAAGAGAGGTTCCTT	41026
7401	GCAATGGAATCCCAAGGAAACATAGT	19059	TCCACGGAATCAGAGGATAGTAAGT	30043	CACCGAGTTGAAAAGCAATGGAATC	41027
7402	TGATCGTGGAAATGGATCTCAAGT	19060	CTGCAATGTGTTGGCTGGGAAT	30044	CAACCACGTAAGTGATCGTGGAA	41028
7403	GAGTCAGCCAATAAAGAGAGACTTGT	19061	CAGAGCCTTGATTGGACAACCCTTA	30045	CAAAGGACTGACCACCTAGAGTCA	41029
7404	GCCACGTTCTTCCCATTGTCA	19062	CAGAACCCACTGCTCTATGAGTGT	30046	GGAGTTCTACAGCCACGTTCTTC	41030
7405	TGCCACCTAAATGAAGACCCTTT	19063	AGTCCACCATCAGGAACAGTAGA	30047	TCTGAATAGTAAACGTTGCCACCTA	41031
7406	CACCCTTTGGTCCTCCTGTATC	19064	CTATCGAGATATTGGGGAAGAGCTTTA	30048	GGCTGGCTTCTCATCACCCTTT	41032
7407	CCCAGTATGTCCCAGGCAATCTGT	19065	GCAGCTCTGGTAGATAAGGCCATACATTA	30049	TGGAGGCTGTCCCAGTATGT	41033

7408	GTCCTTGCTCATGACTCAGAACT	19066	GGAGGGAATGGGCGTTTTCAAC	30050	GAAAAAGGCAAAAGTCCTTGCTCAT	41034
7409	GCTGGTGACTGCACATTTTTCA	19067	ACTTCTCAAGGACAAGTGTTCTCTTC	30051	CCTGTAATTCCATTTGCTGGTGACT	41035
7410	GCTGAGTATCTGCGTGTCTGT	19068	CTCTTGACCACATCTGGAAGTAGAAAC	30052	GTGGTGTCTCCTGAAGCTGAGTATC	41036
7411	GCTTGGCCTTACTGTACTTCTCT	19069	GACCAAGGAAAGGTTGGCTAATAGT	30053	CCTCTCACAACTTGCTTGGCCTTAC	41037
7412	GGAGCAAGTGGGTATGTTGAGAAG	19070	GCCTCCATTTCTTGCTGGTTGTT	30054	GGCCTCTGAGGGAGCAAGT	41038
7413	GGAGGACCGGTTTACATTCCTGATG	19071	AGCTTCTCCCAGATGCCCTACT	30055	CTGTGTTTTTGGAGGACCGGTTTAC	41039
7414	GCATTAGCCTCTTAGTGGTTCCTT	19072	TGTGGCAGGGACAGAGGTTCT	30056	GGCCGAGTCTGTCATTGCAT	41040
7415	ACAACACTTACCTCCCATGCATTT	19073	GGCTCCTAGGGGTGGTAGATTGT	30057	AAGCCCCTACCAAACAACACTT	41041
7416	CACTGTGACTGGCCTGTTCGTT	19074	CCTCATCCTTAGTGGGGAGTAACAGAT	30058	ACAGGTGTGAGCCACTGTGA	41042
7417	AGCCGTTTGGGAAAAGTGGAA	19075	TCTCATCTCGAGACACCCAGAA	30059	AGGAGGGACCAGAGCCGTTT	41043
7418	GTCCCTAAGGTAAGGATGGAAAATCT	19076	GCATGTATACTTGAAGCCCACACAGA	30060	GCCAGTGTTTGTCCCTAAGGTAAG	41044
7419	GCTTTCGAAGGGGTGTTCTTTTC	19077	AGGCCATGGCTCTTCTCCTT	30061	GCCTGGCCTTTGCTTTTGCTT	41045
7420	CAGCTTGTTGGAGAAGCATCTTTAC	19078	GTGAGATGGTATCTAGGTCACTGCTT	30062	CCAGCCCTTTCATCAGCTTGT	41046
7421	CTGTTTGACACCGAAACACTGCAT	19079	CCAATTGCTATAGCCAAAAGCCATACA	30063	GCTCGAGGCTGTACAGATGTTTCT	41047
7422	GTGCCTCTGTTGTCCAGAGTT	19080	GCTCTGTTCTTCAGGTGGGAAAG	30064	AGCCTGTGGAGTGCCTCTGTT	41048
7423	GGGCAAAAGAAGTGCAAATGCAACA	19081	CCGTGATGCTGGATCAGCCAAT	30065	GGTGGAAGCCAGATTGGGCAAA	41049
7424	TGCAGCAGAGACTGTCCACTA	19082	TCTCAGGGCCAGCCCCACTT	30066	GCCAGAGCTGCAGCAGAGA	41050
7425	TGGACATCACATCTGCATGGAA	19083	GACTGACGAGAACTGCAATGTGT	30067	GGCTTATACTTAAGGCATCTGGACATC	41051
7426	GCTATCCCTGGACTTCTCGGTTA	19084	CTGGCTTAAGCAAAAGGGGAATTT	30068	AGTTCTACCTGAAGCCAATGCTATC	41052
7427	GGCAGCCTTAATTCCCACCACAA	19085	AGCTCCCTCCTCATGCTACA	30069	AGGCCCTTGGCAGCCTTAATTC	41053
7428	CGTAAGAGGTTGACAGGTGCTAT	19086	TCTCGAAGTAGGAAGATCCCATTCT	30070	AGGTTAAAACACCGTAAGAGGTTGA	41054
7429	TGGCTCCTACCACACCTTGT	19087	CCGCTATGCCTGGCTTAACTTC	30071	CCTGAGTCTCACTCTGGCTCCTA	41055
7430	CACACAGATGCTACTGGAGTGAAC	19088	CGTGTCATTTAGACAGCCCTGTTTTC	30072	GCCGGGGAAATCTTTCTTCACA	41056
7431	AGGTCGGTCTTCCCAGCTAGA	19089	AGGTGACCCTATCTTCAGTGCTA	30073	GCCTATCTCTCAGGTCGGTCTT	41057
7432	CGGGAGAGTTCCTTCCTGTTCA	19090	CCGGTCAGCCAAAGGTCTTCTA	30074	AAGGCCATGGGCGGGAGAGTT	41058
7433	GAGGGAACACCATGTCCTCACA	19091	GGGTTTGAGGGTGTGGGTTTGT	30075	CCTCAGAAGGGAGGGAACACCAT	41059
7434	CCACAGTGAGGTCAAATGGATCA	19092	CCGCGTCGGATGAACAAGAAAC	30076	AAACTGGCCTAAACCACAGTGA	41060
7435	CATGAACGAGACTCCAAGGTAATTCT	19093	CCTCCCTCCCTATGGCTACTT	30077	GGCACCATGACATGAACGAGACT	41061
7436	CTGTAAGTAAGCTTGACTCCCTGTAT	19094	GGTGCATGATTTGAGAGGAACGAA	30078	GTGACTTCCTGCAGTTACCAATCTGT	41062
7437	CTGGAAAGATAGAGCTGCCACGAT	19095	CACCTTTTAGCTCAGAAGATGCAGAT	30079	CCCACACCTTTTCTGGAAAGATAGA	41063
7438	GTGGTTTACCAACTGCACTACCAA	19096	CAAGGATATGGAGGACCAGGATTC	30080	CCAAAGACCTCACCATAGTGGTTTAC	41064
7439	TGAGGCCCTGCACTTACTGA	19097	GGTCCATGGTGTTGAGGGAATTG	30081	CCCCACTAAACCTCCCAGTGA	41065
7440	CAGCTTCCTGGTTACTGTGTGA	19098	CAGCTAAGGGAAAGTCAATTCAGGTTA	30082	GCCATCACAGCTTCCTGGTT	41066
7441	GTGTGCCCAAGACCCTCTTC	19099	TGCACGGGGAAACTCACATTC	30083	GGCTGCTGTGTCCAGTGTGT	41067
7442	GGCAGCATGGTCTCAGGAGGATA	19100	TGAAACGATACCGCCCTTAAAAGT	30084	CTGCTCATAAACCAGGCAGCAT	41068
7443	CCCCAAATGGTAGTGAACTCTGCAATA	19101	TGTCACCAAACTTTCTGTGCATCT	30085	GACTTTCTCCCCAAATGGTAGTGAAC	41069
7444	TCTGCTCCAGCTGTCTAGACTTT	19102	CCAAAAGGAGCATGGCTGTCT	30086	TCCCCTGGCTCTTCCCTTT	41070
7445	CTGTACAGCATCACAGCTACCTAAAC	19103	AACGCCTACTTTCCCTGTCTTC	30087	GCGGAAGGTACTGTACAGCATCACA	41071
7446	GATGTGTCTCCAGTATTGTGTCTGT	19104	GACAACACGGCTGTCCACAA	30088	CGAACCAAAGATGTGTCTCCAGTAT	41072
7447	GGAGGAGAGAGGCAGAACTGTA	19105	CGAATAGTGCTGGGAATCTCTGA	30089	CCGGGAGCTAGGAGGAGAGA	41073
7448	GCGGTTAGCGCTAGACAACA	19106	GCTTCTGATCCTTGGAACATTTGGTTCAT	30090	ATGGGACCAGTTGCGGTTAG	41074
7449	CCCAGAGGTTCTGCATGTTTTG	19107	CCAGTAAACCTTTTCCATGAAAGCCTGAA	30091	TCGCGGAGCCCAGAGGTTCT	41075
7450	CCCCAGACAGGTAGTCCTGAGATT	19108	TGATTGGCTCCCAGAAGTTGTAAT	30092	CCCTTTGACCCCAGACAGGTAGT	41076
7451	GCTGGAAAGATAATCGTTGAGGCCAAA	19109	CTCTGAAAGATCGTGTCTTCCTTTGT	30093	GCCCAATTTGGCTGGAAAGATAAT	41077
7452	GGCGGTTGAGAGAAATGGTAGA	19110	TGTCAGCACTGCCTTCAAAATG	30094	TGACAGTGGCGGTTGAGAGA	41078
7453	AAGTATCTCAAAACAGCGGGGTTA	19111	CCCTAAAACATGCAACCTTTCCTTATC	30095	GCAACTGAGGGTGTGGAAGT	41079
7454	GCCGTAAAGTCCTGCCACTTAC	19112	AACAAAGGATACCTCCGGGTAATATG	30096	CATGGAGGTACACTGCCGTAAAG	41080
7455	GCCGGGGTTTTGCATAGCTTCA	19113	GCTCAAAGAGGATTAGGCTGCTTCA	30097	TTCCAGGAAGGCCGGGGTTTT	41081
7456	AGTGACTATCAGTGCCCACAAAC	19114	CACCTGCAGACTAGCACAAGA	30098	GCCATCTCAGGGTCAGTGACTATC	41082
7457	TGTCCTTGGTTGAGTCACTCTTTG	19115	CTTGCTTCAGGCCAAGCAAAA	30099	CCATCTCCTATGTCCTTGGTTGAGT	41083
7458	TGGATCAACTCTCCCCTTGACT	19116	GTCCATAAGGGCATATGGGTGTTTC	30100	TCTGGTACCCTTCAGTAATGGATCA	41084
7459	AGGACACAGGCTGACCACACA	19117	AACCGAGGGGAGTCCCAGTT	30101	GGGAACTGGATTTCAGGACACA	41085
7460	GTCTCTTGTTCACAATCCAAGGAGAT	19118	GCCACCCTGGTTCCTCATCATT	30102	CACTCCACCTCCAGTCTCTTGTTC	41086
7461	GAGGATCTATCGGAATCCTCTGTCT	19119	GGCAGGTCCTTAAGTGAGTGACTGT	30103	CAGCCGAACAAGGATGAGGATCT	41087
7462	TCCAGCAAAGCTACTTCTGTGAAA	19120	CAGCTCCTCTCCTGTCCCATTT	30104	GCGGCTCTAACACTTCCAGCAA	41088
7463	CTCTCCAGCGTTACCCGATTTC	19121	GGCGACTCGACACCTGAAAACT	30105	GTGTCGGTTCTCTCCAGCGTTA	41089
7464	CGTTCCCTCTCTTCTTCCCCTTT	19122	TGGGGAACCTTAGAGAGCTTGT	30106	CCTGTTTGAGTCTAATCGTTCCCTCTCT	41090
7465	GGGAAAGCAAGTAGAGGTACTAACATGA	19123	GCAGGTAGACGTGATTCAGCTT	30107	GGGGAGAGAAAATGGGAAAGCAAGTAG	41091
7466	AGCACCGTTTCTGGATGTGAT	19124	CAGGATAGCTCCCTAAGGCTCAAATG	30108	CCTTCAACAGGATAGCACCGTTTC	41092
7467	GAGGAGACAGGCAATTAGCACAT	19125	CTCCAGTGCCATACCCAGTTGT	30109	GCAGATCTTTCACTGGAGGAGACA	41093
7468	CCAGGTGGGACTGTCTTATCTCT	19126	GCTAAACAATGGTGTAGTCTTGGGATCT	30110	TTGCCTCCAGGTGGGACTGT	41094
7469	TCACACCTGGAACCCTGCTT	19127	GAGGCAACAGCAGACATCCATCA	30111	AGCACCATCCATCGGTCACA	41095
7470	CCCTACTCACTCCCTTCTCAGT	19128	TCGGATTCCTTGTTCTAGACCTTTC	30112	TCCCTGGGGAGACCCTACTCA	41096
7471	CCCGCAAGGTGGAAGGTAATAGTAG	19129	AGGCTCTGTCCCCATGACCTAA	30113	TCTCACCCGCAAGGTGGAA	41097
7472	GCTAGCAGTTGATGAGAGTGGATTTC	19130	ATCCGTGACAGGCCACACAAC	30114	CATAAGCACCCAAGTGCTGCTA	41098

7473	GGGGCCACAAATGGGGAAT	19131	CCCCAGCAGAGAGGAGACCATATT	30115	AGCTGGGATGTGGCTGTTTG	41099
7474	GGGTGGTTTGCGCTTCTTATCACA	19132	GAGATCAGGACAGAACAGGTCTAAAG	30116	GAGACAAATGTAACAGGGTGGTTTG	41100
7475	CTAGCAGCTCTTAACAGATGATGTTG	19133	GAGTACAGTCAGCCTCTTGTTTCCTT	30117	AACCATGCCAGCCTGCTCTA	41101
7476	GGTGGTGTGAGGGCTGGATATT	19134	CCTTCCTCATCATGGTAGCATCTCA	30118	GGGGATTGTTGAAGGTGGTGTGA	41102
7477	CTGGTTGGCATTATTTGCTCCAA	19135	GCAGAGCAGCATTGACGAACA	30119	AGAAAGGGCTGGTTGGCATT	41103
7478	GTGTCTGTCCTCTCCACATGTCTTG	19136	TAGCGGCACCCTGTGCTT	30120	GGGACCTCCCCTGTGTCTGT	41104
7479	CACACCCTGAAGTCACTATCCTTTC	19137	AGCCATGGTCCCAGGATACA	30121	GGTCCAGCACACCCTGAAGT	41105
7480	GCTCGAATTCACTACTGGGCTAAA	19138	CGTGCAATCCGTGGTGAGGAA	30122	CTGGCATCGCTCGAATTCACT	41106
7481	CCATCTTCTTTGCCATGCAGCAAT	19139	AGCTGGCCGCATTCCAGTTT	30123	TCGCTGGCCCTTCATCCAT	41107
7482	CTCAGAAATCACCTCCTCCCTGAAG	19140	ACTGAGATTCTCGCCCTGAAAATG	30124	CGAGGAAGACCTGACTCAGAAATC	41108
7483	GCTGTTCACCGCACATTCAGT	19141	GCAGAGAGGTTAGTTCACTAATAGAAGCAT	30125	ACGCAGACCTGGCTGTTCA	41109
7484	CTCTGTCTCCTGTCCCTGGAATAC	19142	GGCCTTCAAAAGGCAGGACTGT	30126	CAGTTTCTTGTATCTGGCTTCTCTGT	41110
7485	GGCTCTTGCACTGCTCCTATTTG	19143	CCTTGGACCAGAGACTATGTGGAA	30127	AGGGTTTGGCTTGGCTCTTG	41111
7486	TGACCCCATGTTCCAGCTACT	19144	TCCCTGTCTACATGAAGCAGATAAAG	30128	CCCAGTTACACTCTTGACCCCATGT	41112
7487	CATCTGTGAGACAACTGGTGAACT	19145	GCCAGAAATCTGCCCTCTAGGTT	30129	GCAGAGTGCATCTGTGAGACAAC	41113
7488	GTGCAGTGAGAGGCGAAGTACA	19146	GGACAGGCTCCAGATGCTAACAA	30130	GCAGGATTTGCCTGTGCAGTGA	41114
7489	GAAGTACATAGGGGTGACCATGAAA	19147	CTCCCCAGACAGAGAGAACTGA	30131	AGGTGCTTCGACAGAAGTACATAG	41115
7490	AGCGCTCTGCGATTTCCTT	19148	GTCACACACAGGCATGGCTACA	30132	ACAGCGTGGCATGCTTTCT	41116
7491	TCACACCACCCATCTGGAGTCA	19149	AGGGAGGGGCATCTGCGTAT	30133	TCTAGAAGCCTCCCTCCTCACA	41117
7492	GGAGGCAAGGAAACATAGTCTTTAAC	19150	CCTTTGCTTTTAGCAGGACACTTG	30134	TCTCTGAAAAGGAGGCAAGGAAAC	41118
7493	CAGTTTTCAGGCCACGTTCTAAACAAG	19151	CTGGGACACCAGGGAATGATGTTC	30135	CTCATTATCAGTTTTCAGGCCACGTT	41119
7494	GCAGGCATGGTATAAAACCCATTCCATT	19152	GACCATGTGGCTTGTGCAAAGTT	30136	GGGTGAATGTTGCAGGCATGGTATAAAAC	41120
7495	CCCTCTGTGGGGTACTTGGTAGA	19153	GGTCCTGATAGGAAGGCAAAAGT	30137	TGGAAACCTCCTTACCCTCTGT	41121
7496	GGTGAGAAATGCTGTTATCCACCTTTAG	19154	AGTGCCCCTCAGCACATGAAG	30138	CTGGAAAGAGGTGAGAAATGCTGTT	41122
7497	GCGCAGATCACCTTGTTCTCCAT	19155	GGTCGCCTTTGGAGCAGAGA	30139	ACCAGGGCGCAGATCACCTT	41123
7498	GGAAGGGGCTGTCCTTCTTCTA	19156	CAGACACGGCATCTTCCTGGAT	30140	TCAGGCGGTTGAAGGAGGAA	41124
7499	CCACACCAAACGGGTGTGAAAC	19157	GCTGCGTCTTTCCTGGATGT	30141	GGCAACCACCACACCAAAC	41125
7500	TGGATCGTGATATGGCGTTTTTAAG	19158	GCCCCAGTTATCACCTAATGAACACA	30142	GCAGTTGGCTTGATTGGATCGTGATA	41126
7501	GGGTTTTGGCTTAAAGCAGTCTGAGT	19159	AGTTTGTCTCCCATAAGCTCTGTAG	30143	GTAGCCCTTGGGTTTTGGCTTA	41127
7502	GGGAAGAGGACAAAGTGGGACTGT	19160	CCACCCTTAAAAACTCCTCTCATCAAC	30144	ACAAGTTTGGTGACAAGGGAAGA	41128
7503	CCTCCCAATCCCTGACCTGTAA	19161	CCACACTTCAAGGCTTCAAATCATCCTT	30145	TCTGTGCCTGTCCTCCCAAT	41129
7504	ACACCTCCATCTCGGCCACAA	19162	CCCGGCCACAAACTGCTTTCTA	30146	TGGCTCCCACACCTCCATCT	41130
7505	GGAGTTTTACCCACTCCACCTGATTC	19163	GATGGAGACCCCTCTCTCCACTA	30147	GGCTCTGTGAATACCAGGAGTTT	41131
7506	GGCAGCATGTGTGTGTTTCCATCT	19164	CCCATTTCCCACCCACAGGAA	30148	GGGAAAGGCAGCATGTGTGT	41132
7507	CCTGAATGCCAACTGGAGGGAAT	19165	CTCACAGCCTTTCTCAGCCTTTC	30149	CAGACGCCTGAATGCCAACT	41133
7508	TCTCCATTTCTGACGGGACATTT	19166	GCTAGGCTTGGTCTCTGCAACT	30150	AGGGTGTATTGCTTTCTCCATTTCT	41134
7509	CCAGGACCCAGACATAGGTGGAT	19167	CACGGTTCATGTAAAAGAGCGAAT	30151	AGGTCCCAGGACCCAGACA	41135
7510	GCTCAGACACACTCCTGGAACA	19168	TGTGGCGGACGCCATTATCA	30152	ATGCTGGGCTCAGACACACT	41136
7511	CAGTTTCACCTCTGAGCATTGATTTC	19169	GTTTGGTGCTGGCTTTGGTT	30153	GGTGAGTGCTCAGTTTCACCTCTGA	41137
7512	GGTTTCTACAGTTGCCTGTTATAGAGAAG	19170	CAGCTCCAGGCTGAGACATTCA	30154	CACATGGTTTCTACAGTTGCCTGTT	41138
7513	AGGACAACCAGAGAGGAACAATG	19171	CCAGGCCAACCTTAGCCATATT	30155	GGGCTGTGTGAGAAGGACAA	41139
7514	GAGAGGCCACTTTCAGCCACTA	19172	GGAGATGGAGGGAGCTTTGTGA	30156	GGCTGTTAGTGAGAGGCCACTTT	41140
7515	GGTAGCTGGGAGAAAAGAAGGTTAG	19173	GTCCTGCTTTAGGACTTTTGCACTTAC	30157	CCAAGCAGGTAGCTGGGAGAAA	41141
7516	GGGATGAAGATCGAAAATAGGACCAA	19174	GAATGGGGTTGTCCACAATTAGTATG	30158	TGGAACAATGGGATGAAGATCGAA	41142
7517	GCCAACGGGAAACACATTGAA	19175	CCACCAACCCGATCAGTGCAAA	30159	CCTCAATGTATGCCAACGGGAAA	41143
7518	GTGACTTCCTCACACTCCTGGATTC	19176	ACTTTTTCAGCAGCTCTCTTCATCT	30160	CTGGGAGTGACTTCCTCACACT	41144
7519	GTGGAGCCAAACCAAGCCAAT	19177	TCTTCCATTCTCCCCTTCCAAAATG	30161	CACACTGTACAATAGTGGAGCCAAAC	41145
7520	CAGGCTCAGTTGAGGTGAGGTT	19178	GCTGACAGACTTGCTTTCCCAGTA	30162	AGACAGCAGCAGGCTCAGTTG	41146
7521	GCAAAATGGCCAGGTACGATGT	19179	GCACCAGGTTGGATGTGCTA	30163	GAAGACATGGGCAGAGAAGCAA	41147
7522	GTTTCCAGTCTCTGCTCATTAGGAA	19180	TCTGTGGGTGTAAGGAATACACAATG	30164	ACTTCCAAATGTGTTTCCAGTCTCT	41148
7523	AGATGGGCTCTTCCTGCTCATC	19181	CTGAGGCTAAAGATCACCCATGGTA	30165	ACCCACACAGATGGGCTCTT	41149
7524	CCAGCCTTGATTCTTTGGAATCCTGTTAC	19182	GCTCCTCACGGATGGTCTGAAA	30166	CTGTCCAGCCTTGATTCTTTGGAA	41150
7525	TCCATCCCCTGGCCTCAAATCT	19183	CTCACCAGCCTGAGAAAAGGAAAC	30167	TGTCGTGTTTCTCCATGTTCCAT	41151
7526	GTGTGCCAGTGTACTTCATTCATCTGT	19184	CAGGGAGTCTGTCCCTTGCATAC	30168	GAGTATCTGTGTGCCAGTGTACTTC	41152
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7532	GTCCTGTAGCATCAGACCTTTAAGACA	19190	GACAGCAGAATGCAAGTGACCTT	30174	GCTGAAACCAAGTCCTGTAGCATCA	41158
7533	CCCTGCATCTTTCAAAGGCATCTTTC	19191	CTGTTTCTTCGCTGGCTACAGTTTG	30175	GCTGTTGACACAGTCCCTGCAT	41159
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7535	GTGACTCCTAATGAAGGCAGACTCCTA	19193	TGTGCCCAAGAAGTAAATTGGGATA	30177	ACGCTATGTGCTGTGACTCCTA	41161
7536	GTGGGCTTTGCAGTCAGGACAT	19194	GGTGTGGCCTTCACAGGTAAGA	30178	CGGTTTAGAGCATGTGGGCTTTG	41162
7537	GAAGAGGGGATTGAATAGTCAGGTTAC	19195	CCTCAAGAGCATGCTACACACT	30179	CTTCCCATTTGAAGAGGGGATTGA	41163

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7605	TGGCCAGGCAGCTCTGTCA	19263	GAACAGGCTGCTTCCGATCA	30247	TCAGCTGACAGGGCCAAAAC	41231
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7607	GCTGTGGAAAGGTCATCTGAGACATC	19265	GATGAGTGCAGACTGGAAACCATT	30249	TGGGCTGTGGAAAGGTCATC	41233
7608	TCACATCCACTGGCCGCATTC	19266	GCCTGAAGAAACTCGGCTCTGT	30250	AGCAACGGAAGGAACTCACATC	41234
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7613	GGTTGGCTTGTCACCCTAGAGA	19271	GGAGCAGGGAAGGATGCAACTA	30255	CCTCCTGTCTTGGTTGGCTTGT	41239
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7615	CCGAATTTTCCGAGGACCAAGT	19273	GGAGCCGCGCAGGTTTACA	30257	GCTGGGACACACCTTCCGAAT	41241
7616	GCTCTGCTAGATGCTGAGGATGA	19274	GTGAGCTCTGTGAGGATGGAGAT	30258	TGCCGGGTGCTCTGCTAGAT	41242
7617	CTGGGAAGAGGTGGATAAATGTTCA	19275	GTGATAACAGGAAGGGGATGTTTCTCT	30259	CAGTTATTTGTCTGGGAAGAGGTGGAT	41243
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7624	AGAGGGGTATTGCCCTGTCTAA	19282	GGGAAGAATCCTGGACCTCACCAT	30266	GGTGAAGCAGCATCTTAGAGGGGTAT	41250
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7626	GTGGGCTAGTTTATCCTTCCAAAATC	19284	GGAAGTAACACCCTACAGAACTGGAA	30268	CCGTGGATGTGGGCTAGTTT	41252
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7634	CCCTGCAGAACCACTTCCGTTA	19292	CAGCATCGGGGAGTAGTTTAGGTTTC	30276	ACCTGCGTCCCTGCAGAA	41260
7635	GCCACCTGAGACCATGCTTCTA	19293	GGAGGCAGAGAAATCCAGTTTGT	30277	TGTCTTGCTGCCACCTGAGA	41261
7636	GGCTTATCCTGGCACCGATTCT	19294	GCACCCAAGCCTGCGAGTAA	30278	AGCCTCTCCAGCTGGGCTTAT	41262
7637	GGGAGACAAACTCATTGGAGTCA	19295	CCGTCTGTCTAGGAAAGGAAGCTCTA	30279	TGGTGAGGAATAAAGGGAGACAAAC	41263
7638	GGAAAGCACATTGTCCAGATGGTT	19296	CTCACAGAGCTATAAGCAGGCAAA	30280	AAGAAGTCACTGGAAAGCACATTG	41264
7639	GGCCCACACATGCATGAAGA	19297	CCTGGCAGGGCGTCTTTTC	30281	TGGTCCCAGGCCCACACAT	41265
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7641	CCCAAAGCTCCCTCATTACCTTCTT	19299	GCTCAGCAAATGCCTACTAACTTG	30283	ACCTTCACAGGATCATCCCAAAG	41267
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7645	CCTCCAGAGAAGTCGGCAATGTT	19303	AATGAGGTATAAGGCAAGCCACTT	30287	CTTTGCAACCCCTCCAGAGAAG	41271
7646	GAGCGAAAGTGCGAGGTCATAG	19304	GCATCATTACAGGCAGACTTTACAAAC	30288	TGCGCACAGAGCGAAAGT	41272
7647	ACAGTGAACCAGCCCCATCTTC	19305	GAAGATCATCTGAAATTTGCCGGATTG	30289	AGCCTCTGGGCACAGTGAAC	41273
7648	GACCAGCTTCCTTATGACTTTTGGTT	19306	GAGAAGCCAAGGTGGCTACAAG	30290	GCCATCTCTTTACTGACCAGCTT	41274
7649	CCCATACACGTCACTGTCTCTCACA	19307	GGCAGGCTTATGTACCCACTGAAA	30291	GCTGTTCTTTCCCATACACGTCACT	41275
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7651	GTGGTAGCAGCAAAGACTGTAATC	19309	GCACTCTAGGTTGAATAGGTGCCTAGA	30293	CTGAAACTGTGTGGTAGCAGCAA	41277
7652	GCACACACAAGGACTTGCTCACA	19310	CTGCCAGGAAGTAGGATTCATTCATT	30294	ACAGGGCTGGCACACACAA	41278
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7656	GGTGTCATCTCTGTGGCAATATTGTGATG	19314	GCAGTTAACTACTTCTCTTAGCAGTGA	30298	CAGGTGTCATCTCTGTGGCAAT	41282
7657	GCTGCCGGGAAAACCATGTAGA	19315	TGCCGGTTCCTCTCTCTTCT	30299	GAGACAATGCTGCCGGGAAA	41283
7658	CGGCAGTGATCTCAATGACAGGAT	19316	GAACATTTGGGTTGCAGAAGTACTGA	30300	GCTTTGCTCCGGCAGTGATCT	41284
7659	GCCAAGGAAGCTCGAGGGATT	19317	GGAAAAACCGAAGGACCAAAAGATGA	30301	TCCAAGCGCGCAGACTGTAT	41285
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7661	GACTTCAAACAATGAAGAGGGGACTA	19319	CCTCTTAGGTGTTGGTTGGCAGAA	30303	CACCTGCTGGTGATATATGACTTCAAAC	41287
7662	GGTGTTGTACGTGGGAAATTTGGGATA	19320	CCTCCCTGGAAGGCTGTAAAATC	30304	CCAGGAAGGAGTCTGGTGTTGT	41288
7663	GGAGAGATGAGAAGGGAACAGATGATTTT	19321	AAACCGTGTGTGAAACCTGAAC	30305	GCGACTGTCACGGACACTTCT	41289
7664	GCACATCGTTTCTGCGTCTTG	19322	TGGGCCTTCCCCATGTCT	30306	CTCACCAGAGGCACATCGTTTC	41290
7665	TCCCTCCTGTTGGTCTCTCCTT	19323	GCAACATTTGGAGGTCAGGGAAGT	30307	CCTGGTTCTTTCTCTCCCTCCTGTT	41291
7666	GACAGCCCAAATCCCAGAGAGT	19324	TGCGTGCTGGGAGAGCTAAG	30308	AGCTCAAGGGACAGCCCAAATC	41292
7667	GCAAGCGGGAAGATGGAAGA	19325	GTGTGTGACGCTGAGAGATCCAA	30309	GGCAAAGGCAGGCAGAAAGCAA	41293

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7669	GCTATTGTTCTCACCTATGTTGTGTTG	19327	CACCCTAGGATGATGGGATGACTGAA	30311	GGGACTCACAAACTGCTATTGTTC	41295
7670	GCTGGCTTTAGAGCTGGGCATA	19328	TGAAGAGGAAGTGGCAGAGTTG	30312	GCTCTGCCAAGAGCTGGCTTTA	41296
7671	GGATTTTCCTCTACAGAGTGGTTTCT	19329	CAGGAGGCTCACAGAAGCTTTA	30313	TCCAACCTCTAGGATTTTCCTCTACA	41297
7672	CTGCTTGTGAGAAGGAGACCAA	19330	GGGAGAGGCCAGGTGGAAGT	30314	GCTGGCAACTGCTTGTGAGA	41298
7673	GCTTGGCCATGTTCTTGGAAGCTA	19331	GGGCTGGAATGTTGTGTGGGAAA	30315	AGGCTGCTTGGCCATGTTCT	41299
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7676	AGAGGAATTGCGAAACCATGACT	19334	TTCAGCGAGACCTGCCCTTTC	30318	CCCAGATGGAGCTGAAAAGAGGAATTG	41302
7677	TGAGCGTGGAGATGGTGATG	19335	GGGAAAAATCCTGGGTAACTACTTCTTC	30319	GGGGTCCAGCACTATTCTCTTG	41303
7678	TCGGAAGGGACTTTCTAGTCTGT	19336	GCACAAAGACAGACGTAAGATGAAAC	30320	CGAGGTTGTCGGAAGGGACTTT	41304
7679	CTCCCAGCACCTTCTGACAAAC	19337	CCAAGCAAACAGTGTACTTCAGACAA	30321	CCAAGGACTCCCAGCACCTT	41305
7680	TGACCCGCACAGCATCTTG	19338	GCCTCCCTGCTTCCTTCTGAT	30322	AATGTCAGGCAGCCCAGAGA	41306
7681	GGATCCTGCTTTCCAGTCTGTCA	19339	GACCCTCCTTCAACTCGTGGTA	30323	AACCGGTGGATCCTGCTTTC	41307
7682	GGAGTTCTTTGCTCCGTGTCTGT	19340	GAGTAACCACAGCTCATCCTCATAG	30324	AGTCCCTCCCTGGAGTTCTTTG	41308
7683	GTGGTGGTGTTATGGCTAGGTTAG	19341	GATCAAGCAGAAAGGTCCCTTGTTTA	30325	TGGAGAATTTGTTGTGGTGGTGTT	41309
7684	CTTCCAAACCCCAGATTCTTTAGTCT	19342	CCTGGTATAGCATAAAGGTTGAGAGTGAT	30326	AAGTTACATCTTCCAAACCCCAGAT	41310
7685	TCCTGCCCTTAGAGTCAGAAAGA	19343	TCTTCAATCCCTTGTGTGCCAATA	30327	GACAGAATCCAAATCCTGCCCTTAG	41311
7686	CCCCTGATTGACAGCCAGCAA	19344	GCAATTCCATGGGGCTGTAGCATAG	30328	CGAGGGTGACCCCTGATTGA	41312
7687	GTCTTCTGTCTACTTGGACCTTTAAGTCA	19345	GAAGGAAGGGATGGCCATTAGGAA	30329	CCAACTGGTGTCTTCTGTCTACTTG	41313
7688	CCTCAGGACCTTTGAGCAGTCCAA	19346	CACCACCCCAACCAATCCCTTT	30330	GCTTCTGTCCCTCAGGACCTTTGA	41314
7689	CCAGCTGCTGTTTCAAGAGTGCAT	19347	CACAGCCAAACCATATCAATAGGTGAA	30331	TCCTTCTCCAGCTGCTGTTTC	41315
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7691	GGAGACAAACAAGACAGAGGAGAT	19349	CTGCACTGCGTTTCCAGCTA	30333	GGGTAGAGGCCATGGAGACAAAC	41317
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7864 CACCCAGGCTTTCTGGCAGATT	19522 GCGTATTCCTAGGGCCTGAAGT	30506 ACTCCACTCACCCAGGCTTT 41490
7865 CGTGCCACAATAGTTTTTAGAGGGTTT	19523 GACCAAATGGAATGCTCAGCTACA	30507 CTTTGCATTCGTGCCACAATAGTT 41491
7866 TTACCGCGTCCGGAACTTCA	19524 GGAATGTGTAAGAGGTTAAGATCCCATTTC	30508 ACACTTGCCGTCTTCCTGTTAC 41492
7867 GGCATGCAAGTCAAGAGTCAAG	19525 AGGATGTTATGTGGCAAGGATGAT	30509 CAAGACCTAAGGCATGCAAGTCA 41493
7868 AGTAAGAGAGGGAAGGGATATTGCAT	19526 ACCACTTGAAAGCAGGGATAGTTG	30510 CCCTTACAACTCCATCAAGTAAGAGA 41494
7869 GCCCTCTACCCCTCTCTGCAA	19527 GGCAGAACTCAAGGGGCAAAAC	30511 CAGTCGGAAGGCGAGAGAGA 41495
7870 GGAATTCAGTCTGTACAAGGGATCA	19528 CCTGGCAACCTTCATTTAACCCAAA	30512 CGGTTGATCCCATGGAATTCAGTCTGT 41496
7871 GCACGTCCAAATCTTGCTCAATC	19529 GCTTTGTGAAGGGGTGTCCTA	30513 CCACATCCTTGCACGTCCAAATC 41497
7872 CCACACTGCATTTGGAATTTGGTATTG	19530 GGAAAAAGAAGTGGACTGACATCGGAAT	30514 CCAACTTTGCTCCACACTGCAT 41498
7873 GACACCTGACTCCCTAAAAGAAACTTGA	19531 AATCCCAACCCTCTGTGCTTTT	30515 GGAGAGACACCTGACTCCCTAA 41499
7874 GGCCCTCCAGAATAAGTGTGAAGA	19532 CTGCATCTGCTGTATCTCTAGTGTCT	30516 ATCCACTTGGCCCTCCAGAA 41500
7875 CCACTTCTACCTGGCCGATTTC	19533 GAAAAGGAAGGGAATGTACCCAGTA	30517 GCCATGTCCCGTACCACTTCTAC 41501
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7877 AGTGTCCATCCAGTCCACGTA	19535 CCTAAGAATGCACAAGGCTATTGAAGATG	30519 GCGTAGGCCACCAACAGT 41503
7878 TCTTCCCACACGTTTATGGACAAT	19536 GCCCTGCATCAGTGCTACTAAG	30520 TGGTGTTTCTTCCCACACGTT 41504
7879 GCAAAACAGACAGGCAGCCAATAG	19537 GCCCGGCCAAGATCATGACTTTTAT	30521 GGCACATCTGGGGCAAAACA 41505
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7882 GGCAGTTCTCAACTTGCAGTAATAG	19540 GGCATTGCCAACCTTTACAGACCTATC	30524 ACCCAATTGGCAGTTCTCAACTT 41508
7883 GCATGAAAGGGGCTAAGGTCCTAGT	19541 CCCTGGAGTCAACATCAGAGTTTTGT	30525 CCTGGCATGAAAGGGGCTAA 41509
7884 GGCATTGCACCATCCCCTAAGA	19542 GTGGAGTCACTAGGCTGACCAA	30526 AGTGCTGCAGATGGGCATT 41510
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7886 CTGGGTTTCTGGGTTCCCTTTC	19544 GCAGAGTCATCAAAAGCCACTTTC	30528 TTTGCTGGCATACTGGGTTTCT 41512
7887 GGGTTTAGGGACAGAATTCAACTTTC	19545 GGCCCTCCAAACATCATTAGTCCAAA	30529 GTCGGTGGGTTTAGGGACAGAA 41513
7888 GGGAGGCTAAGTGATACACACACA	19546 GGCCTCCCTTGAAGAATCGTTGT	30530 GCTGGAGGGAGGCTAAGTGA 41514
7889 CTCCACCAAAGCGATTAGGAGACT	19547 GGCTCACCAGAATCCTAGCAAAA	30531 CCACTTCTGTACCGTCTCCACCAA 41515
7890 GGGTGACCAAAGACGGAAAGTAGA	19548 TTGCTATAACCACTCAGTCCTTTGT	30532 CCCTCAAAACTAGGGTGACCAAA 41516
7891 CTCCAAGAGGTTTCCCTAGTTGTGA	19549 GGGAATGTCTTCCAGGGGATGTTT	30533 CCCACTAGACTCCAAGAGGTTTC 41517
7892 GTCCTGCCTGGATTGGGCTTTT	19550 GCATTACCATGCCCTGTGATTAAG	30534 CCATTCGTAGTCCTGCCTGGAT 41518
7893 GGTGGGCTTCATGCTAGGGTTTT	19551 GGGGACTACTACCAGTGTTAAGTGAGA	30535 ACCTACTGATGGTGGGCTTCA 41519
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7895 GCAAAAGATGCCTGTGGACAAAGA	19553 CCCTTCAGTGTCAACCTTATGGATCTTC	30537 GGCAGCAGGTCTCTGCAAAA 41521
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7897 CTCGTACAGTACTCTTGGGTGTAAAAATTG	19555 ACCAAAACGCCTCCAGAAAAGT	30539 GGACACAGATAAGTTGCCATTCTCGTA 41523
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7899 GCTGGGAGAAGGGTTTGTGTCA	19557 AGAGAGGACATCAGGGGAACAA	30541 AGGCTCTGGGCTGGGAGAAG 41525
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7903 GGTGAAAGGTGACTTGTGGTCTGA	19561 GCAAAGATTCAGGCATCGGAATAA	30545 ACCAACAGGTGAAAGGTGACTT 41529
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7906 CTGCCATGACAGCCCATTTCTCA	19564 CCAGAGTCGGTATTTTCAATCACCAAA	30548 AGGAGGGGTGGCTGCCATGA 41532
7907 GGACGCGCAAAGGTGTTTTC	19565 GTGTGTCATATTCTCCCCTAGGACTT	30549 TTCGGTGAAGGACGCGCAAA 41533
7908 CGGCAGCCAAAATATAGCAGAGA	19566 CCCAGACATGCCCTTGACCTTT	30550 CAGAAGATAAGTTAAACGGCAGCCAAA 41534
7909 GCCAGAAGATGCTGGCAAAAA	19567 GGCTAGAACTGAAAAACAGAGGCTGTA	30551 GGAAAGGACACAATTGCCAGAAGA 41535
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7914 GCCCTAACACCTACAAACCCCTTTT	19572 TCCAGTGGCGAAATGTTTCTGT	30556 TCTGTGGCCCTAACACCTACA 41540
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7916 GCAAACCAGAAACTAGAGAAAGGTATC	19574 TGATTGCCACTTCCATGCCATT	30558 GGACTGGAAGCAAACCAGAAACTA 41542
7917 GTCATTGCTCTTCTGTAATCCTCAGTTTTC	19575 CCACGTTGTAGGCTGTGTTATTC	30559 TGCCAAGTCATTGCTCTTCTGT 41543
7918 CCACCATCAGGGAGAAGGGATA	19576 GCCCAAAACCTCTCTCTCAGAA	30560 TCAAAGCCTGGCCACCATCA 41544
7919 CGTCCCTAAAACCATCTTCCTTCTTCA	19577 TCAGGATTTTGTGAGTGTTCGGTTA	30561 GTCCCGTCCCTAAAACCATCTT 41545
7920 CCCTCTGCATGCGTGTCTATC	19578 AGGCATGGAGCCTCTTCTTGA	30562 TGCCCAACGATCCCTCTGCAT 41546
7921 GCCAACAGTGTAGTGGACACAAGT	19579 CCTTGTGTGAGATCCTTAGGCCAAAAC	30563 GAAGGCAGCCAACAGTGTAGT 41547
7922 CTTTGCTAATCAACCCTCAGCAACT	19580 GCCCTAGATAGTCACCCCTTATCTGTCT	30564 CGGTTGCACTTTCCCTCCTT 41548
7923 CCAGACTCCCTCCCAGCTAGA	19581 CTGTCCTAGCTCCTGGTAAAAGTTC	30565 ACCCTCCCTTCCCCAGACT 41549
7924 CTCAGTCCTGAACAGGTCACCATT	19582 TTTCCCTGAAATGATGGCACTACA	30566 CCAGCCTCTCAGTCCTGAACA 41550
7925 GACCACCACACTGATGTCAAGAGA	19583 AAGGCTGGCTTCGGGTAGAGA	30567 GCTTGTGACCACCACACTGA 41551
7926 CCTAATGGACCTAACTCAAGAGTCATAG	19584 GGTACTGCTACCGTGCTGTT	30568 GCCCATGGAGTTACCTAATGGACCTA 41552
7927 TCAGCCATGACAGAAGCAAATCTA	19585 AGCACACTTGGCATGAAAAGAAAG	30569 GAGCGGGTCACTTCACCTTTT 41553

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7929	GGCTTGTATGTGGACTGCAAGGTT	19587	GGAATGAAGAGGGCTTCTCACA	30571	GCTCAGTAGCTGGGGCTTGTATG	41555
7930	GTCTCCAGCTCAATGCAAAGGAT	19588	GGGCAGCATGAATGACATTGTATCTCT	30572	CAGCTAGATTTGTCTCCAGCTCAA	41556
7931	GGTCAGAGAAGGGGAGTTGGAAAAC	19589	GGATGCCATGGCTGCCAAAC	30573	GCCTCCTGTTGGGTCAGAGAA	41557
7932	TGGGCTCCAAGAGGCAACAA	19590	GGAGGAGAAGCAGAGAGCATGAGA	30574	CAAAAGAAACACTCTGGGCTCCAA	41558
7933	CCCATTCTCAGTATAGGGTTTCCACTCA	19591	CCTCCTTGCTGGCTGAATTGTGA	30575	CTGCTGCTTTACCCATTCTCAGT	41559
7934	CCCTTTGCATCAAATCTATCAGGAAGT	19592	CCTGGAAACAGCTTGCTTGTGAAG	30576	CCTGTCTTTTTAACCCTTTGCATCA	41560
7935	CCCTCAAAACAATGGAGCTGGAA	19593	TGCCTTTTATTCAGTCCTGAGGTT	30577	CTGAGATGGTCACTGTCCCTCAA	41561
7936	CATTCCTGCTGGTCTTGGTTAGT	19594	GTAAAGCCCGGGTCCTAACATT	30578	GCCTCACTTCCCTTTTTCTTTCATTC	41562
7937	GTTCTGGGCCACGACATAGAGA	19595	CCTGTAAGATGTCTGAGTGGACAATG	30579	GGAACGATGTCACCTCTCTGGTT	41563
7938	GGCTGGTTGTCTTGAGGAAATGT	19596	GGCCAGCAATCTGGCTTTCA	30580	TTCCTTAAATTGGCTGGTTGTCTTG	41564
7939	GGCCCTTGAGAAGGTGAAGGAT	19597	ACCCACCTTCCTCTGTAACTATCT	30581	TTCATTCAATCAGGCCCTTGAGAA	41565
7940	GCATCAGTTTCCCTTTCTCTGGTTGA	19598	AAAGCAGTTTCTTACCCTGTGAGAT	30582	CAGCCAATGGGCATCAGTTTC	41566
7941	GCCCTGTGGAAAGATTCTAACAGTTC	19599	TGTGCAAAAGGGCTTGCTTATC	30583	CAGAACTGCCCTGTGGAAAGA	41567
7942	GCACCACACTTTCCCGAGTGTA	19600	ATGTGGTCTCAAATAGCTCCAACA	30584	TCCTACCGCACCACACTTTC	41568
7943	CCCCACAACTACTCCCCTTTCT	19601	GGCCACATGCAGGAACAAATG	30585	CAGGTCTCACCCCACAACTACT	41569
7944	GCCTTGACAAACTGAATTCCAGAGA	19602	CCTCTTCATCTCCTGGCTCACA	30586	GCAGAGGATTCTAAGCCTTGACAAACT	41570
7945	GGCAGTAGCATCACCAGAGAAG	19603	CTTGCTGGAGGGTCACTGTAG	30587	TCTGGAGGGGCAGTAGCATCA	41571
7946	TGCTTAGTGGTCATCTTTTCCTTGA	19604	GAGAATGCATCTCCCTTGGAAATAGT	30588	CGTAGCAGATGCTTAGTGGTCATC	41572
7947	CCAAAGTCAGAGCAGGGAAGTTCA	19605	CCGTGTGGTTTTACCATCCTTCAAC	30589	CACATGGTCAAGTCCAAAGTCAGA	41573
7948	GGCTACACAGAACCTATATAACCATCTTG	19606	CACTAAAAGGTGTGACAGGAGTATAGA	30590	CACTGTGGGCTACACAGAACCTA	41574
7949	GTGAGGTCAAATCATAGACAGGTGGAA	19607	TCCCACCCACCATGAGAACTAC	30591	GCTAGCTATGGGGTGAGGTCAA	41575
7950	CGGTAGATCTGAACAACCAAAAGATGCAAA	19608	GCAGCTACCTCTCCGTCTCA	30592	CCCACTTAACTGAGTATCGGTAGATCTGAA	41576
7951	CCTTGTTAGGCAAATGATGCAGTCTTC	19609	GCTGTTCTTGTCTACACAGTTTTCTAC	30593	GTGATGGGGCAGAGTTAATCCTTGT	41577
7952	GGAGACTGAATGAGAATCAAGGTTGT	19610	GCGGGGAAAAGAGAACCATTTG	30594	GCTTGCCAAAGGTCATAATGGAGACT	41578
7953	CTGACACTTGCCTCCTCCATGAAA	19611	TGCTCTATTCAAAGTGTGACTGTCT	30595	GCCAACTGGCAGCATCTGA	41579
7954	ACCTGTCACGTAGGGCTGCTT	19612	GCCCACCTCACTGGAGCAAAA	30596	GGCAAAAGGAAACCTGTCACGTA	41580
7955	CGTGTGCTTTAGGCTCCTCTGAAA	19613	CCAGACAGGCTGTTCAAAGAGGATAC	30597	CGAACATGGCCGTGTGCTTTAG	41581
7956	GAAGATGAGCTGAATAGCAGTCAAACA	19614	ACCCTTGTCAGGGTACGGTCAA	30598	GACCTTGCAAGAAGATGAGCTGAA	41582
7957	CGCTCAGATTAGGTGATCAGAAGCATT	19615	ATAAGAGCCGGCCCAGAACT	30599	GACTTGAGATCAGCCGCTCAGAT	41583
7958	CTGTGGCTGTACACAAGGACTCA	19616	ACATGTGCGCTTCTGGAGTTC	30600	AGGGAAAGGCTGTGGCTGTA	41584
7959	GACCTTTATACGAAGTCCCTTCCCTTTG	19617	GGACTGGACCTCAGTGTTGCTCTA	30601	CCACCTAGTAGGCGTTGACCTT	41585
7960	GTGTACTAAATCCCAACTCTCCTTACA	19618	GCGAGAGGGGAATTGCTGAATG	30602	CTCCACTTGTGTACTAAATCCCAACT	41586
7961	GGTAGCGATTCTCAAATCACATGGAAAG	19619	TTGGGAGGTGGGTCGGCTAA	30603	CACTCTGTATGGTAGCGATTCTCAA	41587
7962	GGTCAACTGAGCGGAGCAT	19620	CCAGAGGACACCAACGATCAGA	30604	TGGAGTCACCTGTGAGGTCAAC	41588
7963	GTTCTCCCACCAAAAATTCCACTTC	19621	TGTATATTTGTGCGCTCCACGTA	30605	GCTCCTTTGTTCTCCCACCAA	41589
7964	AGCAAGCCAATCCGGGAAGAAG	19622	GCAATCAGGTGTTTCTCACTCTAAC	30606	GTCAGCCACAGCAAGCCAAT	41590
7965	CCAAGAGCATAACCACAGAGGTCCTT	19623	GCAGTGGGGTGTCCAGCAA	30607	CCCCATTGGTGCCAAGAGCATA	41591
7966	GCAGTTCATGGCAGGATTTTGAAC	19624	TCTCTCTAGACCAACCCACATTCT	30608	TGCTTGGGAATTACAGCAGTTCA	41592
7967	GCCATAGCTGGAGATTGGACTTAC	19625	GGACTACTGTTTCTCTTCACCAACTA	30609	GGTTAGGGTGTCCCAGCCATA	41593
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7970	CAGAGGAGAAATGTGTGGAACCCAAT	19628	TCACCCCTTGCAGGACCCTTA	30612	AAGGTGTTCAGAGGAGAAATGTGT	41596
7971	GGGCTGTGAAAGGCTTCTCT	19629	CACTCCTGTTTAGTCTCCAGGTTT	30613	CCCATCCTTATGGGCTGTGAAA	41597
7972	CCTTGACGGTTGAACGGGAGTT	19630	CCTCCATTCTCTCTGACTGGGATGAAC	30614	TGGATATAGGCCTTGACGGTTGA	41598
7973	CTTGGTCCACATTTAAGGCACTTTTC	19631	TGGGTCAAAGATTACCAAACACTGA	30615	GAGAACCCTTATTTCTTGGTCCACAT	41599
7974	GGCCCTGAACACACATGCTA	19632	CTCCTTACTCACTTGTCTGACTGAAG	30616	AAAGAGAGGAAGGGCCCTGAAC	41600
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7976	GCATCTTAAGTGCCACAGGTTGT	19634	GGTGACCCAGAGACCCAAGAAA	30618	CAGGATGGCTCTGGGCATCTTA	41602
7977	GGTGAGGATGCTACCCCAACTT	19635	GACTGAGAGCAAGACAGGATAGATGATACT	30619	CCTCAGATGGGGTGAGGATGCTA	41603
7978	CTGGCCACATTCCGGAAATATAAAC	19636	GGGGCATTGCCTCCTTGAA	30620	CCATTTGGTCCTGGCCACATTC	41604
7979	GAGACTAGGTCTCCCTTACATTTGTT	19637	CACAGAAAGGCATGGTCAGAAAAATC	30621	TTCTTTGATCCGGGGAGAGACT	41605
7980	GGCTAATTGGCTGGATCCCTCACT	19638	GCCCCTGGTTTCATGACAGTGTTC	30622	CCTGGATAACTATCGGTTGGCTAATTG	41606
7981	GTGACCTGCTAAAGAAATACAGCCTACA	19639	CGAAGTATCATTTTCCCCAGTAGTCA	30623	GTTCAGGCATGTGACCTGCTA	41607
7982	CAGATCGGCAGCCAACAGA	19640	CTCTTCATCGCACCAGCAACT	30624	GGTGGGAAACGGGTGCAGAT	41608
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7984	GCAGACCTTTGGGCCTAGAGAA	19642	AGCCTTTTGGGGTCCACTTTG	30626	ACCACCCTCGCAGACCTTTG	41610
7985	GGGATCCAAACTTACTCTTTTGCATGTGA	19643	TCAGCAAATGGACTCAGACAACT	30627	TGAGGTAGGGATCCAAACTTACTCT	41611
7986	GGCAAAGGATGATATAGAGGAGTCCAA	19644	CTAATCCCTCCAGCCTCTCCTT	30628	GTGGCTGGCAAAGGATGATATAGA	41612
7987	GCCTGGAATAGAGGCTTGTGGAA	19645	GGGAGCTGCTTTGCATGAGT	30629	GAGGCAGCAAAGCCTGGAAT	41613
7988	CTCCTGTGGAGCCTCCTTTTCA	19646	GGCAAACTTCTGGAGGGACAA	30630	CAGCCATTCAATCACCCTGTTTC	41614
7989	GCCAGTTGGGAGCCAGAACAA	19647	TCCTGTTACAGTAGCCAAAAGCAT	30631	TGGAATGGTGGTGCCAGTTG	41615
7990	GGAGGGTTGCTGTTGCTCTGA	19648	GAGCTACCAACCTTCTCCACCTT	30632	TCCAGTTTCTGAGGCCAGAGT	41616
7991	CACCCAGGGGTATAGAAGTGTTTCTTC	19649	GAAATCAGCTCACCCATTGCTACT	30633	ACCTTCACCCAGGGGTATAGAAG	41617
7992	TCTTACTAGCAAAACAAGGGGTTCA	19650	GAACTTAAGGAGTCGATGGTCGTT	30634	CATCTCCGGATCTCCATTTTCTTACT	41618

7993	GCTGTATAAGCTCCACCTCCCAAT	19651	GCCACAGACATCACTAATGAACACA	30635	CCACAGTCCCAAAAGGGCTGTAT	41619
7994	GCACCCTTAGCACAGGGTTCAA	19652	GAGGAGCTTGCATAATGGGGAGTAG	30636	CCATCGTGTCCTTGCACCCTTAG	41620
7995	AGGCCCTATACGCCCCTGTTT	19653	GGTCACTGTTAGGCAAGAAGCCATAC	30637	CCTGTGCACTCAGGCCCTATAC	41621
7996	GGAGGACATGACCCGAACCTT	19654	CCTATACATGCAGCTTAACATGTGACT	30638	CACACACAAGTGGAGGACATGA	41622
7997	GGAAAAGCCTAACTAAGCTCTCGAT	19655	GGTGGCATAGTTTTACTAGGTTCAACA	30639	GAGGCTTAAGGGAAAAGCCTAACTA	41623
7998	GACTGTGACTGGATCTGGATTTTAGACA	19656	CACTCCCCTGTCCATGCTACTTT	30640	GATGTTAGTGACTGTGACTGGATCT	41624
7999	GAGAGCCTGTGATGAGGAAGACA	19657	GGAGACCGTCCTGTGATACTGT	30641	TCGAGGTTGAGAGCCTGTGATG	41625
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8004	GCCACACACGTCCCTGATGAAA	19662	AGGGCACTGACGGCTGCTT	30646	TTGCACGGAGGCCACACA	41630
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8018	CCTCATTCTCACTGTTGGACGTTCT	19676	CCAGGAGGCAAGCAGGTAGTTT	30660	GCCCATGAAATTGCACCTCATTC	41644
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8021	GCTCTGTCAATACCCCTGCAA	19679	ACGGTGTCTAAGAATGAGCTTGT	30663	GCAGATGCTCAGGCTCTGTCAATA	41647
8022	GTGCTGCATCAAGGAGTCTGAAG	19680	GGGGCAGATTCCGTTCCATTCA	30664	CCAGGGCTGAGTGCTGCAT	41648
8023	AGCCTGAGTTCCAGCCTTTG	19681	CCCTTCTCTACTCAGAATGGAACAGT	30665	TGGGATCAGAAAGCCTGAGTTC	41649
8024	CCCACCTCTCCAAATAAAGGAGATTC	19682	CTAGGCATGGCATGGTAAACAAG	30666	GCCCTTCCCACCTCTCCAAATA	41650
8025	GGCCATCCTCTTCATTTCAGATCAGTT	19683	GCAAACAGTAAGCAGCTTTGAGA	30667	TCCTTGAGGCCATCCTCTTCAT	41651
8026	CAGAGAGTCCTCGAATTTGGTGAA	19684	CTTGAGATTTTCTCAGGCTCTTGAATC	30668	GGGAATAATGGACAGAGAGTCCTCGAA	41652
8027	GAAGCAACTGGAGCAGGCTTA	19685	CTCCCTAAGTGTTTTTATGGGTCTCTGAA	30669	CCTGTGGGGAGTAGAAGCAACT	41653
8028	TGGCCAATGAGATAAAAGGAGTTGA	19686	CTTGAAAGCTTTATGACCAGGCTTAG	30670	GATTGGGTTCTGGCCAATGAGAT	41654
8029	GAGAGGATGAGCAATAGTTAAAGAGACTGA	19687	GGGACACTCACTGCCTTTGGTT	30671	CGGTGTCTTGGAGAGAGGATGA	41655
8030	CGATGAGACAAATACCGGCTGAA	19688	ACCTGCTTCAGATCCTACAGGAAA	30672	AGGTGCAGCCTTCGATGAGA	41656
8031	CCAACTCTGAAAGGTGCTGTGGAA	19689	TTGGGGCACCGAGGGATTTGT	30673	GAAACAGGCATCATACCAACTCTGA	41657
8032	CGTGTTGCTAGGTACTGGGATTCAT	19690	TTGTAAACCGTGAGGGAGAGATG	30674	GCCAGGCGTGTTGCTAGGTA	41658
8033	GGAGCCTTTGATGGACTGGCATT	19691	GTTCATGCTGATGCCCGTTTG	30675	GCTGCTAACAAAATCAGGAGCCTTTG	41659
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8669	CCCCAGGTATTCTCAGCTTCAATG	20327	AGAGGTTTGCACTTCAGAGACATC	31311	GAGTCCTTACCCCAGGTATTCTCA	42295
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8672	CACTGCTCAGATCACCCTTAAAGA	20330	GGAAGCTGCCAGTCTACTGTGT	31314	TGGAGGTGCACTGCTCAGAT	42298
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8677	GGTGATTGCTGCAGATGGGCTAAG	20335	CCCAAGAAAGGTCTGTTCTGACTGTA	31319	GTGCGAGGAAATAGGGGTGATTG	42303
8678	GCAGCATTAACATTGCTGAACGTTTCT	20336	AGCTCAGATCCACCCCAGCAT	31320	TGACCAGAAAGATTGGCAGCATT	42304
8679	GAGCAACACTGATGGAGAAGAAAGAAATGT	20337	CTGTGTCTGTTGGATGCGTAGTGA	31321	CTTTGAGCAACACTGATGGAGAAGA	42305
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8683	GAGGGTTTATTCACCATCCATCACA	20341	CCAATTGGAGGAAGGATGACCTATC	31325	CAGTTGCTATACCAAGAGGGTTTATTC	42309
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8689	GCATGGAGTATATTGCTGCTGAAAG	20347	GGGTCAGAGAAGCTCAACCTTGGAA	31331	GGGAACAGATAGGTAGCATGGAGTA	42315
8690	GGTCAGAGAGAGAACAAACACTTCCTT	20348	GGCTCTAACAGCTTTTGTCATGTTT	31332	GCACAGCACTTAGGTCAGAGAGAGA	42316
8691	CTTCCAAGCCAGTCCACCAACA	20349	GCTGAAAGTTAAAGGAACTGGTGGATTC	31333	GCTTCCAAACTGAACTGCTTCCAA	42317
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8693	AGCTCCATAGCAACCTCTCTGAA	20351	GGACACTGTTTGCTTTGTGTGAGTAAA	31335	GCCAGGCACAGATAGCTCCATA	42319
8694	ACCCTTCCTTGCAAGACATTGAT	20352	GGAGAGTGTCCCAGACTGATCT	31336	TTCCCAAATCAAGAACCCTTCCTT	42320
8695	GGGAGAATAGCAAGTCTGGTAGCAAAG	20353	CCACGCCGAGCTGTGAAATAGT	31337	AGCATGAATGGGAGAATAGCAAGT	42321
8696	GGATCTGTGGCTGTTTTCAAGAAG	20354	TCTTCTCCCTGTCCTGTCTTTCT	31338	GCAAAGCTGGATCTGTGGCTGTT	42322
8697	AGGGCTATTTCATAGGCTTGGATTC	20355	GCCTGTGGACACAGCTTTGTTTCT	31339	CAGAAAGGTCTGCTTAAGGGCTAT	42323
8698	GAGACAGCAGGTATTTGTTCAGTTG	20356	CGGCTACTTCCTTTTGCCTTAAAA	31340	GGAGGTATTCAATGAGACAGCAGGTAT	42324
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8700	GCTCTCCATAGCCTCATTTCCCACTA	20358	CCATAGTCCAGTGTGGCTGCAT	31342	TTCTCTAGCACAAAGCTCTCCATAG	42326
8701	GCAACAACCTTGTGTAAATCCCTTCA	20359	AAAATTCAGGCTGACAGGTAAGCTA	31343	GTAACTCTGCAACAACCTTGTGTAA	42327
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8703	TGGTTTGCACTTCACTCGTTTC	20361	GCAGGCTAGAAGGGCAAAGGGATA	31345	GCATCTGCCCTACATTCTATGGTTTG	42329
8704	GCTTTTGCACCATTGTGAAGTTG	20362	CCTGTCTACAAATGGTCCCTGACT	31346	TGCTTGTTGCTTTTGCACCAT	42330
8705	CTGAACCGTCTGTGGGAAAGTTCT	20363	CACATTGTTACCCTATAACGGTGTTGT	31347	AAGGTAAAACTCTGAACCGTCTGT	42331
8706	CTCCCATAGGCACATCAATTCTGA	20364	GGAAATAGCTGGGTGTTGCTACTAAG	31348	CCTGCTATTCCTCCCATAGGCACAT	42332
8707	CTGTTCAGAATCAGGACTGACTACTCTCT	20365	CTGAAGTTGGTATCCCTTTAGCTTCAAAC	31349	TCCACAGTGGCTGTTCAGAATC	42333

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8709	GCTGGCATCTACTGGCACACA	20367	ATTGGCTAAGCAGGCCAATCT	31351	TGTTACTACTTCAGCTGGCATCTAC	42335
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8712	GAGGAAAGGGTGGCCATCATCA	20370	CCACCAGGAGCTCAGTTGTGTT	31354	CGACTACCAAGTTTCTGGAGGAAAG	42338
8713	GCTGCACAGGATTTTCCACAATG	20371	GTGGTTACTGCATCGGACAGT	31355	AGGGCTGCTGCACAGGATTT	42339
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8718	GCAGATCTTGGAGATGACCCATAATTC	20376	CCTAAGCCTTCTGCAGCTGAAAT	31360	CCTCCGCAGATCTTGGAGATGA	42344
8719	GCAAAGAGCCAACTTCTGATCTGT	20377	GTGAAGCAGCTGGAAGTCTCT	31361	GGCAAACCAATAGCAAAGAGCCAACT	42345
8720	AGGTAAGGAGGGCAGTTTCTATGA	20378	ACTGCCTCCCATTTCCATTTTCT	31362	TGCAAACAACCCAGGAAGGTAAG	42346
8721	CGTGGTAACACCGCACTCAAG	20379	CTTCACTACGTAGATGCCATCCTATT	31363	TTGTGGCGGCGACGTGGTA	42347
8722	GGGTCCAAAGGACATTTCAGACTA	20380	GACCCTCTGTGTCTTTGTGCAT	31364	TGACCTGAGAGAGGGTCCAAAG	42348
8723	AGGGCTGTGTGAGGTTCCAT	20381	AGCTGCCTCCCAGAGAAGAT	31365	GATGGCATTATATCAGGGCTGTGT	42349
8724	GCCTTGAAGTTGCCTTCAAAACACT	20382	AGTCGCTGTGGGGAAAGGAA	31366	TGCCCCTAGCCTTGAAGTTG	42350
8725	GCCTGACCAATTCTGTGGCTCTT	20383	GGCTTCACGGCTGAAAGGAT	31367	CCACCGTGCCTGACCAATTCT	42351
8726	GGTCTCATCTCACTGTGTCTCAAC	20384	TGGCAAGGCATCCCCAAGA	31368	CGCTTTCCCAATGTACCAGGTCTCA	42352
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8728	GTGTCATCCCACATTGAGTGAAAACT	20386	GGACTCCAGAAGCTTCAGACAAC	31370	GGTTTGACCCTCAACATGTGTCAT	42354
8729	CCCTCGTCAGCTTTTCCACTCT	20387	GGCAACAGAACAAGACCTGGTGAA	31371	GTCGAATCTACCCTCGTCAGCTT	42355
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8731	CGTGGGCCTAAGTTTTCAACTCATT	20389	CTATAACTTCCAGTAACTGTGCTCCTT	31373	TGTGTAGAATTTTACGTGGGCCTAA	42357
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8738	GTGGATCTTTGCTCTTGGTGAAGATGTA	20396	TCCAGGGCAGTGCCTCCTTT	31380	GGCATTTTGACTGTGGATCTTTGCTCTTG	42364
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8744	CCTGCACCGTGCCTTCTCATT	20402	GCCAAACAAAGGGAGCACTGGTT	31386	GGCTTCTGTGCACCCTGGAA	42370
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8748	GCAGGAGTCAGAAAAGTAACACAAA	20406	CTTCCCTATCCAGCTGGTCACT	31390	CCAGAGGAATGCAGGAGTCAGA	42374
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9233	CCTGGTTGGGTGTCTTTGTGT	20891	GCACAGGATGGACATCAGGTTCACT	31875	CCCAGTGGACAAGTACCTGGTT	42859
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9235	GGGCAAAGGAGTGGCATAATCT	20893	GTGCACAGCCAGAATCATCCTT	31877	GAGACAGGAGCCACTGGAATTTTG	42861
9236	GGCTCATGGTAGCTACACCTTTC	20894	GCAGCCTGGTGGACTATTGGAT	31878	GTACTGTGTGTGGCTCATGGTA	42862
9237	GGCTTCAGCAGAGTAAAGTAGGAA	20895	GCCCCTGCCTGCATCTCTA	31879	AGGTCCCAAGACTGTGGCTTCA	42863
9238	CCAACTCTCCTCCAAACAGTGATTC	20896	GGAGGGGACTCAGGGACCTAAAAT	31880	TCTTGAGACCAACTCTCCTCCAA	42864
9239	CAGATTACACGCGATTCACAAATCA	20897	AGTCAGTAGGGTTGCAGAAAGTATAAC	31881	GCGCTCAGATTACACGCGATT	42865
9240	GAAGGAAGTAGAATCCACCAGATCAAC	20898	GGCCTCTGTCCCTTCTCTTTAC	31882	ACAGCTGTGAAGGTATTGAAGGAA	42866
9241	GGAGGAGGAATCAATGATGGTGTAGAT	20899	CTCCCTGCAACACCCTCTTATC	31883	GGAGCAGGAGGAGGAATCAATG	42867
9242	GTCTGCTAGGGTTAAACAGGGCTTT	20900	CAAGGTGGTAACCTGTGATTTGGTT	31884	GCCCAGGTCTGCTAGGGTTAAA	42868
9243	CAGCACAGCCATCATTTCTTCCAATG	20901	GGAGGAATAATGTTTGCACCAGGGAAT	31885	CCATCCTCAGCACAGCCATCA	42869
9244	GAGATGTGCCTATACTGCGTTTGAAG	20902	GGCCCCTCCCTCTTTACAACT	31886	CAGACAGGTTGAGATGTGCCTATAC	42870
9245	TGGGAGGGAATGAGGTGTTATCT	20903	CCGGGTTCGGGTCACCTTT	31887	ACGACAGTAATGGGAGGGAATGA	42871
9246	GAGACAAAATCCCACTGTCAGGATAAC	20904	AAGAAGACCCACCCCTCAGAGA	31888	GGGAATGGCTGATGTTAAGAGACA	42872
9247	GGCACAGAAGTAGAGGCTGTAG	20905	AGTGGGGCCTGTCCTCAAGT	31889	TTGGGAGAAGCAGGCACAGA	42873
9248	ACACCCCAGGTCCTGACTGATA	20906	AAGGACGGCGCAGTGAGGAA	31890	ACGCTGGAGCTGGTGAAACA	42874
9249	CCTCTTGTTTGCCCCAAGCTTTC	20907	GGTCACTCCCTCATCAGCTTAG	31891	GTGGCAAAATTGCCCTCTTGT	42875
9250	GCTTTTCAAGAACGGGAGACACACA	20908	CCCTGAGCCTAGGGTATTGTAATTT	31892	GGACCACGGATGATGCTTTTCA	42876
9251	CCAGGAAGGAATGTTTTTACCTCTTC	20909	GGTGCAACTGATGGCTGTAGT	31893	ACCACATAAAACCAGGAAGGAATGT	42877
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9270	ACTCACCATGGGTGGGTCAA	20928	ACCCTCACCATCCTCAGTTGAT	31912	GTGGCAGCAGTAGACTCACCAT	42896
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9283	GTTAGGAGGGTGTAAATCTGTGAATGA	20941	ACTTTTAGCTCAGGGTCATGCTT	31925	CCTGCATAGTGGTTAGGAGGGTGTA	42909
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9289	CTGCCCCTGGTTAGAATCAACT	20947	GCATTGCAAGTGTTGTGACAGA	31931	TGCTGTGCTGCCCCTGGTTA	42915
9290	GCTGTGACTCATCCACACTGTCT	20948	TTTCCTGGAGGCCATTTTGTCA	31932	GGGTGATGGATCTGCTGTGACT	42916
9291	GGGCAGGTGGATGCTAAGTTTG	20949	TCCAGAGTCCCTTGCTTCATCA	31933	TGGCTGAATGGGCAGGTGGAT	42917
9292	CCTTACATCAAAGAGGGTGAACTGT	20950	CACTGCCAGAGCAACAAGAAAC	31934	GTCCCCACTGCACCTTACATCA	42918

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9294	AGGCCAGAGTTTGTCCAGGAT	20952	GGCTGAGGGATGGGGAAGAAGA	31936	CACGGCAAAGGCCAGAGTTT	42920
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9307	CCAGCCATAACCAGAGGTCAAGTTC	20965	AGCTGTCAGGACTTGGGTAACT	31949	CCACACAACAAGGATTCCAGCCATA	42933
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9314	GGGCAGCCAACATAGCCTAGAA	20972	GGTGAGAAGCCCATGGAAGTATTGA	31956	TGCTACAGGGCAGCCAACAT	42940
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9398	CTGTGGCTTTAGCTGAGAGTTCA	21056	CACTGGAGTGCTTACTGAATGAAAACA	32040	CCTGGTTCTCAGTCTGTGGCTTTAG	43024
9399	GAGGAAGAGTTGCAATACAGCCAAT	21057	AAAGCTGAGGGGTGGAGAGA	32041	AGGAAGGGGATGAGGAAGAGTT	43025
9400	GGGTAATCCCAGCATGAAATGAGGTAAA	21058	GCAGGTGCTAAGGACTGTAAGAAGT	32042	CCTCAAAGGGTAATCCCAGCATGA	43026
9401	GGACAGCCACTCCTTAGTTGTTTGA	21059	CACCCCTTTAGAGCTAAGGAAACAAG	32043	TTAAAACTGGACAGCCACTCCTT	43027
9402	GGGTCATGTCTCCATCCAGTGA	21060	GAGACAGAGGTCCAGAGGTGTT	32044	CCCATGGGTGGGTCATGTCT	43028
9403	CCAGGAGTGTCAAATCAGCTCTT	21061	TGTCAGTCATAACCTTGGATGCTAA	32045	GGTTCCTTTCCAGGAGTGTCAA	43029
9404	CTCCTCTCCCTCATTACCTCTCAA	21062	GGAACCAGAGCTGAGGCTGTTA	32046	GCTCTTGAAACTCCTCTCCCTCATT	43030
9405	CGCATGTCCCTAATGCGTGGAT	21063	ACTCTGGGTGTGTCGGCATT	32047	GGCAGTGAACGCATGTCCCTAA	43031
9406	GCACAGCCAGCATTCCCATAGT	21064	ACCCTCGTGGCATCAGAGATGA	32048	ACCTCTCTGCACAGCCAGCAT	43032
9407	CGTTGCCTACAGTTCTCCTCTATC	21065	AGTGGGCCCAGTGGAGGAA	32049	CACACTTGAATTCGTTGCCTACAGTT	43033
9408	CCGAGAATGTCACTTCTGCTGTATC	21066	GTGGGCTGGGCTTAATGGTATG	32050	CCATAGAAGTCCGAGAATGTCACTT	43034
9409	GCCAAGTCTGTGGCTAGAGGAAGT	21067	CCCAGTACGCTAGGCAGAAGA	32051	CCCAAATAGCTCAAGCCAAGTCTGT	43035
9410	GGCTGCCCGATTCTTAAATCATTC	21068	CTGCCAAGCTCACTAGAGGTAAC	32052	CCATCTTGGCTGCCCGATTCTT	43036
9411	ACCGGAAGCCTTAACCAATTTTTG	21069	GCCAGGTCGACACGTAACAT	32053	AGGCACCGGAAGCCTTAAC	43037
9412	CCCTGGAACCCCTACCTGTCTTAT	21070	CCACCCTCTTTCTCATCACAGTT	32054	CCCATGAGCTTAACAGCCCTGGAA	43038
9413	GCCCACAACCCACTGAGCAT	21071	GGACATAAGACCCATCCACCAATG	32055	GGTTTTAGCCGAAGCCCACAAC	43039
9414	GTATTTACCCCAGATGGGATGTTTCA	21072	GGTCTGTAAAGTTGCCATGAATACTGA	32056	GACCAGAAGCTGTATTTACCCCAGAT	43040
9415	CAGGGCTGGAGTTGTGATGAGT	21073	TGTCCTTGGGAAGAAGTGAGTTTAAG	32057	ATGGAGGGCAGGGCTGGAGTT	43041
9416	TCCCACTATCAGAACATTACCTGGAT	21074	CCCTGAAAATAATACCCAGCTTTCATC	32058	CTTCTAGCATTCCCACTATCAGAACA	43042
9417	GTGGAGCTTCCACGTGATCCTT	21075	CATCTGAGCTGAGGTCATGTTCTTC	32059	CTGTGGAGCTTGTGGAGCTT	43043
9418	GTTCCTACCCCATAGGATCAAGATG	21076	GGGCAGGTGTTTCAGGAAGT	32060	GCCTAAGCAGGTTCCTACCCCATA	43044
9419	CTGAATGGTGAACAGGACAGTCT	21077	AGTAACTATCTCAAGTCACAGGAGTCT	32061	GTCCTAGCCTTACTGAATGGTGAAC	43045
9420	TCAGCCTTCGACATCTTTCATTGT	21078	CATAGTTGCTAAATAACGGGAGCTACT	32062	GAGTCATAGAATTTTCAGCCTTCGACATC	43046
9421	GGAAAGGGCTCACAGAAAATTAAGAGA	21079	GGTCATTCAGACCAGCTCCAATAA	32063	TGCCTAGGAAAGGGCTCACAGA	43047
9422	GGGCTATTTCCATTGTAAGCACTTT	21080	GGCCAGTGGCACATTAGCAAAC	32064	AACAGAAGAGGGCTATTTCCATTGT	43048

9423	CTTGGTATGGTCAAGAAAACGGAAAC	21081	CAGTCACCCTGGGATGAGAGTA	32065	GGAGCTTAACATCTTGGTATGGTCAAG	43049
9424	GGCTGAGACTCTAGGACAGGTAATG	21082	TGGTGCTGCTTAGCATTTCGTA	32066	GCATTGTTCAAGGCTGAGACTCTA	43050
9425	GCATCGAAAGGGATGTAGTCACTTA	21083	AGAACAGGCAAGCCCACTCA	32067	GCAGTAATTTGCATCGAAAGGGATGT	43051
9426	CCTCCCCAGAGGTAATAACGATGTAG	21084	ACAACCTGTGGTCTACCTGAGT	32068	AGCAGCCTCCCCAGAGGTAATA	43052
9427	CAAAACCACACACAACACACCAT	21085	GATCTCTGGAGAATTTGGGAGTATGT	32069	CGCATATGCATCAAAACCACACACA	43053
9428	GCCAAAGACCAAAACCACCTAGA	21086	GGCCAGAGCACCTGTTCTCTAAA	32070	GTCTGACAGTTGCCAAAGACCAAA	43054
9429	CATCTCAAGCATTCACACCCAGAT	21087	CAGGGAGTCTCGCCATCTGTAAC	32071	CCGACCAGCTAAGCTCATCTCAAG	43055
9430	ACCACTCCTTTCACACAAATCCTATG	21088	CAGGTTAGGGAGATTATCTTGCTTTTC	32072	TTGGAACCACTCCTTTCACACA	43056
9431	CCACGTTAGATACCTGACAACCTCAGA	21089	GCCCTAAAACCCTGCTGAGATG	32073	ACCTATTGCTTCATTCCACGTTAGA	43057
9432	CCCTGCCAAATTTTCCCTGGTT	21090	CCTGAATCAGCCAGCATAGAGTT	32074	ATCAGTATGACATCCCTGCCAAAT	43058
9433	GGAGTCAAGCCCAGCCAAGTTT	21091	CTTTCTTCCATGGATCAGCTGAGAT	32075	GACCTAACATGCAGTTTGGAGTCAAG	43059
9434	TGAGAGGCATTGGGAGATAGCAA	21092	GTTGCAGTCTAGGTTTTGTTGGTT	32076	CAGCAGTGTTTTTGAGAGGCATTG	43060
9435	GGAAGATCCTAGCAAGAGGTGAAA	21093	GGCTAGGGTAGGAGTAGAGCAGTATC	32077	GCCAGAAAAGGAAGATCCTAGCAA	43061
9436	CCTTGCCATAGCAGGTAGTAGAAG	21094	CCGGAGGCCCAAACGTTAAA	32078	GATCACAGTTTGTTCCTTGCCATAG	43062
9437	CACAACCTGAGTATTCCCCTGTATTC	21095	CCTTGGAGATTCATTCGATAGCTACTTGT	32079	TGTCTATCCACCACAACCTGAGT	43063
9438	CCCATGCTCATCACTGCCCAAT	21096	TGACAGCCCACTCTCACCTT	32080	GCCTCTGTCCCATGCTCATC	43064
9439	CTGAAGCAAGGCACCTATGAACTCT	21097	ACCTGGTCAGCACTGGCTTCT	32081	ACCCTCCTCAACAGAAGTAACTGAA	43065
9440	GCATGTCCCAAACACTTCCTGTGT	21098	GGAGAGAACTGGGCTCTGAAAAC	32082	CCACACAGGCATGTCCCAAA	43066
9441	CTGCATTCCATTCGCTTTTCCAAAT	21099	GAGGCACAGAACATGCTACCAAAG	32083	AGGAACTGTGCTGCATTCCAT	43067
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9447	TGTTGCCAGTAATATACGCCATCT	21105	TGGTTTAGCAGTGCAGATAAGGAA	32089	CCATCTGAGACATGTTGCCAGTA	43073
9448	GCCCATAGCTTCCTAAACCTCCAT	21106	AGCAGAATGGGGTGAAAAAGGTT	32090	GACTGTTTAATTGGCCCATAGCTTCCTA	43074
9449	CCAAACCTGTAATTTGCTGAGGTACA	21107	GAGGGCACCCCTGAAAAATGT	32091	CCCAATAGGACTTTCCAAACCTGTA	43075
9450	GCCATTGTGTGGAAGACATAGTGTGA	21108	ACTCAATAGCTGCCCTTCCTACT	32092	ACTTTGAGCCATTGTGTGGAAGA	43076
9451	GATTCAGACCCAGACTCCAGATTT	21109	GATGTCTGGAAAGATGTTGGATGATG	32093	GGAGGCAAAGCCAGGATTCA	43077
9452	CTCCTGCGGTGTTTTTGTCTCT	21110	GAGGGTGTCCTGGGCATTAC	32094	TGGGACTCCTGCGGTGTT	43078
9453	CCTGGTTTACATCTACTCCCAACTTTC	21111	CAAATGCAATGGTAGGTGATGAGTT	32095	CTCACACTGTACCCTCCTGGTT	43079
9454	GCTAAGGAGGATTCTAAAGGTGCTAA	21112	AGCCGCAAGGAGTGCATTT	32096	CCTGGACTCAAAAGCTAAGGAGGATTCTA	43080
9455	CAGTCACTGCTTGAACACCATGT	21113	GGACATTTGGGTTTAACCAGCCTAATTC	32097	ATGATGGCCTCGGTATTAAACAGT	43081
9456	GCCAGGAGCTTTGAGAGCTTGTA	21114	CCTGTTGCACCAAATCAATGTGTCT	32098	GGCCAAATTGCCAGGAGCTT	43082
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9458	GCATTTAAGGGGTATTCGTGGGACTA	21116	GGGTAGTCCTGATGGGTAACAGA	32100	TCTGTTGCAAGCATTTAAGGGGTAT	43084
9459	GGGCAGTTTGTGTCACAGTCTTT	21117	ACCAACCAGCATCTCTCTCAGT	32101	CCGTCCAAAGGGCAGTTTGTGT	43085
9460	CTGCAAGCTGTGGTTTTCAGTTTC	21118	TGGTTTGATCTTAGACCGTGACAA	32102	GCCTTCTGCAAGCTGTGGTT	43086
9461	CAGATTCCAGCCACAAATATCTCATC	21119	GTCTTCACAGTAGGGCTTTTGCTATG	32103	GGGAAGTTCAGATTCCAGCCACAA	43087
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9465	AGGGTAAGCTGAGGCAAGTTTAG	21123	CTCAGACTGTGGCTTATGATGTCT	32107	GGATAGAGTATGGGAATGAAAGGGTAAG	43091
9466	GGCTTGTTCTTATGGTAGGGTTCCAAA	21124	CCTTAAGGGGCCTTGGGTACATC	32108	GGCTAGCCCAGGCTTGTTCTTA	43092
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9469	CTGTGGACTCTTTCATCTTGCATGT	21127	GCCAAAGCCAGTGCATAATTAGTTG	32111	TGTTTAAGAGCTGTGGACTCTTTCA	43095
9470	CAAGAGGGGAAAAATCCTCTTTAGACA	21128	CTCTGCTTTAGCATTTGCCACTAATAC	32112	CAGTAATCCTGATTCAAGAGGGGAAA	43096
9471	CCAAGTAACACTGCAGCTTTAAGAA	21129	CACAGAAGTCCAGCGGCATTGA	32113	CCACCTTGAGGCTCCAAGTAAC	43097
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9474	CTGGGGTAAAAGGAGGTTATCAAGCAT	21132	GAGTGGGAGCTCTAACTTGACACTA	32116	AGGATTCTGGGGTAAAAGGAGGTT	43100
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9479	GAACACAGGTCAGTTTCAGGTAGATG	21137	AGGCTGCGACCTTCTTCAGAGA	32121	GGTAGGATGAACACAGGTCAGTT	43105
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9482	CCACAGCTCAGATTGACTCACA	21140	CCAGAAACAACCTTAGTATTGCAACCTTTC	32124	TGTCCACCCACAGCTCAGAT	43108
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9484	GGAGCACATGGTGACTCTCTGA	21142	GAAAATAGTCTCTCTGAAGGAGCAAGTAG	32126	AGTTGGCCTCTGGAGCACAT	43110
9485	CGGTGGGTCCTACACCTGAGAATA	21143	TGGTGATCGCAGCAGAAACAA	32127	GCTTAATGACGGTGGGTCCTACA	43111
9486	CCCCAATCTGTGCCTTCATGTT	21144	GTGGGCAGAGGCATGCATATAA	32128	GGATGTGTTGCCCCAATCTGT	43112
9487	CCTCAGGCATAAAGCCAGGTCAGT	21145	GTGAGCCTCAAGAGACGGGAAA	32129	GGGCAGAAAATGTCCTCAGGCATAA	43113

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9493	GTAGCTGGAATGCACAGCCTTA	21151	AGTAGCCACTAGATGGCAGAATTG	32135	CCCATCCTGTTTGTAGCTGGAATG	43119
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9504	GGCTGATGGCTAAAACTTACTGTTTCCTA	21162	GCAGAGACCAGGTAAGCAGACA	32146	CCTTCCTCAATTGGCTGATGGCTAA	43130
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9508	GCTGTGAACTGTGGAAAACACAGA	21166	GACTCTTTGGAGGACCAAGGTCAAG	32150	ATATCTACTGTGTGCTGTGAACTGT	43134
9509	GGCATTGGGAAACTTGAGCTATGTACT	21167	TGCTCAAGGACAAAAATGGTCACT	32151	ACAGTAGGCATTGGGAAACTTGA	43135
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9513	GCTCCTGTACTTGCCAATAGCAT	21171	AGAGCGCTTGGGTAGAGAGA	32155	GTGGGAATGGAGCTCCTGTACTTG	43139
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9515	GCTGCCTTGCTTTCTTGGTGACA	21173	ACACTGGGGCTCACTCACT	32157	CTTTCTACTGCTGCCTTGCTTTC	43141
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9518	GTCACCACAGAAACACACCTTTAAC	21176	CCTATAAGAGGAGAAACAGAGCATGT	32160	ACCAGGACGTCACCACAGAAAC	43144
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9530	TGCTGGAGTAGACCAAGAGTCA	21188	GCCACTTTTACTGTTGCAAACACATC	32172	GCAGCTGTCATTTTGCTGGAGTAG	43156
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9537	TCTGGACTTGACAGTGACAGATG	21195	TGCGAAGCAGCGAGCACAT	32179	GCAAAATCGATGTGAATTCTGGACTTG	43163
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9539	CGGCTCATTTGCAATGGGCATCA	21197	CCGGCCCTGGGTTTACTTCTTA	32181	ACTGCAGCACTCGGCTCATT	43165
9540	GGGAACTGCAGGCCATAGACA	21198	GGGCTTGGCAGTCCCAAATACA	32182	GAGGAATGAACAGTTTAGGGGAACT	43166
9541	CCCTGCCTCTAATCCTCCAGTGT	21199	CTGACAAAGGGTGCCAGCTTCT	32183	ACAGGTCTCTCCCTGCCTCTAATC	43167
9542	TTTCCCTGCCCTGGGCTCACA	21200	CACTAAGATGGATAAGGCGGAATGT	32184	CAAGCTCTTTGTCCACCTGTTTC	43168
9543	GGCTCTGCCTCACTGTGACT	21201	TGGCCCAGAGTTCCATAAAACAT	32185	TGTGCTCCTCTGGAGGCAATC	43169
9544	TCACCCTCTTACTGCCTCCAA	21202	GCTTATCTGATTTCCCAGCACACAGAA	32186	GCCTGCCTCTAACATCACCCTCTTA	43170
9545	GAGCCAGAGTGTAAAGAGGGATCCTA	21203	CAGCGGGTGAGCTTTCGTTT	32187	GACGCAGAGCCAGAGTGTAAAG	43171
9546	CAGGAGGGATTGTGTGGTCTGA	21204	CCCCTAGAAACTCCCTTCTCTTGT	32188	CCCATGTTTTTGAGCAGGAGGGATTG	43172
9547	GCTCCAATCCCGGACTAGCCTTA	21205	CCCGGCTGCTCTCACTACAATAGA	32189	TCCGGACTCCTCTGCTCCAAT	43173
9548	GCCACGTTAAGGGCCCAAGT	21206	CCATCCCCACAGCTCTCTTTTC	32190	GAGTTATGATTGCAGGCCACGTT	43174
9549	GAAAAATAGTCTGCCCCATCCATCT	21207	TGAGTCCCTGAGGCATAATCAGT	32191	AACTGTCACAGGAAGGAACAGAAA	43175
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9551	GAGGTGTGTTGATTGTGCTACCTA	21209	CCTGGGAGCACTCCTTGTCTTT	32193	ACAATGTGGTGAGGTGTGTTGA	43177
9552	GGTGCAGATCGGTGCTCACAA	21210	AATGGGAAATAGGCAGACATACACA	32194	CGGACATAGCTGTGGTGCAGAT	43178

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9556	TGGAGTGGATTCAGGCTTGCTA	21214 TGCCATTCTTTAATCCCTTGGAGTTAG	32198 CCCAACGACATATGTTGGAGTGGATTC 43182
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9561	GTGCCTGCTGATAAAAACTCACTAC	21219 GTGGTATGGTGTTTGCATTATGCTGTA	32203 GTCAGTAAATGAAGTGCCTGCTGAT 43187
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9563	GACCAGGGAGCATATTACTGGATCA	21221 TGGGCCATAATGGGACAAAGATG	32205 CAGAGAAGGACCAGGGAGCAT 43189
9564	GCTGGGAACAGGGTTTCACA	21222 GGAGGCCAAAGGAGCTTATGGTTAC	32206 AGCCAGTGCTAGCTGGGAACA 43190
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9927 ACATTTGGGGAGATGAGACCTACTAA	21585 AAACCCTGTATTATGCTGCTGTAGA	32569 GGGCATATGATAACATTTGGGGAGATG 43553
9928 ACACAGGCATCCTTAACCAATAACA	21586 GGGAAGCTGAAACTAGGCTAACT	32570 CGAACGCTACACAGGCATCCTT 43554
9929 TGCCTTCACATCAAGGGTGATTC	21587 CAGCTAAACCAACTTTGACAAGGAA	32571 GTAACTACGCAATATGCCTTCACATC 43555
9930 GGTAAAGACAAGGACTTTCTCCATGATGT	21588 GGGAAAGATGGGCATGGCTGAA	32572 CGTGACAAGGCTGAAGGTGGTA 43556
9931 AGAACCCATGTCTAGTAGCCTTCA	21589 TGATCTTTGAGCTGGACCTCTCT	32573 GGGACATTTCAGAACCCATGTCT 43557
9932 CCAACTACAGGGAAGGCTGGAA	21590 CTCCATGTGCTTTTCCTCATACACTTAGA	32574 ACACGGCCTACCCAACTACA 43558
9933 GTCATTGCCTAGATCGTCCAGTAG	21591 GCAGGGAGGCAAAGATGACT	32575 GGCTCAGAGAGCAATTTGGGAATGT 43559
9934 CAGGTGTACACAGCACACCCTTAG	21592 AGGTGGTGCCTTTAAGCTAGAAAA	32576 TTGCCTAGCTGGCAGGTGTA 43560
9935 AAAAGCAACAGTGAGGGAGATCA	21593 CTCGAGGGCTTTCCATCAGCAA	32577 GCAGGAGGTGATAAAAGCAACAGTGA 43561
9936 CAGGTCCCATGTGAAGTGTTAGGAT	21594 GGTCAGTTGGGCCAGGTTTCTA	32578 AAAACAGCATCAGGTCCCATGT 43562
9937 CGGGAGGACAATTTCTGGCAATG	21595 GCCCAGCCTAGCTGCATACAA	32579 GGTCATTTGATTACTTTCGGGAGGACAA 43563
9938 CCAGATGGCTGTGAGTGTCTGT	21596 AAGGCTGAATAGTGGGAAGAGTTAC	32580 AGGAGGCCAGACAGCCAGAT 43564
9939 GGTCACGTAACTTCCTAAAAGTCCAT	21597 ACACACACGTAACTAGGGAAGATG	32581 CCCTGTGTCTTTGGTCACGTAACTT 43565
9940 CAAGAACTGAGAAAAGGCCTTCAAAGA	21598 AGGTCTTAGATAGCAAGGACCATGT	32582 GTCTGGCTGTGACAAGAACTGA 43566
9941 CCGCATGCTTTATTCTAGCCACTT	21599 CTGACTGTCCAGTGCAGTTTAGA	32583 CCGTTATTTTTGACCGCATGCTT 43567
9942 CCACAAAGTGCTCTGGGCTTTC	21600 CACATAGGTGGTATCATCTCCCTTCAGA	32584 GCCTAGAACCTGTTCCCACAAAGT 43568

9943	GAGTGTTGAGGACGGAAAGTCA	21601	ACCTAACCCTCACATTCATTTGCTT	32585	GCCCAGTGATTAGCACAAAGTGA	43569
9944	CCCTATGGGAGGCAACCCTTCT	21602	ACCCTGACCACTTGTGACTCT	32586	TCGGGCCAGCTTACCCTATG	43570
9945	TGAGGATCGCATTGAGCAACA	21603	GTGCACGGGAGCAAGAAAAAC	32587	TGCCCCAGGCTGACCATGA	43571
9946	CCAGGCTGCTAAACATTTTCCAGAGA	21604	GCTCTGGTGCACCCTTGACTTA	32588	CAAGGATGTTTTTCCAGGCTGCTA	43572
9947	GGAGTCATGCCTTCTGCAATTTT	21605	ACACGTGCTGAAATCCAGGAA	32589	CAAACAGCAGGTGTAGGAGTCA	43573
9948	GAAGTGGGTAGAGATGGAAGAAGGAA	21606	AGGAAGACCAGGATACAGACATAAGA	32590	GGAGGAGGAGAAGTGGGTAGAGATG	43574
9949	CTTGACTGATAGTTCCCGGCTTT	21607	TCCTTGGCTTGGGACCTCCTT	32591	GAAGTGTGAGAGGAACTTGACTGA	43575
9950	GGAACACTGGGGACTTGTCTGA	21608	CCACACTGAGCACTTGCCTACT	32592	GGACCCAGTCAAAGAACTGGAACA	43576
9951	GGAAGTCCAGGAGTGGAGATGTCA	21609	GTGCTTGCTGCAATTCCCCAAA	32593	CGCTCTGGGACACTGGAAGT	43577
9952	TCCTTTGGGTACCAGGAGATGA	21610	TGTTGGATGCCATGATGAAGAGA	32594	TCGCCAGTCACTGCTCCTTTG	43578
9953	GGCTTCATCTCCACCCATGAA	21611	CCTCAGTCCCTGTCAGTTAGTGT	32595	CCTTTCCTCAGACAGGCTTCATC	43579
9954	GCCTCCCAATATAACTGACTGCTTTC	21612	GGGGCTCCGAACGGAAAAAC	32596	GCCTCCTCCAGCCTCCCAATATAA	43580
9955	GACATGAGGGAGAGAGGTGTTCTCA	21613	GCCCTCAGAGAGGCACTCAAT	32597	ACCCTGTGACATGAGGGAGAGA	43581
9956	GGAGACACTACCTGTAGGTCAAAAG	21614	TGAGAACCAGGAGCCAGAGAAT	32598	GAGCTGATTGCAGGAGACACTAC	43582
9957	GCTATCACTGGGTTCTCCGAACT	21615	GTCCAGAGCCAGGAGAGAGA	32599	GGCCAAAGGCCCTGTGCTAT	43583
9958	GGTCTTATTAACCCTAAGAAGCTGTGAAC	21616	CTCTTCTTCTCAGAATTCCACCCTGAA	32600	CCGGCTGGTCTTATTAACCCTAAG	43584
9959	ACTTCTCGCAGCTTTCCTAAATCA	21617	AGGGCCTCAGACCCATTGAACA	32601	CCCCATAGTATAACTTCTCGCAGCTT	43585
9960	GACAGCCTTACAGAGACTGCTT	21618	AGGATTTGTTACTGGGCTACACAA	32602	CTGGCATTAGATGAGTTGACAGCCTTA	43586
9961	AGTGAGTGGCTCCTGCATGT	21619	CCAACGCTTTCATAAACGGAGTTAGT	32603	GGGTGCTTTCCCTTGAGTGAGT	43587
9962	GAAGCTGTAGCAAGGAGGAGAAATG	21620	GAAGGTTGCTTTCTGTGTTTGAGA	32604	GCATGATTGTTCCTTAGAAGCTGTAG	43588
9963	AGGCTCACCTTGCATCCACTTG	21621	GCAAAGATGAAAACTGGAAGCTACAAC	32605	CCCATTGGAGAGGCTCACCTT	43589
9964	ACTCACCAAAGTGTGCCCTAAAC	21622	TCTCGGAGACCAGAGGGAGATAC	32606	CCCCGACCTTGAACTCACCAAA	43590
9965	AGAAGACATGGAGTTGGGATTTGT	21623	GAAGCTGAAAGTGGCAGCTAGT	32607	CCTGGGCTGGTCCAACAACTAAG	43591
9966	GCGTCACTGTTGGCATCACA	21624	TCTTCTGCCTTAGCAATTGAACAGT	32608	GGCTCTAACATAACACACGCGTCACT	43592
9967	ACTGCCTGGCCTGCTATTTC	21625	TGCTCTTGTGTACATATGTCTGCTT	32609	AGCGGAGCCCAGTGAGTTA	43593
9968	GGATTGTGACAGCACAGTAACAGTTG	21626	CAGGAATAAGGATTTGGATCTCTGGCTTT	32610	GTCTGACCCCACTGGATTGTGA	43594
9969	TCACAATCTGGAGTCACAGACATTATC	21627	CACATGACGTCCCTGTGTCTGT	32611	GACATTCAGTTCACAATCTGGAGTCA	43595
9970	GGAGTCAGTGGCCTGAGTTCTA	21628	TGAGGCTCAAAGTGATACAGCTATT	32612	GAAAACACTGGACAAGGAGTCAGT	43596
9971	CGCCACCAGACAAATGGTGTTG	21629	CTGCTCGACTCTTCAGTAGGTGAT	32613	GCCATCACGCCACCAGACA	43597
9972	CCCAGTTTCTGCTTCAACTCACTGT	21630	CCTGAGTATGGGAGAAGGGGATTTG	32614	CCACAGACCCCAGTTTCTGCTT	43598
9973	CCTTGATCTGTCTGACTGAGTGGATTT	21631	GGCAACAAGAGCGAAACTGCAT	32615	GCCCAGATCTCCCTTCCTTGATCT	43599
9974	GGGGTTGAGAATGAACTTGTCACT	21632	CCACAGCTGCCAGTGGTCTT	32616	GCAAAAGGCTGGGGTTGAGA	43600
9975	CCTGCAATTCTTGACTGAATGGTTTG	21633	CCCCAGAAGCTGACCCTGAAGATA	32617	GTCTCCTGGTGTTACCTGCAA	43601
9976	GAGGGAAGAAGGTTCAGGTCAGT	21634	TGGCCCCACTCCATCTCTAAG	32618	GTGGAGTCAGCAGGAGATGTGAA	43602
9977	CCCACTGTGCCCCATGTTGTTT	21635	CCTGAGCTACAGGAGTGAGACA	32619	AGTGCCCACTTTCCCACTGT	43603
9978	GGTGGAAGGCTACCCTGACATAC	21636	CCAAGCCGCGAGTGGTTTTATG	32620	GGCTCTGTGCTAAAGGGTGGAA	43604
9979	GCCTGATGTGATGGGAGGTTGT	21637	CCAGGAGCGCAGCTACAGA	32621	CATGCTGTGAGCCTGATGTGA	43605
9980	TGCGGGAAATTCCAGTGATTAGA	21638	TGGGAGCCAAAGAACTTGTGT	32622	CACAGGAGCATATGCGGGAAA	43606
9981	CCCTCCACACAGATTGGTAAGCAAA	21639	ACGCTGTGCTCAAACACTTCT	32623	ACTCTATGCCCTCCACACAGAT	43607
9982	GGTGTGTCTGAGGATAGACGTTAC	21640	TGGGCAAACATGGGAGATGATAG	32624	AGCTCCTGGTGTGTCTGAGGAT	43608
9983	GTCAGCGTCTCTCTCCCTGATACA	21641	GATGTGCTGAGAAGGGTTCAGA	32625	GCTTTCAACAGTCAGCGTCTCTCT	43609
9984	CTCTCCATGTCTGGTCCCTTGT	21642	AAGAATTCAGCTGCCAGGAACA	32626	GGTACCATCTTAGCCTCTCCATGTCT	43610
9985	CTCCACCATGCAACTTGTGAAAG	21643	GCTTAAGGCCAGAAGTTTGAAGGTT	32627	CGATATCTCACTCCACCATGCAA	43611
9986	GCCTGGTCCACCCTAGAAGGAATA	21644	GCCATGGCCAGGACAAAAAC	32628	CTTGCCGGGTCTGTCTGTATTG	43612
9987	CAAGACTGAGCTGGTCTGCAA	21645	GGCTCACCCAAACCTTGGAA	32629	GGTCACTGCTTTGCCCAAGACT	43613
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9989	CTGGGTGCTCTGAGAAAGGTGAT	21647	ATGGTCTTTGGGTTGGCAAAATAC	32631	GGTGAATAGCTGGGTGCTCTGA	43615
9990	GCCCGCTGAAGATGAGATTTCAAG	21648	CCATAACAAGAGAGACTAAAGGCCGTATC	32632	ACTGAATGAATAAGCCCGCTGAA	43616
9991	CCTGCCAAAAAGAGAAGGGAACT	21649	GCTGCTGGCCCCAGTAACATT	32633	GCCAGAGGAAGAGATCCTGCCAAA	43617
9992	GACCTTGCTAGGCACTGTGATTTG	21650	CTTAGGCAGAAACTTGCCCAAAG	32634	GGCTTGAATTCCTGCCTAGACCTT	43618
9993	CAGGATGCAGTAAATGGCAAAGACTAC	21651	TGGTTGCAAATTTGGCAGTGAA	32635	GCTAGGTAGGCTACAGGATGCAGTAAA	43619
9994	TTTACAGGCTAGATGCGGAAACA	21652	GGTAATCACCCAGCCATAACCAATCA	32636	GGTGAGCAGCGAGCTTTTACA	43620
9995	TGAGGACGAGGGATGAGAAGATG	21653	GCAGCTGCCTCTGAGTGTTTG	32637	CGCGTGAGCTGTTTTTAATGTTG	43621
9996	GGTTTGCTCTGCAGTCTGGTCAA	21654	AAGTACTGACAATACAGGCAGGTTT	32638	AGGGTCAGATGCGTGGTTTG	43622
9997	TCCATGGGACTTGAGGTGGAA	21655	GCAGAGGGAGCCTCTAGTGTA	32639	GGAAATAGCCTCTTCCATGGGACTT	43623
9998	GCCAAGCAGATCTCCACTGT	21656	CCCACAGCACTGCTCAAGATG	32640	TGGGGTCAGAGCCAAGCAGAT	43624
9999	CATACCCACCTCAGGCTACTGT	21657	ACTCCTCGCAGACATCAGTCTT	32641	TGTCAGTCAGGGCCAATCATAC	43625
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10006	CTGCCTGCAAGTCAGGATGAGA	21664	GATCAGTGTGGTGGTGAATTGGAT	32648	AAGCTGGCTGCCTGCAAGT	43632
10007	CTCTCCTTCCCCTTTAAACCTTGTGA	21665	GGCAGAGTGGGGACACACA	32649	CTCAGAGCTCTCCTTCCCCTTT	43633

10008	GGTCCTGGCCCAGTTCCAATTT	21666	ATGCAAGGGAGCAATTTGCATAG	32650	CCCCAATGCAGGCGATGA	43634
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10017	GGTTTTGAGAGGGTAGGTAGGTTTG	21675	TGTCTTGGCCCCATCTCCTT	32659	GGGAGGATTACCCACCTAGGTTTTG	43643
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10027	GCTTGTTACACAGCTGCCTATTATTTC	21685	GCAGAGGAGCTCAGCTGTTT	32669	GTTTTGGAGGGAGTGTGATGCTT	43653
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10047	CTGCTGAAATTAGAGGTTAGGCTGAGA	21705	AGCTGCTGCAGACCCATGT	32689	ACACACCCCATCTCTGCTGAA	43673
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10049	GGGGATTAGGGTGCTAATGGATTC	21707	GGCCCCTCTGTAAAAGTGCAT	32691	CACACACTCCTTGCTAATGGGGATT	43675
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10051	GACCCTGTTGTGAGGCTGTT	21709	CCTATTGCACACGGTCACAATTT	32693	CCTAGAGACCATGACCCTGTTGT	43677
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10065	CCCAGTTAGTAACAAAGAGTGGTTTC	21723	GTGTGACCTATTTTAGGAGGCACTGT	32707	CAGAGTGTTCAGAACCCAGTTAGT	43691
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10084	TGCCTCACTCCAGGGCTTTAC	21742	GCTCCATGGGGAAGGACTCTTA	32726	TTTGGGGTTCCTGCCTCACT	43710
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10098	GGCATATCCCACCAGAAATGGGTTT	21756	AGTATACTGCGGGGCCATTTC	32740	CTGTGGCACTGGAAGGCATATC	43724
10099	CCGGGGCCTATCCTAGCATGT	21757	AAAAGGTTCGCTCTGGATGGAT	32741	TCCACGGGATGACGCAGATG	43725
10100	TTCCTCCAATCCAGTTGTCTCTTC	21758	TTTGAAGCCACTGCAGTCTGA	32742	ACCGGTCTGTCCTTCCTCCAATC	43726
10101	GGAGGAAGGTGTGGGCTACATA	21759	CTCAACAAGCACTGCAGTTGTAA	32743	GGATCTCACCATCCAGGAGGAA	43727
10102	GGGTTTGTAGTCGGAGCTCTTG	21760	CGGCTGTGTTTGCTGCTTGT	32744	CCCATCAGCACTGGGGTTTGTA	43728
10103	GCTCCACCTAAGTGTAAATGGAGTTCA	21761	AGCAGAAGATCCTTAATGGCAGAA	32745	ACTCTTCCCTTGCTCCACCTAA	43729
10104	CTGTTGTGGTCTTATGGAAACTTGATCTGA	21762	GCTCAGTGCATCCCTTCTACCTAGT	32746	GGCTTTCTGTTGTGGTCTTATGGAAAC	43730
10105	TCCCCAAAACCCAGGTCCATGA	21763	GCTCTGGGCTCTGCAGGATTT	32747	AGGACCGGGCACCTCATTTGT	43731
10106	GAGGAAGGCCAGATTGAGGGTAAC	21764	CCACTTGTCAAGAACACGCAAGGATT	32748	ACTGTCTGAGGAAGGCCAGAT	43732
10107	AGACAGTTGCCAGAAGTCACAA	21765	GGGGACCTATTGCTCACAGGAAGA	32749	GGCTTCCTCGGAAAAAGACAGTTG	43733
10108	GCATCTGCCAACAGATGTGTGA	21766	GCAAAGGAATCAAGTGGTGTCAAGT	32750	ATCTTGCCTTCTGCCAGCAT	43734
10109	GGTGCTACCCACTTCCAAGGTT	21767	TGGCCCGGTTCAGACTGT	32751	TGTCCCAGCAAAGGGTGCTA	43735
10110	TCCACCTCCAATCCACCTCAA	21768	CCGAAAAATGAAGTTACAGGGGAAA	32752	TCATCCACCTATCCACCTCCAAT	43736
10111	ACAGGTGGCCTTTTAGAGAGTTTC	21769	GCGAAAATTCCTTCTTGCCTTCCTTAG	32753	GGGTTCATGCTACAGGTGGCCTTT	43737
10112	CCTCCCCTCTTACCCTGTTCTTCT	21770	GCATTCCTTGGTGCAGGCTTTAAT	32754	AGCCCAGCCCTCCCCTCTTA	43738
10113	TCCAAGTGGAGTGGGGAGAA	21771	GGTTGCAGTGGAGGAGTTCA	32755	CCCTCATGGCTCCATTCCAAGT	43739
10114	GACCCTACAGAAGAAAGTTGTGAACAT	21772	CCACACACTCAACCCACCTAATG	32756	TGTTTGCAATTCTGTGACCCTACA	43740
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10118	TTGGCGGCGTCCACTGAGA	21776	CCTCTCTTCCGTCTCCTTTCCATA	32760	ACTTCCTGTGGGCCAGTGTTG	43744
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10129	GTGCTTATCATCCAAACCCAGTCAAC	21787	CAAAGTGAGGCACGCATACTCATA	32771	CATTGGGACCAAGTGCTTATCATC	43755
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10133	GGCGGCTCTTTAAACAAACTCGTTCT	21791	GCCCGCTTAATAGCTGAGGTCACA	32775	AGCCAGATGGCGGCTCTTTA	43759
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10269	GCCTGGATGGTTCTAGGGCTTTC	21927	CCCCTGTGGACACTCGACTACA	32911	TGCACAGCCTGGATGGTTCT	43895
10270	CTTGCACACAGAGGGTGGTCATT	21928	CCTCATTTCCACCATCCTGTCCTT	32912	GCCATGCCTTGCACACAGA	43896
10271	GACACTGACTCTCTTCTGTCTCAGATT	21929	AGAGTCTGGCCAAATTCCAGTAAA	32913	GGGACCTTGACACTGACTCTCTTC	43897
10272	CCCTCTTCTCCTTCCACCAGTT	21930	GGCAGCAATGCCTGCTACAT	32914	ACATCCGCCCCTCTTCTCCTT	43898
10273	GAGGTGGCAATAGCGTTGTGTT	21931	TGCCAGGGTCAATGGAGGAA	32915	TGGTGGAAAGAGGTGGCAATAG	43899
10274	GCACAACTTCCCCATCTTGCTT	21932	CAGCCAAACAGAATACTCCAGCTT	32916	AGCTAGTGCCCTCTTGCACAAC	43900
10275	GCCACCCTGCATATTGCCTTCA	21933	TCAACCTCCCCTGCCATGGTTA	32917	TCCCCATGCCACCCTGCATA	43901
10276	GTTCCCCACATACCTTGCTTGT	21934	AGCCGGATGGTCCAGAGATGA	32918	CCACTGGATAGAAGAAGTTCCCCACATA	43902
10277	GCGGATGGATGTCTAACCCAGTT	21935	TTTGCCAGAAGTTCAGCAGTCT	32919	GCTGGTTTGCGGATGGATGTCT	43903
10278	AGCGTTTGGCCTTGGGTAAG	21936	GGGGACAAGCAAGGCATTAGTGT	32920	ATGACCAGGTCGGAGCGTTT	43904
10279	CCGGAATATCAGCCCCATATTTATCTACA	21937	TCCCAAATGAGACCAGAGTTGAAG	32921	AGTCCGGAATATCAGCCCCATA	43905
10280	CTGTGTGGTTGCATTCCCTTGA	21938	GACGAATGTCCTCAGCACTACA	32922	GCGTCTGACCCTGTGTGGTT	43906
10281	CTGGCTGTCTACTGCAAGCAA	21939	TGTTTAACACGAGACAGAACCCAAA	32923	TCCTCCCTTCTGGCTGTCTAC	43907
10282	GGGACACAGAACAATTCAGGACTCT	21940	CCAGCTCACGTGTGTGGCTTA	32924	ACCCTGACTTTGGAGGGACACA	43908
10283	CCTGGGGCAAGTTTTCAAGCCTATC	21941	CCTCAACAGCCCTGAGTCTGTTTC	32925	GGTAGCCTGGGGCAAGTTTT	43909
10284	GTCACCCAGTTCCTACTTGACAGT	21942	CGAAGAGGGGATCCAGCTCTAAT	32926	GAATCTAACAAGTCACCCAGTTCCTA	43910
10285	GTCACAAACCCCTACAGTTACTCCTT	21943	CCATGCCAGCAGAACTTTCACT	32927	CCCGTAGTCACAAACCCCTACA	43911
10286	AGTGCTTGATTCCACGTATCTGTT	21944	GTAGGATAAATTAAGGCAAGCGTCTTC	32928	GCCTGGCTTAAAATAAGTGCTTGA	43912
10287	GTTAGGTGCCATTAGCAGCAGTA	21945	CATTTCCCATCATCCTGGTTCTTTTC	32929	AGCAACCATGTTAGGTGCCATT	43913
10288	GCCCCTGGTTAGTTACAGGAAGAAG	21946	GGCTTCAGGCACCAGAATACACA	32930	TTTTGTGAGCCCCTGGTTAGTT	43914
10289	CCTACCAGATAAGCCTTGAGCAAT	21947	GGCTGAGGGCCTTCAATCAACA	32931	GGGCACCTTTACTCATCCTACCAGATA	43915
10290	GCAGACCTTGTCTCGCTTAGTTC	21948	GTGCAGTGGCGATGGTAGT	32932	TAGCCACCCTGCAGACCTTGT	43916
10291	GCCAGAAGTGCCTATTCCTAAACCAATC	21949	GTTCACACCAGGCAGTGATCT	32933	ACTGGCCAGAAGTGCCTATTC	43917
10292	GCCTCCAGTTGGTTCAGAAAATGA	21950	GCTCTTGGACAGCACAGGTCTAT	32934	CGTTTTCTTGCCTCCAGTTGGTT	43918
10293	CGCTACCATGTCCAGCTAGGTT	21951	GCCTAGGCAGCATAGTGAGATTC	32935	CTACAGGCACACGCTACCAT	43919
10294	ACAGACACCAGCCAGCTTCA	21952	CACAGAAAACTAAGTCGCCCAGAGT	32936	GCCCAACGCTCAGACAGACA	43920
10295	GTGGACTCTGGTCTACATGGGAAA	21953	ACCTGGACTCCTGCTTGCTT	32937	GAGGGTTAGTGGACTCTGGTCTA	43921
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10297	TGCCAAGAACCTTAGGGACATC	21955	AGCAAGGTTCAGTCTGGAAATGA	32939	CCCCAGATGCCAAGAACCTT	43923
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10299	CTCATTCCTAACCCAGGACTGAAC	21957	CAGCAACTAAGGCTTTGCCATT	32941	CAACCAGAAAGCACTCATTCCTAAC	43925
10300	GCAGGCACCTAGTATCAAGCCAAAG	21958	CCCTCTGGGTCATCCCTTCATTTC	32942	GCACTTGGCAGGCACCTAGTAT	43926
10301	CCATATGACTGGCAGGTCCTCAA	21959	CCTCCCCTCCTCAGCATCATTG	32943	GGTGAAGAGCCAATCAGGCCATA	43927
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10305	GCTAATGTTGGTCCTCCAGTTCT	21963	TCAGACAGGCCATGGGATCTA	32947	GGGAGGTCCTGTTGCTAATGTTG	43931
10306	GTCCTCCTTTTGAAGCACACTTCTTTG	21964	GCAATGGAAGGGTAGTGGGTACT	32948	GGACTATGTTCTGTGTCCTCCTTTTGAA	43932
10307	CTGGGAGGCTGGGAAATTTGATAGT	21965	CTGACGCTGGATGGCCAAAT	32949	TCCACTGGGAGGCTGGGAAA	43933
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10310	GCTGCTGTTGCCCGTTCTTC	21968	GCCGCAGCAGCTTCTCATTC	32952	ACTCGAGCCCGCTGCTGTT	43936
10311	GTCTGTTTGCTGATTCCTCCTCCAT	21969	GTTCTAGCATTGGAGGAAATCCAAAGT	32953	CCCTTGTTGTCCTTCCTCGTCTGT	43937
10312	AGCCACCAAAGGGAAATGTTCT	21970	ACTTGAGTGTTTGGGTCAACAAGA	32954	GCCAACTCTCAGCCACCAAA	43938
10313	CCGGTATAACATTGCCTCCCTAAG	21971	AGAGCCCTGCTCCTAGTCTCT	32955	GGGCGCAAGTACCGGTATAACA	43939
10314	AAGAAGGCAGGACTGAATGTCAA	21972	GCCATCTGGAGGCAGCTGAAAT	32956	AGGCAGTCTCTCAGTGTAAAGAAG	43940
10315	ACAACATGGCTGTCTTCCTGAAA	21973	CCCCAATACCTTTAAAGCTGGAGCAA	32957	CCCTTGGTTCCCATACTGTAAACAACA	43941
10316	TGCTGAGAGTTTTCGCTGTCA	21974	AGACTAGCTGAGGGAAAGTGTGA	32958	CCCCTGTTGTGCTGAGAGTTT	43942
10317	TGTCCCATCTCTGCCAGTGAAC	21975	CCTGGATTGCCCCACATTCCAT	32959	GAACTGATAAACAGTGTCCCATCTCT	43943
10318	AGAGAAAAGCAGGCCTGGAATG	21976	GGCCTCGTAAAAGGCAGGAT	32960	TCCCACGGAGAGTGAAGAGAAA	43944
10319	AGGCGTCACTTTCTGCTGTT	21977	TGACGACTGGATTGTCCAACAAA	32961	TGGCTGCAGGCGTCACTT	43945
10320	CCAATGCTGGGACCGGATCT	21978	TGAGTCCAGGTGACTGTAGGAA	32962	ACTGCCTCGAATCAGCCAATG	43946
10321	TCCCTCCATTCCCATGGCTTCA	21979	AGACCCAGGGCACTTCTGAT	32963	TCCTCACTCCATTCCCTCCATT	43947
10322	CTGCCTTAGAAGAAATCTCTGGGAAA	21980	CTCATCTCTGGGTCTCTCCTTTAGATG	32964	TCCCTGCTGGTTCTGCCTTAGA	43948
10323	ACAGCACATAGTCTCAGGGTTCA	21981	AGGTGTCCTCCTCCCATTCTTAG	32965	GTCACGGGTACAGCACATAGTCT	43949
10324	GGGCCCTGAAATATGGAGCCTTT	21982	TGAGGTTGCAATTCACCCAGAA	32966	CACTGTGTTGGGCCCTGAAATA	43950
10325	GCCATAGGATCACCAGCCATCT	21983	GGTGTGCATCTGTGGGCATATC	32967	AGGCTGGAGCCATAGGATCA	43951
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10327	CCCACGAGATGCATTCCCTCTA	21985	AGCCACAGGCGAGCACATAG	32969	GGCTTTAACATGCCCACGAGAT	43953
10328	GGATTGAGGCAAAAGCCAACAGA	21986	CCCCAAACCCTTCGGTTTTCA	32970	CAAGGCAGGGATTGAGGCAAA	43954
10329	GGGGTGGGCAAAGCAGTTAGA	21987	AGGATAATGCTGGCAAAGACACA	32971	AAAACGGCGGGGTGGGCAAA	43955
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10331	CACATGCTGCCATTTATACCTTGTTC	21989	TGCAGCTGGGCTTAGCAACT	32973	GCTTTAAAGATCACATGCTGCCATT	43957
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10333	CAGCAGCCCAACCTAAGACA	21991	CGGTGCCCAAGGGCATGAT	32975	AGCCTGTGGCGCTTTGTT	43959
10334	TGCAGACACTCAAACTCATTTGTTG	21992	GTTCTGTGTTAGAAGCCCTATTCATTC	32976	CCGGCATGCAGACACTCAA	43960
10335	ATACCACCTGTGTGCTGTTACAT	21993	GTGGCTTTTTGGTAATTGTGCTGAAC	32977	TCGGATAGATCTTATACCACCTGTGT	43961
10336	TCCTTGGCCACACATGTCAAG	21994	GCTCTGCCGAGGCTGGTAAAAA	32978	GAGGCACGGTAGACATTTCCTT	43962
10337	GCAAGCCTCAGTGTGAGTTTTCAA	21995	CCCAAAATGCCCGTCTGTGAAG	32979	TGTTTCTGCAAGCCTCAGTGT	43963
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10339	ACACTTGCCAGTGGTCCCTAAG	21997	ACCTGAAAGAGGGAAGTCCAAAG	32981	TGGCACCTATGACTGACACTTG	43965
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10345	CCACACCCACTTCTATGCCTTTG	22003	GATGGAAAAGAGGGAATTGCAAGTAGA	32987	CCTTCAGTATCTCCACACCCACTTCT	43971
10346	GCTAAACTTAACATCTCAGCCTCCTTTC	22004	CTGGAACCCAGACAAATGACCTTGT	32988	CTGGGCACCCAGCTAAACTT	43972
10347	AGAGACCCCAGGGTGCTAGT	22005	GCAAGGAGGTGGGATGAGACA	32989	CCCCTGACTGATGTGTCCAGAGA	43973
10348	GTCGTCTAGGGAACCATGTCAGT	22006	GGAGATCTTTAGGATGAGCAGCAAT	32990	GCACAGTCTGGAAACCAGTCGTCTA	43974
10349	GCCCTGAACGCATCTTCCTA	22007	CACATTCTGGAACTGCCCTTCT	32991	AGTGCCAGACAGCCCTGAAC	43975
10350	GCCAAGCAATGATGCCTTATGAT	22008	GCAGAAGAGAGCTGAGGTTAGATG	32992	ACAGACTACAGCCAAGCAATGAT	43976
10351	TGCTGTCACTGGAAATAGTTGAGAT	22009	TCTAATGGACAGCATTGGGTCAAA	32993	GGTACTGATCTGCTGTCACTGGAAA	43977
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10353	CAGGTATTAGGGACCTCTGAGCTACT	22011	GCTAGTTGAGGAAGCCCAAGTAT	32995	GATTGAGAAGCAGTAGTAGCAGGTAT	43979
10354	TGCTCGATCCGGGAACTGT	22012	CCAGTCCCTCGTTTCCTCACAA	32996	TCCAGCAGCTGTGCTCGAT	43980
10355	CACTCCACACTTAACGAAGGCTATTC	22013	GGCAGCCTTCCATGTCACTTAC	32997	CTAGAGGCAATACACTCCACACTT	43981
10356	CCGCATTGACTGACACAGTTAAAGACA	22014	AAGGTAGCACGATTCACAGCAT	32998	ACACCGCATTGACTGACACA	43982
10357	GCAGGGATCTAACCCCTAAAACATC	22015	GGAATTGCACGGCAGCCTTTAC	32999	GCTGCACTTGCAGGGATCTAAC	43983
10358	CGGAGTGTGCACAAGAATATTTGGATGT	22016	CCCACATGAGCTGGCTTCATTTTC	33000	TGCTGGAGTGAGCGGAGTGT	43984
10359	CAGCCCACATGGTAATTGCCTTAAA	22017	GCCTTGCTGGAGTGCAAAC	33001	CTGTTTATCTCAGCCCACATGGTA	43985
10360	AGAACCCGAAGAGACCCAGAA	22018	GCTGATCATGTCACCGCTTTG	33002	CCACCCAAAGAACCCGAAGA	43986
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10362	GCACTTGTGTGATACCTGCACTTCA	22020	CCCAGATGCCTGTGGAGTCACTA	33004	GGCCCATCAGATTCTGCACTTG	43988
10363	GTCTCAGGGTGCCTGATCTGTT	22021	AGAGAAAGTGGCCCTGAAGATAAG	33005	TTGTGCCTCCCAGGTCTCA	43989
10364	TCAGCTCCGGACCCATGT	22022	GGAGAAGACTACTCGTACCGTGACAAT	33006	TTGCCATGAACCGCCTTCT	43990
10365	CGGCCCTATCTCATCTCTGTCTGA	22023	CGCCAGGCTTGCAGTTGTAT	33007	CCGCACGGCCCTATCTCATC	43991
10366	GGAACCACTAAAACAAATTGCCGAGTA	22024	GATCATGCTCCCAACCGTAGT	33008	CAGAGAGCCACAGGAACCACTAA	43992
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10368	GCTCTCACGAGGACATTTCTCACA	22026	GGGAGCTGATGGGCTCTCTTTAG	33010	GGAAGAGTGTTGGTTTGCTCTCA	43994
10369	GGGCACAGTCCTCCCAGTTTC	22027	TGGTCTGTTAGCACACCCAATTAT	33011	AGGCCTATGGGGCACAGT	43995
10370	GGCCAGGGAGTGAACATAAATCCAA	22028	GAACACATCTTAGGAGGGAATATGTCT	33012	CCAGGCCAGGGAGTGAACA	43996
10371	CACCTTAGCACCCTGAAGAATAAAATC	22029	GCAGTGCAGGGTTCTTACATTC	33013	TGGCACACACCACACCTTAG	43997
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10373	CCCAGTCCTCTTTCTCCTGAAACCAA	22031	CTGTAAAAGGCCTGCTTTGTTGA	33015	CCCAACTTGCCCAGTCCTCTTT	43999
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10377	TCCTCCTCTTTTAAGACTTAGCACATC	22035	GCCTCTGCTGCTCGCATGTAT	33019	CGGGGATAAACACTTCCTCCTCTT	44003
10378	ACCTGCTGACTAATACAAGCTGAAG	22036	ACTATTCAGACATCACCTCCTCCAT	33020	CGAACTTTCAGTAAATACCTGCTGACT	44004
10379	GCCCTGGCTGAAATGGTGAGT	22037	ACCCTCTGGGGTTCTGGCTAT	33021	ACCCTGGGCAAGGTCATCT	44005
10380	CACAAGAGGTTGATGACCCATACA	22038	CACAGTAAACAACCCAGCACATATT	33022	CTGGAAGAGAGCACAAGAGGTTGAT	44006
10381	TGGTGCATCCGATGAGAATGAAAA	22039	TTGCAAGAACACCCATAACCTCTTA	33023	CCGCTGCATGTTTGGTGCAT	44007
10382	CCTCCTAATTAGCAAGGGAGCTAAC	22040	GAGCTAACAATGCAAGGTAAAGCGAAT	33024	GGAGACTGGTCAGGAATACCTCCTA	44008
10383	CCAGAGACTTCGGACACCAGAGA	22041	GCTGTTTATCAGTGAAATCCCAGTCACTT	33025	GTTCATGTGTTTGCCAGAGACTTC	44009
10384	TGCCCAAGGCCATGGAACTA	22042	GGACAGACACGGGGTCAAATTC	33026	GAGGCTCAGAGGAGTTGACTTG	44010
10385	CGACCTTCCAAACAACCATGCAAAG	22043	GGAGTGACGCTCCTTCCTACCTA	33027	TGGCCACCTCACGACCTT	44011
10386	CGTCAAAACAAAGTGACTGCTCATT	22044	GGTAGGAATGTTTCCCAAGCTATTCA	33028	GCTCCAGCTGTACCTACGTCAA	44012
10387	GCCAGAAATGGGGAGACCAGTT	22045	GGATACACATTCTCTCCTATCTGCAT	33029	GCAGAGAGAGGTGCCAGAAAT	44013
10388	AGCCTGCCTCGTATATTAAGTTTCA	22046	CCCTCAGACCTCAGCTGGTTT	33030	GCATTTTTATCTAGCCTGCCTCGTAT	44014
10389	CCTCCCCACTTTCGCCTTTTCA	22047	CTGGTCCCGGCTACTGACATTT	33031	CCACCCACCTCCCCACTTT	44015
10390	CCCATTAGAAGGTAGCTTTTGACTGAAG	22048	TGTTAGCCTGTAACTTGTCCTTCTTG	33032	TGTCACACCCACCTCCCATTAG	44016
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10392	GACCCACACTGCCCAATTAAGA	22050	GCACAGCCCCTTCACTTCTCT	33034	TGCAGCGTTTGACCCACACT	44018
10393	TGGGGAACTCAACCTTCATTTTACA	22051	GCAGGCAAGCATGCACCTTT	33035	ATTGGATCATCAGATTTGGGGAACT	44019
10394	TGCCTGATAGGGATGCTGTTTG	22052	GGCCTCCGCTGTGATTCACTT	33036	GTGAGAGACCTGGAAATGCCTGATAG	44020
10395	GACCCAAAATGCCCTTATTTTAGTGGAGTA	22053	GCATTAAGAGTCATACAGCAGAGTCAT	33037	CAAGGGACCCAAAATGCCCTTA	44021
10396	GGCCTTTGCTTGTCCTAGTAAACTCT	22054	ATGCGCCAGGAGGGTTCTTG	33038	CACCAGGCCTTTGCTTGTCCTA	44022
10397	GTCCCTTGGAAAAGGTGGACAA	22055	GTCTAGCACTGGTAGCACTGGAT	33039	GCAGGTAAGGAGTCCCTTGGAA	44023

10398	GCATGAAACACTTGGGAAAGCACATA	22056	CTCTAGACACACAACTGTCTCTTTTTC	33040	TGAACAGTCTGCATGAAACACTTG	44024
10399	CGTGGGGTGTATAGTCTAAGGATTGAGA	22057	GACATTTGACAGTGGTTTGTGTGA	33041	TCCCTCGTGGGGTGTATAGTCT	44025
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10401	GAAGAAGGTGCTTCTGAGAAATGTAGT	22059	AAGCTGGACCCACTAATTTGAAGT	33043	TACCTGGGCACCTGTGAAGA	44027
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10417	CCAGTGACCTACTTGGGAGATTTCTGT	22075	GAAACAGCTCTCAGAATCTAAGGTTTC	33059	CACCATTACTCCCAGTGACCTACT	44043
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10419	TCTGTGTGGACCCCTGGCAATA	22077	CCCCAGAGGAGGATTGGGAGAATAAG	33061	TGCTCCATGCAGGTCTGTGT	44045
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10430	CAAGGTGTCAGTGGGTTTGCTT	22088	TGCAACAATTTGCAAGCCAAGA	33072	ACCCAGGATCAAGGTGTCAGT	44056
10431	CCTTGTAACTGGGATAGAAAAGCACAT	22089	GGTCTGACTTGGCAGGAAATACA	33073	GGAAATGTGTTCTACCTTGTAACTGGGATA	44057
10432	AGATCCACAAAGCATTTGGTCCTA	22090	CCACTCCTCAGAACCAGTGATGAT	33074	CCTGGTAGGAAGATCCACAAAGCAT	44058
10433	GCAATTCCTGGACAGCTCCTAGT	22091	AGCCACCCTAGTCCTCTCCTTCT	33075	CAGTCTAGGACACAAAGAGTGCAA	44059
10434	TCAGAGGCCATTTCTAGGGTCTT	22092	TGATGTGGAGGAGGCAACAGA	33076	CCAGCATGTCTCTCAGAGGCCATTT	44060
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10436	ACACACCTGGAAGAGAAAACCTT	22094	GCAGTTCTGAAGAGAGGCTTTGAAC	33078	AACTACAAACACACACCTGGAAGA	44062
10437	GAGCTTGAACTCGAAGTATTAGGTGTA	22095	GCTTTTCAGCTAGCATTCATTCACT	33079	TCTGCATGAGCTTGAACTCGAA	44063
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10611	GTTGCCTCACACACTAGACCTTTG	22269	TGCAGACCATCACACATCTCAAC	33253	GTCTTTTAAACAGTTGCCTCACACA	44237
10612	TCCATCAGGGAAGAACCACTTATG	22270	CAGACACTAAGCCAAGCTCTGAAG	33254	CTGTGAGTTCCATCAGGGAAGAA	44238
10613	CCTGTGTATTGGGGCAATGCTT	22271	CAAGAGCCCCAAGTAATGTGTGT	33255	GGATGATGGGGAGAGTCCTGTGTATT	44239
10614	GACAGGATTCCATAGGGTCTTGTTT	22272	CCCCACCTTAAAGGCACAGAGTAG	33256	GGTCTCTTGAAGACAGGATTCCAT	44240
10615	CAGAGAGCAAGTGACAGAGTCAT	22273	GGTGGGGAGAGGCAGGATTG	33257	GCCTGACACAGACAGAGAGCAA	44241
10616	TCTGCATGTCCAACACTGAACT	22274	AAATGTGTGTTTGCGTGTGGAA	33258	TGTACTTCAAACTCTGCATGTCCAA	44242
10617	GCAGTGGCAATGTGGCAAGA	22275	GGCCTAAACGCTGCTCACCTAT	33259	CTGGAGCTAGAGCAGTGGCAAT	44243
10618	GCTCTCCACAAGAAGATGGCAGTT	22276	GGAAACCGGCATTTGGACACA	33260	GCTGGGTTGCTCTCCACAAGAA	44244
10619	ACTTTGGGTCTCTGGTTCCTGTA	22277	GTGACTGTGGCTTCAGAGTGATG	33261	GCCCTACAGAAAACTTTGGGTCTCT	44245
10620	GGCTCCCAATGCAAAGACCTTCA	22278	GAGCACAAAAGGTAGGAGTGAGAA	33262	GCCATCTTGGCTCCCAATGCAA	44246
10621	GGGTGCAGGAACATTACCTGGAGTA	22279	TCATAACGTGGAGGAGAACTGTTG	33263	CCTCACTAAAGTTTGGGTGCAGGAA	44247
10622	GCAAGGGGTATATTGAGTTTCAGTGACTA	22280	GCCTAAAACTTTCATGGGGTGTTTG	33264	TTCCATGCAAAGCAAGGGGTAT	44248
10623	CCAGGGGCATTGATGGGTTT	22281	ACAGACCTCAAGCTCGCTCAGA	33265	GCAAGGGAATCCAGGGGCATT	44249
10624	CAGCAAAACCAATGCAGTGTGA	22282	AGCCTGGGGCCTTGTCTGA	33266	ACCCTGAGTGAGCCAGCAAA	44250
10625	AGATCTTAGGATCAAGGGGAACACTA	22283	CACTGGAACATGACCTGTGAGA	33267	AGGAAGCAAAGAGATCTTAGGATCAAG	44251
10626	GGCCCAGTCTCACTATCTAAGTACAGA	22284	GACGTTTAGGTTTCCATGGCCAAA	33268	AACAGGCCCAGTCTCACTATCT	44252
10627	TGGAAGCAGGCTGGATCACT	22285	TCCCAGGAGAGGAAGGCCAAT	33269	CCCGAGGGAAAACAAGAGCTGGAA	44253
10628	AGCAGGAAATGGGCCTGAAG	22286	CTCCTTAGGGTGGCATACACAAAC	33270	GGCAGGTTAGGTATTAGCAGGAAATG	44254
10629	GGCCTCATTTACTTTTGTTGCCTTT	22287	CAGAGAAGGAATCCCTGCTATCAAA	33271	GGCTGCTGGCCTCATTTACT	44255
10630	CTGTAGAAGACGGACCTCAACAATCA	22288	GGTTTGAGAGGCACCTAGAATCACT	33272	GTCTTGACAGACTAGCCTTCTGTAG	44256
10631	GGGTGACTGCCAAGTCCAGAAA	22289	GTGTGAAAGCCAGGTGAGCAT	33273	TGTCAGAAGGAAGGGGTGACT	44257
10632	AGGTGCAAGGTATGCACTGATT	22290	GGACCACAATCTTCATGAGAGCAA	33274	GTGCTGTGCTAGGTGCAAGGTA	44258
10633	ACGGCCTTGAAAACACCATTCA	22291	CTCATTAGATGCCTTCGTCTTTTCAGT	33275	ACAAGGTTTATGTAACGGCCTTGAA	44259
10634	CCATGAGGACACAATCAGTCTCAA	22292	GTATTGGCCGTAGGTTTATTGACTTC	33276	GGGAAAATCTATGCCATGAGGACACA	44260
10635	GCCCATATCGGTGAGGATGGAT	22293	CCAGATAGTGGCCAGATTGCTT	33277	TGAGACACAGGGAGGCCCATATC	44261
10636	GCCAAGTTCCTCTGGTAACAAAGTT	22294	AGTAGGCAGCAACAGTTGTCAT	33278	GGGATTGGGATTGTTGCCAAGTTC	44262
10637	GGTGGTGGCTACTACCAAAATGT	22295	TCTCCAGCACTGGCTCAGAT	33279	GACTAGCATATGGTGGTGGCTACT	44263
10638	CAGAAACAGCCACCTAGCTGGTAA	22296	ATGTCAAGACAGCCTAGGTTCAAA	33280	GGTGGGGTACCTAGATTCAGAAACA	44264
10639	CCCAGGACATCTCTTCCACTGCTT	22297	TGGAGGGAGCTGCTTCTAGCTT	33281	CCACAAACTCCCAGGACATCTCT	44265
10640	ACGAGCCACGACGGCTCTGA	22298	CGTGACCGGCCAACACTGA	33282	TACCACCGCCGAGAATGGAA	44266
10641	GTCAAGCAGGTAACAGCAGACT	22299	TGCTGTGGTTGGGTCCCATT	33283	AGGGTCCCAGTCAAGCAGGTAA	44267
10642	CCACAAATAGGCAGTTTACAGCATGAA	22300	GTGAAATCCCTGAAGTGTTTGCTT	33284	GCCATGGCCACAAATAGGCAGTT	44268
10643	GGGCTTTGACAGTCAGCAGAT	22301	AGGGTAAATGGGAGTGCTTCTCT	33285	GGGCAGAGGGGCTTTGACA	44269
10644	GGTGCTGCCTTTAGGGGAGTTT	22302	GCAGCTGGTCAGGAGATAAAGT	33286	CAGATGAGGGTGCTGCCTTT	44270
10645	CCAGCATCTGTGAGAAAACTTCGTT	22303	CGCTGGCTTCCAGAAGGGTTT	33287	GGACTCCTTCCAGCATCTGTGAGA	44271
10646	GAGTGGCCAGCTGCATAAAAG	22304	TGGGTCTCTTGAAGACAGAAGATG	33288	TGCCCACATTTAAAAGGCATAGAGT	44272
10647	GCACAGTTTGCAAGCCACTTC	22305	CTCTGAGAAGACGTGTCTCATTTCCAA	33289	CTGGTTTGGGGAAGCACAGT	44273
10648	CGTGTTTCTGGGATGAGTTAGTCTGT	22306	CCCTCTTCAGCTAGGCACACA	33290	CCTGCTCGTGTTTCTGGGATGA	44274
10649	GGGACATTGGTAGAGCAAAGAGT	22307	ATCGGCCCTCCTCACCTTTCA	33291	GGGAGAACAGGGTAGGGACATTG	44275
10650	GCAGCGAGAGCTTAGCACATCT	22308	CCCAGGCCCATGACTACTCCTT	33292	TGGCTGGCAGCGAGAGCTT	44276
10651	GTTTGGGTCAGTGGCAAGAGA	22309	CCTTAAGGATTCAGCCCTCTTCTAAA	33293	CTGAGGCAGAAAGTTTGGGTCAGT	44277
10652	GACAGAGGAAACCGGGGAAACT	22310	CCCTCCCTTCCCTCTAAATAGACTT	33294	TGTCCCAGTTTTGACAGAGGAAAC	44278
10653	GTGGAGGGGATGATGGAGTTCAGA	22311	GTCCAGGAAGACTGGAGCCTAGAA	33295	GGGGAATGTGGAGGGGATGA	44279
10654	GGGCAGAAGATTAGGGAGGTCTT	22312	GGCTTCTGGTTTTGGAAACAGGTTGA	33296	GACAGTTGCTTAGGGCAGAAGATT	44280
10655	TGGGCTTGGATCCTATGGGAAA	22313	GGGGCTTTGTACTCACAAGTCA	33297	CCCCAGATAACTATGGGCTTGGAT	44281
10656	CATTCAGGACTTGGTTTTTCGTACA	22314	CCTTGCGCTTGGCATTGGTT	33298	CAAGGAAAACATTCAGGACTTGGTT	44282
10657	CACAGGGGTCTAGGCAAATCTCA	22315	CAGAGGACACTGACAATCAAGTCA	33299	CAACGGAAACACAGGGGTCTA	44283

10658	CCTGTTCCTTCCACTCCTTTTCA	22316	CCTGACCAGGGTACAGTCACTAAG	33300	GAGTTTCAAGGCTTAACCTGTTCCTT	44284
10659	CCACAAAGCTCCCCTACCATGT	22317	CAGCAACCAAGCCAATCCTGAA	33301	GGGTGAGTTGAAATGCCACAAA	44285
10660	CAAAGGCTGTTTCAGAACTGATCTCT	22318	GGGTAAAGGTGGGAGAGAAGAGAGTTTC	33302	TCCCATTACAAAGGCTGTTTCAGA	44286
10661	GCCCTACCCAGTGAGGAGTAGTAG	22319	TGTAAAAGTGGCCAGTCTGTTCT	33303	AGAGGGCCCTACCCAGTGA	44287
10662	ATCCAGAAGGCAGCAAGCAA	22320	AGGCAGAGCAGCTCCACAA	33304	TGCGGGCCAAGAATCCAGAA	44288
10663	CCTGCTGTAGACATTCAACAACCAA	22321	GGTGCTTGAAGTGGGCTTTTC	33305	GGACTCTACAAGATCCTGCTGTAGACAT	44289
10664	CACGGGAAGTCAAATCAATTACGAT	22322	GGCCAGTTCGCTACCAAGGAA	33306	GGAGAAGATTTCACGGGAAGTCAAA	44290
10665	GGTTTTGACCTCCCGGAATATTAGGAAA	22323	CACCTCAGCCTCCTGCATAG	33307	CTTGGTTTTGACCTCCCGGAAT	44291
10666	GGCTTTTGGGTGTCAAGAACACACT	22324	AGTAGTCCCCAACCAGCAGAGA	33308	GAGTTGAGGCTTTTGGGTGTCA	44292
10667	TGCACGTAGTTGCTAAGAGAGTTATG	22325	CCTCTTCTTCATCCTGGTTAATTTGGAAGT	33309	GTGATTGGTTGCACGTAGTTGCTAAG	44293
10668	GACTGATTATGAGGCAACGTGTGT	22326	AGACGAAGGCTCAATGTAGACAAG	33310	ACCAATGTTCCCTTTCTGACTGAT	44294
10669	TGGCCATCTTGGGCGTAGAA	22327	CGTCTGTCACAGTTAACGGCAAA	33311	GCTTCCGGCTGGCCATCTT	44295
10670	CCGGAGCACAAAGCAAGTGAA	22328	TCCACACCTGCGGCCAAAAT	33312	CTGTTCACAAACCGGAGCACAA	44296
10671	ACACACCAACAGGCACAGAAG	22329	TGCTGGGCCCATGTGCTT	33313	CACACCATCGTATACACACCAACA	44297
10672	CGCTTCATCAACCTCAATGGTAAG	22330	TGCTGGGTGCCAGGACATC	33314	GGAGAGCTGCATTCGCTTCATC	44298
10673	GTCTTAGGAGGAAAGTGTCCAGGTTT	22331	CTGCAGAAGACCTACAGGTAACATC	33315	GCCTTGTTCCCAGTCTTAGGAGGAA	44299
10674	CCATCAGTGGAGCTTTCAGTTCTTC	22332	GCCCTGTGGCATGGTCATTT	33316	ACCCTCCATCAGTGGAGCTTT	44300
10675	GCACAGCATGGAAATGAGAGACA	22333	GTGAGAGGAAATCTTGGGCTATGA	33317	CTTTGGTTAATGCACAGCATGGAA	44301
10676	AGGCACTAAAAGCTGACCTAAAGT	22334	GGAGCAAGGCACAGTTTTCAGA	33318	TGCTGAGGTCAAGGCACTAAAAG	44302
10677	AGTGGCTCCAGTGTGGATGA	22335	GCCCTTTAAGCCAACCCTACA	33319	GGGGAAGCCCACTGGAAAAA	44303
10678	GCTTGCCTTGTAGCTTGGTGTT	22336	GCCCAGCCACTGGTGAACT	33320	CCTGCTGTATTTGCTTGCCTTGTAG	44304
10679	AGGGTAAGGGGAGAGAGGAGTCT	22337	TTGTCACCACCGGGCACATT	33321	CCACGGAGCTCAGAGGGTAAG	44305
10680	AGAGTCTTGGTCCCGGAGTT	22338	GCGGAGCAGATGTGATGCCTAT	33322	GGCCAGTCACGCTAGAGTCTT	44306
10681	TGGAAGACAGGGGTGGAAAAAG	22339	CAATCTCACCCAAACCTGCTTCT	33323	AGAGGGTCACCCAGATTGGAA	44307
10682	TGTGCCTGCTAGAAGGGTAAAG	22340	CCCCGTACCCAATGAGGAGCTAT	33324	AACAAACACTGTGCCTGCTAGA	44308
10683	CACTTCGTTTTTGCAGGACACA	22341	ACAGAAAGTGTGTGCTTCACTGATA	33325	CACACCTCCTGCACTTCGTTT	44309
10684	TGCAGCCATTTGGAGGAGAGA	22342	CAGTGCAAGAACACTGAAGACTCA	33326	GCAGAGGTGATGCAGCCATT	44310
10685	CTCGTCTGTTGGCAGAGACAACT	22343	ACTGATCTCCCAGTCAGGGTCAAG	33327	GCCCTCAACGATCTCGTCTGTT	44311
10686	GGGGAAGAGTAATCTATGGGAGCTAAC	22344	CGAGCCCTGCTGCTTCACATTA	33328	GCCTGAGGAAGGGGAAGAGTAATCTATG	44312
10687	CCCTGACCATGTCCCTTTTGACA	22345	CCCGAGGCTCCTCCGGTTATTA	33329	ACAGCTGACCCTGACCATGT	44313
10688	GCTGTAGGGTCAAAGAATGTGACT	22346	CCCAGAGGACCTCACACCTCAT	33330	TGGAGAGCATGGGACTCCTTCA	44314
10689	GCAGCATGCAAGCCATTGTCA	22347	CACTCTGAATGTGAGGCTGTAGT	33331	AGCCAGAAAGCAGCATGCAA	44315
10690	TGGTGACTAAGATTAAGGGCAGTATCT	22348	GTTCAGCAAGGTGTGCTTCTGT	33332	CTGAGCCCACTTATGGTGACTAAGAT	44316
10691	CAACTTGCCAGTCTTCCACGTATC	22349	TGGAGGGCCAGTGGGATGT	33333	CCCACCTCAACTTGCCAGTCTT	44317
10692	CTGGTCCTCTGCATAGACCTAAC	22350	GCATCACCAAGAGTCTCCCTTGT	33334	TCCTGCTGGTCCTCTGCAT	44318
10693	GCCTGTGTCACATCCCTGAGAAG	22351	GGACAGGGTGGATACTGTGTTTGTAG	33335	CCACAGTGCCTGTGTCACATC	44319
10694	TCCTCCTTCACTCCCTGTCATAG	22352	CTTAGCACAGAGTAAACCCACACA	33336	GTTTTCCCCAATTATCCTCCTTCACT	44320
10695	GGAGAAGGTCTCATTAAGGGAGTAGA	22353	CCCTCCCAAACCTGGACTGTTCT	33337	CCCAGGAGTTGGAGAAGGTCTCATT	44321
10696	GGAGGGATGATGGAGGTGGATT	22354	CCCCTCCCCGCAGGACTTAAATA	33338	TGCACGTCTGGAGGGATGAT	44322
10697	CAAGCCTTATTCAGTTCTTGGCATCA	22355	CAGACCCAAACCATATCACCTTCTTTTC	33339	CCAACCTCACTCAAGCCTTATTCAGT	44323
10698	GTCAATAGCCAGGAAACATGGGTAA	22356	GGGTCAGTCTGTAGAGTCCGTTTC	33340	GGGCAGTTTCTAATTGTCAATAGCCAGGAA	44324
10699	GCAACCTGCACCGGTCTTTTG	22357	TAGCAGGGCCCAGAGCAA	33341	GCCTCTCTGGCTTCACAGCAA	44325
10700	CTCCCCTAGTTATTAAGGGTGAGGTATC	22358	CTGCAGACATTCTGTGTACTGTGAGA	33342	CTGCTGGTAAACCTCCCCTAGT	44326
10701	GGGCTTGTATCCCACTCCAACTAC	22359	AAGTGTGGTCTTCATGGTTCCAA	33343	TGGGAATAAAAGATCTGGGCTTGT	44327
10702	TTGGGGCTGAGAATACCTCTCT	22360	GTGCCCACAGAGGGAGCATTTA	33344	CCGGGGCTTGGATTTCTCTTGTT	44328
10703	CCTGTGTACTGTTTGCTTGGGATA	22361	GCACAATTTTCAGCAAGGAGAGATG	33345	CTGAGCCTTTGATCCTGTGTACTGT	44329
10704	GGGCACTAAGAAGGGCTCAGAGAT	22362	CTGTGAGCATCTAGGGTCACGAT	33346	TCCCTGAGCTGGGCACTAA	44330
10705	CCTAGATCCAGCCTCTATCGAGTTC	22363	CACAATGGTCCCAGACAGAAGCAA	33347	CAGGCTAACTCAAGACACTCCTAGA	44331
10706	TGCCACCAGGATATTGATTGAGATT	22364	GTGCCCATACTACCCAAAGCAATAC	33348	CTGTGAAGAATGCCACCAGGATATT	44332
10707	CAGCCCTAAGGGTGGGATTTCA	22365	TGGGCAGGGTACCCAGTGA	33349	CCCACCATGACACAGCCCTAA	44333
10708	ACGCCAGGCCAGAAAAATATTACA	22366	CATTTTCCCTGGGCTACACTCT	33350	CATCAACGCCAGGCCAGAAA	44334
10709	GACCCAAGTCGCGTCAATTCT	22367	GGTGGTGATGAATGATGGACATACT	33351	CTTTATATCCAACCCAGACCCAAGT	44335
10710	GGGAGAGCTGGTCTATAAGAGGAACTTG	22368	TCTGAAGATTTCACGACAGGAAGAA	33352	GGGTGGGAGAGCTGGTCTATAA	44336
10711	TGCCTGTAGCATTTGACAACCAT	22369	GAACCACAAACCTTTGGAATGGAA	33353	CCCTGCCTTGCCTGTAGCATTT	44337
10712	GGTGGGTAAATCTGCCTATTTCAGA	22370	GCTCTGCTAGCAAACTGTGCTTGTT	33354	GAGTATTGTGACTAGGTGGGTAAATCT	44338
10713	CCACCCTAGTCAATGTCACTATCCTTTC	22371	ACCAGGTAGGAGGCTGCTTT	33355	TGCTACCACCCTAGTCAATGTCA	44339
10714	GCCTGTCTGCCAATTTGAACAT	22372	GGGGAAGGATGGAGGTATTAAGTGA	33356	CTGTGGGACCTTTAGCCTGTCT	44340
10715	CAGTAGGCCTGAAGAACCACTCT	22373	TCGAGAGGAAAGGGCTGTGT	33357	TGCCAACAGTAGGCCTGAAG	44341
10716	CCACTGACAGGTCTGCTGTT	22374	AAGGCCAGGTCACCTACAGA	33358	GGCTTGAAGGGTTTCCACTGACA	44342
10717	TGGGGACTCTGCAGGTCAAA	22375	TGTGCCCAGGCCTAAACTGT	33359	CGCTGAAGCTTGGGGACTCT	44343
10718	TGGGTTGTCAGTCACCCAGGTA	22376	AGCCTCCAGCTACCCGGAAT	33360	AGGCTCCTGGGTTGTCAGT	44344
10719	GCCTCAAGGTCTCATTTGCATAGAAAG	22377	GTGTGGTGAGGACCATGGATT	33361	CTCTGCAGCCTCAAGGTCTCAT	44345
10720	GCTGCTGGTCCATTCTGAGTTG	22378	GGGAGGGCTTCCCACAAAG	33362	TGTCACTGCTGCTGGTCCAT	44346
10721	GTGCAGAATCAAAGACAGTGTAGAAAC	22379	CCCCGTAGAGAACTGTCTCACATC	33363	TGGGAACATGTGCAGAATCAAAGA	44347
10722	GCCCTGAGACCAAGCAATCA	22380	ACTCAAAATGCAGCCTAGATAGCAA	33364	GCAGCAAGTTAGCCCTGAGA	44348

10723	GTGGTATGCCTTCCTTCCATACTAAACA	22381	GCAGCTACCAGATAGACTTAGCTGTAA	33365	GTAGCAAGTGGTATGCCTTCCTT	44349
10724	GTGCCCTTCCTTCCCAGTTTCA	22382	GACAGAACCCCTTGTATAACAATCAGACT	33366	CCAAGTCCAAGTGCCCTTCCTT	44350
10725	CACACTAGAATAGGTAGGGTGCTCTT	22383	GTCCAGGCAGAGCCATACATC	33367	GGAAGCCTCCCACACTAGAATAGGTA	44351
10726	TGCTTCGGAGTAAGACCTCCAT	22384	TAGGGCCAAGAGCTCCTGCAA	33368	GAGGACAAATACTATGCTTCGGAGTAA	44352
10727	GACATGTGGCCAGTTGGATCAGT	22385	GAAGCCATGCAGAAAACATTTCCTTAC	33369	CCTCCCCAGCCTCTTTTGACAT	44353
10728	TGTTTCCCCACTCCCCAAGA	22386	CCTGCCCCGCCTCCATATTTA	33370	GGTTTGCTGCTGCGCTTTGT	44354
10729	GCCAGATGCTATGTAGTGGCAAA	22387	GTGGAAATAGAAGCTCACTCTCAACT	33371	GCACTGTGCCAGATGCTATGT	44355
10730	TGATCAGGCACAATACTCTAGTCTCA	22388	CAACACACCAATGCAGCCAATC	33372	CCCAGACAAAAATGATCAGGCACAATACT	44356
10731	CCAGGTACTATCAGTGCCGACTGT	22389	CCTGCAGAGTTATTGGGAAGATGA	33373	GGAGGTAGGCCAGGTACTATCA	44357
10732	CTGTTGTTTGGAGCCAAAAGTGT	22390	TGGGCAGCACGTCACAGAATC	33374	GGGGTCAGTCGTATTTTCTGTTGT	44358
10733	CCTTCCACAATGCAGAGCAGTGA	22391	AGCAGGCTGGGCCAAAATCA	33375	AGTATCAGGGGCCTTCCACAA	44359
10734	CTCTGCCCTGTAAGTTTTCTACTGACTA	22392	CACTTGCGAAGGAATCACCATCA	33376	TCTCCTCTGCCCTGTAAGTTTTC	44360
10735	GGAGAAGGTCTTTCTACAGTGAAACT	22393	TGGTGGAGCAGTCACCTCTTCT	33377	CCCCACCATAGGAGAAGGTCTTT	44361
10736	CGGCAAGACTAGGAATACCAGAGGAA	22394	GCCTGGCTTTGCAGAGCTTTT	33378	GCGCTCGGCAAGACTAGGAATA	44362
10737	GCATCTTCACATACAGGAAGGAGCAAA	22395	AGGGCCTTTACAAAAGAGTCTTGA	33379	AATGGCACCTTTTTGCTGCAT	44363
10738	GGTAAAGCTGCTCAATGGGCAATAG	22396	CAGCCCAGGACCCTCTTTTGAT	33380	TTGTCCAGGTAAAGCTGCTCAAT	44364
10739	GCACCCATGTTGCTCTCAGTGT	22397	GCAGGATATGAGTGATGCTCCAT	33381	CCAAAATCTCTCTTGGCACCCATGTT	44365
10740	CCTGTTTCCTAGCTTGGCTCTCTTT	22398	GCCGCCATCTCTGAACTGT	33382	GCCGATCCGTCTATCAACCTGTTTC	44366
10741	TCACCAACACCTTCCTGGAATTAAG	22399	GTCATAACTACATGGCTGGAACCTA	33383	CAAGCAATTCTTCACCAACACCTT	44367
10742	TGCATGGATCACAGTTTCTTCTGTA	22400	ACCATGGACTAATGAAATCTGGCTATG	33384	CCCTGGTGCATGGATCACAGTT	44368
10743	CCATGTTTCCTCTCAATTCTCTGAAAG	22401	ATCCACAGCCAGCCATCAAG	33385	CAGTTGCAACCATGTTTCCTCTCA	44369
10744	GGTGATGTGGCCAGACTTGT	22402	GAGTGCTCACTCAGCATATCTGCTATC	33386	GAAGGAACCTGTCTGGTGATGT	44370
10745	CTTGTGTAGGGACAAAAGCATACCAA	22403	TTCTGGGGACACCTGATAGACA	33387	CCCAAGGTAAAAGCAATTCTTGTGT	44371
10746	CCAAAGGCCCAGGAGAGGTTT	22404	AGCAAAAATACCGAGGTAGCCAAT	33388	ACTGGGCTTCAAAGACCCAAAG	44372
10747	GAGAACTGACTCTCCCAGATATAAAGAGTT	22405	GCCCAATAGGTGTGAAACTTTGAA	33389	AAGGAGAACTGACTCTCCCAGAT	44373
10748	TCTCTGTGATTTAGGTTCCTGCTTTAG	22406	GGATAGGCAAGTACTGGCTAAAAGT	33390	GGCTCGTCAGTCTCTTCTCTGT	44374
10749	GCCTGGCCTCAATTCTCATTTTT	22407	CCCCTACCATGACTGATGAAGAAA	33391	CCACTGTGCCTGGCCTCAAT	44375
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10753	GGCAGAGTCAAAAGGAAGTCAAC	22411	GAGTCTGATCATACCAGACCATCCTT	33395	GCCCTTTGAAATAAGGCAGAGTCA	44379
10754	TGCTGGACTCAGGGTTGAATATG	22412	GAGCTCGTGATAACAATATCCCTTTG	33396	TCCATAGGCTTGCTGGACTCA	44380
10755	ACACCTGCGAAACACTTTATTGTAG	22413	CACGCTCAATTTTCTCCTGTTTTCA	33397	GACACCACACACCTGCGAAA	44381
10756	CCTGTCATTACCAGTGACTGCAT	22414	GGAAAGTGGGAAACTTGAAACTGAGA	33398	AGGTCACACTCTAGACCTGTCATT	44382
10757	GGCATCCTTTGACCCATGATGAGA	22415	CCAGCAGGACCCTGACTGTACT	33399	GGGTTGGCTTGGCATCCTTTGA	44383
10758	AGGACCAGGCCTCCTTGTTT	22416	CAGGACCTGACATTAACCTTGGTTTTAG	33400	AGCAGCTTTGTGGCCTGGTA	44384
10759	CCACAATGACCTAGCAGACCTT	22417	GCATCTATGGGGAATGGGAGCAA	33401	GAAATACAGTGGAACCACAATGACCTA	44385
10760	CCAGCTTCACCATTGGCTTTC	22418	CAGCAAGGCGTTTTCCACTGA	33402	GGCACGAATCATTCCAGCTTCA	44386
10761	GTGTCTACTAATAAGTACCAGGGCAAA	22419	GCCTGCTTGGTATCCCCTGAAT	33403	GGCAGCTGCACTACAAAGTGTCT	44387
10762	TCTGAAAGTACCCCATTCAAGTCAAG	22420	CAGGACACAATTGGAAACACTGGTTA	33404	GAGTCTGTATCTGAAAGTACCCCATT	44388
10763	GTGGTCATTTCCTCAGTGCCAGAA	22421	CTGCCAGCTACTAAGTATGTCTATGCTA	33405	GGACCCCATCCTGTGGTCATTTC	44389
10764	GGTTTTGCTTCCAAGGGACTGT	22422	GCCTAAGAGCAGGCATGCAA	33406	CCTCCCTGTAGCTTTGAGGTGGTT	44390
10765	GGCATATTCAGCACAATCTGCTTTAC	22423	GGCAGCTGTGGTAGACCTTTGT	33407	GCTAAGAGAAGCCGTTTGGCATA	44391
10766	GCCATGAGTTGATGATTGCGAGGAA	22424	CCATTAGTGGCTCCATACCCTTCA	33408	AGGGAGGTGCCATGAGTTGA	44392
10767	GCAGCAATGCCCAGGTACTACA	22425	CCTGAAGCCATCAAGTTTCAGGTTTA	33409	GCCCTGAATAACTGGCAGCAATG	44393
10768	CTTGTGGGGTCTGGAATTTGGAT	22426	CTGGGGACTGGTCGTATAGGTATC	33410	GCTGAACTCCCGCTTTCCTTGT	44394
10769	TGGTCCTGGGTGTAGTCTGGATTC	22427	GGCCATGGACCTTTGGCAAT	33411	GGACTTCATTGGTCCTGGGTGTA	44395
10770	GGAGGCACAAACATGCATTCAGA	22428	GAATGTGGATTTAGCTTCTCCCTAGA	33412	ACTCTGCAGGAGGCACAAAC	44396
10771	GTACTTGTGCTTGGATGTTTTGCTA	22429	CACCATGAGTTTGACTTTGTACCTGAT	33413	ACTTACTGTGTACTTGTGCTTGGAT	44397
10772	TGGGAGTTAAGAGTGAGTGGAATG	22430	TTCCTGACTCCACCGTCCTT	33414	GGTGACCCTCTTGGGAGTTAAGAGT	44398
10773	CCAGAGCACACAGCTTCACAAC	22431	GGAGAAATCTCTGTTTCTCACCATGACTCT	33415	GTGGAAATACCCAGAGCACACA	44399
10774	TCTCACCGATGTTGGGCTCTA	22432	TTCCCTCACCCTCAGGGATCTT	33416	GCAGCACGTATATCTCACCGATGT	44400
10775	GACACAATGCTGCGTGTTGCTT	22433	AGGGCCAGAGGTGCGCAAA	33417	GGCAGGGCTAATTTGACACAATG	44401
10776	GAGTGAGTGAGGGTGGGAAGTT	22434	CAAGCTGCCCCTACTACAACA	33418	GAGGAAAAAGGTACAGAGTGAGTGA	44402
10777	TGCCCAAAGCTCCTGCTAGT	22435	AGCCAGTGGGAGGAGCAGATA	33419	ACACAACGTCCTGCCCAAAG	44403
10778	CAGAGGCCTGAGTTTGTTTAGTTG	22436	TGTCACGGAAACTATCACCTTTGA	33420	TTCCTCCAGAGGCCTGAGTT	44404
10779	AGGCCAGCCTCCACACATACT	22437	GGGACTAAAGCCTGAGAGCATGAGA	33421	CCTGTGGGCCCAGACTTCA	44405
10780	GCCATTTTGAGAGCTGCCCTAT	22438	GTTGAGCCACCTGCATTTTCTTAC	33422	AGCCAGCTGCCATTTTGAGA	44406
10781	CCCACACAATCACACCTTACCTTTC	22439	AACCAGGGGACCAGGATATGT	33423	CCTGAAAGCCCACCCACACAAT	44407
10782	GGGACTTCTGTTTCTCAGAGTAGTTC	22440	GGGTGAGGTGTCCTGGTCTGT	33424	GGGTAGTTCAAGGGACTTCTGTTTC	44408
10783	TGAGAAGGCAGGAGCAGTGATA	22441	CTCATTCCCTCTCTCCCACCTAAC	33425	GCTTCAGCAGTGGCTAAGAATGA	44409
10784	GGGTCTGTATAGCCCCATTTCACA	22442	GAGCCAAGCCATGACACACT	33426	GGTCGCTGTCTCATAGGGTCTGTA	44410
10785	GGATGCACTTAATTCGCCCAACAA	22443	GGAGGAGCTTCCCTGATTGGTAT	33427	GGGAGCTATGCAGGATGCACTT	44411
10786	GCAAGTTTTGGCTTGAGAGCTT	22444	GCACATGAACTGCCACGTCCTA	33428	AGCCCACCCACTCTCACTTA	44412
10787	GCACTGTCTGCTTCCTTCTAGACT	22445	GGTGGTTGTAGCTGTGATTTAACAAGA	33429	CGCCTTGAAGTTAGATGCACTGT	44413

10788	GCGGGCAGTGATCATTGGAA	22446	TCCTTCTTCAGTCCTGTTGAAAGTAG	33430	AACTGCTATGCGGGCAGTGA	44414
10789	GGTGAGGGTGAAATCAATAGCAGAAG	22447	ATCCTCAGGGGCTCCAGGATCT	33431	GCAGCTAGAGAGGTGAGGGTGAAATC	44415
10790	CACAGACCCAAAACGCAACACA	22448	GGTGGAGACCAGTAGGTTTTATTGT	33432	CCCTTGTTCCTTATCTCACAGACCCAAA	44416
10791	GACCTCCTGGATTTTCTCCCTTTG	22449	CCAGGAAAATATCAGGCTCAGTTTCA	33433	TCCCCAATTGACCTCCTGGAT	44417
10792	GCATGGGAGGGGTAGTCTATTAGCAA	22450	GGTGAAATGTAAGCTCTCTGGTTCTTTC	33434	GTGGCATGGGAGGGGTAGT	44418
10793	GTGGTGGTTAGGACAGTTGAACCAA	22451	GGGTGCTGAAATGGTTGGAATGAGT	33435	GCATCTGGGAAAGAAGTGGTGGTT	44419
10794	ACAGCTACCCCTCCCATTACA	22452	GGCCCAGCCTATAAAACCACAT	33436	ATGTCAGAGGTCTCCACAGCTA	44420
10795	GGACAGTTGTTGACCTAGGCTGCTA	22453	GGTTAAAAACATGGGCCTTAGAGTCA	33437	ACCTAACAGAGCTGGACAGTTG	44421
10796	GGGGCAGACTGAGTGTATAGGAA	22454	CCCACAGCTAGGGCAATCTGA	33438	AGAGCTTGGGGCAGACTGA	44422
10797	GCCACATGACAACATGTAGCTGGAA	22455	GCAGAGGGCACTCATAAGTCAGT	33439	CCCACCTTGCCACATGACAACA	44423
10798	CCTGACTGGGTCAGCTTTCAA	22456	GTCCGGGGACACATTCTAGTTTC	33440	TGTAACGTGGGTCCCTGACT	44424
10799	CCTGGTTTCCCCTTCATGCAAA	22457	CTAAAGACCTAAGCCTGTTCACATCT	33441	CCTGGTTTGGGTCACCTGGTTT	44425
10800	GGAACACAGGACTGGTTCAGAAA	22458	GTGCCACCTTACTGGCCAAAGA	33442	CACCAAACCTAGCATGGAACACA	44426
10801	CCGCTCTTCAGAGTGACTACCCTAAC	22459	CCAGCTGCAGATGAAGCTCAA	33443	CCAAGGCTAACAATTCCGCTCTTC	44427
10802	TGGTCCTTGTAGATGGGAAGACA	22460	GTAAGTCAAGTCACACCAATTCCTTAC	33444	AGGGCCTTGATGGTCCTTGTAG	44428
10803	CCAGAGGACTTCAGCCAGTTGT	22461	CAGATCCCAGTGTTCTGAACTTGA	33445	GGTTTATTTAAAGCCCCAGAGGACTTC	44429
10804	CCTGGCCCTCATAGGGAGATAGA	22462	GCCTGTACCACCTCCTGCTAAT	33446	GTATAGTATTGTACCTGGCCCTCATAG	44430
10805	CTGGCTGCGTATTTTGGCATGT	22463	CTGAACATGCACCCGAGACAGA	33447	TAGGTGGCTGGCTGCGTAT	44431
10806	GGCAAAACTGTTATGGTGACAAGACAGA	22464	AGCCAGGCCTCCTGTTCT	33448	AGGGGCTTGGATCCTGGCAAA	44432
10807	GGGGCACAAAATGCACCACAGA	22465	GCAAGCCCCATCAACCATCT	33449	CGGAGATCTCTGGGGCACAAAA	44433
10808	TGGATGGAGTTCACCACTCAATG	22466	GATGCCTCAAAACGCCTCATAGAT	33450	CACAAAGATGCCTTATGGATGGAGTTC	44434
10809	CCCCGGTTTTTCACAGTACAGA	22467	GAGTCAATGAATACTGCACCCTCTT	33451	ACACGCCCCGGTTTTTCA	44435
10810	CCAGACAGACGCCTTCCAGTT	22468	CCCAGGGAATCTGCTGGAATGTAG	33452	ACAAGCCAGAGACCCAGACA	44436
10811	CAGCCTCAAAATATATTACAGGGACCATAC	22469	AAAATGGACCCTAACTGCCAGAA	33453	GGCAACCTTTGACAGCCTCAAAAT	44437
10812	GCGTGCTCCAACCAGTCTTG	22470	GGATCCCTGCCTCTAACTCTGA	33454	AACTGAGTGCGTGCTCCAA	44438
10813	CGTGTGCAATCTGGAGCATGT	22471	GTTCCAGAAATCTATGAGGCCATGA	33455	CCTGAGCACGTGTGCAATCT	44439
10814	GGGTTCAAGTCCTCATCGTGTCT	22472	GGGAAGGCCAGAGAGTAAGCTACA	33456	GCTTCCCAAGCATGGGTTCAAG	44440
10815	TCTGTGTCTGACCTTTCACTTTTCT	22473	GATTGGAAGGACATAGGCAGTTCA	33457	GCCCCTGGTTCCTGTTTGTTT	44441
10816	CGGTACAGTGAGTTTCTGCATAGATTT	22474	GTCCCTTGACAAAGTTCCCCTAGA	33458	ACCAATTCGGTACAGTGAGTTTCT	44442
10817	CCTGCTACTAAAGTTACTCTCCCATTG	22475	GAGAGTCTGGGAAGGCTGAACT	33459	TGGCCACAGCAGCACTAATTC	44443
10818	AGTCCAGGGAAAGCATTGATGATAG	22476	CACTATTTTCCACACTGAGGGCATCT	33460	GACTGACTTAGTCCAGGGAAAGCAT	44444
10819	ACCCATCCCACAAAACCAGATG	22477	CGTAGAACCAAGCCTGAGATATGA	33461	GACTTTTCAAAACCCATCCCACAA	44445
10820	CCGACTGTCCAGCTTTTTCGTAAG	22478	TCGAGGAGCCCAGCACATCTA	33462	GACTTTTCCGACTGTCCAGCTT	44446
10821	GGAGCATCTTTTCCAACACTATGAATC	22479	TGATGTCTTTGGCAGAGTGTTTTAC	33463	GTCACGCTTGGAGCATCTTTTC	44447
10822	ACCTAAGCCACAGAGTGCTTATC	22480	CTAGGCCTCAGCATCAAGCAT	33464	GCAACCTGACAGCAGCTCTA	44448
10823	CCCAACTGTGTTCTGCCTACAAG	22481	CCCTTGTAGCCTATGTTTATCCTTGA	33465	AAAAGCAAGACCCAACTGTGTTC	44449
10824	CTGCAGGCAGATCATCCCAAT	22482	CCCAAACTGGGTCTCCTCTTTGAT	33466	CTGCAGTGGGTAGCTCCTTTCT	44450
10825	GGAGTAGCAGGTTTCCGTGATG	22483	CCCACCTCCCAGACAGCATTT	33467	TCCCCTTGGAGTAGCAGGTT	44451
10826	GACTGGAACAGGTAAGGGGAAAG	22484	TTCACATTACTCGCAAGGTCAGT	33468	CACCTCTAATAGAAGACTGGAACAGGTA	44452
10827	TCAATTCAGTCTTCCTGAGTGCAA	22485	TGATGACCATGCCACATCTGTAA	33469	GTCAGGACTGCAACACACTCT	44453
10828	CCACAGTCAGTCCAGTCTCCAA	22486	GGGGAAAACAGTGACAAGTCACGAA	33470	TCAGGCTGCCACAGTCAGT	44454
10829	CTCATAGCCAGGATTCACAGATCCAT	22487	GAGTGGTGCCACTTCTGCTTCA	33471	GGTACTATTTCTCTCATAGCCAGGATTC	44455
10830	GACAGTGCAGGATTGAGTAAATGCAA	22488	GCACTTGGGAGAGGGAAAAATGCAA	33472	CTGCTATGACAGTGCAGGATTGA	44456
10831	GCAGGTATGCTGGCTGGTGAAA	22489	CCCTCATTTGTAGGTCTACCCTGAT	33473	GGGTTAGTTTTCATGCAGCAGGTATG	44457
10832	GACAAATGGCTTTGCATATCCCCATAC	22490	CCCTGCCATGTTGCCAGATA	33474	GTCTGCCAATTAAGACAAATGGCTTTGCAT	44458
10833	ACCAACGGGAAATCCCAGTTAATC	22491	GGGTCGGACTCTTGGCATCTTC	33475	GGCAGAAAAACACCAACGGGAAATC	44459
10834	GCTCCAGGCTTGCTTAGGCTAGAA	22492	AGGAGCTACCACGGGGTATGAA	33476	TCTCGCTCCAGGCTTGCTT	44460
10835	GCTTGGAAAGGCAGTAGGAAAGTGT	22493	GCTTTCCCCTTTTCCTGAAAAACCAA	33477	CACTCAAAGTAGCTTGGAAAGGCAGTA	44461
10836	TGGCCATGAAAGGAGGTACAATTC	22494	CAGCTGAGATCATTCTGCCCTTCAA	33478	GCGTAAGTCCAATGGCCATGAAAG	44462
10837	GCCGTGTGTTTCCAGAGCTT	22495	ACAAATCCTAACACAGCACGTGAA	33479	CCCTAGTTTGGGCCGTGTGTTT	44463
10838	GGGACTTATTGTAGCCCCTTAGTCA	22496	CTGTTTTCGAGCAGCTAGGGATT	33480	GGGGTGAGTAAGGGACTTATTGTAG	44464
10839	ATAGGAGGTGCGGGCAGATTC	22497	TGGAATCCTTTCCCCTGTAAGATAAC	33481	ATAGGCCCAGTGGCCAGGATA	44465
10840	AATGTGATGAGGAAGTGGCATCT	22498	GCCAGACCACTTCAGGAACA	33482	GTGCAGCAAGAATGTGATGAGGAA	44466
10841	GGAAGGGATTAAGTGGCAGGTT	22499	CTGTTAGAAGGTTTCCTCTCATGGTT	33483	GAGGGTTGGTTGGAAGGGATTAAG	44467
10842	GCAGACACTGATATCCCCTACCCATT	22500	CCAGCTGAATGCTTGGAGATGGTT	33484	GGCTTCTCCTACATGGCAGACA	44468
10843	CTCCCTCAGCTAAGCTATACAGTCAGT	22501	CACTGCAGAGAAGTTTGGGCTTT	33485	GGTCTCCAGATCTCCCTCAGCTA	44469
10844	CCTCCAAAGAGTCTGTCCACAATGA	22502	GTGAGATGACTCACCCTTTTTGCTTAG	33486	TGCCAAACTGGCCCTCCAAAG	44470
10845	GCCAGCACCATGGAAGAGGTA	22503	CTGTGGGGCAGTCAGTTTTTCT	33487	GTTGCCAGCCAGCACCAT	44471
10846	GTCATGGAATCCTCTTCTTTAGCCATA	22504	ACCTAACCCTATCTCAGCTGTTTTC	33488	GCTGATGAAGTCATGGAATCCTCTTCT	44472
10847	ACTCCACCAACCTGCCCTTTG	22505	CAGATACAGAGATCAATTCCAGCTAGT	33489	GCTTGCACCACAACTCCACCAA	44473
10848	AGAACCGTGGTCTTCAAATAGCAT	22506	GCGTCTCAGATGGGTTTTATGTAACTCT	33490	GTAGTTTTGTTAGAACCGTGGTCTTC	44474
10849	AGCAAGAGTTGAGGGGCATTG	22507	GCTCCTCTCTGAAGTTGGGTTT	33491	GGTGGTCTGAATATTAAGCAAGAGTTG	44475
10850	CCCACTAACAGCGCCAAGTT	22508	GGCCTGAGGCAGGTTTGAGTT	33492	CCCACAGTGCCCCACTAACA	44476
10851	GACTCCTTGGCTTTCACAAAGAGA	22509	TACCCAGGAAGCCGCCTGAA	33493	GGCCTTTTGACTCCTTGGCTTT	44477
10852	CAGCCGTGCATAATTTGGGTGTAG	22510	AGGCTGCCTAGGGGCTATTT	33494	CTGGCCAGCCGTGCATAAT	44478

10853	CCCTCCTGTATCTCATCCCCATTC	22511	TGGGGAGGAGGGACTCTAGTTT	33495	CCTTCTGCCCTCCTGTATCTCA	44479
10854	CACCATGCCATTTTGCTGTGA	22512	AGGTGCCAGAGACTGCAGAAAC	33496	CGACATCCTTCACCATGCCATT	44480
10855	CAGACATGAAGCTCACTGAAGGTGTAA	22513	CCACTGCACCTGGCCTATCA	33497	TGAAGCAGACATGAAGCTCACT	44481
10856	GCACAGCTAACATACTCAGTGGTGAA	22514	TCCTTGTCATGTTCCTTCCTCTTTT	33498	ACACATACGCACAGCTAACATACT	44482
10857	CAGAGCCTTTGCTCATCCCACTAC	22515	CAGATATCCATGGGAATCAACAGTTTC	33499	GTACTGACCTTGCAGAGCCTTT	44483
10858	GTGAATCCCAGCGGGTTACAAG	22516	TGTGCTTTCACTTCCTGGAAACTT	33500	GCTGGAGGTGTTGTGGTGAATC	44484
10859	GCACGTGCTGGTGCTCAGA	22517	GCCCCAGTTCAAAATCTCTTGTCA	33501	CCCTGGCTTTGGGTTGGTGAT	44485
10860	CCCTACTCCCAGCCAATTTCCAT	22518	GGAAGACCAAAAAGGGATAAAGGTTGA	33502	CCAGCCTCTTCTAGCACCCTACT	44486
10861	GTCTACACACACAGCCTCACTATG	22519	GCCTAGAGGGGTGGAAAGCAAAG	33503	CACAGAGCAGTCTACACACACA	44487
10862	GGCACATACTGTACTCTTGCATGTTG	22520	TGCGTCTGCATGGGTGGTTT	33504	GGGTGGAGGCACATACTGTACT	44488
10863	CCTAATCCACGATCTCCTCATCTGT	22521	GACAGCCCTGTGACGTAGGAAT	33505	GGCACTGCTTCCTAATCCACGAT	44489
10864	GCAATTCCCTGAAGATACCTGCTA	22522	GAGAAACAGTAGGGTCAAAGGAACAGA	33506	CCTTGACCTTTGCAATTCCCTGAA	44490
10865	CGGGTACACAATTGTCCCCAGTT	22523	GGAGCAAATTTGGTGGATTAAGGATAG	33507	TGTCCCCTCGGGTACACAAT	44491
10866	ACTTGGGCGAGGTGGCTAAG	22524	CGCTCCTGCCGCAAAACTCTA	33508	GGCTACAGGCCTGGCACTT	44492
10867	TCACTGGTATGTCTGGGAAAGTTG	22525	TGCCTCCACCTTGCTGCTA	33509	GGTTTCAAGCCTCACTGGTATGTCT	44493
10868	GCACCCAAGTGGAGAGGGTAAATC	22526	GAGCAAAGAGGTTAAGGTCACAGT	33510	GAAGGACAGTGCACCCAAGT	44494
10869	CCCAAGAAGCTCTCTGTGCTATG	22527	GGCACCTGCAGACCAGTTCTT	33511	GGGCTAAGGCTCCCTACACAT	44495
10870	GCTCCTGCACAAGCACAGATG	22528	CGATCCCCTTGGTGTGAGAGTTG	33512	CCTGTTAGCTTTGCTCCTGCACAA	44496
10871	CTGAACACCTCCTTGAGTACAGTTC	22529	CCTAGGATAGCCCACTGGATGTAG	33513	CCTTGGATCACTGAACACCTCCTT	44497
10872	CCCAGTTTGCAAGAAGCATGAAAG	22530	CCCTCCAAGGACGTCTTCCCTAA	33514	GCCTGTAAGACTTTAACCCAGTTTG	44498
10873	GGTTATTCCGTGTTCTTGATCTTGCTT	22531	CTTTGTGTCTCAGGCTCACCAT	33515	GCACCTCTGTGGTTATTCCGTGTTC	44499
10874	GCAGTGGCAGAAGGGCATTTG	22532	AGTCCCGCCTCCTCTATAGTCATC	33516	CCGGATAGGCAGTGGCAGAA	44500
10875	CTCCTCTAAGAAACTAAGGGCAACA	22533	GCCAGGGATAGCTAACAATGACA	33517	CTGCAAACTCTTTCTTCTCCTCTAAGAAAC	44501
10876	CGCTAGGTTGTGTACTGGGAACA	22534	GTGGACTCCTCTAGGTCAAGCTATTTG	33518	AGCCGTACGCTAGGTTGTGT	44502
10877	GCCCATAGCTTGTCCCATTGACTGTT	22535	ACATCATGTGGATAGAATGGGGATTAG	33519	GTATCCAGATTGCCCATAGCTTGT	44503
10878	CCCTAAGCTTTACCTTAGATGGAGAGAAAG	22536	CCAGCCTGAGGTTCCTTCAGAT	33520	GAGGATCAGGAACCCTAAGCTTTAC	44504
10879	CACACTGGAACCAAAGACCTTTATC	22537	GCGAGCTATCTTGCAGGCTAGTAA	33521	AGTTACCCACACTGGAACCAAAG	44505
10880	GGGCTGAGTAACAAGAGTGGTTCT	22538	AAGTAATGCAGGATGAGTGATGGAT	33522	AGAATCTTGTGGGCTGAGTAACAA	44506
10881	GGTTTCCCTGTAGCTTAAGAACCAT	22539	GGGGTGGCTCAGAAACCCTTT	33523	CCCTCTGTATAGGTTTCCCTGTAGCTT	44507
10882	GGTGGAGAATGACAACATGCAGAA	22540	CTCTACTGAGAGACAAGCAAAGTTTCA	33524	GCTAGGCTATGTAAGTAGGTGGAGAATG	44508
10883	ACCAGTGGGATTGATGAGACAAAA	22541	CGCCCTACTTCACTTGTTTCTTG	33525	GCCCCTGTATTCTAACCAGTGGGATTG	44509
10884	CCACAGAGCCTAGCAAGGTGTT	22542	GGGAGGCTATCCAACCAGTAAGT	33526	AGAGCACAGGGACCACAGA	44510
10885	CCCTTCTGTGCAATGGGACTTG	22543	CCCACCCCTGCCCATCTTAC	33527	CAGCTTCCCCTTCTGTGCAA	44511
10886	TGTTGGCTTCTGCAGTGTGA	22544	TGGGCTCCCACACCTGTTTC	33528	AGGACCTTTCTGGCATGTGTTG	44512
10887	CCAACTGAGAACTTGCTTCCCAAAAG	22545	GGCTGTGAAGAAGCAGTGAGGAT	33529	GCCACAACCCTACCAACTGAGA	44513
10888	CTGCCCAGGCTTTTGACTTTTG	22546	CCTCAATGAATAGTTCCCACCTCAA	33530	AAGGTTTCTGCCCAGGCTTT	44514
10889	GTGAAAGCTAGCATCTGGGGTTT	22547	CTTTAAACTGGGATCGCTACAGAAAAG	33531	GCAAAAGTACTCGCATTGTGAAAGCTA	44515
10890	CAAAATCCTGGGGATACAGACTCTT	22548	TGCCCAGGCAGAGGGAGCAA	33532	CCTCTGCCCCAGCATATATCAAA	44516
10891	GAACCCACTGGAATACGTCCTT	22549	CCCAAAGTGCCTTGTTTTCTCTGATAC	33533	CACACCCAAGAACCCACTGGAA	44517
10892	GACATGATCTGACCCTTGACACA	22550	GGCTGCAGCAGAGTGAATAAGA	33534	GCCCAGAGGCTTGACATGATCT	44518
10893	AGGGAGCTTAGAGGAAGGTCAA	22551	CACTCTGACAGTGAGGTTCTTGGTA	33535	GTCATTGCCTCAAAGGGAGCTTAG	44519
10894	CAGCCACATTCCAGAGCCAATC	22552	GCCATCTCCTGCTGGGTTACT	33536	CTAGCCTCTCTCAGCCACATTC	44520
10895	GTGTGTGTGGGCTTTATGAAGAAAA	22553	TGCATACAGCATACTCTGTTCACAT	33537	GCTCACAGTGTGTGTGGGCTTT	44521
10896	GAAGTGAAGAAGAGTGGTCAGCTA	22554	TCCTACAGGCCCCTGAACTT	33538	CCTACTTTTGGGAGGGGAAGTGA	44522
10897	ACCATGGTTTCCTTGTCAGCATAA	22555	CCGAAAAGAGGTAGGCAGAGCTAAT	33539	GCCCCTTGATTCCTCTAAACCAT	44523
10898	GAAATCAGCTTCTCTCTCACTCAGTT	22556	GTGATTGGGGCACAGAACTCTCTA	33540	GAGCCCTGAAATCAGCTTCTCTCT	44524
10899	TGTGGTAGAAGAGTTGTCTCCATTG	22557	CTGAAGGTAGGTCAGATCAAGCAA	33541	GGTGGTTCAGTGTATTGTGGTAGAAG	44525
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10901	GTGGCTTACCCACCAGCAAACT	22559	ACTGTCATGTGTCTCTCTGCTTTT	33543	AACGGGCAGCAGGTGGCTTA	44527
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10903	CAGAGGGCTAGTGTATCTCCAAGA	22561	GGGGAGTATTACAAAGACCTAAGGAGGTA	33545	TCACCCATCAGAGGGCTAGTGT	44529
10904	GTCTCTGGGCTTAATGTGCAAGT	22562	GCAATGTGTACCGGTTCTGTACT	33546	TCCCAGCTTGTCTCTGGGCTTA	44530
10905	CACAGTGGACTTGTTTGTTCTTGAGT	22563	GGGTCTGTTCATCACTCAATCATTC	33547	CAGATTCTGACTCACAGTGGACTTGTT	44531
10906	GCTCCATTTATGTTCCCATTGTGTCT	22564	GACACTGCCTAGCCTTTGTCATTG	33548	CCTGCCACCTTGATTGCTCCAT	44532
10907	GCTTATTTGGTAACGACTAGGCAGAT	22565	TGCTGGGCTGTGGATTGGTA	33549	CCACATACATTACCGTCTGCTTATTTG	44533
10908	TCTCCAGAGCCTTTGCTTCCTA	22566	AGCCAAGAGGAGTTGGGACTTG	33550	CGAACCCAAGCAGTCTTCTGTCT	44534
10909	CCACCAGCATTCAAGTGCAATGTA	22567	GCACTCACAGAAGGGCAGGTAA	33551	GCTAGGACATCCACCAGCATTCA	44535
10910	ACCTTCCAGGTCTCAGCTCTCT	22568	GCTGGTGGGGAGAGGAGTATAGAT	33552	CTGTGTTTGCCATGGACACCTT	44536
10911	CACCCTTGAGACAATGGGTTGTAG	22569	GACCCACTGGGAAGGTATGGTA	33553	GCCCTCAATCACCCTTGAGACA	44537
10912	GGACATGGAAAGATTGGGACAGGTT	22570	TCCTATGGGGTCCCATCATTTTCT	33554	GCTGGCTGAATAGGGACATGGAA	44538
10913	CCAGTGTCAGCTGGGTTTTGT	22571	CATTAAACTCAGTGCTGTCCTGTTAG	33555	TCCTTCCATGGGCCAGTGTCA	44539
10914	AGGTAAAGGGGTGGAAAAAGACATC	22572	GTGTCTGATATTCCTGCTCACTTTTG	33556	CACAAACTTAAGGTAAAGGGGTGGAA	44540
10915	CCTTCTAACACCCCTTCTCTGTGAGAT	22573	CATTCCCAACAGCATGCCTAGATA	33557	AGAGTTTCCTTCTAACACCCCTTCT	44541
10916	GAGTGAAGACCAGCAGACAGAAG	22574	GGTTAGCTTCAGTTCTTGGGAGTA	33558	GGAAATGATCCACCATGAGTGAAGA	44542
10917	GGTGGAACCAAAGAAGAAAGGGTTT	22575	GCATCTCAGCCTTTGGGTTTTG	33559	TTTGGACAGGTGGAACCAAAGA	44543

10918	GCTGCCACCTAGGAAACAAAGT	22576	CCTAGCCCTATGGCAGAGTTTTC	33560	CCAGACCAAAGCTGCCACCTA	44544
10919	GGGGACATCGTGGTCTTGCAT	22577	AACTGTTGCCCTATCCTCCATTAC	33561	GGAGAGGGAAGAGTGGGGACAT	44545
10920	GCCACTTGCAAAGAACACTTTTGT	22578	CTGGTTGTCTTCATGGGATTTCCTTA	33562	GACGTCTAAATTGTTCTTGCCACTTG	44546
10921	GCCTTTCTGCCTGGCTCTTG	22579	CAGCCCTGGAGGAGTTCTGA	33563	TCCTTTTCCTTGGTGCCTTTCT	44547
10922	GGACCATTTAGACTCACTGCCATCA	22580	CCAGGGTGCTGGAGACATTT	33564	CTATTTCACCTGGACCATTTAGACTCA	44548
10923	ACCCGTGTATTATGACTTTCAGAGTTC	22581	CCAGAGGATATCCCACAGTTCATCTCA	33565	GCCCCACCCGTGTATTATGACT	44549
10924	TGGGGAGGTAACCAAATCTTTTGA	22582	CCACATCAACACTATTATCCCCAATTC	33566	ACGAAGCCTTGGGGAGGTAAC	44550
10925	CCAAGGACTTGGGACGACTTT	22583	CGGCCTGAAATTTCTTTACAACCAT	33567	AGGGCTGGCACCCTACCAA	44551
10926	GACGTTGCTGTGTCCTTTGCTT	22584	CAGCCTGGTGGACACCTTGATT	33568	TGGCTATGACGTTGCTGTGT	44552
10927	GCCACTGGATAGCATCATTGGATCT	22585	GTCCGAGCTGTTCTGCCTTTTG	33569	GGCTATCTGCCACTGGATAGCAT	44553
10928	CCGCTTCCATAACCTGGAGATACCTA	22586	GGAGCCTCCTACATACATCATTCCAA	33570	CCTGACCTACCGCTTCCATAAC	44554
10929	CCCCTCTATTCAAGGCTATGTTGT	22587	CCAGGAGTAAAATCAGGGAATCTGAGT	33571	TGACACTTTGTTGTCCCCTCTATTC	44555
10930	CATGCCGAACAGCCTTCTTTC	22588	ACACACTGTGACATGATGGGATT	33572	GGACAAAGCTCATGCCGAACA	44556
10931	CCTGAGGGGAGGTAGGAGGAAAAA	22589	TCCACTCTGACTCAGTCTTCAGTA	33573	CAGTTGCCTGAGGGGAGGTA	44557
10932	TGAAGCCTCAGATCCTAGAGTCA	22590	AGTGACCTTTGGGGTTCTTCTTG	33574	TTTCCTCATTTCCCAGGCTTGAA	44558
10933	GGGCATCTAACAACTACCCATGCATTT	22591	CTGTCTCTGGATCCTGTGAGTCT	33575	GGGAACTTTGGGGCATCTAACAAC	44559
10934	GAGAGCCATCACATTCTGGTCTTC	22592	GCCCAGACAGCAAGAAAGGAAGT	33576	ACCTGAGCTGAGAGCCATCACA	44560
10935	GAATCCACCCATTCAAGGAAAGAAG	22593	GAACCCGCTGGCATTCTCTT	33577	TGGTAGGCAGAATCCACCCATT	44561
10936	GGCAGTAGTGCTCCAAATATGCTA	22594	ACAGGAAGGGCGGGAGTAG	33578	CGACATCCTCACGATGGCAGTAG	44562
10937	TGGCTACAGACAAGTGAGGATGA	22595	GACCTATTGAGGCTTGGGCAAAGT	33579	CCCCAGCTATTAACTACTTGGCTACAGA	44563
10938	ACTCAAAGCAAAAGAGGGACAACT	22596	ACCTACCTGTGGCCTGGAAGAT	33580	AGGTGGAGCAACTCAAAGCAA	44564
10939	ACACATGGGGTCTCAGGAACT	22597	GACAGAGCTGCAGCCAGTAAT	33581	GCCACCACTGAGGAACACAT	44565
10940	CCTCACTAGACCTTAACTGGGATGACT	22598	AGGCAGAGCCAGGGCCTAAAT	33582	GGCCAATGTCCTCACTAGACCTT	44566
10941	GCCATCCTTGCTTGTATCCTTCTCTT	22599	GAGCATGGCATGGACCTGGAAT	33583	GTGACAAAGCCATCCTTGCTTGTA	44567
10942	CTGAGTCACACAGTCCATTGAAAC	22600	AGCGCCACATGGCTGATG	33584	TCTCCACCAGCTGAGTCACA	44568
10943	TGCTGACAGCCCTCCTAAGCTA	22601	GCAAAGCTTGATGGTGGAAGAAAG	33585	GAAAAGTTGTTTAACCCTGCTGACA	44569
10944	GCCTCCATTGTCTCTCTCCTGGATAA	22602	GGGAAGCAAGGAGAGCAGCTAGTA	33586	TCCAAGCCTCCATTGTCTCTCT	44570
10945	GGCCGGAGAAAGGTTTGGCATT	22603	GGCTCAGGGGCAAAAGTTGT	33587	GAACTAACTCAAGGCCGGAGAA	44571
10946	GCCACCAATTCCTTTGGATGATCCTTT	22604	GTCACCAGGAAATGCACATCTTTT	33588	CCTCATATGCCACCAATTCCTTTG	44572
10947	TCCATTCCATGTTGGAGCAAAAC	22605	GCCCTGAAAATTCGTGCTATCAAATG	33589	CCACCTAATTTGCTCCATTCCATGT	44573
10948	TGAGTAGGAATGGCAACAGCTAAT	22606	TGACAGGGGCTCTAGAAGTAGTAAG	33590	GTGGAGTAGACAGCAAATGAGTAGGAA	44574
10949	CCCATTCAGTGGGGTTTCGTT	22607	GGCCAACTTCCTGCTAAGGATTG	33591	GTGCAGTAAGCCCCATTCAGT	44575
10950	CGGCACAGAGTGGTTAAGAATAAGA	22608	GCTAGGCATTTTGCCAGAGGCTTT	33592	CACCTATATAATCGGCACAGAGTGGTT	44576
10951	GCATCAGAACTCATAGACACTTGATTG	22609	AGAACCCGAGAGGCCTATCCAA	33593	CATGTTTCCAAACACATGCATCAGA	44577
10952	GTGGCATGGAAGAATCATGTGCTTAG	22610	GCTTTCCTGCTCTGGTGCTGAA	33594	GGGAGTGGCATGGAAGAATCA	44578
10953	ACCTTCTCATAGCCCAGTCTGT	22611	AACTGATCTGGCCTGCTAAAACT	33595	CAGGCTTCCCAACCTTCTCATAG	44579
10954	GCTCTTAAATAGCTCACACGCTAGT	22612	TGTGCTTAACCTCCTAGCCTCAT	33596	ACCCGTGGCATCTGCTCTTA	44580
10955	CGTTTGTGGAATGGCTCAAGATT	22613	GGTGATATTAGTGAAGCCCTCTGTTG	33597	CAGCAAAGAGACGTTTGTGGAATG	44581
10956	TCCCCACCTTGAGTGCTCAGT	22614	CCAAAGTAGCAAAAGGTGTCTGCATTC	33598	GCCCTTATCTCAATGATCCCCACCTT	44582
10957	GCTCTATACATTGTGGTGCCTGCAA	22615	GGATGATGCTGGCTAATGGGAGAAA	33599	ACCCCAGTGTTGCTCTATACATTG	44583
10958	GAGTATGGGATCAGGTCCATGTCA	22616	ACCTTGTCTCCCACTCCAGAT	33600	CTGGGTACCGAGTATGGGATCA	44584
10959	GGCAAACCCTACATTCTGTGTAAAAAC	22617	CACATGCATAGCTTAGCCAGTGA	33601	CCAGAAAAGGCAAACCCTACATTC	44585
10960	GCTGGTGACTGGTTCATCCTAGA	22618	CGAGAGATTGGCAGTCCCAGTT	33602	AGCAGTGAGGCCAGAAACTTAG	44586
10961	GCGTGCTAGCCAAACTGTTG	22619	CCCCATTCAAAGGGCTCTCAGT	33603	TGGATTCCAAACTTTGCGTGCTA	44587
10962	GGTTGGGAAGACTGTGGGACAA	22620	AGCCTGAGTAACCAGGGACTCT	33604	TGGTCTGTGGGTTGGGAAGACT	44588
10963	CCAAGTGGTGGCAATTACAGGACTA	22621	CACAGCCTGCTGCTAATTGTTC	33605	GGCTTCCAAGTGGTGGCAAT	44589
10964	TGGTCACTCAGGGTGGGTAAG	22622	GCTGCAGGAGTGAAACTAGGAACA	33606	AGGCAGGGGTGGTCACTCA	44590
10965	ACACACTCCCCACCCCAAAC	22623	TCTTCCAGAATTCCCTTCAAAGCAT	33607	TCGCCACTGCCACACACT	44591
10966	GCTTCCCACTCTGCCCATCAAT	22624	GTGAAAGCAGGTGGTCATACTCT	33608	AGTAGAAGATCAGCTTCCCACTCT	44592
10967	CACCACCGTAGATCCAAAAGTGT	22625	CTGCAGGTAGCAGAATTAACTGACA	33609	GCCTATAGTGAACACCACCGTAGA	44593
10968	GGTGCTATTGCTCCCTGTTCA	22626	CTGTGCTCAGATCTGCATTGGAT	33610	CCATGTCCATTGCTAGGTGCTATTG	44594
10969	GAACCTCTCCTTTTACGGTGCAA	22627	CACTCATTTGACTTCCCGCTGTT	33611	CGACGGAACAGAACCTCTCCTT	44595
10970	CTCTCTGGCCAGGTGACATTGTAG	22628	ACAGGGATCCACTGGTATGATAAAGA	33612	GCTCACTGCAGCTTGCTCTCT	44596
10971	GCTCAAAACCGGCATTGATGGTA	22629	CTGAGCAGGCTATTCCAGTCTGT	33613	GCCAATAGCTAATGCTACAAGTAGCTCAAA	44597
10972	TCAACCAACCAGCGGTCAGA	22630	TCGCCAAGGACCTAGGAACA	33614	TTCTCCATAGACGTGACCATCAAC	44598
10973	GAGTTTCCGTCACAACGTTCCTT	22631	CGGTGGTGCGCAAGTAGT	33615	GCAGCTGGAGTTTCCGTCACA	44599
10974	AGGGAGGGAGTTATTGTGCTACT	22632	GAGTTGTGCAGATGGGTTGAAAA	33616	CCTCAGCCAAGGGAGGGAGTTAT	44600
10975	GAAGCTGGGTTGAAGTGCTTTG	22633	CCTGGCATCACAGTGGCTACAA	33617	GCAAAATCTTAGAGAAGCTGGGTTGA	44601
10976	GGAGAGTTATCAATGAGCCTTCTGT	22634	AGTGCCCCACCGGTGTTT	33618	GGTGTCCATGTACTAGAAGGAGAGTTATC	44602
10977	TGGCAAAGTGAGAACCTGCAT	22635	CCTTATAGTGGGTTCTGGGACTTAGTA	33619	GCTGTTTTTGAAGCTGGCAAAGT	44603
10978	CGTGTGTCCTGGAAGAGATGGAA	22636	GGGTAGCCCTGGGAATGCTT	33620	TGCTCACGTGTGTCCTGGAA	44604
10979	GAGAGACAGCAATTCTGGGGAACAT	22637	GAACCTCGCCTCATTGTGATTTC	33621	GCCCCATCTGAGAGACAGCAATTC	44605
10980	AGGGTTGGACTAGGGGAAGTGA	22638	AGCACCGGCAGGCGACTTT	33622	TAGGGCGGCAGGGTTGGACTA	44606
10981	GGTTTCTCCCTGAAATCGTGTGTGA	22639	CTCCAGAATTCTACGGAAACTGGAAGT	33623	CCAGGGTGGTTTCTCCCTGAAA	44607
10982	CTGCACCTTGGCAACACTTTGA	22640	GCTCTGTACTGGCCACTTTGTTC	33624	ATCACCTCTCCCTGCACCTT	44608

10983	GGACCCCAGGATTGAGGAGACAA	22641	CCAGCCTAGACCTAAACCCTTGA	33625	CAACTAAGATGGACCCCAGGATTG	44609
10984	TGCACATGCGCTCTTCTCA	22642	GTGATGCCAGACAGAGCTGACA	33626	CCTGTGCCTGCCTTCCGAAT	44610

FIG. 37

Description	MAPPED_READS	TOTAL_READS	%Mapped Reads
gMother	3,633,900	3,647,446	100%
gMother	3,567,685	3,578,549	100%
gMother	3,733,706	3,747,692	100%
gChild	3,725,554	3,737,742	100%
gChild	3,816,989	3,829,450	100%
gChild	3,524,956	3,536,569	100%
1 mother	3,467,493	7,564,869	46%
1 mother	3,868,210	7,107,028	54%
1 mother	4,918,140	7,613,240	65%
1 child	361,390	6,507,434	6%
1 child	1,885,864	6,496,348	29%
1 child	2,789,647	6,259,288	45%

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FIG. 38

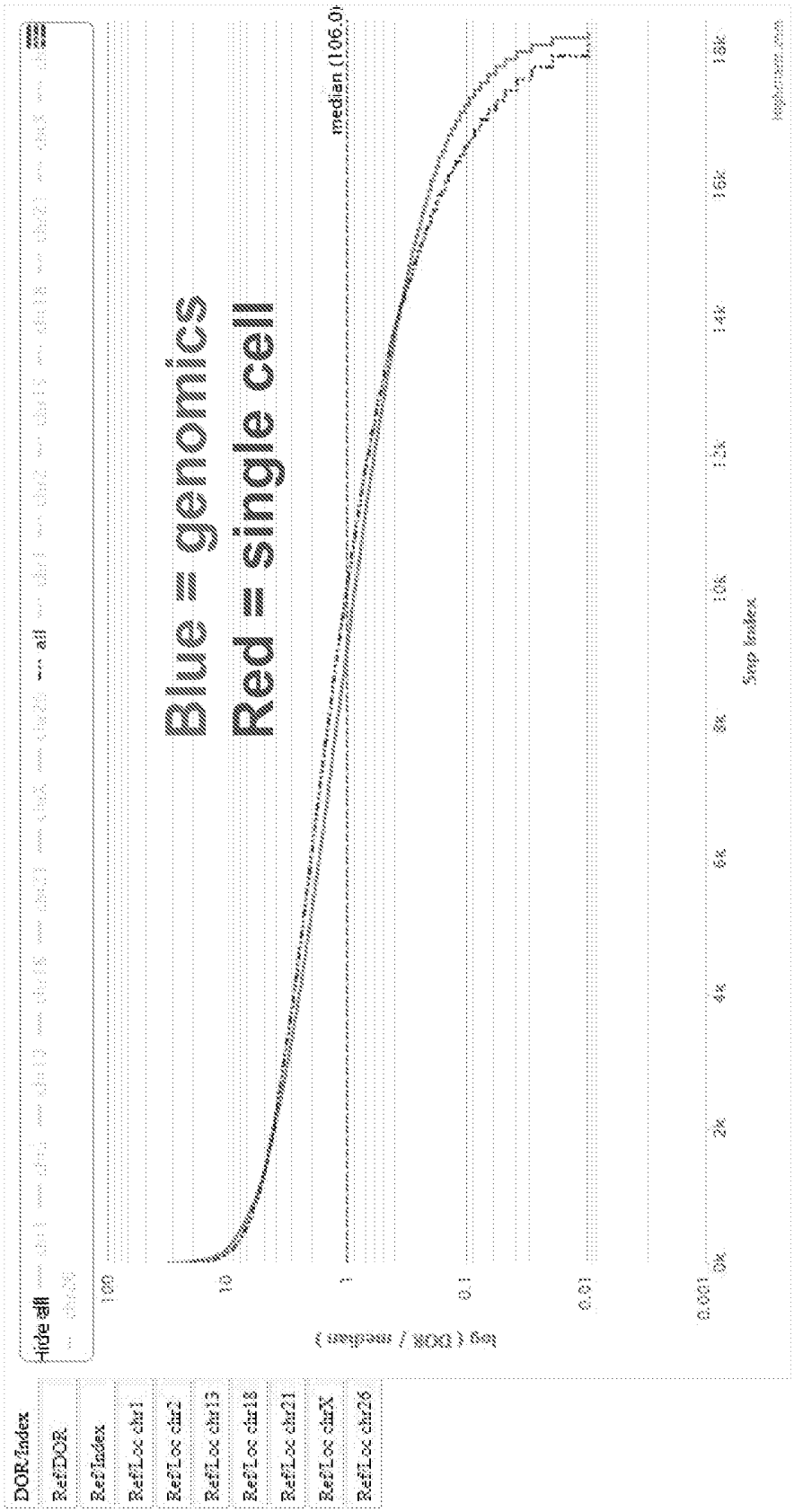
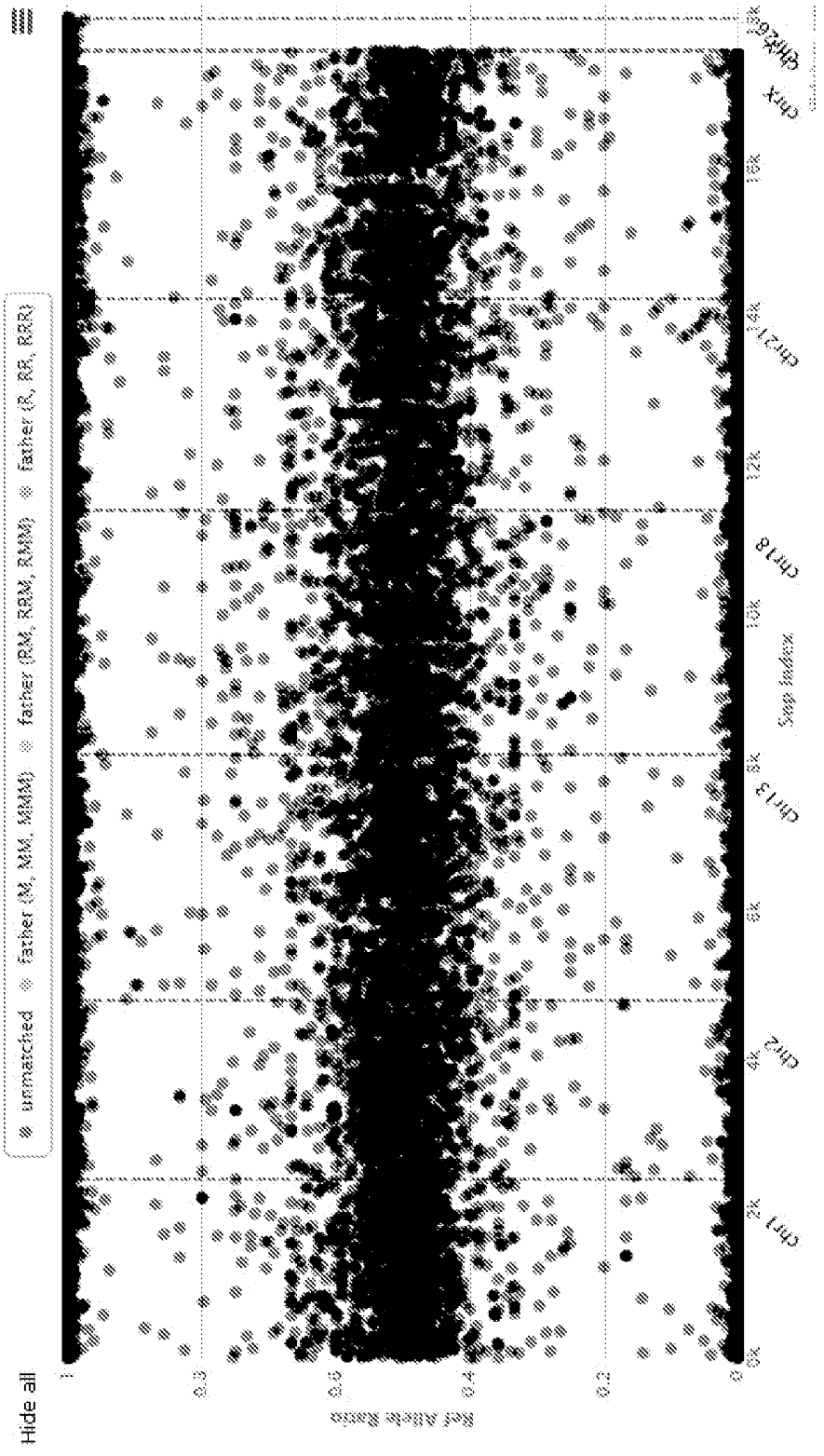

DOR.index
RefDOR
RefIndex
RefLoc chr1
RefLoc chr2
RefLoc chr13
RefLoc chr18
RefLoc chr21
RefLoc chrX
RefLoc chr26
Hide all
Blue = genomics
Red = single cell
median (106.0)
log (DOR / median)
Snap Index
0.0001
0.01
0.1
1
10
100
0%
2%
4%
6%
8%
10%
12%
14%
16%
18%

FIG. 39

Description	MAPPED_READS	TOTAL_READS	%Mapped Reads
case 1 blastoceol	68,123	8,470,872	1%
case 1 cell 1	4,340,613	8,248,598	53%
case 1 cell 2	5,480,580	8,230,870	67%
case 1 cell 3	4,664,577	7,846,040	59%
case 2 blastoceol	45,794	6,302,957	1%
case 2 cell 1	6,988,854	8,622,495	81%
case 2 cell 2	7,083,600	8,843,495	80%
case 2 cell 3	5,811,364	8,256,310	70%

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FIG. 40


Hide all
unmatched
father (M, MM, MMM)
father (RM, RRM, RMM)
father (R, RR, RRR)
Ref Allele Ratio
1
0.8
0.6
0.4
0.2
0
chr1
chr2
chr3
chr18
chr21
chrX
chrX.6
Snp Index

TITLE OF THE INVENTION

HIGHLY MULTIPLEX PCR METHODS AND COMPOSITIONS

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This work was supported by Grant No. 5R44HD60423-3, awarded by the National Institutes of Health. The U.S. government may have rights in any patent issuing on this application.

FIELD OF THE INVENTION

The present invention generally relates to methods and compositions for simultaneously amplifying multiple nucleic acid regions of interest in one reaction volume.

BACKGROUND OF THE INVENTION

To increase assay throughput and allow more efficient use of nucleic acid samples, simultaneous amplification of many target nucleic acids in a sample of interest can be carried out by combining many oligonucleotide primers with the sample and then subjecting the sample to polymerase chain reaction (PCR) conditions in a process known in the art as multiplex PCR. Use of multiplex PCR can significantly simplify experimental procedures and shorten the time required for nucleic acid analysis and detection. However, when multiple pairs are added to the same PCR reaction, non-target amplification products may be generated, such as amplified primer dimers. The risk of generating such products increases as the number of primers increases. These non-target amplicons significantly limit the use of the amplified products for further analysis and/or assays. Thus, improved methods are needed to reduce the formation of non-target amplicons during multiplex PCR.

Improved multiplex PCR methods would be useful for a variety of application, such as Non-Invasive Prenatal Genetic Diagnosis (NPD). In particular, current methods of prenatal diagnosis can alert physicians and parents to abnormalities in growing fetuses. Without prenatal diagnosis, one in 50 babies is born with serious physical or mental handicap, and as many as one in 30 will have some form of congenital malformation. Unfortunately, standard methods have either poor accuracy, or involve an invasive procedure that carries a risk of miscarriage. Methods based on maternal blood hormone levels or ultrasound measurements are

non-invasive, however, they also have low accuracies. Methods such as amniocentesis, chorion villus biopsy and fetal blood sampling have high accuracy, but are invasive and carry significant risks. Amniocentesis was performed in approximately 3% of all pregnancies in the US, though its frequency of use has been decreasing over the past decade and a half.

Normal humans have two sets of 23 chromosomes in every healthy, diploid cell, with one copy coming from each parent. Aneuploidy, a condition in a nuclear cell where the cell contains too many and/or too few chromosomes is believed to be responsible for a large percentage of failed implantations, miscarriages, and genetic diseases. Detection of chromosomal abnormalities can identify individuals or embryos with conditions such as Down syndrome, Klinefelter's syndrome, and Turner syndrome, among others, in addition to increasing the chances of a successful pregnancy. Testing for chromosomal abnormalities is especially important as the mother's age: between the ages of 35 and 40 it is estimated that at least 40% of the embryos are abnormal, and above the age of 40, more than half of the embryos are abnormal.

It has recently been discovered that cell-free fetal DNA and intact fetal cells can enter maternal blood circulation. Consequently, analysis of this genetic material can allow early NPD. Improved methods are desired to improve the sensitivity and specificity and reduce the time and cost required for NPD.

SUMMARY OF THE INVENTION

In one aspect, the invention features methods of amplifying target loci in a nucleic acid sample. In some embodiments, the method involves (i) contacting the nucleic acid sample with a library of test primers (such as non-immobilized primers) that simultaneously hybridize to at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci to produce a reaction mixture; and (ii) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products that include target amplicons. In some embodiments, the method also includes determining the presence or absence of at least one target amplicon (such as at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target amplicons). In some embodiments, the method also includes determining the sequence of at least one target amplicon (such as at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target amplicons). In some embodiments, the method involves multiplex PCR and sequencing

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(such as high throughput sequencing). In some embodiments, the method includes selecting the test primers from a library of candidate primers by removing one or more of the candidate primers based at least in part on the likelihood of dimer formation between candidate primers (such as ΔG values, undesirability scores, or interaction scores) prior to contacting the nucleic acid sample with the library of test primers.

In various embodiments of any of the aspects of the invention, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified. In some embodiments, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold. In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the amplified products are target amplicons. In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target loci are amplified. In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold. In various embodiments, less than 60, 50, 40, 30, 20, 10, 5, 4, 3, 2, 1, 0.5, 0.25, 0.1, or 0.05% of the amplified products are primer dimers. In some embodiments, the library of test primers includes at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 test primer pairs, wherein each pair of primers includes a forward test primer and a reverse test primer that hybridize to the same target locus. In some embodiments, the library of test primers includes at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 individual test primers that hybridize to different target loci, wherein the individual primers are not part of primer pairs.

In various embodiments of any of the aspects of the invention, the concentration of each test primer is less than 100, 75, 50, 25, 10, 5, 2, 1 , 0.5, 0.1, or 0.05 nM. In various embodiments, the guanine-cytosine (GC) content of the test primers is between 30 to 80%, such as between 20 to 70%, 40 to 70%, or 50 to 60%, inclusive. In some embodiments, the range of GC content (*e.g.,* the maximum GC content minus minimum GC content, such as 80% - 60% = a range of 20%) of the test primers is less than 30, 20, 10, or 5%. In some embodiments, there are at least 2 (such as 3, 4, or 5) guanines or cytosines in the last 5 bases at

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the 3' end of the primers. In some embodiments, there are at least 1 (such as 2 or 3) guanines or cytosines in the last 3 bases at the 3' end of the primers. In some embodiments, the melting temperature (T_m) of the test primers is between 40 to 80 °C, such as 50 to 70 °C, 55 to 65 °C, 54 to 60.5 °C, or 57 to 60.5 °C, inclusive. In some embodiments, the range of melting temperatures of the test primers is less than 20, 15, 10, 5, 3, or 1 °C. In some embodiments, the length of the test primers is between 15 to 100 nucleotides, such as between 15 to 75 nucleotides, 15 to 40 nucleotides, 17 to 35 nucleotides, 18 to 30 nucleotides, or 20 to 65 nucleotides, inclusive. In some embodiments, the test primers include a tag that is not target specific, such as a tag that forms an internal loop structure. In some embodiments, the tag is between two DNA binding regions. In various embodiments, the test primers include a 5' region that is specific for a target locus, an internal region that is not specific for the target locus and forms a loop structure, and a 3' region that is specific for the target locus. In various embodiments, the length of the 3' region is at least 7 nucleotides. In some embodiments, the length of the 3' region is between 7 and 20 nucleotides, such as between 7 to 15 nucleotides, or 7 to 10 nucleotides, inclusive. In various embodiments, the test primers include a 5' region that is not specific for a target locus (such as a tag or a universal primer binding site) followed by a region that is specific for a target locus, an internal region that is not specific for the target locus and forms a loop structure, and a 3' region that is specific for the target locus. In some embodiments, the range of the length of the test primers is less than 50, 40, 30, 20, 10, or 5 nucleotides. In some embodiments, the length of the target amplicons is between 50 and 100 nucleotides, such as between 60 and 80 nucleotides, or 60 to 75 nucleotides, inclusive. In some embodiments, the range of the length of the target amplicons is less than 50, 25, 15, 10, or 5 nucleotides.

In various embodiments of any of the aspects of the invention, the primer extension reaction conditions are polymerase chain reaction conditions (PCR). In various embodiments, the length of the annealing step is greater than 3, 5, 8, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, or 180 minutes. In various embodiments, the length of the extension step is greater than 3, 5, 8, 10, or 15 minutes.

In various embodiments of any of the aspects of the invention, the test primers are used to simultaneously amplify at least 300 different target loci in a sample that includes maternal DNA from the pregnant mother of a fetus and fetal DNA to determine the presence or absence of a fetal chromosome abnormality. In various embodiments, the method includes ligating a

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universal primer binding site to the DNA molecules in the sample; amplifying the ligated DNA molecules using at least 300 specific primers and a universal primer to produce a first set of amplified products; and amplifying the first set of amplified products using at least 300 pairs of specific primers to produce a second set of amplified products.

In various embodiments of any of the aspects of the invention, the test primers are used to simultaneously amplify *e.g.*at least 100 (*e.g.*, at least 300 or 1,000) different target loci in sample includes DNA from an alleged father of a fetus and to simultaneously amplify the target loci in a sample that includes maternal DNA from the pregnant mother of the fetus and fetal DNA to establish whether the alleged father is the biological father of the fetus.

In various embodiments of any of the aspects of the invention, the test primers are used to simultaneously amplify *e.g.*at least 100 (*e.g.*, at least 300 or 1,000) different target loci in one cell or multiple cells from an embryo to determine the presence or absence of a chromosome abnormality. In various embodiments, cells from a set of two or more embryos are analyzed, and one embryo is selected for *in vitro* fertilization.

In various embodiments of any of the aspects of the invention, the test primers are used to simultaneously amplify *e.g.*at least 100 (*e.g.*, at least 300 or 1,000) different target loci in a forensic nucleic acid sample. In various embodiments, the length of the annealing step is greater than 3, 5, 8, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, or 180 minutes

In various embodiments of any of the aspects of the invention, the method involves using the test primers to simultaneously amplify *e.g.*at least 100 (*e.g.*, at least 300 or 1,000) different target loci in a control nucleic acid sample to produce a first set of target amplicons and to simultaneously amplify the target loci in a test nucleic acid sample to produce a second set of target amplicons; and comparing the first and second sets of target amplicons to determine whether a target locus is present in one sample but absent in the other, or whether a target locus is present at different levels in the control sample and the test sample. In various embodiments, the test sample is from an individual suspected of having a disease or phenotype of interest (such as cancer), or an increased risk for a disease or phenotype of interest; and wherein one or more of the target loci include a sequence (*e.g.*, a polymorphism or other mutation) associated with an increased risk for the disease or phenotype of interest, or associated with the disease or phenotype of interest. In various embodiments, the method involves using the test primers to simultaneously amplify *e.g.*at least 100 (*e.g.*, at least 300 or 1,000) different target loci in a control sample that includes RNA to produce a first set of target

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amplicons and to simultaneously amplify the target loci in a test sample that includes RNA to produce a second set of target amplicons; and comparing the first and second sets of target amplicons to determine the presence or absence of a difference in the RNA expression levels between the control sample and the test sample. In various embodiments, the RNA is mRNA. In various embodiments, the test sample is from an individual suspected of having a disease or phenotype of interest (such as cancer) or an increased risk for the disease or phenotype of interest (such as cancer); and wherein one or more of the target loci includes a sequence (*e.g.*, a polymorphism or other mutation) associated with an increased risk for the disease or phenotype of interest or associated with the disease or phenotype of interest. In some embodiments, the test sample is from an individual diagnosed with a disease or phenotype of interest (such as cancer); and wherein a difference in the RNA expression level between the control sample and test sample indicates a target locus includes a sequence (*e.g.*, a polymorphism or other mutation) associated with an increased or decreased risk for the disease or phenotype of interest.

In some embodiments of any of the aspects of the invention, the test primers are selected from a library of candidate primers based on one or more parameters, such as the selection of primers using any of the methods of the invention. In some embodiments, the test primers are selected from a library of candidate primers based at least in part on the ability of the candidate primers to form primer dimers.

In one aspect, the invention features methods of selecting test primers from a library of candidate primers. In various embodiments, the selection involves (i) calculating on a computer a score (such as an undesirability score) for most or all of the possible combinations of two candidate primers from the library, wherein each score (such as an undesirability score) is based at least in part on the likelihood of dimer formation between the two candidate primers; (ii) removing the candidate primer with the highest score (such as an undesirability score) from the library of candidate primers; and (iii) if the candidate primer removed in step (ii) is a member of a primer pair, then removing the other member of the primer pair from the library of candidate primers; and (iv) optionally repeating steps (ii) and (iii), thereby selecting a library of test primers. In some embodiments, the selection method is performed until the score (such as the undesirability score) for the candidate primer combinations remaining in the library are all equal to or below a minimum threshold. In some embodiments, the selection method is performed until the number of candidate primers remaining in the library is reduced

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to a desired number. In various embodiments, a score (such as an undesirability score) is calculated for at least 80, 90, 95, 98, 99, or 99.5% of the possible combinations of candidate primers in the library. In various embodiments, the candidate primers remaining in the library are capable of simultaneously amplifying at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci. In various embodiments, the method also includes (v) contacting a nucleic acid sample that includes target loci with the candidate primers remaining in the library to produce a reaction mixture; and (vi) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products that includes target amplicons.

In one aspect, the invention features methods of selecting test primers from a library of candidate primers. In various embodiments, the selection of test primers are selected from a library of candidate primers involves (i) calculating on a computer a score (such as an undesirability score) for most or all of the possible combinations of two candidate primers from the library, wherein each score (such as an undesirability score) is based at least in part on the likelihood of dimer formation between the two candidate primers; (ii) removing from the library of candidate primers the candidate primer that is part of the greatest number of combinations of two candidate primers with a score (such as an undesirability score) above a first minimum threshold; (iii) if the candidate primer removed in step (ii) is a member of a primer pair, then removing the other member of the primer pair from the library of candidate primers; and (iv) optionally repeating steps (ii) and (iii), thereby selecting a library of test primers. In some embodiments, the selection method is performed until the score (such as the undesirability score) for the candidate primer combinations remaining in the library are all equal to or below the first minimum threshold. In some embodiments, the selection method is performed until the number of candidate primers remaining in the library is reduced to a desired number. In various embodiments, a score (such as an undesirability score) is calculated for at least 80, 90, 95, 98, 99, or 99.5% of the possible combinations of candidate primers in the library. In various embodiments, the candidate primers remaining in the library are capable of simultaneously amplifying at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci. In various embodiments, the method also includes (v) contacting a nucleic acid sample that includes target loci with the candidate primers remaining

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in the library to produce a reaction mixture; and (vi) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products that includes target amplicons.

In various embodiments of any of the aspects of the invention, the selection method involves further reducing the number of candidate primers remaining in the library by decreasing the first minimum threshold used in step (ii) to a lower second minimum threshold and optionally repeating steps (ii) and (iii). In some embodiments, the selection method involves increasing the first minimum threshold used in step (ii) to a higher second minimum threshold and optionally repeating steps (ii) and (iii). In some embodiments, the selection method is performed until the score (such as the undesirability score) for the candidate primer combinations remaining in the library are all equal to or below the second minimum threshold, or until the number of candidate primers remaining in the library is reduced to a desired number.

In various embodiments of any of the aspects of the invention, the method involves, prior to step (i), identifying or selecting primers that hybridize to the target loci. In some embodiments, multiple primers (or primer pairs) hybridize to the same target locus, and the selection method is used to select a one primer (or one primer pair) for this target locus based on one or more parameters. In various embodiments, the method involves, prior to step (ii), removing a primer pair from the library that produces a target amplicon that overlaps with a target amplicon produced by another primer pair. In various embodiments, a candidate primer is selected out of a group of two or more candidate primers with equal scores (such as undesirability scores) for removal from the library of candidate primers based on one or more other parameters. In some embodiments, the candidate primers remaining in the library are used as a library of test primers in any of the methods of the invention. In some embodiments, the resulting library of test primers includes any of the primer libraries of the invention.

In various embodiments of any of the aspects of the invention, the selection method selects candidate primers and divides them into different pools (*e.g.*, 2, 3, 4, 5, 6, or more different pools). Each pool can be used to simultaneously amplify a large number of target loci (or a subset of target loci) in a single reaction volume. In some embodiments, a graph coloring algorithm is used to divide candidate primers into different pools. If desired, this method can be used to minimize the number of different pools needed to amplify most or all of the target loci.

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In some embodiments, most or all of the target loci (such as at least 70, 80, 90, 92, 94, 96, 98, 99, or 100% of the target loci) are amplified by at least 2, 3, 4, 5, 6, or more different pools. In some embodiments, most or all of the bases in the target loci (such as at least 70, 80, 90, 92, 94, 96, 98, 99, or 100% of the bases in the target loci) are amplified by at least 2,3, 4, 5, 6, or more different pools. In some embodiments, most or all of the bases in the target loci (such as at least 70, 80, 90, 92, 94, 96, 98, 99, or 100% of the bases in the target loci) are amplified by at least 2, 3, 4, 5, 6, or more different primers or primer pairs in different pools. For example, a particular base in a target locus may be amplified by at least 2, 3, 4, 5, 6, or more different primers or primer pairs; wherein each different primer or primer pair is in a different pool. Using different primers or primer pairs to amplify each base allows multiple independent measurements of the base to be made, thereby increasing the accuracy of the method. Dividing the different primers or primer pairs that amplify the same base into different pools prevents interference due to overlapping amplicons being formed by different primers or primer pairs.

In one aspect, the invention features methods of selecting test primers from a library of candidate primers to form 2 or more different primer pools. In various embodiments, the selection involves (i) calculating on a computer a score (such as an undesirability score) for most or all of the possible combinations of two candidate primers from the library, wherein each score (such as an undesirability score) is based at least in part on the likelihood of dimer formation between the two candidate primers; (ii) removing the candidate primer with the highest score (such as an undesirability score) from the library of candidate primers; and (iii) if the candidate primer removed in step (ii) is a member of a primer pair, then removing the other member of the primer pair from the library of candidate primers; and (iv) optionally repeating steps (ii) and (iii), thereby selecting a first pool. In some embodiments, the selection method is performed until the score (such as the undesirability score) for the candidate primer combinations remaining in the library are all equal to or below a minimum threshold for the first pool. In some embodiments, the selection method is performed until the number of candidate primers remaining in the library is reduced to a desired number for the first pool. In some embodiments, after the first pool is selected those primers are removed from further consideration and steps of the method (such as steps (ii) and (iii)) are repeated with the remaining primers to select a second pool. If desired, this method may be repeated to select the desired number of primer pools. In various embodiments, a score (such as an

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undesirability score) is calculated for at least 80, 90, 95, 98, 99, or 99.5% of the possible combinations of candidate primers in the library. In some embodiments, the score is based at least in part on the current coverage of the bases in the target locus (such as the number of other primer pools that have a primer or primer pair that amplifies a particular base in the target locus). In various embodiments, one or more of the pools are each capable of simultaneously amplifying at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci. In various embodiments, the method also includes separately contacting a nucleic acid sample that includes target loci with two or more of the pools to produce separate reaction mixtures; and (vi) subjecting the reaction mixtures to primer extension reaction conditions to produce amplified products that includes target amplicons.

In one aspect, the invention features methods of selecting test primers from a library of candidate primers to form 2 or more different primer pools. In various embodiments, the selection of test primers are selected from a library of candidate primers involves (i) calculating on a computer a score (such as an undesirability score) for most or all of the possible combinations of two candidate primers from the library, wherein each score (such as an undesirability score) is based at least in part on the likelihood of dimer formation between the two candidate primers; (ii) removing from the library of candidate primers the candidate primer that is part of the greatest number of combinations of two candidate primers with a score (such as an undesirability score) above a first minimum threshold; (iii) if the candidate primer removed in step (ii) is a member of a primer pair, then removing the other member of the primer pair from the library of candidate primers; and (iv) optionally repeating steps (ii) and (iii), thereby selecting a first pool. In some embodiments, the selection method is performed until the score (such as the undesirability score) for the candidate primer combinations remaining in the library are all equal to or below the first minimum threshold for the first pool. In some embodiments, the selection method is performed until the number of candidate primers remaining in the library is reduced to a desired number for the first pool. In various embodiments, the selection method involves further reducing the number of candidate primers remaining in the library by decreasing the first minimum threshold used in step (ii) to a lower second minimum threshold and optionally repeating steps (ii) and (iii). In some embodiments, the selection method involves increasing the first minimum threshold used in step (ii) to a higher second minimum threshold and optionally repeating steps (ii) and (iii). In

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some embodiments, the selection method is performed until the score (such as the undesirability score) for the candidate primer combinations remaining in the library are all equal to or below the second minimum threshold, or until the number of candidate primers remaining in the library is reduced to a desired number for the first pool. In some embodiments, after the first pool is selected those primers are removed from further consideration and steps of the method (such as steps (ii) and (iii)) are repeated with the remaining primers to select a second pool. If desired, this method may be repeated to select the desired number of primer pools. In various embodiments, a score (such as an undesirability score) is calculated for at least 80, 90, 95, 98, 99, or 99.5% of the possible combinations of candidate primers in the library. In some embodiments, the score is based at least in part on the current coverage of the bases in the target locus (such as the number of other primer pools that have a primer or primer pair that amplifies a particular base in the target locus). In various embodiments, one or more of the pools are each capable of simultaneously amplifying at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci. In various embodiments, the method also includes separately contacting a nucleic acid sample that includes target loci with two or more of the pools to produce separate reaction mixtures; and (vi) subjecting the reaction mixtures to primer extension reaction conditions to produce amplified products that includes target amplicons.

In some embodiments, at least 70, 80, 85, 90, 95, or 100% of the nucleotides in a region of interest (such as an exon) are included in at least 1, 2, 3, or 4 different amplicons (*i.e.*, amplicons with non-identical sequences that are formed by different primers or primer pairs). In some embodiments, at least 70, 80, 85, 90, 95, or 100% of the nucleotides in at least 70, 80, 85, 90, 95, or 100% of the regions of interest are amplified by at least 1, 2, 3, or 4 different amplicons. In some embodiments in which 2-fold coverage is desired, the primers are divided into at least two different pools such the amplicons in each pool do not overlap with each other (which would cause interference during amplification).

In various embodiments of any of the aspects of the invention, the score (such as the undesirability score) are based at least in part on one or more parameters selected from the group consisting of heterozygosity rate of the target locus, disease prevalence associated with a sequence (*e.g.*, a polymorphism) at the target locus, disease penetrance associated with a sequence (*e.g.*, a polymorphism) at the target locus, specificity of the candidate primer for the

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target locus, size of the candidate primer, melting temperature of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, GC content of the 3' end of the candidate primer, homopolymer length in the candidate primer, amplification efficiency of the target amplicon, and size of the target amplicon.

In various embodiments of any of the aspects of the invention, the score (such as the undesirability score) are based at least in part on one or more parameters selected from the group consisting of heterozygosity rate of the target locus, specificity of the candidate primer for the target locus; size of the candidate primer, melting temperature of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, GC content of the 3' end of the candidate primer, homopolymer length in the candidate primer, amplification efficiency of the target amplicon, and size of the target amplicon; and the test primers are used to simultaneously amplify *e.g.*at least 100 (*e.g.*, at least 300 or 1,000) different target loci in a sample that includes maternal DNA from the pregnant mother of a fetus and fetal DNA to determine the presence or absence of a fetal chromosome abnormality. In various embodiments, the method includes ligating a universal primer binding site to the DNA molecules in the sample; amplifying the ligated DNA molecules using *e.g.*at least 100 (*e.g.*, at least 300 or 1,000) specific primers and a universal primer to produce a first set of amplified products; and amplifying the first set of amplified products using *e.g.*at least 100 (*e.g.*, at least 300 or 1,000) pairs of specific primers to produce a second set of amplified products. In various embodiments, at least 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different primer pairs are used. In various embodiments, at least 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified. In some embodiments, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold. In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.5, or 100% of the target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold.

In various embodiments of any of the aspects of the invention, the score (such as the undesirability score) are based at least in part on one or more parameters selected from the

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group consisting of heterozygosity rate of the target locus, specificity of the candidate primer for the target locus; size of the candidate primer, melting temperature of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, GC content of the 3' end of the candidate primer, homopolymer length in the candidate primer, amplification efficiency of the target amplicon, and size of the target amplicon; and the test primers are used to simultaneously amplify *e.g.*at least 100 (*e.g.*, at least 300 or 1,000) different target loci in sample includes DNA from an alleged father of a fetus and to simultaneously amplify the target loci in a sample that includes maternal DNA from the pregnant mother of a fetus and fetal DNA to establish whether the alleged father is the biological father of the fetus. In various embodiments, at least 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified. In some embodiments, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold. In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.5, or 100% of the target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold.

In various embodiments of any of the aspects of the invention, the score (such as the undesirability score) are based at least in part on one or more parameters selected from the group consisting of heterozygosity rate of the target locus, specificity of the candidate primer for the target locus; size of the candidate primer, melting temperature of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, GC content of the 3' end of the candidate primer, homopolymer length in the candidate primer, amplification efficiency of the target amplicon, and size of the target amplicon; and the test primers are used to simultaneously amplify *e.g.*at least 100 (*e.g.*, at least 300 or 1,000) different target loci in one cell or multiple cells from an embryo to determine the presence or absence of a chromosome abnormality. In various embodiments, cells from a set of two or more embryos are analyzed, and one embryo is selected for *in vitro* fertilization. In various embodiments, at least 300, 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified. In some embodiments, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different

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target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold. In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.5, or 100% of the target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold.

In various embodiments of any of the aspects of the invention, the scores (such as the undesirability scores) are based at least in part on one or more parameters selected from the group consisting of heterozygosity rate of the target locus, specificity of the candidate primer for the target locus; size of the candidate primer, melting temperature of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, GC content of the 3' end of the candidate primer, homopolymer length in the candidate primer, amplification efficiency of the target amplicon, and size of the target amplicon; and the test primers are used to simultaneously amplify *e.g.*at least 100 (*e.g.*, at least 300 or 1,000) different target loci in a forensic nucleic acid sample. In various embodiments, the length of the annealing step is greater than 3, 5, 8, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, or 180 minutes. In various embodiments, at least 300, 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified. In some embodiments, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold. In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.5, or 100% of the target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold.

In various embodiments of any of the aspects of the invention, the scores (such as the undesirability scores) are based at least in part on one or more parameters selected from the group consisting of heterozygosity rate of the target locus, disease prevalence associated with a sequence (*e.g.*, a polymorphism) at the target locus, disease penetrance associated with a sequence (*e.g.*, a polymorphism) at the target locus, specificity of the candidate primer for the target locus, size of the candidate primer, melting temperature of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, GC content of the 3' end of the candidate primer, homopolymer length in the candidate primer, amplification efficiency of the target amplicon, and size of the target amplicon; and the method involves using the test primers to simultaneously amplify *e.g.*at least 100 (*e.g.*, at least 300 or 1,000) different target loci in a control nucleic acid sample to produce a first set of target amplicons

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and to simultaneously amplify the target loci in a test nucleic acid sample to produce a second set of target amplicons; and comparing the first and second sets of target amplicons to determine whether a target locus is present in one sample but absent in the other, or whether a target locus is present at different levels in the control sample and the test sample. In various embodiments, the test sample is from an individual suspected of having a disease or phenotype of interest, or an increased risk for a disease or phenotype of interest; and wherein one or more of the target loci include a sequence (*e.g.*, a polymorphism) at the target locus associated with an increased risk for the disease or phenotype of interest, or associated with the disease or phenotype of interest. In various embodiments, at least 300, 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified. In some embodiments, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold. In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.5, or 100% of the target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold.

In various embodiments of any of the aspects of the invention, the scores (such as the undesirability scores) are based at least in part on one or more parameters selected from the group consisting of heterozygosity rate of the target locus, disease prevalence associated with a sequence (*e.g.*, a polymorphism) at the target locus, disease penetrance associated with a sequence (*e.g.*, a polymorphism) at the target locus, specificity of the candidate primer for the target locus, size of the candidate primer, melting temperature of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, GC content of the 3' end of the candidate primer, homopolymer length in the candidate primer, amplification efficiency of the target amplicon, and size of the target amplicon; and the method involves using the test primers to simultaneously amplify *e.g.*at least 100 (*e.g.*, at least 300 or 1,000); different target loci in a control sample that includes RNA to produce a first set of target amplicons and to simultaneously amplify the target loci in a test sample that includes RNA to produce a second set of target amplicons; and comparing the first and second sets of target amplicons to determine the presence or absence of a difference in the RNA expression levels between the control sample and the test sample. In various embodiments, the RNA is mRNA. In various embodiments, the test sample is from an individual suspected of having a disease or

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phenotype of interest (such as cancer) or an increased risk for the disease or phenotype of interest (such as cancer); and wherein one or more of the target loci includes a sequence (*e.g.*, a polymorphism or other mutation) associated with an increased risk for the disease or phenotype of interest or associated with the disease or phenotype of interest. In some embodiments, the test sample is from an individual diagnosed with a disease or phenotype of interest (such as cancer); and wherein a difference in the RNA expression level between the control sample and test sample indicates a target locus includes a sequence (*e.g.*, a polymorphism or other mutation) associated with an increased or decreased risk for the disease or phenotype of interest. In various embodiments, at least 300, 500; 750; 1,000; 2000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified. In some embodiments, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold. In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.5, or 100% of the target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold.

In one aspect, the invention features libraries of primers (such as non-immobilized primers). In some embodiments, the primers are selected from a library of candidate primers using any of the methods of the invention. In some embodiments, the library includes primers that simultaneously hybridize to at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci. In some embodiments, the library includes primers that simultaneously amplify at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci. In some embodiments, the library includes primers that simultaneously amplify at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci such that less than 60, 40, 30, 20, 10, 5, 4, 3, 2, 1, 0.5, 0.25, 0.1, or 0.05% of the amplified products are primer dimers. In some embodiments, the library includes primers that simultaneously amplify at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci such that at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the

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amplified products are target amplicons. In some embodiments, the library includes primers that simultaneously amplify target loci such that at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target loci out of at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified. In some embodiments, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold. In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.5, or 100% of the target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold. In some embodiments, the library of primers includes at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical primers or primer pairs. In some embodiments, the library of primers includes at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 primer pairs, wherein each pair of primers includes a forward test primer and a reverse test primer where each pair of test primers hybridize to a target locus. In some embodiments, the library of primers includes at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 individual primers that each hybridize to a different target locus, wherein the individual primers are not part of primer pairs. In some embodiments, the primers in the library are not immobilized (such as not immobilized to a solid support) or not part of a microarray. In some embodiments, the primers are dissolved in solution (such as dissolved in the liquid phase). In some embodiments, the library of primers consists essentially of, or consists of primers.

In some embodiments, ΔG values for each possible combination of two primers (each possible primer dimer) in a library are all equal to or greater than -20, -18, -16, -14, 12, -10, -9, -8, -7, -6, -5, -4, -3, -2, or -1 kcal/mol. In some embodiments, ΔG values for at least 80, 85, 90, 92, 94, 96, 98, 99, or 100% of the primers in the library for possible combinations of that primer with other primers in the library are all equal to or greater than -20, -18, -16, -14, 12, -10, -9, -8, -7, -6, -5, -4, -3, -2, or -1 kcal/mol.

In various embodiments of any of the aspects of the invention, the library of primers includes one or more primers (*e.g.*, at least 10; 20; 50; 100; 300; 500; 750; 1,000; 2,000; 5,000;

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7,500; 10,000; 15,000; 20,000; 25,000; 30,000; 40,000; or 50,000 different primers) with at least 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.5, or 100% identity to the corresponding region (*e.g.*, a region of at least 10, 20, 30, 40, 50, or more contiguous nucleotides) of a primer in FIG. 34 (SEQ ID NOs. 1-3,600), FIG. 35 (SEQ ID NOs. 3,601-11,658), or FIG. 36 (SEQ ID NOs 11,659-44,609). In some embodiments, the library of primers includes one or more primers (*e.g.*, at least 10; 20; 50; 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 20,000; 25,000; 30,000; 40,000; or 50,000 different primers) with at least 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.5, or 100% identity over the entire length of a primer in FIG. 34 (SEQ ID NOs. 1-3,600), FIG. 35 (SEQ ID NOs. 3,601-11,658), or FIG. 36 (SEQ ID NOs 11,659-44,609). In some embodiments, the library of primers includes at least 10; 20; 50; 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 20,000; 25,000; 30,000; 40,000; or 50,000 different primers from the primers in FIG. 34 (SEQ ID NOs. 1-3,600), FIG. 35 (SEQ ID NOs. 3,601-11,658), or FIG. 36 (SEQ ID NOs 11,659-44,609). In some embodiments, the library of primers includes one or more primers (*e.g.*, at least 10; 20; 50; 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 20,000; 25,000; 30,000; 40,000; or 50,000 different primers) that hybridize to the complement of a primer in FIG. 34 (SEQ ID NOs. 1-3,600), FIG. 35 (SEQ ID NOs. 3,601-11,658), or FIG. 36 (SEQ ID NOs 11,659-44,609). under very high stringency hybridization conditions or under high stringency hybridization conditions.

In various embodiments of any of the aspects of the invention, the concentration of each primer is less than 100, 75, 50, 25, 10, 5, 2, 1, 0.5, 0.1, or 0.05 nM. In various embodiments, the GC content of the primers is between 30 to 80%, such as between 40 to 70%, 20 to 70%, or 50 to 60%, inclusive. In some embodiments, the range of GC content of the primers is less than 30, 20, 10, or 5%. In some embodiments, there are at least 2 (such as 3, 4, or 5) guanines or cytosines in the last 5 bases at the 3' end of the primers. In some embodiments, there are at least 1 (such as 2 or 3) guanines or cytosines in the last 3 bases at the 3' end of the primers. In some embodiments, the melting temperature of the primers is between 40 to 80 °C, such as 50 to 70 °C, 55 to 65 °C, 54 to 60.5 °C, or 57 to 60.5 °C, inclusive. In some embodiments, the range of melting temperature of the primers is less than 15, 10, 5, 3, or 1 °C. In some embodiments, the length of the primers is between 15 to 100 nucleotides, such as between 15 to 75 nucleotides, 15 to 40 nucleotides, 17 to 35 nucleotides, 18 to 30 nucleotides, or 20 to 65 nucleotides, inclusive. In some embodiments, the primers

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include a tag that is not target specific, such as a tag that forms an internal loop structure. In some embodiments, the tag is between two DNA binding regions. In various embodiments, the primers include a 5' region that is specific for a target locus, an internal region that is not specific for the target locus and forms a loop structure, and a 3' region that is specific for the target locus. In various embodiments, the length of the 3' region is at least 7 nucleotides. In some embodiments, the length of the 3' region is between 7 and 20 nucleotides, such as between 7 to 15 nucleotides, or 7 to 10 nucleotides, inclusive. In various embodiments, the primers include a 5' region that is not specific for a target locus (such as another tag or a universal primer binding site) followed by a region that is specific for a target locus, an internal region that is not specific for the target locus and forms a loop structure, and a 3' region that is specific for the target locus. In some embodiments, the range of the length of the primers is less than 50, 40, 30, 20, 10, or 5 nucleotides. In some embodiments, the length of the target amplicons is between 50 and 100 nucleotides, such as between 60 and 80 nucleotides, or 60 to 75 nucleotides, inclusive. In some embodiments, the range of the length of the target amplicons is less than 50, 25, 15, 10, or 5 nucleotides. In some embodiments, the target loci are on two or more different chromosomes, such as two or more of chromosomes 13, 18, 21, X and Y. In some embodiments, the target loci are target human loci. In some embodiments, the target loci includes a sequence (*e.g.*, a polymorphism or other mutation) associated with an increased risk for the disease or phenotype of interest (such as cancer), or associated with the disease or phenotype of interest (such as cancer). In some embodiments, the polymorphism or mutation is a driver mutation that has a causative role in the disease or phenotype of interest (such as cancer). In some embodiments, the polymorphism or mutation is not a causative mutation. For example, in some cancers, multiple mutations accumulate but some of them are not causative mutations. Polymorphisms or mutations (such as those that are present at a higher frequency in subjects with a disease or phenotype of interest such as cancer than subjects without the disease or phenotype of interest such as cancer) that are not causative can still be useful for diagnosing the disease or phenotype.

In one aspect, the invention provides a composition that includes any of the primer libraries of the invention (such as non-immobilized primers). In some embodiments, the composition includes one or more free nucleotides (such as deoxynucleotides, ATP, CTP, GTP, TTP, UTP, dATP, dCTP, dGTP, dTTP, dUTP an activated nucleotide or deoxynucleotide, or a non-naturally occurring nucleotide or deoxynucleotide). In some

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embodiments, the composition includes at least one primer (*e.g.*, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical primers) with a polynucleotide sequence of a human nucleic acid and at least one primer (*e.g.*, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical primers) with a polynucleotide sequence that is not found in a human (such as a universal primer, a primer that comprises a region or consists entirely of random nucleotides, or a primer with a region such as a tag or barcode of one or more nucleotides that are not found in a human or are not found in nature as part of the polynucleotide sequence of the primer). In some embodiments, the composition includes at least one primer (*e.g.*, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical primers) with the polynucleotide sequence of a human nucleic acid and one or more non-human enzymes (*e.g.*, ligase or polymerase from a species other than a human, such as a bacterial or non-naturally-occurring ligase or polymerase). In some embodiments, the composition includes at least one primer (*e.g.*, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical primers) with the polynucleotide sequence of a human nucleic acid and a buffer or additive that is non-naturally-occurring or is not found in a human. In some embodiments, the composition comprises, consists essentially of, or consists of one or more of the following: primer(s), amplicon(s) free nucleotide(s), non-human enzyme(s), buffer(s), additive(s), or any combination thereof. In some embodiments, the composition comprises, consists essentially of, or consists of primers and one or more non-human enzymes.

In one aspect, the invention provides a composition comprising at least 100 different amplicons (*e.g.*, at least 300, 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical amplicons) in solution in one reaction volume. In some embodiments, the amplicons are produced from the simultaneous PCR amplification of at least 100 different target loci (*e.g.*, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical target loci) using at least 100 different primers or primer pairs (*e.g.*, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000;

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75,000; or 100,000 non-identical primers or primer pairs) in one reaction volume. In some embodiments, (i) less than 60% of the amplified products are primer dimers and at least 40% of the amplified products are target amplicons, (ii) less than 40% of the amplified products are primer dimers and at least 60% of the amplified products are target amplicons, (iii) less than 20% of the amplified products are primer dimers and at least 80% of the amplified products are target amplicons, (iv) less than 10% of the amplified products are primer dimers and at least 90% of the amplified products are target amplicons, or (v) less than 5% of the amplified products are primer dimers and at least 95% of the amplified products are target amplicons. In some embodiments, the length of the target amplicons is between 50 and 100 nucleotides, such as between 60 and 80 nucleotides, or 60 and 75 nucleotides, inclusive. In some embodiments, the range of the length of the target amplicons is less than 50, 25, 15, 10, or 5 nucleotides. In some embodiments, the range of the length of the target amplicons is between 5 to 50 nucleotides, such as 5 to 25 nucleotides, 5 to 15 nucleotides, or 5 to 10 nucleotides, inclusive. In some embodiments, the composition includes at least 1,000 different amplicons in solution in one reaction volume. In some embodiments, the amplicons are produced from the simultaneous PCR amplification of at least 1,000 different target human loci using at least 1,000 different primers in one reaction volume; wherein (i) less than 20% of the amplicons are primer dimers, and (ii) at least 80% of the amplicons comprise one of the target human loci and are between 50 and 100 nucleotides in length, inclusive. In some embodiments, the composition consists essentially of, or consists of one or more of the following: amplicons, primers (such as any of the primers disclosed herein), free nucleotide(s), non-human enzyme(s), buffer(s), or any combination thereof.

In one aspect, the invention provides a composition comprising at least 100 different primers or primer pairs (*e.g.*, at least 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical primers or primer pairs) and at least 100 different amplicons (*e.g.*, at least 300, 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical amplicons) in solution in one reaction volume. In some embodiments, the amplicons are produced from the simultaneous PCR amplification of at least 100 different target loci (*e.g.*, at least 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical target loci) using the primers or primer pairs in one

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reaction volume. In some embodiments, (i) less than 60% of the amplified products are primer dimers and at least 40% of the amplified products are target amplicons, (ii) less than 40% of the amplified products are primer dimers and at least 60% of the amplified products are target amplicons, (iii) less than 20% of the amplified products are primer dimers and at least 80% of the amplified products are target amplicons, (iv) less than 10% of the amplified products are primer dimers and at least 90% of the amplified products are target amplicons, or (v) less than 5% of the amplified products are primer dimers and at least 95% of the amplified products are target amplicons. In some embodiments, the length of the target amplicons is between 50 and 100 nucleotides, such as between 60 and 80 nucleotides, or 60 and 75 nucleotides, inclusive. In some embodiments, the range of the length of the target amplicons is less than 50, 25, 15, 10, or 5 nucleotides. In some embodiments, the range of the length of the target amplicons is between 5 to 50 nucleotides, such as 5 to 25 nucleotides, 5 to 15 nucleotides, or 5 to 10 nucleotides, inclusive. In some embodiments, the composition comprising at least 1,000 different primers and at least 1,000 different amplicons in solution in one reaction volume. In some embodiments, the amplicons are produced from the simultaneous PCR amplification of at least 1,000 different target human loci with the primers in one reaction volume; wherein (i) less than 20% of the amplicons are primer dimers, and (ii) at least 80% of the amplicons comprise one of the target loci and are between 50 and 100 nucleotides in length, inclusive. In some embodiments, the composition consists essentially of, or consists of one or more of the following: amplicons, primers (such as any of the primers disclosed herein), free nucleotide(s), non-human enzyme(s), buffer(s), or any combination thereof.

In one aspect, the invention provides kits that include any of the primer libraries or compositions of the invention for amplifying target loci in a nucleic acid sample. In some embodiments, the kits consists essentially of, or consists of primers, primers and instructions for using the primers, a composition of the invention, or a composition of the invention and instructions for using the composition. In some embodiments, the kit includes instructions for using the library to amplify the target loci.

In one aspect, the invention features methods for determining a ploidy status of chromosome in a gestating fetus. In some embodiments, the method involves contacting a nucleic acid sample with a library of primers that simultaneously hybridize to at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different polymorphic loci to produce a reaction

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mixture; wherein the nucleic acid sample includes maternal DNA from the mother of the fetus and fetal DNA from the fetus. In some embodiments, the reaction mixture is subjected to primer extension reaction conditions to produce amplified products; the amplified products are measured with a high throughput sequencer to produce sequencing data; allele counts at the polymorphic loci are calculated on a computer based on the sequencing data; a plurality of ploidy hypotheses each pertaining to a different possible ploidy state of the chromosome are created on a computer; a joint distribution model for the expected allele counts at the polymorphic loci on the chromosome is built on a computer for each ploidy hypothesis; a relative probability of each of the ploidy hypotheses is determined on a computer using the joint distribution model and the allele counts; and the ploidy state of the fetus is called by selecting the ploidy state corresponding to the hypothesis with the greatest probability.

In one aspect, the invention features methods for determining a ploidy status of a chromosome in a gestating fetus. In an embodiment a method for determining a ploidy status of a chromosome in a gestating fetus includes obtaining a first sample of DNA that comprises maternal DNA from the mother of the fetus and fetal DNA from the fetus, preparing the first sample by isolating the DNA so as to obtain a prepared sample, measuring the DNA in the prepared sample at a plurality of polymorphic loci on the chromosome, calculating, on a computer, allele counts at the plurality of polymorphic loci from the DNA measurements made on the prepared sample, creating, on a computer, a plurality of ploidy hypotheses each pertaining to a different possible ploidy state of the chromosome, building, on a computer, a joint distribution model for the expected allele counts at the plurality of polymorphic loci on the chromosome for each ploidy hypothesis, determining, on a computer, a relative probability of each of the ploidy hypotheses using the joint distribution model and the allele counts measured on the prepared sample, and calling the ploidy state of the fetus by selecting the ploidy state corresponding to the hypothesis with the greatest probability.

In one aspect, the invention features methods of testing for an abnormal distribution of a chromosome in a sample that includes a mixture of maternal and fetal DNA. In some embodiments, the method involves (i) contacting the sample with a library of primers that simultaneously hybridize to at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci to produce a reaction mixture; wherein the target loci are from a plurality of different chromosomes; and wherein the plurality of different chromosomes include at least

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one first chromosome suspected of having an abnormal distribution in the sample and at least one second chromosome presumed to be normally distributed in the sample; (ii) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products; (iii) sequencing the amplified products to obtain a plurality of sequence tags aligning to the target loci; wherein the sequence tags are of sufficient length to be assigned to a specific target locus; (iv) assigning on a computer the plurality of sequence tags to their corresponding target loci; (v) determining on a computer a number of sequence tags aligning to the target loci of the first chromosome and a number of sequence tags aligning to the target loci of the second chromosome; and (vi) comparing on a computer the numbers from step (v) to determine the presence or absence of an abnormal distribution of the first chromosome.

In one aspect, the invention provides methods for detecting the presence or absence of a fetal aneuploidy. In some embodiments, the method involves (i) contacting a sample that includes a mixture of maternal and fetal DNA with a library of primers that simultaneously hybridize to at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different non-polymorphic target loci to produce a reaction mixture; wherein the target loci are from a plurality of different chromosomes; (ii) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products that includes target amplicons; (iii) quantifying on a computer a relative frequency of the target amplicons from the first and second chromosomes of interest; (iv) comparing on a computer the relative frequency of the target amplicons from the first and second chromosomes of interest; and (v) identifying the presence or absence of an aneuploidy based on the compared relative frequencies of the first and second chromosome of interest. In some embodiments, the first chromosome is a chromosome suspected of being euploid. In some embodiments, the second chromosome is a chromosome suspected of being aneuploidy.

In one aspect, a method is disclosed for determining presence or absence of fetal aneuploidy in a maternal tissue sample comprising fetal and maternal genomic DNA, the method including (a) obtaining a mixture of fetal and maternal genomic DNA from said maternal tissue sample, (b) conducting massively parallel DNA sequencing of DNA fragments randomly selected from the mixture of fetal and maternal genomic DNA of step (a) to determine the sequence of said DNA fragments, (c) identifying chromosomes to which the sequences obtained in step (b) belong, (d) using the data of step (c) to determine an amount of

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at least one first chromosome in said mixture of maternal and fetal genomic DNA, wherein said at least one first chromosome is presumed to be euploid in the fetus, (e) using the data of step (c) to determine an amount of a second chromosome in said mixture of maternal and fetal genomic DNA, wherein said second chromosome is suspected to be aneuploid in the fetus, (f) calculating the fraction of fetal DNA in the mixture of fetal and maternal DNA, (g) calculating an expected distribution of the amount of the second target chromosome if the second target chromosome is euploid, using the number in step (d), (h) calculating an expected distribution of the amount of the second target chromosome if the second target chromosome is aneuploid, using the first number is step (d) and the calculated fraction of fetal DNA in the mixture of fetal and maternal DNA in step (f), and (i) using a maximum likelihood or maximum a posteriori approach to determine whether the amount of the second chromosome as determined in step (e) is more likely to be part of the distribution calculated in step (g) or the distribution calculated in step (h); thereby indicating the presence or absence of a fetal aneuploidy.

In various embodiments of any of the aspects of the invention, the target loci include one or more SNPs in the homologous non-recombining region of chromosome X and/or chromosome Y. In some embodiments, the method includes determining the relative amount of chromosome X and chromosome Y. In some embodiments, the method includes determining the number of copies of chromosome X and/or chromosome Y.

In some embodiments, the method also includes obtaining genotypic data from one or both parents of the fetus. In some embodiments, obtaining genotypic data from one or both parents of the fetus includes preparing the DNA from the parents where the preparing comprises preferentially enriching the DNA at the plurality of polymorphic loci to give prepared parental DNA, optionally amplifying the prepared parental DNA, and measuring the parental DNA in the prepared sample at the plurality of polymorphic loci.

In various embodiments of any of the aspects of the invention, building a joint distribution model for the expected allele count probabilities of the plurality of polymorphic loci on the chromosome is done using the obtained genetic data from the one or both parents. In some embodiments, the sample (*e.g.*, the first sample) has been isolated from maternal plasma and where the obtaining genotypic data from the mother is done by estimating the maternal genotypic data from the DNA measurements made on the prepared sample.

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In one aspect, a diagnostic box is disclosed for helping to determine a ploidy status of a chromosome in a gestating fetus where the diagnostic box is capable of executing the preparing and measuring steps of any of the methods of the invention.

In various embodiments of any of the aspects of the invention, the allele counts are probabilistic rather than binary. In some embodiments, measurements of the DNA in the prepared sample at the plurality of polymorphic loci are also used to determine whether or not the fetus has inherited one or a plurality of disease linked haplotypes.

In various embodiments of any of the aspects of the invention, building a joint distribution model for allele count probabilities is done by using data about the probability of chromosomes crossing over at different locations in a chromosome to model dependence between polymorphic alleles on the chromosome. In some embodiments, building a joint distribution model for allele counts and the step of determining the relative probability of each hypothesis are done using a method that does not require the use of a reference chromosome.

In various embodiments of any of the aspects of the invention, determining the relative probability of each hypothesis makes use of an estimated fraction of fetal DNA in the prepared sample. In some embodiments, the DNA measurements from the prepared sample used in calculating allele count probabilities and determining the relative probability of each hypothesis comprise primary genetic data. In some embodiments, selecting the ploidy state corresponding to the hypothesis with the greatest probability is carried out using maximum likelihood estimates or maximum a posteriori estimates.

In various embodiments of any of the aspects of the invention, calling the ploidy state of the fetus also includes combining the relative probabilities of each of the ploidy hypotheses determined using the joint distribution model and the allele count probabilities with relative probabilities of each of the ploidy hypotheses that are calculated using statistical techniques taken from a group consisting of a read count analysis, comparing heterozygosity rates, a statistic that is only available when parental genetic information is used, the probability of normalized genotype signals for certain parent contexts, a statistic that is calculated using an estimated fetal fraction of the sample (*e.g.*, the first sample) or the prepared sample, and combinations thereof.

In various embodiments of any of the aspects of the invention, a confidence estimate is calculated for the called ploidy state. In some embodiments, the method also includes taking a

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clinical action based on the called ploidy state of the fetus, wherein the clinical action is selected from one of terminating the pregnancy or maintaining the pregnancy.

In various embodiments of any of the aspects of the invention, the method may be performed for fetuses at between 4 and 5 weeks gestation; between 5 and 6 weeks gestation; between 6 and 7 weeks gestation; between 7 and 8 weeks gestation; between 8 and 9 weeks gestation; between 9 and 10 weeks gestation; between 10 and 12 weeks gestation; between 12 and 14 weeks gestation; between 14 and 20 weeks gestation; between 20 and 40 weeks gestation; in the first trimester; in the second trimester; in the third trimester; or combinations thereof.

In various embodiments of any of the aspects of the invention, a report displaying a determined ploidy status of a chromosome in a gestating fetus generated using the method. In some embodiments, a kit is disclosed for determining a ploidy status of a target chromosome in a gestating fetus designed to be used with any of the methods of the invention, the kit including a plurality of inner forward primers and optionally the plurality of inner reverse primers, where each of the primers is designed to hybridize to the region of DNA immediately upstream and/or downstream from one of the polymorphic sites on the target chromosome, and optionally additional chromosomes, where the region of hybridization is separated from the polymorphic site by a small number of bases, where the small number is selected from the group consisting of 1, 2, 3, 4, 5, 6 to 10, 11 to 15, 16 to 20, 21 to 25, 26 to 30, 31 to 60, and combinations thereof.

In one aspect, the invention features methods for establishing whether an alleged father is the biological father of a fetus that is gestating in a pregnant mother. In some embodiments the method involves, (i) simultaneously amplifying a plurality of polymorphic loci that includes at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different polymorphic loci on genetic material from the alleged father to produce a first set of amplified products; (ii) simultaneously amplifying the corresponding plurality of polymorphic loci on a mixed sample of DNA originating from a blood sample from the pregnant mother to produce a second set of amplified products; wherein the mixed sample of DNA includes fetal DNA and maternal DNA; (iii) determining on a computer the probability that the alleged father is the biological father of the fetus using genotypic measurements based on the first and second sets of amplified products; and (iv) establishing whether the alleged father is the biological father

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of the fetus using the determined probability that the alleged father is the biological father of the fetus. In various embodiments, the method further includes simultaneously amplifying the corresponding plurality of polymorphic loci on genetic material from the mother to produce a third set of amplified products; wherein the probability that the alleged father is the biological father of the fetus is determined using genotypic measurements based on the first, second, and third sets of amplified products.

In one aspect, the invention provides methods of estimating relative likelihoods that each embryo from a set of embryos will develop as desired. In some embodiments, the method involves contacting a sample from each embryo with a library of primers that simultaneously hybridize to at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci to produce a reaction mixture for each embryo, wherein the samples are each derived from one or more cells from an embryo. In some embodiments, each reaction mixture is subjected to primer extension reaction conditions to produce amplified products. In some embodiments, the method includes determining on a computer one or more characteristics of at least one cell from each embryo based on the amplified products; and estimating on a computer the relative likelihoods that each embryo will develop as desired, based on the one or more characteristics of the at least one cell for each embryo.

In one aspect, the invention features methods of measuring the amount of two or more target loci in a nucleic acid sample. In some embodiments, the method involves (i) using PCR to amplify a nucleic acid sample that includes a first standard locus, a second standard locus, a first target locus, and a second target locus to form amplified products; wherein the first standard locus and the first target locus have the same number of nucleotides but have a sequence that differs at one or more nucleotides; and wherein the second standard locus and the second target locus have the same number of nucleotides but have a sequence that differs at one or more nucleotides; (ii) sequencing the amplified products to determine a standard ratio that compares the relative amount of the amplified first standard locus compared to the amplified second standard locus; wherein the standard ratio indicates the difference in PCR efficiency for the amplification of the first standard locus and the second standard locus; (iii) determining a target ratio that compares the relative amount of the amplified first target locus compared to the amplified second target locus; and (iv) adjusting the target ratio from step (iii) based on the standard ratio from step (ii) to determine the relative amount of the first target

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locus and the second target locus in the sample. In various embodiments, the method involves determining the absolute amount of the first target locus and the second target locus in the sample. In various embodiments, the method further includes determining the presence or absence of a target locus (*e.g*, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci) in the sample. In various embodiments, the method involves using any of the primer libraries of the invention. In various embodiments, the method involves simultaneously amplifying at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci.

In one aspect, the invention features methods of quantitatively measuring a plurality of genetic targets in a sample for analysis. In some embodiments, the method includes (i) mixing genetic material derived from the sample for analysis with a plurality of target specific amplification reagents, and a plurality of standard sequences corresponding to the target specific amplification reagent targets; (ii) amplifying target regions of the genetic material and the standard sequences to produce target amplicons and standard sequence amplicons; and (iii) measuring the quantity of target amplicons and standard sequence amplicons produced. In some embodiments, the genetic material is present in a genetic library. In some embodiments, the genetic targets are polymorphic loci (such as SNPs). In some embodiments, the measuring of quantity is achieved by counting sequences. In some embodiments, the method further includes determining the estimated copy number of at least one chromosome in a sample from which the genetic library was derived, wherein the determination involves comparing the number of sequence reads of a target amplicon with the number of sequence reads of a standard amplicon. In some embodiments, the standard sequences and the genetic library include universal priming sites cable of being primed by the same primer. In some embodiments, the mixing step includes at least 10; 100, 500; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target specific amplification reagents and at least 10; 100, 500; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 standard sequences. In various embodiments, the method involves using any of the primer libraries of the invention. In various embodiments, the method involves simultaneously amplifying at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000;

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15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target regions. In some embodiments, the relative amounts of each of the standard sequences are known. In some embodiments, the relative amounts of each of the sequences is has been calibrated with respect to a reference genome. In some embodiments, the sample for analysis includes a mixture of fetal and maternal genomes. In some embodiments, the sample for analysis is derived from the blood of a pregnant woman or derived from blood plasma. In some embodiments, the reference genome has at least one aneuploidy, such as an aneuploidy at chromosome 13, 18, 21, X, or Y. In some embodiments, the reference genome is diploid.

In one aspect, the invention features a mixture that includes a plurality of genetic standard sequences, wherein the relative amount of each genetic standard sequence in the mixture has been determined by calibration to a reference genome. In various embodiments, the mixture includes at least 10; 100, 500; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 genetic standard sequences. In various embodiments, the genetic standard sequences include a first universal priming site, a second universal priming site, a first target specific priming site, a second target specific priming site, and a marker sequence located between the first and second target specific priming sites, wherein the first target specific site and the second target specific priming site are located between the first and second universal priming sites. In various embodiments, the calibration involves using any of the primer libraries of the invention. In various embodiments, the calibration involves simultaneously amplifying at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target regions. In some embodiments, the reference genome has at least one aneuploidy, such as an aneuploidy at chromosome 13, 18, 21, X, or Y. In some embodiments, the reference genome is diploid.

In one aspect, the invention features methods of producing a set of calibrated genetic standard sequences. In some embodiments, the method includes (i) forming an amplification reaction mixture that includes a genetic library prepared from a reference genome, a plurality of target-specific amplification primer reagent sets, and a plurality of genetic standard sequences corresponding to the target specific amplification reagent sets, (ii) amplifying the genetic library and the genetic standard sequences to produce amplicons from the target sequences and amplicons from the genetic standard sequences, (iii) measuring the quantity of the amplicons from the target sequences and amplicons from the genetic standard sequences,

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and (iv) determining the relative amount of each of genetic standard sequences with respect to each other, whereby the plurality of genetic standard sequences is calibrated. In various embodiments, at least 10; 100, 500; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 genetic standard sequences are used. In various embodiments, the method involves using any of the primer libraries of the invention. In various embodiments, the method involves simultaneously amplifying at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different sequences. In some embodiments, the reference genome has at least one aneuploidy, such as an aneuploidy at chromosome 13, 18, 21, X, or Y. In some embodiments, the reference genome is diploid.

In one aspect, the invention provides a set of genetic standard sequences that have been calibrated according to any of the methods of the invention. In one aspect, the invention provides a set of genetic standard sequences that may be calibrated either before, during or after the method is performed.

In one aspect, the invention features methods of measuring the number of copies of a gene of interest having at least one allele that has a deletion. In some embodiments, the method includes (i) mixing genetic material derived from a sample for analysis with an amplification reagent specific for the gene of interest and not capable of significantly amplifying the deletion comprising allele of the gene of interest, a standard sequence corresponding to gene of interest, an amplification reagent specific for a reference sequence, and a standard sequence corresponding to the reference sequence; (ii) amplifying the gene sequence of interest, the standard sequence corresponding to the gene of interest, the reference sequence, and the standard sequence corresponding to the reference sequence to produce gene of interest amplicons, reference sequence amplicons, and standard sequence amplicons; and (iii) measuring the quantity of target amplicons and standard sequence amplicons produced. In some embodiments, the measuring of quantity is achieved by counting sequence reads. In some embodiments, the method further includes determining the estimated copy number of at least one chromosome in the sample from which the genetic library was derived, wherein the determination involves comparing the number of sequences of target amplicons with the number of sequences of a standard amplicons. In some embodiments, the standard sequences and the genetic library include universal priming sites capable of being primed by the same

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primer. In some embodiments, the relative amounts of each of the sequences have been calibrated with respect to reference genome. In various embodiments, at least 10; 100, 500; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 genetic standard sequences are used. In various embodiments, the method involves using any of the primer libraries of the invention. In various embodiments, the method involves simultaneously amplifying at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target regions. In some embodiments, the reference genome is diploid. In some embodiments, the sample for analysis is derived from blood.

In some embodiments of any of the aspects of the invention, preferentially enriching the DNA in the sample (*e.g.*, the first sample) at the target loci (*e.g.*, the plurality of polymorphic loci) includes obtaining a plurality of pre-circularized probes where each probe targets one of the loci (*e.g.*, polymorphic loci), where the 3' and 5' end of the probes are preferably designed to hybridize to a region of DNA that is separated from the polymorphic site of the locus by a small number of bases, where the small number is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 to 25, 26 to 30, 31 to 60, or a combination thereof, hybridizing the pre-circularized probes to DNA from the sample (*e.g.*, the first sample), filling the gap between the hybridized probe ends using DNA polymerase, circularizing the pre-circularized probe, and amplifying the circularized probe.

In some embodiments of any of the aspects of the invention, the preferentially enriching the DNA at the target loci (*e.g.*, the plurality of polymorphic loci) includes obtaining a plurality of ligation-mediated PCR probes where each PCR probe targets one of the target loci (*e.g.*, the polymorphic loci), and where the upstream and downstream PCR probes are designed to hybridize to a region of DNA on one strand of DNA that is preferably separated from the polymorphic site of the locus by a small number of bases, where the small number is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 to 25, 26 to 30, 31 to 60, or a combination thereof, hybridizing the ligation-mediated PCR probes to the DNA from the sample (*e.g.*, the first sample), filling the gap between the ligation-mediated PCR probe ends using DNA polymerase, ligating the ligation-mediated PCR probes, and amplifying the ligated ligation-mediated PCR probes.

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In some embodiments of various aspects of the invention, preferentially enriching the DNA at the target loci (*e.g.*, plurality of polymorphic loci) includes obtaining a plurality of hybrid capture probes that target the loci (*e.g.*, the polymorphic loci), hybridizing the hybrid capture probes to the DNA in the sample (*e.g.*, the first sample) and physically removing some or all of the unhybridized DNA from the sample (*e.g.*, the first sample) of DNA.

In some embodiments of any of the aspects of the invention, the hybrid capture probes are designed to hybridize to a region that is flanking but not overlapping the polymorphic site. In some embodiments, the hybrid capture probes are designed to hybridize to a region that is flanking but not overlapping the polymorphic site, and where the length of the flanking capture probe may be selected from the group consisting of less than about 120 bases, less than about 110 bases, less than about 100 bases, less than about 90 bases, less than about 80 bases, less than about 70 bases, less than about 60 bases, less than about 50 bases, less than about 40 bases, less than about 30 bases, and less than about 25 bases. In some embodiments, the hybrid capture probes are designed to hybridize to a region that overlaps the polymorphic site, and where the plurality of hybrid capture probes comprise at least two hybrid capture probes for each polymorphic loci, and where each hybrid capture probe is designed to be complementary to a different allele at that polymorphic locus.

In some embodiments of any of the aspects of the invention, preferentially enriching the DNA a plurality of polymorphic loci includes obtaining a plurality of inner forward primers where each primer targets one of the polymorphic loci, and where the 3' end of the inner forward primers are designed to hybridize to a region of DNA upstream from the polymorphic site, and separated from the polymorphic site by a small number of bases, where the small number is selected from the group consisting of 1, 2, 3, 4, 5, 6 to 10, 11 to 15, 16 to 20, 21 to 25, 26 to 30, or 31 to 60 base pairs, optionally obtaining a plurality of inner reverse primers where each primer targets one of the polymorphic loci, and where the 3' end of the inner reverse primers are designed to hybridize to a region of DNA upstream from the polymorphic site, and separated from the polymorphic site by a small number of bases, where the small number is selected from the group consisting of 1, 2, 3, 4, 5, 6 to 10, 11 to 15, 16 to 20, 21 to 25, 26 to 30, or 31 to 60 base pairs, hybridizing the inner primers to the DNA, and amplifying the DNA using the polymerase chain reaction to form amplicons.

In some embodiments of any of the aspects of the invention, the method also includes obtaining a plurality of outer forward primers where each primer targets one of the target (*e.g.*,

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polymorphic loci), and where the outer forward primers are designed to hybridize to the region of DNA upstream from the inner forward primer, optionally obtaining a plurality of outer reverse primers where each primer targets one of the target loci (*e.g.*, polymorphic loci), and where the outer reverse primers are designed to hybridize to the region of DNA immediately downstream from the inner reverse primer, hybridizing the first primers to the DNA, and amplifying the DNA using the polymerase chain reaction.

In some embodiments of any of the aspects of the invention, the method also includes obtaining a plurality of outer reverse primers where each primer targets one of the polymorphic loci, and where the outer reverse primers are designed to hybridize to the region of DNA immediately downstream from the inner reverse primer, optionally obtaining a plurality of outer forward primers where each primer targets one of the target loci (*e.g.*, the polymorphic loci), and where the outer forward primers are designed to hybridize to the region of DNA upstream from the inner forward primer, hybridizing the first primers to the DNA, and amplifying the DNA using the polymerase chain reaction.

In some embodiments of any of the aspects of the invention, preparing the sample (*e.g.*, the first sample) further includes appending universal adapters to the DNA in the sample (*e.g.*, the first sample) and amplifying the DNA in the sample (*e.g.*, the first sample) using the polymerase chain reaction. In some embodiments, at least a fraction of the amplicons that are amplified are less than 100 bp, less than 90 bp, less than 80 bp, less than 70 bp, less than 65 bp, less than 60 bp, less than 55 bp, less than 50 bp, or less than 45 bp, and where the fraction is 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 99%.

In some embodiments of any of the aspects of the invention, amplifying the DNA is done in one or a plurality of individual reaction volumes, and where each individual reaction volume contains more than 100 different forward and reverse primer pairs, more than 200 different forward and reverse primer pairs, more than 500 different forward and reverse primer pairs, more than 1,000 different forward and reverse primer pairs, more than 2,000 different forward and reverse primer pairs, more than 5,000 different forward and reverse primer pairs, more than 10,000 different forward and reverse primer pairs, more than 20,000 different forward and reverse primer pairs, more than 50,000 different forward and reverse primer pairs, or more than 100,000 different forward and reverse primer pairs. In various embodiments of any of the aspects of the invention, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500;

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10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different primer pairs are used.

In some embodiments of any of the aspects of the invention, preparing the sample (*e.g.*, the first sample) further comprises dividing the sample (*e.g.*, the first sample) into a plurality of portions, and where the DNA in each portion is preferentially enriched at a subset of the target loci (*e.g.*, plurality of polymorphic loci). In some embodiments, the inner primers are selected by identifying primer pairs likely to form undesired primer duplexes and removing from the plurality of primers at least one of the pair of primers identified as being likely to form undesired primer duplexes. In some embodiments, the inner primers contain a region that is designed to hybridize either upstream or downstream of the targeted locus (*e.g.*, the polymorphic locus), and optionally contain a universal priming sequence designed to allow PCR amplification. In some embodiments, at least some of the primers additionally contain a random region that differs for each individual primer molecule. In some embodiments, at least some of the primers additionally contain a molecular barcode.

In some embodiments of any of the aspects of the invention, preferential enrichment results in average degree of allelic bias between the prepared sample and the sample (*e.g.*, the first sample) of a factor selected from the group consisting of no more than a factor of 2, no more than a factor of 1.5, no more than a factor of 1.2, no more than a factor of 1.1, no more than a factor of 1.05, no more than a factor of 1.02, no more than a factor of 1.01, no more than a factor of 1.005, no more than a factor of 1.002, no more than a factor of 1.001 and no more than a factor of 1.0001. In some embodiments, the plurality of polymorphic loci are SNPs. In some embodiments, measuring the DNA in the prepared sample is done by sequencing.

In some embodiments, the nucleic acids in the sample are non-specifically amplified prior to amplification of the target loci (such as specific amplification of the target loci with a primer library of the invention). In some embodiments, the non-specific amplification includes whole genome application (WGA), such as ligation-mediated PCR (LM-PCR), degenerate oligonucleotide primer PCR (DOP-PCR), or multiple displacement amplification (MDA). In some embodiments, the non-specific amplification includes universal PCR, such as adaptor-mediated universal PCR.

In some embodiments of any of the aspects of the invention, the target loci are present on the same nucleic acid of interest (*e.g*, the same chromosome or the same region of a chromosome). In some embodiments, at least some of the target loci are present on different

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nucleic acids of interest (*e.g*, different chromosomes). In some embodiments, the nucleic acid sample includes fragmented or digested nucleic acids. In some embodiments, the nucleic acid sample includes DNA, such as genomic DNA, cDNA, cell-free DNA (cfDNA), cell-free mitochondrial DNA (cf mDNA), cell-free DNA that originated from nuclear DNA (cf nDNA), cellular DNA, or mitochondrial DNA. In some embodiments, nucleic acid sample includes RNA, such as cfRNA, cellular RNA, cytoplasmic RNA, coding cytoplasmic RNA, non-coding cytoplasmic RNA, mRNA, miRNA, mitochondrial RNA, rRNA, or tRNA. In some embodiments, the nucleic acid sample includes DNA from a single cell, 2 cells, 3 cells, 4 cells, 5 cells, 6 cells, 7 cells, 8 cells, 9 cell, 10 cells, or more than 10 cells. In some embodiments, the nucleic acid sample is a blood or plasma sample that is substantially free of cells. In some embodiments, the nucleic acid sample includes or is derived from blood, plasma, saliva, semen, sperm, cell culture supernatant, mucus secretion, dental plaque, gastrointestinal tract tissue, stool, urine, hair, bone, body fluids, , tears, tissue, skin, fingernails, blastomeres, embryos, amniotic fluid, chorionic villus samples, bile, lymph, cervical mucus, or a forensic sample. In some embodiments, the target loci are segments of human nucleic acids. In some embodiments, the target loci comprise or consist of single nucleotide polymorphisms (SNPs). In some embodiments, the primers are DNA molecules.

In some embodiments of any of the aspects of the invention, the DNA in the sample (*e.g.*, the first sample) originates from maternal plasma. In some embodiments, preparing the sample (*e.g.*, the first sample) further comprises amplifying the DNA. In some embodiments, preparing the sample (*e.g.*, the first sample) further comprises preferentially enriching the DNA in the sample (*e.g.*, the first sample) at the target loci (*e.g.*, a plurality of polymorphic loci).

In various embodiments, the primer extension reaction or the polymerase chain reaction includes the addition of one or more nucleotides by a polymerase. In some embodiments, greater than or equal to 5, 10, 20, 30, 40, 50, or 60 cycles of PCR are performed. In some embodiments, the amplification of loci is performed using a polymerase (*e.g.*, a DNA polymerase, RNA polymerase, or reverse transcriptase) with low 5′→ 3′ exonuclease and/or low strand displacement activity. In some embodiments, a DNA polymerase is used produce DNA amplicons using DNA as a template. In some embodiments, a RNA polymerase is used produce RNA amplicons using DNA as a template. In some embodiments, a reverse transcriptase is used produce cDNA amplicons using RNA as a template.

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In various embodiments, the primer extension reaction or the polymerase chain reaction does not include ligation-mediated PCR. In various embodiments, the primer extension reaction or the polymerase chain reaction does not include the joining of two primers by a ligase. In various embodiments, the primers do not include Linked Inverted Probes (LIPs), which can also be called pre-circularized probes, pre-circularizing probes, circularizing probes, Padlock Probes, or Molecular Inversion Probes (MIPs). In some embodiments, the primers are not loopable primers. In some embodiments, the primers do not form a loop structure, for example, the primers do not comprise a 3' target specific portion, a stem (comprising a first loop forming region and a second loop forming region), and a loop portion. In various embodiments, the primer library, composition, kit, or method does not include an array (such as a microarray) or do no use an array (such as a microarray). In some embodiments, multiplex PCR and/or sequencing is performed without use of an array (such as a microarray).

It is understood that all of the aspects and embodiments of the invention described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments. It is understood that aspects and embodiments of the invention described herein include combinations of any two or more of the aspects or embodiments of the invention.

Definitions

Single Nucleotide Polymorphism (SNP) refers to a single nucleotide that may differ between the genomes of two members of the same species. The usage of the term should not imply any limit on the frequency with which each variant occurs.

Sequence refers to a DNA sequence or a genetic sequence. It may refer to the primary, physical structure of the DNA molecule or strand in an individual. It may refer to the sequence of nucleotides found in that DNA molecule, or the complementary strand to the DNA molecule. It may refer to the information contained in the DNA molecule as its representation *in silico*.

Locus refers to a particular region of interest on the DNA (or corresponding RNA) of an individual, which may refer to a SNP, the site of a possible insertion or deletion, or the site of some other relevant genetic variation. Disease-linked SNPs may also refer to disease-linked loci.

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Polymorphic Allele, also "Polymorphic Locus," refers to an allele or locus where the genotype varies between individuals within a given species. Some examples of polymorphic alleles include single nucleotide polymorphisms, short tandem repeats, deletions, duplications, and inversions.

Polymorphic Site refers to the specific nucleotides found in a polymorphic region that vary between individuals.

Allele refers to the genes that occupy a particular locus.

Genetic Data also "*Genotypic Data"* refers to the data describing aspects of the genome of one or more individuals. It may refer to one or a set of loci, partial or entire sequences, partial or entire chromosomes, or the entire genome. It may refer to the identity of one or a plurality of nucleotides; it may refer to a set of sequential nucleotides, or nucleotides from different locations in the genome, or a combination thereof. Genotypic data is typically *in silico*, however, it is also possible to consider physical nucleotides in a sequence as chemically encoded genetic data. Genotypic Data may be said to be "on," "of," "at," "from" or "on" the individual(s). Genotypic Data may refer to output measurements from a genotyping platform where those measurements are made on genetic material.

Genetic Material also "*Genetic Sample"* refers to physical matter, such as tissue or blood, from one or more individuals comprising DNA or RNA

Noisy Genetic Data refers to genetic data with any of the following: allele dropouts, uncertain base pair measurements, incorrect base pair measurements, missing base pair measurements, uncertain measurements of insertions or deletions, uncertain measurements of chromosome segment copy numbers, spurious signals, missing measurements, other errors, or combinations thereof.

Confidence refers to the statistical likelihood that the called SNP, allele, set of alleles, ploidy call, or determined number of chromosome segment copies correctly represents the real genetic state of the individual.

Ploidy Calling, also "Chromosome Copy Number Calling," or "Copy Number Calling" (CNC), may refer to the act of determining the quantity and/or chromosomal identity of one or more chromosomes present in a cell.

Aneuploidy refers to the state where the wrong number of chromosomes (*e.g.,* the wrong number of full chromosomes or the wrong number of chromosome segments, such as

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the presence of deletions or duplications of a chromosome segment) is present in a cell. In the case of a somatic human cell it may refer to the case where a cell does not contain 22 pairs of autosomal chromosomes and one pair of sex chromosomes. In the case of a human gamete, it may refer to the case where a cell does not contain one of each of the 23 chromosomes. In the case of a single chromosome type, it may refer to the case where more or less than two homologous but non-identical chromosome copies are present, or where there are two chromosome copies present that originate from the same parent. In some embodiments, the deletion of a chromosome segment is a microdeletion.

Ploidy State refers to the quantity and/or chromosomal identity of one or more chromosomes types in a cell.

Chromosome may refer to a single chromosome copy, meaning a single molecule of DNA of which there are 46 in a normal somatic cell; an example is 'the maternally derived chromosome 18'. Chromosome may also refer to a chromosome type, of which there are 23 in a normal human somatic cell; an example is 'chromosome 18'.

Chromosomal Identity may refer to the referent chromosome number, *i.e.* the chromosome type. Normal humans have 22 types of numbered autosomal chromosome types, and two types of sex chromosomes. It may also refer to the parental origin of the chromosome. It may also refer to a specific chromosome inherited from the parent. It may also refer to other identifying features of a chromosome.

The State of the Genetic Material or simply "Genetic State" may refer to the identity of a set of SNPs on the DNA, to the phased haplotypes of the genetic material, and to the sequence of the DNA, including insertions, deletions, repeats and mutations. It may also refer to the ploidy state of one or more chromosomes, chromosomal segments, or set of chromosomal segments.

Allelic Data refers to a set of genotypic data concerning a set of one or more alleles. It may refer to the phased, haplotypic data. It may refer to SNP identities, and it may refer to the sequence data of the DNA, including insertions, deletions, repeats and mutations. It may include the parental origin of each allele.

Allelic State refers to the actual state of the genes in a set of one or more alleles. It may refer to the actual state of the genes described by the allelic data.

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Allelic Ratio or allele ratio, refers to the ratio between the amount of each allele at a locus that is present in a sample or in an individual. When the sample was measured by sequencing, the allelic ratio may refer to the ratio of sequence reads that map to each allele at the locus. When the sample was measured by an intensity based measurement method, the allele ratio may refer to the ratio of the amounts of each allele present at that locus as estimated by the measurement method.

Allele Count refers to the number of sequences that map to a particular locus, and if that locus is polymorphic, it refers to the number of sequences that map to each of the alleles. If each allele is counted in a binary fashion, then the allele count will be whole number. If the alleles are counted probabilistically, then the allele count can be a fractional number.

Allele Count Probability refers to the number of sequences that are likely to map to a particular locus or a set of alleles at a polymorphic locus, combined with the probability of the mapping. Note that allele counts are equivalent to allele count probabilities where the probability of the mapping for each counted sequence is binary (zero or one). In some embodiments, the allele count probabilities may be binary. In some embodiments, the allele count probabilities may be set to be equal to the DNA measurements.

Allelic Distribution, or 'allele count distribution' refers to the relative amount of each allele that is present for each locus in a set of loci. An allelic distribution can refer to an individual, to a sample, or to a set of measurements made on a sample. In the context of sequencing, the allelic distribution refers to the number or probable number of reads that map to a particular allele for each allele in a set of polymorphic loci. The allele measurements may be treated probabilistically, that is, the likelihood that a given allele is present for a give sequence read is a fraction between 0 and 1, or they may be treated in a binary fashion, that is, any given read is considered to be exactly zero or one copies of a particular allele.

Allelic Distribution Pattern refers to a set of different allele distributions for different parental contexts. Certain allelic distribution patterns may be indicative of certain ploidy states.

Allelic Bias refers to the degree to which the measured ratio of alleles at a heterozygous locus is different to the ratio that was present in the original sample, such as a smaple of DNA. The degree of allelic bias at a particular locus is equal to the observed allelic ratio at that locus, as measured, divided by the ratio of alleles in the original DNA or

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RNA sample at that locus. Allelic bias may be defined to be greater than one, such that if the calculation of the degree of allelic bias returns a value, x, that is less than 1, then the degree of allelic bias may be restated as 1/x. Allelic bias maybe due to amplification bias, purification bias, or some other phenomenon that affects different alleles differently.

Primer, also "PCR probe" refers to a single DNA molecule (a DNA oligomer) or a collection of DNA molecules (DNA oligomers) where the DNA molecules are identical, or nearly so, and where the primer contains a region that is designed to hybridize to a targeted locus (*e.g,* a targeted polymorphic locus or a nonpolymorphic locus), and may contain a priming sequence designed to allow PCR amplification. A primer may also contain a molecular barcode. A primer may contain a random region that differs for each individual molecule. The terms "test primer" and "candidate primer" are not meant to be limiting and may refer to any of the primers disclosed herein.

Library of primers refers to a population of two or more primers. In various embodiments, the library includes at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different primers. In various embodiments, the library includes at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different primer pairs, wherein each pair of primers includes a forward test primer and a reverse test primer where each pair of test primers hybridize to a target locus. In some embodiments, the library of primers includes at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different individual primers that each hybridize to a different target locus, wherein the individual primers are not part of primer pairs. In some embodiments, the library has both (i) primer pairs and (ii) individual primers (such as universal primers) that are not part of primer pairs.

Different primers refers to non-identical primers.

Different pools refers to non-identical pools.

Different target loci refers to non-identical target loci.

Different amplicons refers to non-identical amplicons.

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Hybrid Capture Probe refers to any nucleic acid sequence, possibly modified, that is generated by various methods such as PCR or direct synthesis and intended to be complementary to one strand of a specific target DNA sequence in a sample. The exogenous hybrid capture probes may be added to a prepared sample and hybridized through a deanture-reannealing process to form duplexes of exogenous-endogenous fragments. These duplexes may then be physically separated from the sample by various means.

Sequence Read refers to data representing a sequence of nucleotide bases that were measured using a clonal sequencing method. Clonal sequencing may produce sequence data representing single, or clones, or clusters of one original DNA molecule. A sequence read may also have associated quality score at each base position of the sequence indicating the probability that nucleotide has been called correctly.

Mapping a sequence read is the process of determining a sequence read's location of origin in the genome sequence of a particular organism. The location of origin of sequence reads is based on similarity of nucleotide sequence of the read and the genome sequence.

Matched Copy Error, also "Matching Chromosome Aneuploidy" (MCA), refers to a state of aneuploidy where one cell contains two identical or nearly identical chromosomes. This type of aneuploidy may arise during the formation of the gametes in meiosis, and may be referred to as a meiotic non-disjunction error. This type of error may arise in mitosis. Matching trisomy may refer to the case where three copies of a given chromosome are present in an individual and two of the copies are identical.

Unmatched Copy Error, also "Unique Chromosome Aneuploidy" (UCA), refers to a state of aneuploidy where one cell contains two chromosomes that are from the same parent, and that may be homologous but not identical. This type of aneuploidy may arise during meiosis, and may be referred to as a meiotic error. Unmatching trisomy may refer to the case where three copies of a given chromosome are present in an individual and two of the copies are from the same parent, and are homologous, but are not identical. Note that unmatching trisomy may refer to the case where two homologous chromosomes from one parent are present, and where some segments of the chromosomes are identical while other segments are merely homologous.

Homologous Chromosomes refers to chromosome copies that contain the same set of genes that normally pair up during meiosis.

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Identical Chromosomes refers to chromosome copies that contain the same set of genes, and for each gene they have the same set of alleles that are identical, or nearly identical.

Allele Drop Out (ADO) refers to the situation where at least one of the base pairs in a set of base pairs from homologous chromosomes at a given allele is not detected.

Locus Drop Out (LDO) refers to the situation where both base pairs in a set of base pairs from homologous chromosomes at a given allele are not detected.

Homozygous refers to having similar alleles as corresponding chromosomal loci.

Heterozygous refers to having dissimilar alleles as corresponding chromosomal loci.

Heterozygosity Rate refers to the rate of individuals in the population having heterozygous alleles at a given locus. The heterozygosity rate may also refer to the expected or measured ratio of alleles, at a given locus in an individual, or a sample of DNA.

Highly Informative Single Nucleotide Polymorphism (HISNP) refers to a SNP where the fetus has an allele that is not present in the mother's genotype.

Chromosomal Region refers to a segment of a chromosome, or a full chromosome.

Segment of a Chromosome refers to a section of a chromosome that can range in size from one base pair to the entire chromosome.

Chromosome refers to either a full chromosome, or a segment or section of a chromosome.

Copies refers to the number of copies of a chromosome segment. It may refer to identical copies, or to non-identical, homologous copies of a chromosome segment wherein the different copies of the chromosome segment contain a substantially similar set of loci, and where one or more of the alleles are different. Note that in some cases of aneuploidy, such as the M2 copy error, it is possible to have some copies of the given chromosome segment that are identical as well as some copies of the same chromosome segment that are not identical.

Haplotype refers to a combination of alleles at multiple loci that are typically inherited together on the same chromosome. Haplotype may refer to as few as two loci or to an entire chromosome depending on the number of recombination events that have occurred between a given set of loci. Haplotype can also refer to a set of single nucleotide polymorphisms (SNPs) on a single chromatid that are statistically associated.

Haplotypic Data, also "Phased Data" or "Ordered Genetic Data," refers to data from a single chromosome in a diploid or polyploid genome, *i.e.*, either the segregated maternal or paternal copy of a chromosome in a diploid genome.

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Phasing refers to the act of determining the haplotypic genetic data of an individual given unordered, diploid (or polyploidy) genetic data. It may refer to the act of determining which of two genes at an allele, for a set of alleles found on one chromosome, are associated with each of the two homologous chromosomes in an individual.

Phased Data refers to genetic data where one or more haplotypes have been determined.

Hypothesis refers to a possible ploidy state at a given set of chromosomes, or a set of possible allelic states at a given set of loci. The set of possibilities may comprise one or more elements.

Copy Number Hypothesis, also "Ploidy State Hypothesis," refers to a hypothesis concerning the number of copies of a chromosome in an individual. It may also refer to a hypothesis concerning the identity of each of the chromosomes, including the parent of origin of each chromosome, and which of the parent's two chromosomes are present in the individual. It may also refer to a hypothesis concerning which chromosomes, or chromosome segments, if any, from a related individual correspond genetically to a given chromosome from an individual.

Target Individual refers to the individual whose genetic state is being determined. In some embodiments, only a limited amount of DNA is available from the target individual. In some embodiments, the target individual is a fetus. In some embodiments, there may be more than one target individual. In some embodiments, each fetus that originated from a pair of parents may be considered to be target individuals. In some embodiments, the genetic data that is being determined is one or a set of allele calls. In some embodiments, the genetic data that is being determined is a ploidy call.

Related Individual refers to any individual who is genetically related to, and thus shares haplotype blocks with, the target individual. In one context, the related individual may be a genetic parent of the target individual, or any genetic material derived from a parent, such as a sperm, a polar body, an embryo, a fetus, or a child. It may also refer to a sibling, parent or a grandparent.

Sibling refers to any individual whose genetic parents are the same as the individual in question. In some embodiments, it may refer to a born child, an embryo, or a fetus, or one or more cells originating from a born child, an embryo, or a fetus. A sibling may also refer to a haploid individual that originates from one of the parents, such as a

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sperm, a polar body, or any other set of haplotypic genetic matter. An individual may be considered to be a sibling of itself.

Fetal refers to "of the fetus," or "of the region of the placenta that is genetically similar to the fetus". In a pregnant woman, some portion of the placenta is genetically similar to the fetus, and the free floating fetal DNA found in maternal blood may have originated from the portion of the placenta with a genotype that matches the fetus. Note that the genetic information in half of the chromosomes in a fetus is inherited from the mother of the fetus. In some embodiments, the DNA from these maternally inherited chromosomes that came from a fetal cell is considered to be "of fetal origin," not "of maternal origin."

DNA of Fetal Origin refers to DNA that was originally part of a cell whose genotype was essentially equivalent to that of the fetus.

DNA of Maternal Origin refers to DNA that was originally part of a cell whose genotype was essentially equivalent to that of the mother.

Child may refer to an embryo, a blastomere, or a fetus. Note that in the presently disclosed embodiments, the concepts described apply equally well to individuals who are a born child, a fetus, an embryo or a set of cells therefrom. The use of the term child may simply be meant to connote that the individual referred to as the child is the genetic offspring of the parents.

Parent refers to the genetic mother or father of an individual. An individual typically has two parents, a mother and a father, though this may not necessarily be the case such as in genetic or chromosomal chimerism. A parent may be considered to be an individual.

Parental Context refers to the genetic state of a given SNP, on each of the two relevant chromosomes for one or both of the two parents of the target.

Develop As Desired, also "Develop Normally," refers to a viable embryo implanting in a uterus and resulting in a pregnancy, and/or to a pregnancy continuing and resulting in a live birth, and/or to a born child being free of chromosomal abnormalities, and/or to a born child being free of other undesired genetic conditions such as disease-linked genes. The term "develop as desired" is meant to encompass anything that may be desired by parents or healthcare facilitators. In some cases, "develop as desired" may refer to an unviable or viable embryo that is useful for medical research or other purposes.

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Insertion into a Uterus refers to the process of transferring an embryo into the uterine cavity in the context of *in vitro* fertilization.

Maternal Plasma refers to the plasma portion of the blood from a female who is pregnant.

Clinical Decision refers to any decision to take or not take an action that has an outcome that affects the health or survival of an individual. In the context of prenatal diagnosis, a clinical decision may refer to a decision to abort or not abort a fetus. A clinical decision may also refer to a decision to conduct further testing, to take actions to mitigate an undesirable phenotype, or to take actions to prepare for the birth of a child with abnormalities.

Diagnostic Box refers to one or a combination of machines designed to perform one or a plurality of aspects of the methods disclosed herein. In an embodiment, the diagnostic box may be placed at a point of patient care. In an embodiment, the diagnostic box may perform targeted amplification followed by sequencing. In an embodiment the diagnostic box may function alone or with the help of a technician.

Informatics Based Method refers to a method that relies heavily on statistics to make sense of a large amount of data. In the context of prenatal diagnosis, it refers to a method designed to determine the ploidy state at one or more chromosomes or the allelic state at one or more alleles by statistically inferring the most likely state, rather than by directly physically measuring the state, given a large amount of genetic data, for example from a molecular array or sequencing. In an embodiment of the present disclosure, the informatics based technique may be one disclosed in this patent. In an embodiment of the present disclosure it may be PARENTAL SUPPORT™.

Primary Genetic Data refers to the analog intensity signals that are output by a genotyping platform. In the context of SNP arrays, primary genetic data refers to the intensity signals before any genotype calling has been done. In the context of sequencing, primary genetic data refers to the analog measurements, analogous to the chromatogram, that comes off the sequencer before the identity of any base pairs have been determined, and before the sequence has been mapped to the genome.

Secondary Genetic Data refers to processed genetic data that are output by a genotyping platform. In the context of a SNP array, the secondary genetic data refers to the allele calls made by software associated with the SNP array reader, wherein the software has made a call whether a given allele is present or not present in the sample. In the context

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of sequencing, the secondary genetic data refers to the base pair identities of the sequences have been determined, and possibly also where the sequences have been mapped to the genome.

Non-Invasive Prenatal Diagnosis (NPD), or also "Non-Invasive Prenatal Screening" (NPS), refers to a method of determining the genetic state of a fetus that is gestating in a mother using genetic material found in the mother's blood, where the genetic material is obtained by drawing the mother's intravenous blood.

Preferential Enrichment of DNA that corresponds to a locus, or preferential enrichment of DNA at a locus, refers to any method that results in the percentage of molecules of DNA in a post-enrichment DNA mixture that correspond to the locus being higher than the percentage of molecules of DNA in the pre-enrichment DNA mixture that correspond to the locus. The method may involve selective amplification of DNA molecules that correspond to a locus. The method may involve removing DNA molecules that do not correspond to the locus. The method may involve a combination of methods. The degree of enrichment is defined as the percentage of molecules of DNA in the post-enrichment mixture that correspond to the locus divided by the percentage of molecules of DNA in the pre-enrichment mixture that correspond to the locus. Preferential enrichment may be carried out at a plurality of loci. In some embodiments of the present disclosure, the degree of enrichment is greater than 20. In some embodiments of the present disclosure, the degree of enrichment is greater than 200. In some embodiments of the present disclosure, the degree of enrichment is greater than 2,000. When preferential enrichment is carried out at a plurality of loci, the degree of enrichment may refer to the average degree of enrichment of all of the loci in the set of loci.

Amplification refers to a method that increases the number of copies of a molecule, such as a molecule of DNA.

Selective Amplification may refer to a method that increases the number of copies of a particular molecule of DNA, or molecules of DNA that correspond to a particular region of DNA. It may also refer to a method that increases the number of copies of a particular targeted molecule of DNA, or targeted region of DNA more than it increases non-targeted molecules or regions of DNA. Selective amplification may be a method of preferential enrichment.

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Universal Priming Sequence refers to a DNA sequence that may be appended to a population of target DNA molecules, for example by ligation, PCR, or ligation mediated PCR. Once added to the population of target molecules, primers specific to the universal priming sequences can be used to amplify the target population using a single pair of amplification primers. Universal priming sequences are typically not related to the target sequences.

Universal Adapters, or 'ligation adaptors' or 'library tags' are DNA molecules containing a universal priming sequence that can be covalently linked to the 5-prime and 3-prime end of a population of target double stranded DNA molecules. The addition of the adapters provides universal priming sequences to the 5-prime and 3-prime end of the target population from which PCR amplification can take place, amplifying all molecules from the target population, using a single pair of amplification primers.

Targeting refers to a method used to selectively amplify or otherwise preferentially enrich those molecules of DNA that correspond to a set of loci, in a mixture of DNA.

Joint Distribution Model refers to a model that defines the probability of events defined in terms of multiple random variables, given a plurality of random variables defined on the same probability space, where the probabilities of the variable are linked. In some embodiments, the degenerate case where the probabilities of the variables are not linked may be used.

Percent identity in reference to nucleic acid sequences refers to the degree of sequence identity between nucleic acid sequences.

BRIEF DESCRIPTION OF THE DRAWINGS

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

The presently disclosed embodiments will be further explained with reference to the attached drawings, wherein like structures are referred to by like numerals throughout the several views. The drawings shown are not necessarily to scale, with emphasis instead generally being placed upon illustrating the principles of the presently disclosed embodiments.

FIG. 1: Graphical representation of direct multiplexed mini-PCR method.

FIG. 2: Graphical representation of semi-nested mini-PCR method.

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FIG. 3: Graphical representation of fully nested mini-PCR method.

FIG. 4: Graphical representation of hemi-nested mini-PCR method.

FIG. 5: Graphical representation of triply hemi-nested mini-PCR method.

FIG. 6: Graphical representation of one-sided nested mini-PCR method.

FIG. 7: Graphical representation of one-sided mini-PCR method.

FIG. 8: Graphical representation of reverse semi-nested mini-PCR method.

FIG. 9: Some possible workflows for semi-nested methods.

FIG. 10: Graphical representation of looped ligation adaptors.

FIG. 11: Graphical representation of internally tagged primers.

FIG. 12: An example of some primers with internal tags (SEQ ID NOs: 44,610-44,621).

FIG. 13: Graphical representation of a method using primers with a ligation adaptor binding region.

FIG. 14: Simulated ploidy call accuracies for counting method with two different analysis techniques.

FIG. 15: Ratio of two alleles for a plurality of SNPs in a cell line in Experiment 4.

FIG. 16: Ratio of two alleles for a plurality of SNPs in a cell line in Experiment 4 sorted by chromosome.

FIGs. 17A-17D: Ratio of two alleles for a plurality of SNPs in four pregnant women plasma samples, sorted by chromosome.

FIG. 18: Fraction of data that can be explained by binomial variance before and after data correction.

FIG. 19: Graph showing relative enrichment of fetal DNA in samples following a short library preparation protocol.

FIG. 20: Depth of read graph comparing direct PCR and semi-nested methods.

FIG. 21: Comparison of depth of read for direct PCR of three genomic samples.

FIG. 22: Comparison of depth of read for semi-nested mini-PCR of three samples.

FIG. 23: Comparison of depth of read for 1,200-plex and 9,600-plex reactions.

FIG. 24: Read count ratios for six cells at three chromosomes.

FIGs. 25A-25C: Allele ratios for two three-cell reactions (FIGs. 25B and 25C) and a third reaction run on 1 ng of genomic DNA at three chromosomes (FIG. 25A).

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FIGs. 26A and 26B: Allele ratios for two single-cell reactions (FIGs. 26A and 26B) at three chromosomes.

FIG. 27: Comparison of two primer libraries showing the number of loci with a particular minor allele frequency that are targeted by each primer library.

FIG. 28A: Graph of the electrophoresis of PCR products.

FIGs. 28B-28M are electropherograms of lanes 1-12, respectively, in FIG. 28A.

FIGs. 29A-29E: Cartoon depiction of a method of the invention for the determination of a fetal aneuploidy (FIG. 29A). Maternal and paternal genotype data (from blood or buccal swabs) and crossover frequency data from the HapMap database are utilized to generate (FIG. 29B) multiple independent hypotheses for each potential fetal ploidy state *in silico* (FIG. 29C). Each of these hypotheses is expanded to include sub-hypotheses with take into consideration the different possible crossover points. The data model predicts what the sequencing data would look like (the expected allele distributions) given each hypothetical fetal genotype and at different fetal cfDNA fractions, and is compared to the actual sequencing data; the likelihood for each hypothesis is determined using Bayesian statistics. In this hypothetical example, the hypotheses with the highest likelihoods (euploidy) are determined (FIG. 29D). The individual likelihoods from FIG. 29C are summed for each copy number hypothesis family (monosomy, disomy, or triploidy). The hypothesis with the maximum likelihood is called as the ploidy state, reveals the fetal fraction, and represents the sample-specific calculated accuracy.

FIGs. 30A-30H: Typical graphical representations of euploidy (FIGs. 30A-30C), monosomy (FIG. 30D), and trisomy (FIGs. 30E-30H). For all plots, the x-axis represents the linear position of the individual polymorphic loci along each chromosome (as indicated below the plots), and the y-axis represents the number of A allele reads as a fraction of the total (A+B) allele reads. Maternal and fetal genotypes, as well as the position on the y-axis around which the bands are centered, are indicated to the right of the plots. If desired to facilitate visualization, the plots may be color-coded according to maternal genotype, such that red indicates a maternal genotype of AA, blue indicates a maternal genotype of BB, and green indicates a maternal genotype of AB. If desired, maternal allele contributions may be indicated in color in the "Fetal Genotype" column. Allele contributions are indicated as maternal|fetal, such that alleles for which the mother is AA and the fetus is AB are indicated as AA|AB. FIG. 30A: Generated plots when two chromosomes are present and the fetal cfDNA

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fraction is 0%. This plot is from a non-pregnant woman, and thus represents the pattern when the genotype is entirely maternal. Allele clusters are thus centered around 1 (AA alleles), 0.5 (AB alleles), and 0 (BB alleles). FIG. 30B**:** Generated plot when two chromosomes are present and the fetal fraction is 12%. The contribution of fetal alleles to the fraction of A allele reads shifts the position of some allele spots up or down along the y-axis, such that the bands are centered around 1 (AA|AA alleles), 0.94 (AA|AB alleles), 0.56 (AB|AA alleles), 0.50 (AB|AB alleles), 0.44 (AB|BB alleles), 0.06 (BB|AB alleles), and 0 (BB|BB alleles). FIG. 30C. Generated plot when two chromosomes are present and the fetal fraction is 26%. The pattern, including two red and two blue peripheral bands and a trio of central green bands, is readily apparent. Bands are centered around 1 (AA|AA alleles), 0.87 (AA|AB alleles), 0.63 (AB|AA alleles), 0.50 (AB|AB alleles), 0.37 (AB|BB alleles), 0.13 (BB|AB alleles), and 0 (BB|BB alleles). FIG. 30D: Generated plot when one chromosome is present and the fetal fraction is 26%. The hallmark pattern of one external red and one external blue peripheral band as well as two central green bands indicated maternally-inherited monosomy. Because the fetus only contributes a single allele (A or B) to the allele reads, the internal peripheral red and blue bands are not present, and the center trio of bands condenses into two bands. Bands that are centered around 1 (AA|A alleles), 0.57 (AB|A alleles), 0.43 (AB|B alleles), and 0 (BB|B alleles). FIG. 30E: Generated plot when three chromosomes are present and the fetal fraction is 27%. This pattern of two red and two blue peripheral bands as well as two central green bands indicates maternally-inherited meiotic trisomy. Bands are centered around 1 (AA|AAA alleles), 0.88 (AA|AAB alleles), 0.56 (AB|AAB alleles), 0.44 (AB|ABB alleles), 0.12 (BB|ABB alleles), and 0 (BB|BBB alleles). FIG. 30F: Generated plot when three chromosomes are present and the fetal fraction is 14%. This pattern of three red and three blue peripheral bands, as well as two central green bands, indicates paternally-inherited meiotic trisomy. Bands are centered around 1 (AA|AAA alleles), 0.93 (AA|AAB alleles), 0.87 (AA|ABB alleles), 0.60 (AB|AAA alleles), 0.53 (AB|AAB alleles), 0.47 (AB|ABB alleles), 0.40 (AB|BBB alleles), 0.13 (BB|AAB alleles), 0.07 (BB|ABB alleles), and 0 (BB|BBB alleles). FIG. 30G: Generated plot when three chromosomes are present and the fetal fraction is 35%. This pattern of two red and two blue peripheral bands and four central green bands indicates maternally-inherited mitotic trisomy. Bands are centered around 1 (AA|AAA alleles), 0.85 (AA|AAB alleles), 0.72 (AB|AAA alleles), 0.57 (AB|AAB alleles), 0.43 (AB|ABB alleles), 0.28 (AB|BBB alleles), 0.15 (BB|ABB alleles), and 0 (BB|BBB alleles). FIG. 30H: Generated

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plot when three chromosomes are present and the fetal fraction is 25%. This pattern of two red and two blue peripheral bands as well as four central green bands indicates paternally-inherited mitotic trisomy. This pattern can be distinguished from that of maternally-inherited mitotic trisomy (as in FIG. 30G) by the position of the internal peripheral bands. Specifically, bands are centered around 1 (AA|AAA alleles), 0.78 (AA|ABB alleles), 0.67 (AB|AAA alleles), 0.56 (AB|AAB alleles), 0.44 (AB|ABB alleles), 0.33 (AB|BBB alleles), 0.22 (BB|AAB alleles), and 0 (BB|BBB alleles).

FIG. 31: Graphical representations of (FIG. 31A) euploid, (FIG. 31B) T13, (FIG. 31C) T18, (FIG. 31D) T21, (FIG. 31E) 45,X, and (FIG. 31F) 47,XXY test samples as indicated. Each chromosome is indicated at the top of the plot, fetal and maternal genotypes are indicated to the right of the plots, the x-axis represents the linear position of the SNPs along each chromosome, and the y-axis indicates the number of A allele reads as a fraction of the total reads. Note the altered cluster positioning based on fetal fraction, as described herein. Each spot represents a single SNP locus. Fetal and maternal genotypes are indicated to the right of the plot, and chromosome identities are indicated at the top of the plots.

FIG. 32: The combined at-birth prevalence of sex chromosome aneuploidies is greater than that of autosomal aneuploidies.

FIGs. 33A-33F: Illustrations of the calculation of an interaction score between primers in a primer library. FIG. 33A shows the first two bases (dinucleotide) of a primer that align to the other primer for calculation of ΔG. Iterate over the remainder of the primer that aligns with the other primer by sliding the bases being observing one base to the right. Continue until ΔG has been calculated for all pairs of bases that align (FIG. 33B). Shift the alignment of the two primers (FIGs. 33C and 33D). Determine ΔG for the new alignment (FIGs. 33E and 33F).

FIG. 34: Sequences of an exemplary 1,200-plex primer library (SEQ ID NOs. 1-3,600). The three primers (one from pool A, one from pool B, and one from pool C) in each row all hybridize to the same target locus.

FIG. 35: Sequences of an exemplary 2,686-plex primer library (SEQ ID NOs. 3,601-11,658). The three primers (one from pool A, one from pool B, and one from pool C) in each row all hybridize to the same target locus.

FIG. 36: Sequences of an exemplary 10,984-plex primer library (SEQ ID NOs 11,659-44,609). The three primers (one from pool A, one from pool B, and one from pool C) in each row all hybridize to the same target locus.

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FIG. 37: Table of the percentage of reads that map to target loci for genomic DNA samples and for samples of a single cell from a cell line for both mother and child samples.

FIG. 38: Overlay of depth of read for a genomic (blue) and a single cell (red) sample for different SNPs.

FIG. 39: Table of the percentage of reads that map to target loci for blastoceol fluid and for a single blastocyst cell.

FIG. 40. Graph of reference counts (counts of one allele, such as the "A" allele) divided by total counts for that locus for a single blastocyst cell.

While the above-identified drawings set forth presently disclosed embodiments, other embodiments are also contemplated, as noted in the discussion. This disclosure presents illustrative embodiments by way of representation and not limitation. Numerous other modifications and embodiments can be devised by those skilled in the art which fall within the scope and spirit of the principles of the presently disclosed embodiments.

DETAILED DESCRIPTION

The present invention is based in part on the surprising discovery that often only a relatively small number of primers in a library of primers are responsible for a substantial amount of the amplified primer dimers that form during multiplex PCR reactions. Methods have been developed to select the most undesirable primers for removal from a library of candidate primers. By reducing the amount of primer dimers to a negligible amount (~0.1% of the PCR products), these methods allow the resulting primer libraries to simultaneously amplify a large number of target loci in a single multiplex PCR reaction. Because the primers hybridize to the target loci and amplify them rather than hybridizing to other primers and forming amplified primer dimers, the number of different target loci that can be amplified is increased. It was also discovered that using lower primer concentrations and much longer annealing times than normal increases the likelihood that the primers hybridize to the target loci instead of hybridizing to each other and forming primer dimers (see, *e.g.*, U.S.S.N. 13/683,604, filed Nov. 21, 2012, which is hereby incorporated by reference in its entirety).

During the PCR amplification and sequencing of 19,488 target loci in a genomic sample, 99.4-99.7% of the sequencing reads mapped to the genome, of those, 99.99% of the mapped to target loci. For plasma samples with 10 million sequencing reads, typically at least

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19,350 of the 19,488 target loci (99.3 %) were amplified and sequenced. This primer library has even been used to amplify the nucleic acids in a single cell (FIGs. 37-40).

During the PCR amplification and sequencing of ~28,000 target loci in a genomic sample, 99% of the sequencing reads mapped to target loci. This primer library has also been used to amplify nucleic acids in a single cell.

Being able to simultaneously amplify such a large number of target loci at once greatly decreases the amount of time and the amount of DNA required to analyze thousands of target loci. For example, DNA from a single cell is sufficient to simultaneously analyze thousands of target loci, which is important for applications in which the amount of DNA is low, such as genetic testing of a single cell from an embryo prior to *in vitro* fertilization or genetic testing of a forensic sample with little DNA. In addition, being able to analyze the target loci in one reaction volume (such as in one chamber, well, or vessel) rather than splitting the sample into multiple different reactions reduces variability that can occur between reactions. In addition, methods have been developed to use reference standards to correct for amplification bias that may occur between different target loci. For example, differences in amplification efficiency between target loci due to factors such as GC content may cause differing amounts of PCR products to be produced for target loci that are actually present in the same amount. The use of reference standards similar to the target loci allows the detection of such amplification bias so that it can be corrected for during the quantitation of the target loci.

During sequencing of PCR products, artifacts such as primer dimers are detected and thus inhibit the detection of target amplicons. Because of this limitation, microarrays with hybridization probes are often used for detection since microarrays are less sensitive to interference from primer dimers (for example, microarrays can be used as a target specific detection that uses probes to hybridize to target amplicons but does not have probes to hybridize to undesired primer dimers). The high level of multiplexing with minimal non-target amplicons that has now been achieved allows PCR followed by sequencing to be used as an alternative to microarrays. For example, the present multiplex PCR methods can be used with a non-target specific method of detection, such as sequencing that detects all amplified products (including both target amplicons and primer dimers, if any). The small amount of primer dimers that are produced allows detection of target amplicons by methods that detect all amplicons.

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The multiplex-PCR methods of the invention can be in a variety of applications, such as genotyping, detection of chromosomal abnormalities (such as a fetal chromosome aneuploidy), gene mutation and polymorphism (such as single nucleotide polymorphisms, SNPs) analysis, gene deletion analysis, determination of paternity, analysis of genetic differences among populations, forensic analysis, measuring predisposition to disease, quantitative analysis of mRNA, and detection and identification of infectious agents (such as bacteria, parasite, and viruses). The multiplex PCR methods can also be used for non-invasive prenatal testing, such as paternity testing or the detection of fetal chromosome abnormalities.

Exemplary Primer Design Methods

Highly multiplexed PCR can often result in the production of a very high proportion of product DNA that results from unproductive side reactions such as primer dimer formation. In an embodiment, the particular primers that are most likely to cause unproductive side reactions may be removed from the primer library to give a primer library that will result in a greater proportion of amplified DNA that maps to the genome. The step of removing problematic primers, that is, those primers that are particularly likely to firm dimers has unexpectedly enabled extremely high PCR multiplexing levels for subsequent analysis by sequencing. In systems such as sequencing, where performance significantly degrades by primer dimers and/or other mischief products, greater than 10, greater than 50, and greater than 100 times higher multiplexing than other described multiplexing has been achieved. Note this is opposed to probe based detection methods, *e.g.* microarrays, TAQMAN, PCR etc. where an excess of primer dimers will not affect the outcome appreciably. Also note that the general belief in the art is that multiplexing PCR for sequencing is limited to about 100 assays in the same well. Fluidigm and Rain Dance offer platforms to perform 48 or 1000s of PCR assays *in parallel reactions* for one sample.

There are a number of ways to choose primers for a library where the amount of non-mapping primer dimer or other primer mischief products are minimized. Empirical data indicate that a small number of 'bad' primers are responsible for a large amount of non-mapping primer dimer side reactions. Removing these 'bad' primers can increase the percent of sequence reads that map to targeted loci. One way to identify the 'bad' primers is to look at the sequencing data of DNA that was amplified by targeted amplification; those primer dimers that are seen with greatest frequency can be removed to give a primer library that is

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significantly less likely to result in side product DNA that does not map to the genome. There are also publicly available programs that can calculate the binding energy of various primer combinations, and removing those with the highest binding energy will also give a primer library that is significantly less likely to result in side product DNA that does not map to the genome.

In some embodiments for selecting primers, an initial library of candidate primers is created by designing one or more primers or primer pairs to candidate target loci. A set of candidate target loci (such as SNPs) can selected based on publically available information about desired parameters for the target loci, such as frequency of the SNPs within a target population or the heterozygosity rate of the SNPs. In one embodiment, the PCR primers may be designed using the Primer3 program (the worldwide web at primer3.sourceforge.net; libprimer3 release 2.2.3, which is hereby incorporated by reference in its entirety). If desired, the primers can be designed to anneal within a particular annealing temperature range, have a particular range of GC contents, have a particular size range, produce target amplicons in a particular size range, and/or have other parameter characteristics. Starting with multiple primers or primer pairs per candidate target locus increases the likelihood that a primer or prime pair will remain in the library for most or all of the target loci. In one embodiment, the selection criteria may require that at least one primer pair per target locus remains in the library. That way, most or all of the target loci will be amplified when using the final primer library. This is desirable for applications such as screening for deletions or duplications at a large number of locations in the genome or screening for a large number of sequences (such as polymorphisms or other mutations) associated with a disease or an increased risk for a disease. If a primer pair from the library would produces a target amplicon that overlaps with a target amplicon produced by another primer pair, one of the primer pairs may be removed from the library to prevent interference.

In some embodiments, a score such as an "undesirability score" (higher score representing least desirability) is calculated (such as calculation on a computer) for most or all of the possible combinations of two primers from a library of candidate primers. In various embodiments, a score (such as an undesirability score) is calculated for at least 80, 90, 95, 98, 99, or 99.5% of the possible combinations of candidate primers in the library. Each score (such as an undesirability score) is based at least in part on the likelihood of dimer formation between the two candidate primers. If desired, the score (such as the undesirability score) may

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also be based on one or more other parameters selected from the group consisting of heterozygosity rate of the target locus, disease prevalence associated with a sequence (*e.g.*, a polymorphism) at the target locus, disease penetrance associated with a sequence (*e.g.*, a polymorphism) at the target locus, specificity of the candidate primer for the target locus, size of the candidate primer, melting temperature of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, GC content of the 3' end of the candidate primer, homopolymer length in the candidate primer, amplification efficiency of the target amplicon, size of the target amplicon. number of SNPs within the candidate primer, location of SNPs within the candidate primer, distance from an end of the amplicon to the target bases within the amplicon, and the number of target loci in an amplicon. In some embodiments, the lower the number of SNPs with the candidate primer (such as 2, 1 or 0 SNPs) the better. In some embodiments, there are no SNPs in the candidate primer. In some embodiments, SNPs (if any) are preferably not in the last 5 nucleotides in the 3' end of the candidate primer. In some embodiments, the target bases (the bases of interest in a target locus) are preferably near an end (the 3' or 5' end) of the amplicon; this may improve the quality of sequencing data (since bases near the end of an amplicon are sequenced more accurately), and/or allow shorter sequencing reads to be performed. In some embodiments, a single amplicon includes 2 or more target loci (such as 2 or more nearby SNPs or variants). In some embodiments, the specificity of the candidate primer for the target locus includes the likelihood that the candidate primer will mis-prime by binding and amplifying a locus other than the target locus it was designed to amplify. In some embodiments, one or more or all the candidate primers that mis-prime are removed from the library. In some embodiments to increase the number of candidate primers to choose from, candidate primers that may mis-prime are not removed from the library. In some embodiments, the optimal melting temperature for selection of the candidate primers is 57 °C. In some embodiments, the optimal size for selection of the candidate primers is a length of 24 nucleotides. In some embodiments, the optimal GC content for selection of the candidate primers is 50%. If multiple factors are considered, the score (such as the undesirability score) may be calculated based on a weighted average of the various parameters. The parameters may be assigned different weights based on their importance for the particular application that the primers will be used for. An exemplary score (such as an undesirability score) for a primer is shown below in which the parameters are weighted by various factors.

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score = (1)(total number of targets - number of targets covered) + (100)(number of SNPs in GC clamp) + (10)(number of SNPs in primer binding site) + (10)(number of similar primer pair designs) + (0.1)(distance of target base from amplicon end) + (0.1)(amplicon length) + (100) (interaction score)

where interaction score = max (-1 * ΔG value) as described herein

Another exemplary score for a primer is shown below.

score = (100)(number of SNPs in GC clamp) + (10)(number of SNPs in primer binding site) + (0.1)(distance of target base from amplicon end) + (0.1)(amplicon length) + (100) (interaction score)

where interaction score = max (-1 * ΔG value) as described herein

In some embodiments, the score for a primer pair is the worse score out of the scores for the two primers in the pair. An exemplary score (such as an undesirability score or the score in Experiment 20) for a pairs of designs (in which each design is one primer pair so that a pair of designs includes two primer pairs with a total of 4 primers) is shown below.

score = (10000000)(amplicon overlap) + (100)(distance between designs) + (1)(total number of targets - number of targets covered) + (100)(number of SNPs in GC clamp) + (10)(number of SNPs in primer binding site) + (10)(number of similar primer pair designs) + (0.1)(distance of target base from amplicon end) + (0.1)(amplicon length) + (100) (interaction score)

where interaction score = max (-1 * ΔG value) as described herein;

where amplicon overlap = overlap between the two amplicons formed by a pair of designs

In some embodiments, the score for a pair of designs is the worse score out of the scores for the four primers in the pair of designs.

In some embodiments, the primer with the highest score (such as the undesirability score) is removed from the library. If the removed primer is a member of a primer pair that hybridizes to one target locus, then the other member of the primer pair may be removed from the library. The process of removing primers may be repeated as desired. In some embodiments, the selection method is performed until the score (such as the undesirability score) for the candidate primer combinations remaining in the library are all equal to or below

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a minimum threshold. In some embodiments, the selection method is performed until the number of candidate primers remaining in the library is reduced to a desired number.

In various embodiments, after the score (such as the undesirability score) are calculated, the candidate primer that is part of the greatest number of combinations of two candidate primers with a score (such as an undesirability score) above a first minimum threshold is removed from the library. This step ignores interactions equal to or below the first minimum threshold since these interactions are less significant. If the removed primer is a member of a primer pair that hybridizes to one target locus, then the other member of the primer pair may be removed from the library. The process of removing primers may be repeated as desired. In some embodiments, the selection method is performed until the score (such as the undesirability score) for the candidate primer combinations remaining in the library are all equal to or below the first minimum threshold. If the number of candidate primers remaining in the library is higher than desired, the number of primers may be reduced by decreasing the first minimum threshold to a lower second minimum threshold and repeating the process of removing primers. If the number of candidate primers remaining in the library is lower than desired, the method can be continued by increasing the first minimum threshold to a higher second minimum threshold and repeating the process of removing primers using the original candidate primer library, thereby allowing more of the candidate primers to remain in the library. In some embodiments, the selection method is performed until the score (such as the undesirability score) for the candidate primer combinations remaining in the library are all equal to or below the second minimum threshold, or until the number of candidate primers remaining in the library is reduced to a desired number.

If desired, primer pairs that produce a target amplicon that overlaps with a target amplicon produced by another primer pair can be divided into separate amplification reactions. Multiple PCR amplification reactions may be desirable for applications in which it is desirable to analyze all of the candidate target loci (instead of omitting candidate target loci from the analysis due to overlapping target amplicons).

In various embodiments of any of the aspects of the invention, the selection method selects candidate primers and divides them into different pools (*e.g.*, 2, 3, 4, 5, 6, or more different pools). Each pool can be used to simultaneously amplify a large number of target loci (or a subset of target loci) in a single reaction volume. In some embodiments, a graph coloring algorithm is used to divide candidate primers into different pools. If desired, this

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method can be used to minimize the number of different pools needed to amplify most or all of the target loci.

In some embodiments, most or all of the target loci (such as at least 70, 80, 90, 92, 94, 96, 98, 99, or 100% of the target loci) are amplified by at least 2, 3, 4, 5, 6, or more different pools. In some embodiments, most or all of the bases in the target loci (such as at least 70, 80, 90, 92, 94, 96, 98, 99, or 100% of the bases in the target loci) are amplified by at least 2,3, 4, 5, 6, or more different pools. In some embodiments, most or all of the bases in the target loci (such as at least 70, 80, 90, 92, 94, 96, 98, 99, or 100% of the bases in the target loci) are amplified by at least 2, 3, 4, 5, 6, or more different primers or primer pairs in different pools. For example, a particular base in a target locus may be amplified by at least 2, 3, 4, 5, 6, or more different primers or primer pairs; wherein each different primer or primer pair is in a different pool. Using different primers or primer pairs to amplify each base allows multiple independent measurements of the base to be made, thereby increasing the accuracy of the method. Dividing the different primers or primer pairs that amplify the same base into different pools prevents interference due to overlapping amplicons being formed by different primers or primer pairs.

In one aspect, the invention features methods of selecting test primers from a library of candidate primers to form 2 or more different primer pools. In various embodiments, the selection involves (i) calculating on a computer a score (such as an undesirability score) for most or all of the possible combinations of two candidate primers from the library, wherein each score (such as an undesirability score) is based at least in part on the likelihood of dimer formation between the two candidate primers; (ii) removing the candidate primer with the highest score (such as an undesirability score) from the library of candidate primers; and (iii) if the candidate primer removed in step (ii) is a member of a primer pair, then removing the other member of the primer pair from the library of candidate primers; and (iv) optionally repeating steps (ii) and (iii), thereby selecting a first pool. In some embodiments, the selection method is performed until the score (such as the undesirability score) for the candidate primer combinations remaining in the library are all equal to or below a minimum threshold for the first pool. In some embodiments, the selection method is performed until the number of candidate primers remaining in the library is reduced to a desired number for the first pool. In some embodiments, after the first pool is selected those primers are removed from further consideration and steps of the method (such as steps (ii) and (iii)) are repeated with the

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remaining primers to select a second pool. If desired, this method may be repeated to select the desired number of primer pools. In some embodiments, the score is based at least in part on the current coverage of the bases in the target locus (such as the number of other primer pools that have a primer or primer pair that amplifies a particular base in the target locus).

In one aspect, the invention features methods of selecting test primers from a library of candidate primers to form 2 or more different primer pools. In various embodiments, the selection of test primers are selected from a library of candidate primers involves (i) calculating on a computer a score (such as an undesirability score) for most or all of the possible combinations of two candidate primers from the library, wherein each score (such as an undesirability score) is based at least in part on the likelihood of dimer formation between the two candidate primers; (ii) removing from the library of candidate primers the candidate primer that is part of the greatest number of combinations of two candidate primers with a score (such as an undesirability score) above a first minimum threshold; (iii) if the candidate primer removed in step (ii) is a member of a primer pair, then removing the other member of the primer pair from the library of candidate primers; and (iv) optionally repeating steps (ii) and (iii), thereby selecting a first pool. In some embodiments, the selection method is performed until the score (such as the undesirability score) for the candidate primer combinations remaining in the library are all equal to or below the first minimum threshold for the first pool. In some embodiments, the selection method is performed until the number of candidate primers remaining in the library is reduced to a desired number for the first pool. In various embodiments, the selection method involves further reducing the number of candidate primers remaining in the library by decreasing the first minimum threshold used in step (ii) to a lower second minimum threshold and optionally repeating steps (ii) and (iii). In some embodiments, the selection method involves increasing the first minimum threshold used in step (ii) to a higher second minimum threshold and optionally repeating steps (ii) and (iii). In some embodiments, the selection method is performed until the score (such as the undesirability score) for the candidate primer combinations remaining in the library are all equal to or below the second minimum threshold, or until the number of candidate primers remaining in the library is reduced to a desired number for the first pool. In some embodiments, after the first pool is selected those primers are removed from further consideration and steps of the method (such as steps (ii) and (iii)) are repeated with the remaining primers to select a second pool. If desired, this method may be repeated to select

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the desired number of primer pools. In some embodiments, the score is based at least in part on the current coverage of the bases in the target locus (such as the number of other primer pools that have a primer or primer pair that amplifies a particular base in the target locus).

As discussed above, in some embodiments, a library is formed by starting with a library of candidate primers and removing primers until the primers remaining in the library have the desired characteristics for use as a final primer library.

In other embodiments, candidate primers are added to a library (such as a library starting with no primers) to form a library with the desired characteristics. In some embodiments, the candidate primer or primer pair with the most desirable score (such as the lowest undesirability score) is added to a library (such as a library starting with no primers). The process of adding candidate primers may be repeated as desired. In some embodiments, the selection method is performed until the score (such as the undesirability score) for the candidate primers that have not been added to the library are all above a minimum threshold (such that all the candidate primers that have not been chosen for the library all have worse scores than the threshold). In some embodiments, the selection method is performed until the number of candidate primers that have been added to the library reaches a desired number. The library of selected primers can then be used in any of the methods of the invention.

In some embodiments, most (such as at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5%) or all of the possible sets of two primer pairs (two primer pairs with a total of 4 primers) are considered for inclusion in a library. In some embodiments, the set of two different candidate primer pair with the most desirable score (such as the lowest undesirability score) is added to a first pool (such as a first pool starting with no primers). In some embodiments, the set of two different candidate primer pairs with the next most desirable score is added to the first pool if it is connected to at most two sets of two different candidate primer pairs in the first pool. By "connected" for purposes of this step is meant that a single candidate primer pair in one set of two different candidate primer pairs is the same as a single candidate primer pair in another set of two different candidate primer pairs. If the set of two different candidate primer pairs is connected to more than two sets, it may be added to a different pool than the first pool. The process of set of two different candidate primer pair to pool(s) may be repeated as desired for the next set of two different candidate primer pairs with the next most desirable score. In some embodiments, the selection method is performed until the score (such as the undesirability score) for the candidate primers that have not been added

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to the pool(s) are all above a minimum threshold (such that all the candidate primers that have not been chosen for the pool(s) all have worse scores than the threshold). In some embodiments, the selection method is performed until the number of candidate primers that have been added to the pool(s) reaches a desired number. In some embodiments, the method involves storing designs in N number of doubly linked list data structures with the design pairs. N represents the current number of different primer pools. Initially, N =1, since there is only one primer pool. In some embodiments, a second pool is only created if necessary to include the desired target loci or the desired level of coverage of target loci. The library of selected primers can then be used in any of the methods of the invention.

In some embodiments, the minimum threshold, the first minimum threshold, or the second minimum threshold is an interaction score equal to or about 20, 18, 16, 14, 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 kcal/mol. In some embodiments, the interaction score is calculated as followed as described further herein:

Interaction score = max (-1 * ΔG value); or

interaction_score = max(- deltaG_2, 0.8 * (- deltaG_1))

where

deltaG_2 = Gibbs energy (energy required to break the dimer) for a dimer that is extensible by PCR on both ends, *i.e,.* the 3' end of each primer anneals to the other primer; and

deltaG_1 = Gibbs energy for a dimer that is extensible by PCR on at least one end.

These selection methods minimize the number of candidate primers that have to be removed from the library to achieve the desired reduction in primer dimers. By removing a smaller number of candidate primers from the library, more (or all) of the target loci can be amplified using the resulting primer library.

Multiplexing large numbers of primers imposes considerable constraint on the assays that can be included. Assays that unintentionally interact result in spurious amplification products. The size constraints of miniPCR may result in further constraints. In an embodiment, it is possible to begin with a very large number of potential SNP targets (between about 500 to greater than 1 million) and attempt to design primers to amplify each SNP. Where primers can

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be designed it is possible to attempt to identify primer pairs likely to form spurious products by evaluating the likelihood of spurious primer duplex formation between all possible pairs of primers using published thermodynamic parameters for DNA duplex formation. Primer interactions may be ranked by a scoring function related to the interaction and primers with the worst interaction scores are eliminated until the number of primers desired is met. In cases where SNPs likely to be heterozygous are most useful, it is possible to also rank the list of assays and select the most heterozygous compatible assays. Experiments have validated that primers with high interaction scores are most likely to form primer dimers. At high multiplexing it is not possible to eliminate all spurious interactions, but it is essential to remove the primers or pairs of primers with the highest interaction scores *in silico* as they can dominate an entire reaction, greatly limiting amplification from intended targets. We have performed this procedure to create multiplex primer sets of up to and in some cases more than 10,000 primers. The improvement due to this procedure is substantial, enabling amplification of more than 80%, more than 90%, more than 95%, more than 98%, and even more than 99% on target products as determined by sequencing of all PCR products, as compared to 10% from a reaction in which the worst primers were not removed. When combined with a partial semi-nested approach as previously described, more than 90%, and even more than 95% of amplicons may map to the targeted sequences.

Note that there are other methods for determining which PCR probes are likely to form dimers. In an embodiment, analysis of a pool of DNA that has been amplified using a non-optimized set of primers may be sufficient to determine problematic primers. For example, analysis may be done using sequencing, and those dimers which are present in the greatest number are determined to be those most likely to form dimers, and may be removed.

This method has a number of potential application, for example to SNP genotyping, heterozygosity rate determination, copy number measurement, and other targeted sequencing applications. In an embodiment, the method of primer design may be used in combination with the mini-PCR method described elsewhere in this document. In some embodiments, the primer design method may be used as part of a massive multiplexed PCR method.

The use of tags on the primers may reduce amplification and sequencing of primer dimer products. In some embodiments, the primer contains an internal region that forms a loop structure with a tag. In particular embodiments, the primers include a 5' region that is specific for a target locus, an internal region that is not specific for the target locus and forms a loop

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structure, and a 3' region that is specific for the target locus. In some embodiments, the loop region may lie between two binding regions where the two binding regions are designed to bind to contiguous or neighboring regions of template DNA. In various embodiments, the length of the 3' region is at least 7 nucleotides. In some embodiments, the length of the 3' region is between 7 and 20 nucleotides, such as between 7 to 15 nucleotides, or 7 to 10 nucleotides, inclusive. In various embodiments, the primers include a 5' region that is not specific for a target locus (such as a tag or a universal primer binding site) followed by a region that is specific for a target locus, an internal region that is not specific for the target locus and forms a loop structure, and a 3' region that is specific for the target locus. Tag-primers can be used to shorten necessary target-specific sequences to below 20, below 15, below 12, and even below 10 base pairs. This can be serendipitous with standard primer design when the target sequence is fragmented within the primer binding site or, or it can be designed into the primer design. Advantages of this method include: it increases the number of assays that can be designed for a certain maximal amplicon length, and it shortens the "non-informative" sequencing of primer sequence. It may also be used in combination with internal tagging (see elsewhere in this document).

In an embodiment, the relative amount of nonproductive products in the multiplexed targeted PCR amplification can be reduced by raising the annealing temperature. In cases where one is amplifying libraries with the same tag as the target specific primers, the annealing temperature can be increased in comparison to the genomic DNA as the tags will contribute to the primer binding. In some embodiments we are using considerably lower primer concentrations than previously reported along with using longer annealing times than reported elsewhere. In some embodiments the annealing times may be longer than 3 minutes, longer than 5 minutes, longer than 8 minutes, longer than 10 minutes, longer than 15 minutes, longer than 20 minutes, longer than 30 minutes, longer than 60 minutes, longer than 120 minutes, longer than 240 minutes, longer than 480 minutes, and even longer than 960 minutes. In an embodiment, longer annealing times are used than in previous reports, allowing lower primer concentrations. In various embodiments, longer than normal extension times are used, such as greater than 3, 5, 8, 10, or 15 minutes. In some embodiments, the primer concentrations are as low as 50 nM, 20 nM, 10 nM, 5 nM, 1 nM, and lower than 1 uM. This surprisingly results in robust performance for highly multiplexed reactions, for example 1,000-plex reactions, 2,000-plex reactions, 5,000-plex reactions, 10,000-plex reactions, 20,000-plex reactions, 50,000-plex

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reactions, and even 100,000-plex reactions. In an embodiment, the amplification uses one, two, three, four or five cycles run with long annealing times, followed by PCR cycles with more usual annealing times with tagged primers.

To select target locations, one may start with a pool of candidate primer pair designs and create a thermodynamic model of potentially adverse interactions between primer pairs, and then use the model to eliminate designs that are incompatible with other the designs in the pool.

In an embodiment, the invention features a method of decreasing the number of target loci (such as loci that may contain a polymorphism or mutation associated with a disease or disorder or an increased risk for a disease or disorder such as cancer) that need to be detected for a diagnosis and/or increasing the disease load that is detected (*e.g.*, increasing the number of polymorphisms or mutations that are detected). In some embodiments, the method includes ranking (such as ranking from highest to lowest) loci by frequency or reoccurrence of a polymorphism or mutation (such as a single nucleotide variation, insertion, or deletion, or any of the other variations described herein) in each locus among subjects with the disease or disorder such as cancer. In some embodiments, PCR primers are designed to some or all of the loci. During selection of PCR primers for a library of primers, primers to loci that have a higher frequency or reoccurrence (higher ranking loci) are favored over those with a lower frequency or reoccurrence (lower ranking loci). In some embodiments, this parameter is included as one of the parameters in the calculation of the scores (such as the undesirability scores) described herein. If desired, primers (such as primers to high ranking loci) that are incompatible with other designs in the library can be included in a different PCR library/pool. In some embodiments, multiple libraries/pools (such as 2, 3, 4, 5 or more) are used in separate PCR reactions to enable amplification of all (or a majority) of the loci represented by all the libraries/pools. In some embodiment, this method is continued until sufficient primers are included in one or more libraries/pools such that the primers, in aggregate, enable the desired disease load to be captured for the disease or disorder (*e.g.*, such as by detection of at least 80, 85, 90, 95, or 99% of the disease load).

After the selection process, the primers remaining in the library may be used in any of the methods of the invention.

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Exemplary Methods for Determining Interaction Scores

Exemplary methods of determining a ΔG value or an interaction score that indicates the likelihood of dimer formation between candidate primers are described below. In some embodiments, a thermodynamic Nearest-Neighbors approach is used to calculate the likelihood of dimer formation between any two primers (*see, e.g.*, Rahmann and Grafe (2004), "Mean and variance of the Gibbs free energy of oligonucleotides in the nearest neighbor model under varying conditions" Bioinformatics 20, 2928-2933; Allawi, H. T. & SantaLucia, J., Jr. (1998), "Thermodynamics of Internal C-T Mismatches in DNA", *Nucleic Acids Res. 26*, 2694-2701; Peyret, N., Seneviratne, P. A., Allawi, H. T. & SantaLucia, J., Jr. (1999), "Nearest-Neighbor Thermodynamics and NMR of DNA Sequences with Internal A-A, C-C, G-G, and T-T Mismatches", *Biochemistry 38*, 3468-3477; Allawi, H. T. & SantaLucia, J., Jr. (1998), "Nearest-Neighbor Thermodynamics of Internal A-C Mismatches in DNA: Sequence Dependence and pH Effects", *Biochemistry 37*, 9435-9444.; Allawi, H. T. & SantaLucia, J., Jr. (1998), "Nearest Neighbor Thermodynamic Parameters for Internal G-A Mismatches in DNA", *Biochemistry 37*, 2170-2179; and Allawi, H. T. & SantaLucia, J., Jr. (1997), "Thermodynamics and NMR of Internal G-T Mismatches in DNA", *Biochemistry 36*, 10581-10594; MultiPLX 2.1 (Kaplinski L, Andreson R, Puurand T, Remm M. MultiPLX: automatic grouping and evaluation of PCR primers. Bioinformatics. 2005 Apr 15;21(8):1701-2, which are each hereby incorporated by reference in its entirety).

In some embodiments, the following steps are performed.

Step 1

For each primer in a set of candidate primers, compare to every other candidate primer in the following way. Implement an ungapped thermodynamic alignment between the two primers, matching the 5' end of the first primer to the 3' end of the second primer. Taking the first two bases (dinucleotide) that align to the other primer and vice versa, determine the ΔH and ΔS values for the dinucleotide in one primer hybridizing to the dinucleotide in the other primer (see the "AT" hybridizing to "GA" in FIG. 33A). ΔH and ΔS values for various combinations of dinucleotides are known and can be determined, for example, using a thermodynamic look up table (such as the Unified NN model parameters according to Allawi and SantaLucia (1997) "Thermodynamics and NMR of internal G-T mismatches in DNA".

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Biochemistry, 36: 10581-10594, which is hereby incorporated by reference in its entirety). Use the ΔH and ΔS values to calculate ΔG for that interaction as follows or as described in any known equation for this.

ΔG = (1000.0 * ΔH - (temperature * (ΔS + 0.368 * (numPhosphates/2) *log(saltConcentration)))) / 1000.0

In some embodiments, one or more of the following conditions are assumed for this calculation: temperature: of 60.0 °C, primer concentration of 100 nM, or salt concentration of 100 mM. In some embodiments, other conditions are assumed for this calculation, such as the conditions that will be used for multiplex PCR with the pool. Iterate over the remainder of the primer that aligns with the other primer by sliding the bases being observing one base to the right. Continue until ΔG has been calculated for all dinucleotides that align (FIG. 33B). The ΔG for that alignment of the primer pair is the sum of the ΔG values for the various dinucleotides.

Step 2

Shift the alignment of the two primers (FIGs. 33C and 33D).

Step 3

Repeat step 1 again for the new alignment (FIGs. 33E and 33F).

Step 4

After aligning all possible alignments between the two primers, determine the final ΔG value and the interaction score.

In some embodiments, the ΔG value for a combination of primers is the lowest ΔG value (the lowest numerical value, which is indicative of the greatest likelihood of primer dimer formation) out of the ΔG values for all possible alignments between the two primers. For example, if one alignment has a ΔG value of -12 kcal/mol and another alignment has a ΔG value of -2 kcal/mol then -12 kcal/mol (worse value) is used as the ΔG value for that combination of primers.

For algorithms such as the one in Experiment 16 in which it is easiest to rank primers based on assigning the worse combination of primers (those with the greatest likelihood of

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dimer formation) the highest interaction score, then the interaction score can be calculated as follows.

Interaction score = max (-1 * ΔG value)

For example, if one alignment has a ΔG value of -12 kcal/mol and another alignment has a ΔG value of -2 kcal/mol, then 12 kcal/mol is used as the interaction score. In this case, the interaction score with the largest numerical positive number indicates the least desirable combination of primers due to the greates likelihood of primer dimer formation.

In some embodiments, the interaction score is calculated as follows (this score weights the ΔG values depending on the number of ends that a dimer can be extended by PCR).

interaction_score = max(- deltaG_2, 0.8 * (- deltaG_1))

where

deltaG_2 = Gibbs energy (energy required to break the dimer) for a dimer that is extensible by PCR on both ends, *i.e,.* the 3' end of each primer anneals to the other primer; and

deltaG_1 = Gibbs energy for a dimer that is extensible by PCR on at least one end.

In some embodiments, deltaG_2 is determined by performing steps 1-4 above for all the alignments in which a dimer is extensible by PCR on both ends. The deltaG_2 value is the lowest ΔG value (the lowest numerical value, which is indicative of the greatest likelihood of primer dimer formation) for all the alignments in which a dimer is extensible by PCR on both ends.

In some embodiments, deltaG_1 is determined by performing steps 1-4 above for all the alignments in which a dimer is extensible by PCR on at least one end (such as by PCR on one end or by PCR on both ends). The deltaG_1 value is the lowest ΔG value (the lowest numerical value, which is indicative of the greatest likelihood of primer dimer formation) for all the alignments in which a dimer is extensible by PCR on at least one end.

In some embodiments, possible loop structures or gaps in alignment between primers are also considered.

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In some embodiments, ΔG values from step 4 for each possible combination of two primers (each possible primer dimer) in a library are all equal to or greater than -20, -18, -16, -14, 12, -10, -9, -8, -7, -6, -5, -4, -3, -2, or -1 kcal/mol. In some embodiments, ΔG values from step 4 for at least 80, 85, 90, 92, 94, 96, 98, 99, or 100% of the primers in the library for possible combinations of that primer with other primers in the library are all equal to or greater than -20, -18, -16, -14, 12, -10, -9, -8, -7, -6, -5, -4, -3, -2, or -1 kcal/mol. In some embodiments, possible combinations of two primers in a library that have positive ΔG values are ignored since these values are indicative of no likelihood to for primer dimers. In some embodiments for the possible combination of two primers in a library that have negative ΔG values, the ΔG values are between -20 and 0 kcal/mol, such as between -15 and 0 kcal/mol, -10 and 0 kcal/mol, -8 and 0 kcal/mol, -7 and 0 kcal/mol, -6 and 0 kcal/mol, -5.5 and 0 kcal/mol, -5 and 0 kcal/mol, -4.5 and 0 kcal/mol, -4 and 0 kcal/mol, -3.5 and 0 kcal/mol, -3 and 0 kcal/mol, -2.5 and 0 kcal/mol, -2 and 0 kcal/mol, or -1.5 and 0 kcal/mol, inclusive.

In some embodiments, the interaction scores from step 4 for each possible combination of two primers in a library are all equal to or less than 20, 18, 16, 14, 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 kcal/mol. In some embodiments, the interaction scores from step 4 for at least 80, 85, 90, 92, 94, 96, 98, 99, or 100% of the primers in the library for possible combinations of that primer with other primers in the library are all equal to or less than 20, 18, 16, 14, 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 kcal/mol. In some embodiments, possible combination of two primers in a library that have negative interaction scores are ignored since these values are indicative of no likelihood to for primer dimers. In some embodiments for the possible combination of two primers in a library that have positive interaction scores, the interaction scores are between 20 and 0 kcal/mol, such as between 15 and 0 kcal/mol, 10 and 0 kcal/mol, 8 and 0 kcal/mol, 7 and 0 kcal/mol, 6 and 0 kcal/mol, 5.5 and 0 kcal/mol, 5 and 0 kcal/mol, 4.5 and 0 kcal/mol, 4 and 0 kcal/mol, 3.5 and 0 kcal/mol, 3 and 0 kcal/mol, 2.5 and 0 kcal/mol, 2 and 0 kcal/mol, or 1.5 and 0 kcal/mol, inclusive.

In some embodiments, the score (such as the undesirability score) for candidate primers is based at least in part on the ΔG value or the interaction score that indicates the likelihood of dimer formation between candidate primers as calculated using any of these methods.

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Exemplary Primer Libraries

In one aspect, the invention features libraries of primers, such as primers selected from a library of candidate primers using any of the methods of the invention. In some embodiments, the library includes primers that simultaneously hybridize (or are capable of simultaneously hybridizing) to or that simultaneously amplify (or are capable of simultaneously amplifying) at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci in one reaction volume. In various embodiments, the library includes primers that simultaneously amplify (or are capable of simultaneously amplifying) between 1,000 to 2,000; 2,000 to 5,000; 5,000 to 7,500; 7,500 to 10,000; 10,000 to 20,000; 20,000 to 25,000; 25,000 to 30,000; 30,000 to 40,000; 40,000 to 50,000; 50,000 to 75,000; or 75,000 to 100,000 different target loci in one reaction volume, inclusive. In various embodiments, the library includes primers that simultaneously amplify (or are capable of simultaneously amplifying) between 1,000 to 100,000 different target loci in one reaction volume, such as between 1,000 to 50,000; 1,000 to 30,000; 1,000 to 20,000; 1,000 to 10,000; 2,000 to 30,000; 2,000 to 20,000; 2,000 to 10,000; 5,000 to 30,000; 5,000 to 20,000; or 5,000 to 10,000 different target loci, inclusive. In some embodiments, the library includes primers that simultaneously amplify (or are capable of simultaneously amplifying) the target loci in one reaction volume such that less than 60, 40, 30, 20, 10, 5, 4, 3, 2, 1, 0.5, 0.25, 0.1, or 0.5% of the amplified products are primer dimers. The various embodiments, the amount of amplified products that are primer dimers is between 0.5 to 60%, such as between 0.1 to 40%, 0.1 to 20%, 0.25 to 20%, 0.25 to 10%, 0.5 to 20%, 0.5 to 10%, 1 to 20%, or 1 to 10%, inclusive. In some embodiments, the primers simultaneously amplify (or are capable of simultaneously amplifying) the target loci in one reaction volume such that at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the amplified products are target amplicons. In various embodiments, the amount of amplified products that are target amplicons is between 50 to 99.5%, such as between 60 to 99%, 70 to 98%, 80 to 98%, 90 to 99.5%, or 95 to 99.5%, inclusive. In some embodiments, the primers simultaneously amplify (or are capable of simultaneously amplifying) the target loci in one reaction volume such that at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target loci are amplified. In various embodiments, the amount target loci that are amplified is between 50 to 99.5%, such as between 60 to 99%, 70 to 98%, 80 to 99%, 90 to 99.5%, 95 to 99.9%, or 98 to 99.99% inclusive. In some embodiments, at

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least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold. In some embodiments, the library of primers includes at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 primer pairs, wherein each pair of primers includes a forward test primer and a reverse test primer where each pair of test primers hybridize to a target locus. In some embodiments, the library of primers includes at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 individual primers that each hybridize to a different target locus, wherein the individual primers are not part of primer pairs.

In some embodiments, the library includes primers (such as at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different primers or different primer pairs) that simultaneously amplify (or are capable of simultaneously amplifying) the target loci (such as at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci) in one reaction volume such that one or more of the following: (i) less than 60, 40, 30, 20, 10, 5, 4, 3, 2, 1, 0.5, 0.25, 0.1, or 0.5% of the amplified products are primer dimers, (ii) at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the amplified products are target amplicons, (iii) at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target loci are amplified, (iv) at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold, (v) at least 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.5, or 100% of the amplified products map to the human genome, or (vi) any combination thereof.

In some embodiments, the library includes at least 1,000 different primers or different primer pairs (such as at least 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different primers or different primer pairs) that simultaneously amplify (or are capable of simultaneously amplifying) at least 1,000 different target loci (such as at least 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci) in one reaction volume such that one or more of the following: (i) less than 60% of the amplified products are primer dimers and at least 40% of the amplified products are target

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amplicons, (ii) less than 40% of the amplified products are primer dimers and at least 60% of the amplified products are target amplicons, (iii) less than 20% of the amplified products are primer dimers and at least 80% of the amplified products are target amplicons, (iv) less than 10% of the amplified products are primer dimers and at least 90% of the amplified products are target amplicons, or (v) less than 5% of the amplified products are primer dimers and at least 95% of the amplified products are target amplicons. In some embodiments for the amplification of nucleic acids (such as DNA or RNA) from a single cell (such as a fetal or embryonic cell), the library includes at least 1,000 different primers or different primer pairs (such as at least 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different primers or different primer pairs) that simultaneously amplify (or are capable of simultaneously amplifying) at least 1,000 different target loci (such as at least 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci) in one reaction volume such that one or more of the following: (i) less than 60% of the amplified products are primer dimers and at least 10% of the amplified products are target amplicons, (ii) less than 40% of the amplified products are primer dimers and at least 10% of the amplified products are target amplicons, (iii) less than 20% of the amplified products are primer dimers and at least 10% of the amplified products are target amplicons, (iv) less than 10% of the amplified products are primer dimers and at least 10% of the amplified products are target amplicons, (v) less than 5% of the amplified products are primer dimers and at least 15% of the amplified products are target amplicons; (vi) less than 60% of the amplified products are primer dimers and at least 20% of the amplified products are target amplicons, (vii) less than 40% of the amplified products are primer dimers and at least 20% of the amplified products are target amplicons, (viii) less than 20% of the amplified products are primer dimers and at least 20% of the amplified products are target amplicons, (ix) less than 10% of the amplified products are primer dimers and at least 20% of the amplified products are target amplicons, (x) less than 5% of the amplified products are primer dimers and at least 20% of the amplified products are target amplicons; (xi) less than 60% of the amplified products are primer dimers and at least 40% of the amplified products are target amplicons, (xii) less than 40% of the amplified products are primer dimers and at least 40% of the amplified products are target amplicons, (xiii) less than 20% of the amplified products are primer dimers and at least 40% of the amplified products are target amplicons, (xiv) less than 10% of the amplified products are

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primer dimers and at least 40% of the amplified products are target amplicons, (xv) less than 5% of the amplified products are primer dimers and at least 45% of the amplified products are target amplicons; (xvi) less than 40% of the amplified products are primer dimers and at least 60% of the amplified products are target amplicons, (xvii) less than 20% of the amplified products are primer dimers and at least 80% of the amplified products are target amplicons, (xviii) less than 10% of the amplified products are primer dimers and at least 90% of the amplified products are target amplicons, or (xviiii) less than 5% of the amplified products are primer dimers and at least 95% of the amplified products are target amplicons. In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold. In some embodiments, one or more of these embodiments (*e.g.*, percent of primer dimers, target amplicons, or amplified target loci) is achieved after greater than or equal to 5, 10, 20, 30, 40, 50, or 60 cycles of PCR are performed. In some embodiments for a library that amplifies human target loci, at least 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.5, or 100% of the amplified products map to the human genome.

In various embodiments, the concentration of each primer is less than 100, 75, 50, 25, 20, 10, 5, 2, 1, 0.5, 0.1, or 0.05 nM, or less than 500, 100, 10, or 1 uM. In various embodiments, the concentration of each primer is between 1 uM to 100 nM, such as between 1 uM to 1 nM, 1 to 75 nM, 1 to 20 nM, 1 to 10 nM, 2 to 50 nM or 5 to 50 nM, inclusive. In various embodiments, the GC content of the primers is between 30 to 80%, such as between 20 to 70%, 40 to 70%, or 50 to 60%, inclusive. In some embodiments, the range of GC content of the primers is less than 30, 20, 10, or 5%. In some embodiments, the range of GC content of the primers is between 5 to 30%, such as 5 to 20% or 5 to 10%, inclusive. In some embodiments, there is a high GC content in the 3' end of the primers. In some embodiments, there are at least 2 (such as 3, 4, or 5) guanines or cytosines in the last 5 bases at the 3' end of the primers. In some embodiments, there are at least 1 (such as 2 or 3) guanines or cytosines in the last 3 bases at the 3' end of the primers. In some embodiments, the maximum length of a homopolymer (the same base in a row) in the primers is 12, 10, 8, 6, 5, 4, 3, or 2 consecutive nucleotides. In some embodiments, the melting temperature (T_m) of the test primers is between 40 to 80 °C, such as 50 to 70 °C, 55 to 65 °C, 54 to 60.5 °C, or 57 to 60.5 °C, inclusive. In some embodiments, the T_m is calculated using the Primer3 program (libprimer3 release 2.2.3) using the built-in SantaLucia parameters (the world wide web at

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primer3.sourceforge.net). In some embodiments, the range of melting temperature of the primers is less than 15, 10, 5, 3, or 1 °C. In some embodiments, the range of melting temperature of the primers is between 1 to 15 °C, such as between 1 to 10 °C, 1 to 5 °C, or 1 to 3 °C, inclusive. In some embodiments, the length of the primers is between 15 to 100 nucleotides, such as between 15 to 75 nucleotides, 15 to 40 nucleotides, 17 to 35 nucleotides, 18 to 30 nucleotides, 20 to 65 nucleotides, inclusive. In some embodiments, the range of the length of the primers is less than 50, 40, 30, 20, 10, or 5 nucleotides. In some embodiments, the range of the length of the primers is between 5 to 50 nucleotides, such as 5 to 40 nucleotides, 5 to 20 nucleotides, or 5 to 10 nucleotides, inclusive. In some embodiments, the length of the target amplicons is between 50 and 100 nucleotides, such as between 60 and 80 nucleotides, or 60 to 75 nucleotides, inclusive. In some embodiments, the length of the target amplicons is between 30 and 400 nucleotides, such as between 30 and 200 nucleotides, or 100 and 400 nucleotides, inclusive. In some embodiments, the range of the length of the target amplicons is less than 50, 25, 15, 10, or 5 nucleotides. In some embodiments, the range of the length of the target amplicons is between 5 to 50 nucleotides, such as 5 to 25 nucleotides, 5 to 15 nucleotides, or 5 to 10 nucleotides, inclusive.

In some embodiments, library includes at least at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different primers that each includes a target specific sequence, such as a sequence that binds a target locus but does not substantially bind to other nucleic acids (such as non-target loci) in a sample, *e.g.*, a biological sample, which naturally includes other nucleic acids. In some embodiments, each primer binds and amplifies a target locus by at least 2, 4, 6, 8, 10, 20, 50-fold or more than one or more (or all) other nucleic acids (such as non-target loci) in a sample. In some embodiments, library also includes a universal primer or a random primer.

In some embodiments, the composition includes at least one primer (*e.g.*, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical primers) with a polynucleotide sequence of a human nucleic acid and at least one primer (*e.g.*, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical primers) with a polynucleotide sequence that is not found in a human (such as a universal primer, a primer that

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comprises a region or consists entirely of random nucleotides, or a primer with a region such as a tag or barcode of one or more nucleotides that are not found in a human or are not found in nature as part of the polynucleotide sequence of the primer). In some embodiments, at least one primer (*e.g.*, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical primers) includes a region of one or more nucleotides that is not naturally part of the primer sequence (such as a region added to the 5' end of the target specific sequence in the primer or an internal region added between the 5' and 3' ends of the primer). In some embodiments, the primer is free of the nucleic acids (such as genes) which, in the naturally-occurring genome of the organism from which the nucleic acid of the invention is derived, flank the gene. In some embodiments, the primer has been separated from one or more components that naturally accompany the corresponding sequence in nature (such as in the genome of a human). Typically, each primer is at least 90, 95, 98, 99, 99.9, or 100%, by weight, free from the molecules (such as proteins, nucleic acids, and naturally-occurring organic molecules) that naturally accompany the corresponding sequence in nature (such as in the genome of a human). Purity can be assayed by any appropriate method, *e.g.*, by electrophoresis or HPLC analysis.

In some embodiments, the primers in the library are not immobilized (such as not immobilized to a solid support) or not part of a microarray. In some embodiments, the primers are dissolved in solution (such as dissolved in the liquid phase).

In some embodiments, at least one of the primers (such as at least 20, 40, 80, 90, 95, 98, 99, 99.5, or 100% of the primers) in a library are nucleic acid analogs that have a lower likelihood of primer dimerization compared to the naturally-occurring nucleic acids (*see, e.g.,* US Patent Nos. 7,414,118 and 6,001,611; which are each hereby incorporated by reference in its entirety). Exemplary nucleic acid analogs have a modified pyrimidine nucleobase, or a purine or pyrimidine base that contains an exocyclic amine.

In some embodiments, the primers have any combination of two or more of the aspects or embodiments disclosed herein.

These primer libraries can be used in any of the methods of the invention.

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Exemplary Primers

The primer design methods of the invention have been used to generate several exemplary primer libraries to human target loci. For example, the primer design methods of the invention were used to generate the primer libraries shown in FIG. 34 (SEQ ID NOs. 1-3,600), FIG. 35 (SEQ ID NOs. 3,601-11,658), and FIG. 36 (SEQ ID NOs 11,659-44,609). Each of these libraries is composed of three primers (one each from pool A, pool B, and pool C in the same row of each figure) per target locus for 1,200; 2,686; or 10,984 different target loci, respectively. The methods of the invention can also be used to generate libraries to non-human target loci.

For an experiment using the 2,686-plex library FIG. 35 (SEQ ID NOs. 3,601-11,658) for multiplex PCR followed by sequencing, the percent of the amplified products that were primer dimers was 11.13%, the median depth of read per target that was amplified was 799.5x coverage, the percent of amplified products that were target amplicons out of the amplified products that were not primer dimers was 93.15% (this is the percent of on target reads when reads for amplified primer dimers are ignored); the number of target loci that were not amplified (failed assay count) was 246; the percent of target loci that were not amplified (failed assay percentage) was 9.16%; the percent of target loci that were amplified was 90.84%; and the total number of reads was 2,522,742. For this primer library, the ΔG values for each possible combination of two primers (each possible primer dimer) in the library are all equal to or greater than -3.86 kcal/mol. This -3.86 kcal/mol value was used as a threshold value to select candidate primer that all had a value equal to or greater than (more desirable than) this value from an initial library of candidate primers.

For an experiment using the 10,984-plex library FIG. 36 (SEQ ID NOs 11,659-44,609). for multiplex PCR followed by sequencing, the percent of the amplified products that were primer dimers was 5.50%, the median depth of read per target that was amplified was 1,286.5x coverage, the percent of amplified products that were target amplicons out of the amplified products that were not primer dimers was 60.16% (this is the percent of on target reads when reads for amplified primer dimers were ignored); the number of target loci that were not amplified (failed assay count) was 3,712; the percent of target loci that were not amplified (failed assay percentage) was 33.79%; the percent of target loci that were amplified was 66.21%; and the total number of reads was 25,372,858.

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For an experiment using the 1,200-plex library FIG. 34 (SEQ ID NOs. 1-3,600), for multiplex PCR of a sample of only a single cell followed by sequencing, the percent of the amplified products that were primer dimers was 24.13%. This library has primers to human target loci on chromosome 1, chromosome 21, and the X chromosome. For chromosomes 1 and 21, the median depth of read per target that was amplified was 436x coverage; the percent of target loci that were not amplified (failed assay percentage) was 32.69%; and the percent of target loci that were amplified was 67.31%. The total number of reads was 808,106.

The primer design methods of the invention were also used to generate a library for ~11,000 different target loci. For an experiment using this library for multiplex PCR followed by sequencing, the percent of the amplified products that were primer dimers was 14.75%, the median depth of read per target that was amplified was 72.27x coverage, the percent of the amplified products that were target amplicons was 84.32%; the number of target loci that were not amplified (failed assay count) was 118; the percent of target loci that were not amplified (failed assay percentage) was 1.10%; the percent of target loci that were amplified was 98.9%; and the total number of reads was 6,345,782. For this primer library, the ΔG values for each possible combination of two primers (each possible primer dimer) in the library are all equal to or greater than -4.28 kcal/mol. This -4.28 kcal/mol value was used as a threshold value to select candidate primer that all had a value equal to or greater than (more desirable than) this value from an initial library of candidate primers. For the initial candidate primers that were used to select primers for this library, the following interaction cost histogram shows the number of candidate primers for each of the following ranges of ΔG values. This illustrates how the values for the candidate primers compares to the -4.28 kcal/mol threshold value for the final library.

0 to -0.497 kcal/mol : 88357
-0.497 to -0.993 kcal/mol : 30529
-0.993 to -1.49 kcal/mol : 7862
-1.49 to -1.99 kcal/mol : 2639
-1.99 to -2.48 kcal/mol : 1086
-2.48 to -2.98 kcal/mol : 393
-2.98 to -3.48 kcal/mol : 148
-3.48 to -3.97 kcal/mol : 58
-3.97 to -4.47 kcal/mol : 18
-4.47 to -4.97 kcal/mol : 4
-4.97 to -5.46 kcal/mol : 3
-5.46 to -5.96 kcal/mol : 0
-5.96 to -6.46 kcal/mol : 2

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-6.46 to -6.95 kcal/mol : 3

The primer design methods of the invention were also used to generate a library for ~14,000 different target loci. For an experiment using this library for multiplex PCR followed by sequencing, the percent of the amplified products that were primer dimers was 0.56%, the median depth of read per target that was amplified was 69.09x coverage, the percent of the amplified products that were target amplicons was 99.42%; the number of target loci that were not amplified (failed assay count) was 44; the percent of target loci that were not amplified (failed assay percentage) was 0.33%; the percent of target loci that were amplified was 99.67%; and the total number of reads was 7,772,454.

The primer design methods of the invention were also used to generate a library composed of three primers per target locus for 19,488 different target loci. Experiments 15, 18, and 19 describe the use of this library. During the PCR amplification and sequencing of a genomic sample, 99.4-99.7% of the sequencing reads mapped to the genome, of those, 99.99% of the mapped to target loci. For plasma samples with 10 million sequencing reads, typically at least 19,350 of the 19,488 target loci (99.3 %) were amplified and sequenced. For another experiment, the percent of the amplified products that were primer dimers was 1.62%, the median depth of read per target that was amplified was 30x coverage; the percent of the amplified products that were target amplicons was 98.15%; the number of target loci that were not amplified (failed assay count) was 736; the percent of target loci that were not amplified (failed assay percentage) was 0.56%; the percent of target loci that were amplified was 99.44%; and the total number of reads was 6,476,975. For this 19,488-plex library, FIG. 37 is a table of the percentage of reads that map to target loci for genomic DNA samples and for samples of a single cell from a cell line for both mother and child samples using this primer library. There was variability in the single cell data which may have resulted from some dead cells being selected, which may have had most of the DNA leaked out. FIG. 38 is an overlay of depth of read for genomic (blue) and a single cell (red) sample for different SNPs. FIG. 39 is a table of the percentage of reads that map to target loci for blastoceol fluid and for a single blastocyst cell. The blastoceol fluid produced no mapped reads, possibly due to no DNA being detected. For a single blastocyst, 50-80% of the reads mapped to target loci. FIG. 40 is a graph of reference counts (counts of one allele, such as the "A" allele) divided by total counts for that locus for a single blastocyst cell. For this primer library, the ΔG values for each possible

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combination of two primers (each possible primer dimer) in the library are all equal to or greater than -3.86 kcal/mol. This -3.86 kcal/mol value was used as a threshold value to select candidate primer that all had a value equal to or greater than (more desirable than) this value from an initial library of candidate primers.

The primer design methods of the invention were used to generate a library for ~28,000 different target loci. For multiplex PCR and sequencing of genomic DNA samples, 99% of the sequencing reads mapped to target loci. For an experiment using this library, the percent of the amplified products that were primer dimers was 0.63%, the median depth of read per target that was amplified was 20x coverage, the percent of the amplified products that were target amplicons was 99.33%; the number of target loci that were not amplified (failed assay count) was 3,968; the percent of target loci that were not amplified (failed assay percentage) was 14.29%; the percent of target loci that were amplified was 85.71%; and the total number of reads was 4,456,636. For a single cell from a cell line, between 2 and 8% of the reads mapped to target loci.

The primer design methods of the invention were used to generate a library for ~9,600 different target loci. As described in Experiment 10, 7.6 million (97%) of reads mapped to the genome, and 6.3 million (80%) of the reads mapped to the targeted SNPs. The average depth of read was 751, and the median depth of read was 396. As described in Experiment 9, another experiment produced 3.7 million reads mapping to the genome (94%), and of those, 2.9 million reads (74%) mapped to targeted SNPs with an average depth of read of 344 and a median depth of read of 255.

The primer design methods of the invention were used to generate a library for ~2,400 different target loci. As described in Experiment 12, when four portions were each amplified with ~2,400 primers, 4.5 million reads mapped to targeted SNPs, the average depth of read was 535 and the median depth of read was 412.

If desired, any of the results may be improved by increasing the number of cells or the amount of nucleic acid template used for the analysis or by optimizing the conditions. For example, if results from single cell samples are not as good as desired for a particular application, a sample with more cells or more nucleic acids may be used instead (such as to decrease the percentage of primer dimers, increase the percentage of target amplicons, or increase the percentage of target loci that are amplified). Samples with more nucleic acids

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have more template molecules for the primers to bind (instead of primers binding each other and forming primer dimers).

These primer libraries can be used in any of the PCR methods of the invention. In some embodiments, primers from one of the primer pools in FIG. 34, 35, or 36 (either pool A, pool B, or pool C) are used in combination with a universal primer to amplify the target loci. In some embodiments, multiple rounds of PCR are performed in which each round of PCR uses primers from one of the primer pools of FIG. 34, 35, or 36 and a universal primer. In some embodiments, primer from two of the primer pools (such as primers from pools C and B or primers from pools A and C) are used to amplify the target loci. In some embodiments, multiple rounds of PCR are performed. In some embodiments, primers from pools C and B are used for the first round of PCR and then primers from pools A and C are used for the second of PCR. In some embodiments, primers from pools C and B are used for the first round of PCR and then primers from pools A and B are used for the second of PCR. In some embodiments, at least 60, 70, 80, 90, 95, or 100% of the primers from one or more of the primer pools in FIG. 34, 35, or 36 (*e.g.*, pool A, pool B, and/or pool C) are used for the PCR amplification. In some embodiments, primers from FIG. 34, 35, or 36 are combined with other primers that are not included in FIG. 34, 35, or 36.

In one aspect, the invention features one or more primers (*e.g.*, at least 10; 20; 50; 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 20,000; 25,000; 30,000; 40,000; or 50,000 different primers) with at least 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.5, or 100% identity to the corresponding region (*e.g.*, a region of at least 10, 20, 30, 40, 50 or more contiguous nucleotides) of a primer in pool A, pool B, and/or pool C in FIG. 34, 35, or 36 (SEQ ID NOs 1-44,609). In one aspect, the invention features one or more primers (*e.g.*, at least 10; 20; 50; 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 20,000; 25,000; 30,000; 40,000; or 50,000 different primers) with at least 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.5, or 100% identity over the entire length of a primer in pool A, pool B, and/or pool C in FIG. 34, 35, or 36. If desired, the primers in FIG. 34, 35, or 36 can be modified by the insertion, deletion, or substitution of one or more nucleotides (*e.g.*, at least 1, 2, 3, 4, 5, 6, 8, 10, 20, or more nucleotides). In some embodiments, a region that is not specific for a target locus (such as a tag, bar code, or universal binding site) is added to one or more primers in FIG. 34, 35, or 36. In various embodiments, the nonspecific region is added to the 5' end of the primer, to the 3' end of the primer, or to an internal region of the primer. In some embodiments, the primers

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are fragments (such as fragments of at least 10, 20, 30, 40, 50 or more contiguous nucleotides that are less than full-length) of primers in FIG. 34, 35, or 36.

In some embodiments, the invention provides a library of primers that includes at least 10; 20; 50; 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 20,000; 25,000; 30,000; 40,000; or 50,000 different primers that comprise the polynucleotide sequence of a primer in pool A, pool B, and/or pool C in FIG. 34, 35, or 36. In some embodiments, the invention provides a library of primers that includes at least 10; 20; 50; 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 20,000; 25,000; 30,000; 40,000; or 50,000 different primers or all of the primer from pool A, pool B, and/or pool C in one of FIG. 34, 35, or 36. In some embodiment, some or all of the primers in two or three of the primer libraries of FIGs. 34, 35, or 36 are combined.

Percent identity in reference to nucleic acid sequences refers to the degree of sequence identity between nucleic acid sequences. Percent identity can be determined in various ways that are within the skill in the art, for instance, using publicly available computer software with the default parameters such as Smith Waterman Alignment (Smith and Waterman J. Mol. Biol. 147:195-7, 1981); "BestFit" (Smith and Waterman, Advances in Applied Mathematics, 482-489, 1981); Basic Local Alignment Search Tool (BLAST, Altschul, S. F., W. Gish, et al., J. Mol. Biol. 215: 403-410, 1990; available through the U.S. government's National Center for Biotechnology Information web site at the world wide web at ncbi.nlm.nih.gov), BLAST-2, BLAST-N, WU-BLAST, WU-BLAST-2, ENTREZ (available through the National Center for Biotechnology Information), CLUSTALW, CLUSTAL Omega, or Megalign (DNASTAR, Inc. Madison, Wis.) software. In addition, those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the length of the sequences being compared. In general, the length of comparison will generally be at least 20, 30, 40, 45, 50, or more nucleotides.

In some embodiments, percent identity is calculated by determining the number of matched positions in aligned nucleic acid sequences, dividing the number of matched positions by the total number of aligned nucleotides, and multiplying by 100. A matched position refers to a position in which identical nucleotides occur at the same position in aligned nucleic acid sequences. The percent identity over a particular length is determined by counting the number of matched positions over that length and dividing that number by the length followed by multiplying the resulting value by 100. For example, if (i) a 500-nucleotide nucleic acid target

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sequence is compared to a subject nucleic acid sequence, (ii) an alignment program presents 200 nucleotides from the target sequence aligned with a region of the subject sequence where the first and last nucleotides of that 200-nucleotide region are matches, and (iii) the number of matches over those 200 aligned nucleotides is 180, then the 500-nucleotide nucleic acid target sequence contains a length of 200 and a sequence identity over that length of 90% (*i.e.*, 180, 200x100=90).

In one aspect, the invention features one or more primers (*e.g.*, at least 10; 20; 50; 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 20,000; 25,000; 30,000; 40,000; or 50,000 different primers) that comprise a polynucleotide sequence that hybridizes to the complement of a primer in pool A, pool B, and/or pool C in FIG. 34, 35, or 36 under very high stringency hybridization conditions, high stringency hybridization conditions, or low stringency hybridization conditions. In some embodiments, one or more primers (*e.g.*, at least 10; 20; 50; 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 20,000; 25,000; 30,000; 40,000; or 50,000 different primers) hybridize to the complement of a primer in pool A, pool B, and/or pool C in FIG. 34, 35, or 36 under very high stringency hybridization conditions, high stringency hybridization conditions, or low stringency hybridization conditions. Hybridization conditions resulting in a particular degree of stringency will vary depending upon the nature of the hybridization method and the composition and length of the hybridizing nucleic acid sequences. Generally, the temperature of hybridization and the ionic strength (such as the Na^+ concentration) of the hybridization buffer will determine the stringency of hybridization. Calculations regarding hybridization conditions for attaining particular degrees of stringency are discussed in Sambrook *et al.,* (1989) Molecular Cloning, second edition, Cold Spring Harbor Laboratory, Plainview, N.Y. (chapters 9 and 11); Nucleic Acid Hybridization, A Practical Approach, Ed. Hames, B. D. and Higgins, S. J., IRL Press, 1985; Ausubel *et al.* Current Protocols in Molecular Biology, Wiley, New York 1994; and U.S Pat. No. 8,357,488, filed May 16, 2008. In some embodiments, very high stringency hybridization conditions includes an overnight incubation at 42° C in a solution comprising 50% formamide, 5xSSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5xDenhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1xSSC at about 65° C. The following is an exemplary set of hybridization conditions and is not limiting:

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Very High Stringency (Detects Sequences that Share at Least 90% Identity)

a) Hybridization: 5xSSC at 65° C for 16 hours

b) Wash twice: 2xSSC at room temperature for 15 minutes each

c) Wash twice: 0.5xSSC at 65° C for 20 minutes each

High Stringency (Detects Sequences that Share at Least 80% Identity)

a) Hybridization: 5x-6xSSC at 65° C-70° C. for 16-20 hours

b) Wash twice: 2xSSC at room temperature for 5-20 minutes each

c) Wash twice: 1xSSC at 55° C-70° C for 30 minutes each

Low Stringency (Detects Sequences that Share at Least 50% Identity)

a) Hybridization: 6xSSC at room temperature to 55° C. for 16-20 hours

b) Wash at least twice: 2x-3xSSC at room temperature to 55° C for 20-30 minutes each

These primers can be used in any of the primer libraries or methods of the invention. In some embodiments, the library of primers consists essentially of, or consists of primers from any one of FIGs. 34-36.

Exemplary Primer Kits

In one aspect, the invention features a kit (such as kits for amplifying target loci in a nucleic acid sample) the includes any of the primer libraries of the invention. In some embodiments, a kit may be formulated that comprises a plurality of primers designed to achieve the methods described in this disclosure. The primers may be outer forward and reverse primers, inner forward and reverse primers as disclosed herein, they could be primers that have been designed to have low binding affinity to other primers in the kit as disclosed in the section on primer design, they could be hybrid capture probes or pre-circularized probes as described in the relevant sections, or some combination thereof. In an embodiment, a kit may be formulated for determining a ploidy status of a target chromosome in a gestating fetus designed to be used with the methods disclosed herein, the kit comprising a plurality of inner forward primers and optionally the plurality of inner reverse primers, and optionally outer

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forward primers and outer reverse primers, where each of the primers is designed to hybridize to the region of DNA immediately upstream and/or downstream from one of the target sites (*e.g.*, polymorphic sites) on the target chromosome, and optionally additional chromosomes. In an embodiment, the primer kit may be used in combination with the diagnostic box described elsewhere in this document. In some embodiments, the kit includes instructions for using the library to amplify the target loci. In some embodiments, the kit consists essentially of, or consists of primers (or of primers and instructions for using the primers).

Exemplary Amplicons

In one aspect, the invention provides a composition comprising at least 100 different amplicons (*e.g.*, at least 300, 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical amplicons) in solution in one reaction volume. In some embodiments, the amplicons are produced from the simultaneous PCR amplification of at least 100 different target loci (*e.g.*, at least 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical target loci) using at least 100 different primers or primer pairs (*e.g.*, at least 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical primers or primer pairs) in one reaction volume. In some embodiments, (i) less than 60% of the amplified products are primer dimers and at least 40% of the amplified products are target amplicons, (ii) less than 40% of the amplified products are primer dimers and at least 60% of the amplified products are target amplicons, (iii) less than 20% of the amplified products are primer dimers and at least 80% of the amplified products are target amplicons, (iv) less than 10% of the amplified products are primer dimers and at least 90% of the amplified products are target amplicons, or (v) less than 5% of the amplified products are primer dimers and at least 95% of the amplified products are target amplicons. In some embodiments, the length of the target amplicons is between 50 and 100 nucleotides, such as between 60 and 80 nucleotides, or 60 to 75 nucleotides, inclusive. In some embodiments, the range of the length of the target amplicons is less than 50, 25, 15, 10, or 5 nucleotides. In some embodiments, the range of the length of the target amplicons is between 5 to 50 nucleotides, such as 5 to 25 nucleotides, 5 to 15 nucleotides, or 5 to 10 nucleotides, inclusive. In some embodiments, the composition includes at least 1,000 different amplicons in solution

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in one reaction volume; wherein the amplicons are produced from the simultaneous PCR amplification of at least 1,000 different target human loci using at least 1,000 different primers in one reaction volume; wherein (i) less than 20% of the amplicons are primer dimers, and (ii) at least 80% of the amplicons comprise one of the target human loci and are between 50 and 100 nucleotides in length, inclusive. In some embodiments, the composition consists essentially of, or consists of one or more of the following: amplicons, primers (such as any of the primers disclosed herein), free nucleotide(s), non-human enzyme(s), buffer(s), or any combination thereof.

In some embodiments, a large percentage or substantially all of the primers used for the multiplex PCR method are consumed during the PCR reaction or are removed from the reaction volume after the PCR amplification. In some embodiments, at least 80, 90, 92, 94, 96, 98, 99, or 100% of the primer molecules are extended to form amplified products. In some embodiments, for at least 80, 90, 92, 94, 96, 98, 99, or 100% of target loci, at least 80, 90, 92, 94, 96, 98, 99, or 100% of the primer molecules to that target loci are extended to form amplified products. In some embodiments, multiple cycles are performed until all or substantially all of the primers are consumed. If desired, a higher percentage of the primers can be consumed by decreasing the initial primer concentration and/or increasing the number of PCR cycles that are performed. In some embodiments, at least 80, 90, 95, 96, 97, 98, 99, or 100% of the nucleic acids in the composition are amplicons (instead of unextended dimers).

In one aspect, the invention provides a composition comprising at least 100 different primers or primer pairs (*e.g.*, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical primers or primer pairs) and at least 100 different amplicons (*e.g.*, at least 300, 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical amplicons) in solution in one reaction volume. In some embodiments, the amplicons are produced from the simultaneous PCR amplification of at least 100 different target loci (*e.g.*, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 non-identical target loci) using the primers or primer pairs in one reaction volume. In some embodiments, (i) less than 60% of the amplified products are primer dimers and at least 40% of the amplified products are target amplicons, (ii) less than 40% of the amplified products are primer dimers and at least 60% of the amplified products are target

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amplicons, (iii) less than 20% of the amplified products are primer dimers and at least 80% of the amplified products are target amplicons, (iv) less than 10% of the amplified products are primer dimers and at least 90% of the amplified products are target amplicons, or (v) less than 5% of the amplified products are primer dimers and at least 95% of the amplified products are target amplicons. In some embodiments, the length of the target amplicons is between 50 and 100 nucleotides, such as between 60 and 80 nucleotides, or 60 to 75 nucleotides, inclusive. In some embodiments, the range of the length of the target amplicons is less than 50, 25, 15, 10, or 5 nucleotides. In some embodiments, the range of the length of the target amplicons is between 5 to 50 nucleotides, such as 5 to 25 nucleotides, 5 to 15 nucleotides, or 5 to 10 nucleotides, inclusive. In some embodiments, the composition comprising at least 1,000 different primers and at least 1,000 different amplicons in solution in one reaction volume; wherein the amplicons are produced from the simultaneous PCR amplification of at least 1,000 different target human loci with the primers in one reaction volume; wherein (i) less than 20% of the amplicons are primer dimers, and (ii) at least 80% of the amplicons comprise one of the target loci and are between 50 and 100 nucleotides in length, inclusive. In some embodiments, the composition consists essentially of, or consists of one or more of the following: amplicons, primers (such as any of the primers disclosed herein), free nucleotide(s), non-human enzyme(s), buffer(s), or any combination thereof.

In some embodiments, the amplification of different target loci is substantially uniform. In some embodiments, target loci (such as nonpolymorphic target loci or polymorphic target loci that are amplified regardless of what allele is present at the polymorphic site) that were present in the same amount (or substantially the same amount) in the initial unamplified sample are also present in substantially the same amount in the PCR-amplified products. In some embodiments, for at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different targets, the target loci that is amplified the most out of these targets (which can be all of the targets or a subset of the targets for a libary) is amplified less than 500, 400, 300, 200, 100%, 50, 20, 10, 5, or 2% more than the target loci that is amplified the least out of these targets. In some embodiments, for at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target amplicons, the target amplicon in greatest abundance out of these target amplicons (which can be all of the target amplicons or a subset of the target amplicons

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produced by a library) is present in an amount that is less than 500, 400, 300, 200, 100%, 50, 20, 10, 5, or 2% more than the target amplicon in least abundance out of these target amplicons. In some embodiments, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target amplicons are present in an amount that is at least 5, 10, 15, 20, 40, 50, 60, 70,. 80, or 90% of the amount of the target amplicon in greatest abundance. In some embodiments, for at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different amplicons that are produced by multiplex PCR and then sequenced, at least 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target amplicons produce a number of sequencing reads within 20, 30, 50, or 80% above or below the mean number of sequences for target amplicons. If desired, the uniformity can be increased by using primers with more similar primer lengths, target amplicon lengths, GC contents, melting temperatures, or any combination thereof. In some embodiments, the uniformity can be increased by using TMAC in the reaction volume during amplification. In some embodiments, having most or all the primers consumed in the PCR reaction increases the uniformity of amplification.

Exemplary Multiplex PCR Methods

In one aspect, the invention features methods of amplifying target loci in a nucleic acid sample that involve (i) contacting the nucleic acid sample with a library of primers that simultaneously hybridize to least 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci to produce a reaction mixture; and (ii) subjecting the reaction mixture to primer extension reaction conditions (such as PCR conditions) to produce amplified products that include target amplicons. In some embodiments, the method also includes determining the presence or absence of at least one target amplicon (such as at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target amplicons). In some embodiments, the method also includes determining the sequence of at least one target amplicon (such as at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target amplicons). In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target loci are amplified. In some embodiments, at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified at least

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5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold. In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, 99.5, or 100% of the target loci are amplified at least 5, 10, 20, 40, 50, 60, 80, 100, 120, 150, 200, 300, or 400-fold. In various embodiments, less than 60, 50, 40, 30, 20, 10, 5, 4, 3, 2, 1, 0.5, 0.25, 0.1, or 0.05% of the amplified products are primer dimers. In some embodiments, the method involves multiplex PCR and sequencing (such as high throughput sequencing).

In various embodiments, long annealing times and/or low primer concentrations are used. In various embodiments, the length of the annealing step is greater than 3, 5, 8, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, or 180 minutes. In various embodiments, the length of the anneal step is greater than 5 minutes (such greater than 10, or 15 minutes), and the concentration of each primer is less than 20 nM. In various embodiments, the length of the anneal step is greater than 5 minutes (such greater than 10, or 15 minutes), and the concentration of each primer is between 1 to 20 nM, or 1 to 10 nM, inclusive. In various embodiments, the length of the anneal step is greater than 20 minutes (such as greater than 30, 45, 60, or 90 minutes), and the concentration of each primer is less than 1 nM.

In some embodiments, most or all of the primers are extended to form amplified products. Having all the primers consumed in the PCR reaction increases the uniformity of amplification of the different target loci since the same or similar number of primer molecules are converted to target amplicons for each target loci. In some embodiment, at least 80, 90, 92, 94, 96, 98, 99, or 100% of the primer molecules are extended to form amplified products. In some embodiments, for at least 80, 90, 92, 94, 96, 98, 99, or 100% of target loci, at least 80, 90, 92, 94, 96, 98, 99, or 100% of the primer molecules to that target loci are extended to form amplified products. In some embodiments, multiple cycles are performed until this percentage of the primers are consumed. In some embodiments, multiple cycles are performed until all or substantially all of the primers are consumed. If desired, a higher percentage of the primers can be consumed by decreasing the initial primer concentration and/or increasing the number of PCR cycles that are performed.

In some embodiments, the PCR methods may be performed with microliter reaction volumes, for which it can be harder to achieve specific PCR amplification (due to the lower local concentration of the template nucleic acids) compared to nanoliter or picoliter reaction volumes used in microfluidics applications. In some embodiments, the reaction volume is

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between 1 and 60 uL, such as between 5 and 50 uL, 10 and 50 uL, 10 and 20 uL, 20 and 30 uL, 30 and 40 uL, or 40 to 50 uL, inclusive.

In an embodiment, a method disclosed herein uses highly efficient highly multiplexed targeted PCR to amplify DNA followed by high throughput sequencing to determine the allele frequencies at each target locus. The ability to multiplex more than about 50 or 100 PCR primers in one reaction volume in a way that most of the resulting sequence reads map to targeted loci is novel and non-obvious. One technique that allows highly multiplexed targeted PCR to perform in a highly efficient manner involves designing primers that are unlikely to hybridize with one another. The PCR probes, typically referred to as primers, are selected by creating a thermodynamic model of potentially adverse interactions between at least 300; at least 500; at least 750; at least 1,000; at least 2,000; at least 5,000; at least 7,500; at least 10,000; at least 20,000; at least 25,000; at least 30,000; at least 40,000; at least 50,000; at least 75,000; or at least 100,000 potential primer pairs, or unintended interactions between primers and sample DNA, and then using the model to eliminate designs that are incompatible with other the designs in the pool. Another technique that allows highly multiplexed targeted PCR to perform in a highly efficient manner is using a partial or full nesting approach to the targeted PCR. Using one or a combination of these approaches allows multiplexing of at least 300, at least 800, at least 1,200, at least 4,000 or at least 10,000 primers in a single pool with the resulting amplified DNA comprising a majority of DNA molecules that, when sequenced, will map to targeted loci. Using one or a combination of these approaches allows multiplexing of a large number of primers in a single pool with the resulting amplified DNA comprising greater than 50%, greater than 60%, greater than 67%, greater than 80%, greater than 90%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, greater than 99%, or greater than 99.5% DNA molecules that map to targeted loci.

In some embodiments the detection of the target genetic material may be done in a multiplexed fashion. The number of genetic target sequences that may be run in parallel can range from one to ten, ten to one hundred, one hundred to one thousand, one thousand to ten thousand, ten thousand to one hundred thousand, one hundred thousand to one million, or one million to ten million. Prior attempts to multiplex more than 100 primers per pool have resulted in significant problems with unwanted side reactions such as primer-dimer formation.

Targeted PCR

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In some embodiments, PCR can be used to target specific locations of the genome. In plasma samples, the original DNA is highly fragmented (typically less than 500 bp, with an average length less than 200 bp). In PCR, both forward and reverse primers anneal to the same fragment to enable amplification. Therefore, if the fragments are short, the PCR assays must amplify relatively short regions as well. Like MIPS, if the polymorphic positions are too close the polymerase binding site, it could result in biases in the amplification from different alleles. Currently, PCR primers that target polymorphic regions, such as those containing SNPs, are typically designed such that the 3' end of the primer will hybridize to the base immediately adjacent to the polymorphic base or bases. In an embodiment of the present disclosure, the 3' ends of both the forward and reverse PCR primers are designed to hybridize to bases that are one or a few positions away from the variant positions (polymorphic sites) of the targeted allele. The number of bases between the polymorphic site (SNP or otherwise) and the base to which the 3' end of the primer is designed to hybridize may be one base, it may be two bases, it may be three bases, it may be four bases, it may be five bases, it may be six bases, it may be seven to ten bases, it may be eleven to fifteen bases, or it may be sixteen to twenty bases. The forward and reverse primers may be designed to hybridize a different number of bases away from the polymorphic site.

PCR assay can be generated in large numbers, however, the interactions between different PCR assays makes it difficult to multiplex them beyond about one hundred assays. Various complex molecular approaches can be used to increase the level of multiplexing, but it may still be limited to fewer than 100, perhaps 200, or possibly 500 assays per reaction. Samples with large quantities of DNA can be split among multiple sub-reactions and then recombined before sequencing. For samples where either the overall sample or some subpopulation of DNA molecules is limited, splitting the sample would introduce statistical noise. In an embodiment, a small or limited quantity of DNA may refer to an amount below 10 pg, between 10 and 100 pg, between 100 pg and 1 ng, between 1 and 10 ng, or between 10 and 100 ng. Note that while this method is particularly useful on small amounts of DNA where other methods that involve splitting into multiple pools can cause significant problems related to introduced stochastic noise, this method still provides the benefit of minimizing bias when it is run on samples of any quantity of DNA. In these situations a universal pre-amplification step may be used to increase the overall sample quantity. Ideally, this pre-amplification step should not appreciably alter the allelic distributions.

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In an embodiment, a method of the present disclosure can generate PCR products that are specific to a large number of targeted loci, specifically 1,000 to 5,000 loci, 5,000 to 10,000 loci or more than 10,000 loci, for genotyping by sequencing or some other genotyping method, from limited samples such as single cells or DNA from body fluids. Currently, performing multiplex PCR reactions of more than 5 to 10 targets presents a major challenge and is often hindered by primer side products, such as primer dimers, and other artifacts. When detecting target sequences using microarrays with hybridization probes, primer dimers and other artifacts may be ignored, as these are not detected. However, when using sequencing as a method of detection, the vast majority of the sequencing reads would sequence such artifacts and not the desired target sequences in a sample. Methods described in the prior art used to multiplex more than 50 or 100 reactions in one reaction volume followed by sequencing will typically result in more than 20%, and often more than 50%, in many cases more than 80% and in some cases more than 90% off-target sequence reads.

In general, to perform targeted sequencing of multiple (n) targets of a sample (greater than 50, greater than 100, greater than 500, or greater than 1,000), one can split the sample into a number of parallel reactions that amplify one individual target. This has been performed in PCR multiwell plates or can be done in commercial platforms such as the FLUIDIGM ACCESS ARRAY (48 reactions per sample in microfluidic chips) or DROPLET PCR by RAIN DANCE TECHNOLOGY (100s to a few thousands of targets). Unfortunately, these split-and-pool methods are problematic for samples with a limited amount of DNA, as there is often not enough copies of the genome to ensure that there is one copy of each region of the genome in each well. This is an especially severe problem when polymorphic loci are targeted, and the relative proportions of the alleles at the polymorphic loci are needed, as the stochastic noise introduced by the splitting and pooling will cause very poorly accurate measurements of the proportions of the alleles that were present in the original sample of DNA. Described here is a method to effectively and efficiently amplify many PCR reactions that is applicable to cases where only a limited amount of DNA is available. In an embodiment, the method may be applied for analysis of single cells, body fluids, mixtures of DNA such as the free floating DNA found in maternal plasma, biopsies, environmental and/or forensic samples.

In an embodiment, the targeted sequencing may involve one, a plurality, or all of the following steps. a) Generate and amplify a library with adaptor sequences on both ends of DNA fragments. b) Divide into multiple reactions after library amplification. c) Generate and

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optionally amplify a library with adaptor sequences on both ends of DNA fragments. d) Perform 1000- to 10,000-plex amplification of selected targets using one target specific "Forward" primer per target and one tag specific primer. e) Perform a second amplification from this product using "Reverse" target specific primers and one (or more) primer specific to a universal tag that was introduced as part of the target specific forward primers in the first round. f) Perform a 1000-plex preamplification of selected target for a limited number of cycles. g) Divide the product into multiple aliquots and amplify subpools of targets in individual reactions (for example, 50 to 500-plex, though this can be used all the way down to singleplex. h) Pool products of parallel subpools reactions. i) During these amplifications primers may carry sequencing compatible tags (partial or full length) such that the products can be sequenced.

Highly Multiplexed PCR

Disclosed herein are methods that permit the targeted amplification of over a hundred to tens of thousands of target sequences (*e.g.,* SNP loci) from a nucleic acid sample such as genomic DNA obtained from plasma. The amplified sample may be relatively free of primer dimer products and have low allelic bias at target loci. If during or after amplification the products are appended with sequencing compatible adaptors, analysis of these products can be performed by sequencing.

Performing a highly multiplexed PCR amplification using methods known in the art results in the generation of primer dimer products that are in excess of the desired amplification products and not suitable for sequencing. These can be reduced empirically by eliminating primers that form these products, or by performing *in silico* selection of primers. However, the larger the number of assays, the more difficult this problem becomes.

One solution is to split the 5000-plex reaction into several lower-plexed amplifications, *e.g.* one hundred 50-plex or fifty 100-plex reactions, or to use microfluidics or even to split the sample into individual PCR reactions. However, if the sample DNA is limited, such as in non-invasive prenatal diagnostics from pregnancy plasma, dividing the sample between multiple reactions should be avoided as this will result in bottlenecking.

Described herein are methods to first globally amplify the plasma DNA of a sample and then divide the sample up into multiple multiplexed target enrichment reactions with more moderate numbers of target sequences per reaction. In an embodiment, a method of the present

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disclosure can be used for preferentially enriching a DNA mixture at a plurality of loci, the method comprising one or more of the following steps: generating and amplifying a library from a mixture of DNA where the molecules in the library have adaptor sequences ligated on both ends of the DNA fragments, dividing the amplified library into multiple reactions, performing a first round of multiplex amplification of selected targets using one target specific "forward" primer per target and one or a plurality of adaptor specific universal "reverse" primers. In an embodiment, a method of the present disclosure further includes performing a second amplification using "reverse" target specific primers and one or a plurality of primers specific to a universal tag that was introduced as part of the target specific forward primers in the first round. In an embodiment, the method may involve a fully nested, hemi-nested, semi-nested, one sided fully nested, one sided hemi-nested, or one sided semi-nested PCR approach. In an embodiment, a method of the present disclosure is used for preferentially enriching a DNA mixture at a plurality of loci, the method comprising performing a multiplex preamplification of selected targets for a limited number of cycles, dividing the product into multiple aliquots and amplifying subpools of targets in individual reactions, and pooling products of parallel subpools reactions. Note that this approach could be used to perform targeted amplification in a manner that would result in low levels of allelic bias for 50-500 loci, for 500 to 5,000 loci, for 5,000 to 50,000 loci, or even for 50,000 to 500,000 loci. In an embodiment, the primers carry partial or full length sequencing compatible tags.

The workflow may entail (1) extracting DNA such as plasma DNA, (2) preparing fragment library with universal adaptors on both ends of fragments, (3) amplifying the library using universal primers specific to the adaptors, (4) dividing the amplified sample "library" into multiple aliquots, (5) performing multiplex (*e.g.* about 100-plex, 1,000, or 10,000-plex with one target specific primer per target and a tag-specific primer) amplifications on aliquots, (6) pooling aliquots of one sample, (7) barcoding the sample, (8) mixing the samples and adjusting the concentration, (9) sequencing the sample. The workflow may comprise multiple sub-steps that contain one of the listed steps (*e.g.* step (2) of preparing the library step could entail three enzymatic steps (blunt ending, dA tailing and adaptor ligation) and three purification steps). Steps of the workflow may be combined, divided up or performed in different order (*e.g.* bar coding and pooling of samples).

It is important to note that the amplification of a library can be performed in such a way that it is biased to amplify short fragments more efficiently. In this manner it is possible to

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preferentially amplify shorter sequences, *e.g.* mono-nucleosomal DNA fragments as the cell free fetal DNA (of placental origin) found in the circulation of pregnant women. Note that PCR assays can have the tags, for example sequencing tags, (usually a truncated form of 15-25 bases). After multiplexing, PCR multiplexes of a sample are pooled and then the tags are completed (including bar coding) by a tag-specific PCR (could also be done by ligation). Also, the full sequencing tags can be added in the same reaction as the multiplexing. In the first cycles targets may be amplified with the target specific primers, subsequently the tag-specific primers take over to complete the SQ-adaptor sequence. The PCR primers may carry no tags. The sequencing tags may be appended to the amplification products by ligation.

In an embodiment, highly multiplex PCR followed by evaluation of amplified material by clonal sequencing may be used for various applications such as the detection of fetal aneuploidy. Whereas traditional multiplex PCRs evaluate up to fifty loci simultaneously, the approach described herein may be used to enable simultaneous evaluation of more than 50 loci simultaneously, more than 100 loci simultaneously, more than 500 loci simultaneously, more than 1,000 loci simultaneously, more than 5,000 loci simultaneously, more than 10,000 loci simultaneously, more than 50,000 loci simultaneously, and more than 100,000 loci simultaneously. Experiments have shown that up to, including and more than 10,000 distinct loci can be evaluated simultaneously, in a single reaction, with sufficiently good efficiency and specificity to make non-invasive prenatal aneuploidy diagnoses and/or copy number calls with high accuracy. Assays may be combined in a single reaction with the entirety of a sample such as a cfDNA sample isolated from maternal plasma, a fraction thereof, or a further processed derivative of the cfDNA sample. The sample (*e.g.*, cfDNA or derivative) may also be split into multiple parallel multiplex reactions. The optimum sample splitting and multiplex is determined by trading off various performance specifications. Due to the limited amount of material, splitting the sample into multiple fractions can introduce sampling noise, handling time, and increase the possibility of error. Conversely, higher multiplexing can result in greater amounts of spurious amplification and greater inequalities in amplification both of which can reduce test performance.

Two crucial related considerations in the application of the methods described herein are the limited amount of original sample (*e.g.*, plasma) and the number of original molecules in that material from which allele frequency or other measurements are obtained. If the number of original molecules falls below a certain level, random sampling noise becomes significant,

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and can affect the accuracy of the test. Typically, data of sufficient quality for making non-invasive prenatal aneuploidy diagnoses can be obtained if measurements are made on a sample comprising the equivalent of 500-1000 original molecules per target locus. There are a number of ways of increasing the number of distinct measurements, for example increasing the sample volume. Each manipulation applied to the sample also potentially results in losses of material. It is essential to characterize losses incurred by various manipulations and avoid, or as necessary improve yield of certain manipulations to avoid losses that could degrade performance of the test.

In an embodiment, it is possible to mitigate potential losses in subsequent steps by amplifying all or a fraction of the original sample (*e.g.*, cfDNA sample). Various methods are available to amplify all of the genetic material in a sample, increasing the amount available for downstream procedures. In an embodiment, ligation mediated PCR (LM-PCR) DNA fragments are amplified by PCR after ligation of either one distinct adaptors, two distinct adapters, or many distinct adaptors. In an embodiment, multiple displacement amplification (MDA) phi-29 polymerase is used to amplify all DNA isothermally. In DOP-PCR and variations, random priming is used to amplify the original material DNA. Each method has certain characteristics such as uniformity of amplification across all represented regions of the genome, efficiency of capture and amplification of original DNA, and amplification performance as a function of the length of the fragment.

In an embodiment LM-PCR may be used with a single heteroduplexed adaptor having a 3-prime tyrosine. The heteroduplexed adaptor enables the use of a single adaptor molecule that may be converted to two distinct sequences on 5-prime and 3-prime ends of the original DNA fragment during the first round of PCR. In an embodiment, it is possible to fractionate the amplified library by size separations, or products such as AMPURE, TASS or other similar methods. Prior to ligation, sample DNA may be blunt ended, and then a single adenosine base is added to the 3-prime end. Prior to ligation the DNA may be cleaved using a restriction enzyme or some other cleavage method. During ligation the 3-prime adenosine of the sample fragments and the complementary 3-prime tyrosine overhang of adaptor can enhance ligation efficiency. The extension step of the PCR amplification may be limited from a time standpoint to reduce amplification from fragments longer than about 200 bp, about 300 bp, about 400 bp, about 500 bp or about 1,000 bp. Since longer DNA found in the maternal plasma is nearly exclusively maternal, this may result in the enrichment of fetal DNA by 10-50% and

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improvement of test performance. A number of reactions were run using conditions as specified by commercially available kits; the resulted in successful ligation of fewer than 10% of sample DNA molecules. A series of optimizations of the reaction conditions for this improved ligation to approximately 70%.

Mini-PCR

The following Mini-PCR method is desirable for samples containing short nucleic acids, digested nucleic acids, or fragmented nucleic acids, such as cfDNA. Traditional PCR assay design results in significant losses of distinct fetal molecules, but losses can be greatly reduced by designing very short PCR assays, termed mini-PCR assays. Fetal cfDNA in maternal serum is highly fragmented and the fragment sizes are distributed in approximately a Gaussian fashion with a mean of 160 bp, a standard deviation of 15 bp, a minimum size of about 100 bp, and a maximum size of about 220 bp. The distribution of fragment start and end positions with respect to the targeted polymorphisms, while not necessarily random, vary widely among individual targets and among all targets collectively and the polymorphic site of one particular target locus may occupy any position from the start to the end among the various fragments originating from that locus. Note that the term mini-PCR may equally well refer to normal PCR with no additional restrictions or limitations.

During PCR, amplification will only occur from template DNA fragments comprising both forward and reverse primer sites. Because fetal cfDNA fragments are short, the likelihood of both primer sites being present the likelihood of a fetal fragment of length L comprising both the forward and reverse primers sites is ratio of the length of the amplicon to the length of the fragment. Under ideal conditions, assays in which the amplicon is 45, 50, 55, 60, 65, or 70 bp will successfully amplify from 72%, 69%, 66%, 63%, 59%, or 56%, respectively, of available template fragment molecules. The amplicon length is the distance between the 5-prime ends of the forward and reverse priming sites. Amplicon length that is shorter than typically used by those known in the art may result in more efficient measurements of the desired polymorphic loci by only requiring short sequence reads. In an embodiment, a substantial fraction of the amplicons should be less than 100 bp, less than 90 bp, less than 80 bp, less than 70 bp, less than 65 bp, less than 60 bp, less than 55 bp, less than 50 bp, or less than 45 bp.

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Note that in methods known in the prior art, short assays such as those described herein are usually avoided because they are not required and they impose considerable constraint on primer design by limiting primer length, annealing characteristics, and the distance between the forward and reverse primer.

Also note that there is the potential for biased amplification if the 3-prime end of the either primer is within roughly 1-6 bases of the polymorphic site. This single base difference at the site of initial polymerase binding can result in preferential amplification of one allele, which can alter observed allele frequencies and degrade performance. All of these constraints make it very challenging to identify primers that will amplify a particular locus successfully and furthermore, to design large sets of primers that are compatible in the same multiplex reaction. In an embodiment, the 3' end of the inner forward and reverse primers are designed to hybridize to a region of DNA upstream from the polymorphic site, and separated from the polymorphic site by a small number of bases. Ideally, the number of bases may be between 6 and 10 bases, but may equally well be between 4 and 15 bases, between three and 20 bases, between two and 30 bases, or between 1 and 60 bases, and achieve substantially the same end.

Multiplex PCR may involve a single round of PCR in which all targets are amplified or it may involve one round of PCR followed by one or more rounds of nested PCR or some variant of nested PCR. Nested PCR consists of a subsequent round or rounds of PCR amplification using one or more new primers that bind internally, by at least one base pair, to the primers used in a previous round. Nested PCR reduces the number of spurious amplification targets by amplifying, in subsequent reactions, only those amplification products from the previous one that have the correct internal sequence. Reducing spurious amplification targets improves the number of useful measurements that can be obtained, especially in sequencing. Nested PCR typically entails designing primers completely internal to the previous primer binding sites, necessarily increasing the minimum DNA segment size required for amplification. For samples such as maternal plasma cfDNA, in which the DNA is highly fragmented, the larger assay size reduces the number of distinct cfDNA molecules from which a measurement can be obtained. In an embodiment, to offset this effect, one may use a partial nesting approach where one or both of the second round primers overlap the first binding sites extending internally some number of bases to achieve additional specificity while minimally increasing in the total assay size.

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In an embodiment, a multiplex pool of PCR assays are designed to amplify potentially heterozygous SNP or other polymorphic or non-polymorphic loci on one or more chromosomes and these assays are used in a single reaction to amplify DNA. The number of PCR assays may be between 50 and 200 PCR assays, between 200 and 1,000 PCR assays, between 1,000 and 5,000 PCR assays, or between 5,000 and 20,000 PCR assays (50 to 200-plex, 200 to 1,000-plex, 1,000 to 5,000-plex, 5,000 to 20,000-plex, more than 20,000-plex respectively). In an embodiment, a multiplex pool of about 10,000 PCR assays (10,000-plex) are designed to amplify potentially heterozygous SNP loci on chromosomes X, Y, 13, 18, and 21 and 1 or 2 and these assays are used in a single reaction to amplify cfDNA obtained from a material plasma sample, chorion villus samples, amniocentesis samples, single or a small number of cells, other bodily fluids or tissues, cancers, or other genetic matter. The SNP frequencies of each locus may be determined by clonal or some other method of sequencing of the amplicons. Statistical analysis of the allele frequency distributions or ratios of all assays may be used to determine if the sample contains a trisomy of one or more of the chromosomes included in the test. In another embodiment the original cfDNA samples is split into two samples and parallel 5,000-plex assays are performed. In another embodiment the original cfDNA samples is split into n samples and parallel (~10,000/n)-plex assays are performed where n is between 2 and 12, or between 12 and 24, or between 24 and 48, or between 48 and 96. Data is collected and analyzed in a similar manner to that already described. Note that this method is equally well applicable to detecting translocations, deletions, duplications, and other chromosomal abnormalities.

In an embodiment, tails with no homology to the target genome may also be added to the 3-prime or 5-prime end of any of the primers. These tails facilitate subsequent manipulations, procedures, or measurements. In an embodiment, the tail sequence can be the same for the forward and reverse target specific primers. In an embodiment, different tails may be used for the forward and reverse target specific primers. In an embodiment, a plurality of different tails may be used for different loci or sets of loci. Certain tails may be shared among all loci or among subsets of loci. For example, using forward and reverse tails corresponding to forward and reverse sequences required by any of the current sequencing platforms can enable direct sequencing following amplification. In an embodiment, the tails can be used as common priming sites among all amplified targets that can be used to add other useful sequences. In some embodiments, the inner primers may contain a region that is designed to hybridize either

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upstream or downstream of the targeted locus (*e.g*, a polymorphic locus). In some embodiments, the primers may contain a molecular barcode. In some embodiments, the primer may contain a universal priming sequence designed to allow PCR amplification.

In an embodiment, a 10,000-plex PCR assay pool is created such that forward and reverse primers have tails corresponding to the required forward and reverse sequences required by a high throughput sequencing instrument such as the HISEQ, GAIIX, or MYSEQ available from ILLUMINA. In addition, included 5-prime to the sequencing tails is an additional sequence that can be used as a priming site in a subsequent PCR to add nucleotide barcode sequences to the amplicons, enabling multiplex sequencing of multiple samples in a single lane of the high throughput sequencing instrument.

In an embodiment, a 10,000-plex PCR assay pool is created such that reverse primers have tails corresponding to the required reverse sequences required by a high throughput sequencing instrument. After amplification with the first 10,000-plex assay, a subsequent PCR amplification may be performed using a another 10,000-plex pool having partly nested forward primers (*e.g.* 6-bases nested) for all targets and a reverse primer corresponding to the reverse sequencing tail included in the first round. This subsequent round of partly nested amplification with just one target specific primer and a universal primer limits the required size of the assay, reducing sampling noise, but greatly reduces the number of spurious amplicons. The sequencing tags can be added to appended ligation adaptors and/or as part of PCR probes, such that the tag is part of the final amplicon.

Fetal fraction affects performance of the test. There are a number of ways to enrich the fetal fraction of the DNA found in maternal plasma. Fetal fraction can be increased by the previously described LM-PCR method already discussed as well as by a targeted removal of long maternal fragments. In an embodiment, prior to multiplex PCR amplification of the target loci, an additional multiplex PCR reaction may be carried out to selectively remove long and largely maternal fragments corresponding to the loci targeted in the subsequent multiplex PCR. Additional primers are designed to anneal a site a greater distance from the polymorphism than is expected to be present among cell free fetal DNA fragments. These primers may be used in a one cycle multiplex PCR reaction prior to multiplex PCR of the target polymorphic loci. These distal primers are tagged with a molecule or moiety that can allow selective recognition of the tagged pieces of DNA. In an embodiment, these molecules of DNA may be covalently modified with a biotin molecule that allows removal of newly formed double stranded DNA

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comprising these primers after one cycle of PCR. Double stranded DNA formed during that first round is likely maternal in origin. Removal of the hybrid material may be accomplish by the used of magnetic streptavidin beads. There are other methods of tagging that may work equally well. In an embodiment, size selection methods may be used to enrich the sample for shorter strands of DNA; for example those less than about 800 bp, less than about 500 bp, or less than about 300 bp. Amplification of short fragments can then proceed as usual.

The mini-PCR method described in this disclosure enables highly multiplexed amplification and analysis of hundreds to thousands or even millions of loci in a single reaction, from a single sample. At the same, the detection of the amplified DNA can be multiplexed; tens to hundreds of samples can be multiplexed in one sequencing lane by using barcoding PCR. This multiplexed detection has been successfully tested up to 49-plex, and a much higher degree of multiplexing is possible. In effect, this allows hundreds of samples to be genotyped at thousands of SNPs in a single sequencing run. For these samples, the method allows determination of genotype and heterozygosity rate and simultaneously determination of copy number, both of which may be used for the purpose of aneuploidy detection. This method is particularly useful in detecting aneuploidy of a gestating fetus from the free floating DNA found in maternal plasma. This method may be used as part of a method for sexing a fetus, and/or predicting the paternity of the fetus. It may be used as part of a method for mutation dosage. This method may be used for any amount of DNA or RNA, and the targeted regions may be SNPs, other polymorphic regions, non-polymorphic regions, and combinations thereof.

In some embodiments, ligation mediated universal-PCR amplification of fragmented DNA may be used. The ligation mediated universal-PCR amplification can be used to amplify plasma DNA, which can then be divided into multiple parallel reactions. It may also be used to preferentially amplify short fragments, thereby enriching fetal fraction. In some embodiments the addition of tags to the fragments by ligation can enable detection of shorter fragments, use of shorter target sequence specific portions of the primers and/or annealing at higher temperatures which reduces unspecific reactions.

The methods described herein may be used for a number of purposes where there is a target set of DNA that is mixed with an amount of contaminating DNA. In some embodiments, the target DNA and the contaminating DNA may be from individuals who are genetically related. For example, genetic abnormalities in a fetus (target) may be detected from maternal plasma which contains fetal (target) DNA and also maternal (contaminating) DNA; the

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abnormalities include whole chromosome abnormalities (*e.g.* aneuploidy) partial chromosome abnormalities (*e.g.* deletions, duplications, inversions, translocations), polynucleotide polymorphisms (*e.g.* STRs), single nucleotide polymorphisms, and/or other genetic abnormalities or differences. In some embodiments, the target and contaminating DNA may be from the same individual, but where the target and contaminating DNA are different by one or more mutations, for example in the case of cancer. (see *e.g.* H. Mamon et al. *Preferential Amplification of Apoptotic DNA from Plasma: Potential for Enhancing Detection of Minor DNA Alterations in Circulating DNA*. Clinical Chemistry 54:9 (2008). In some embodiments, the DNA may be found in cell culture (apoptotic) supernatant. In some embodiments, it is possible to induce apoptosis in biological samples (*e.g.,* blood) for subsequent library preparation, amplification and/or sequencing. A number of enabling workflows and protocols to achieve this end are presented elsewhere in this disclosure.

In some embodiments, the target DNA may originate from single cells, from samples of DNA consisting of less than one copy of the target genome, from low amounts of DNA, from DNA from mixed origin (*e.g.* pregnancy plasma: placental and maternal DNA; cancer patient plasma and tumors: mix between healthy and cancer DNA, transplantation *etc*), from other body fluids, from cell cultures, from culture supernatants, from forensic samples of DNA, from ancient samples of DNA (*e.g.* insects trapped in amber), from other samples of DNA, and combinations thereof.

In some embodiments, a short amplicon size may be used. Short amplicon sizes are especially suited for fragmented DNA (see *e.g.* A. Sikora, et sl. Detection of increased amounts of cell-free fetal DNA with short PCR amplicons. Clin Chem. 2010 Jan;56(1):136-8.)

The use of short amplicon sizes may result in some significant benefits. Short amplicon sizes may result in optimized amplification efficiency. Short amplicon sizes typically produce shorter products, therefore there is less chance for nonspecific priming. Shorter products can be clustered more densely on sequencing flow cell, as the clusters will be smaller. Note that the methods described herein may work equally well for longer PCR amplicons. Amplicon length may be increased if necessary, for example, when sequencing larger sequence stretches. Experiments with 146-plex targeted amplification with assays of 100 bp to 200 bp length as first step in a nested-PCR protocol were run on single cells and on genomic DNA with positive results.

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In some embodiments, the methods described herein may be used to amplify and/or detect SNPs, copy number, nucleotide methylation, mRNA levels, other types of RNA expression levels, other genetic and/or epigenetic features. The mini-PCR methods described herein may be used along with next-generation sequencing; it may be used with other downstream methods such as microarrays, counting by digital PCR, real-time PCR, Mass-spectrometry analysis etc.

In some embodiment, the mini-PCR amplification methods described herein may be used as part of a method for accurate quantification of minority populations. It may be used for absolute quantification using spike calibrators. It may be used for mutation / minor allele quantification through very deep sequencing, and may be run in a highly multiplexed fashion. It may be used for standard paternity and identity testing of relatives or ancestors, in human, animals, plants or other creatures. It may be used for forensic testing. It may be used for rapid genotyping and copy number analysis (CN), on any kind of material, *e.g.* amniotic fluid and CVS, sperm, product of conception (POC). It may be used for single cell analysis, such as genotyping on samples biopsied from embryos. It may be used for rapid embryo analysis (within less than one, one, or two days of biopsy) by targeted sequencing using min-PCR.

In some embodiments, it may be used for tumor analysis: tumor biopsies are often a mixture of health and tumor cells. Targeted PCR allows deep sequencing of SNPs and loci with close to no background sequences. It may be used for copy number and loss of heterozygosity analysis on tumor DNA. Said tumor DNA may be present in many different body fluids or tissues of tumor patients. It may be used for detection of tumor recurrence, and/or tumor screening. It may be used for quality control testing of seeds. It may be used for breeding, or fishing purposes. Note that any of these methods could equally well be used targeting non-polymorphic loci for the purpose of ploidy calling.

Some literature describing some of the fundamental methods that underlie the methods disclosed herein include: (1) Wang HY, Luo M, Tereshchenko IV, Frikker DM, Cui X, Li JY, Hu G, Chu Y, Azaro MA, Lin Y, Shen L, Yang Q, Kambouris ME, Gao R, Shih W, Li H. Genome Res. 2005 Feb;15(2):276-83. Department of Molecular Genetics, Microbiology and Immunology/The Cancer Institute of New Jersey, Robert Wood Johnson Medical School, New Brunswick, New Jersey 08903, USA. (2) High-throughput genotyping of single nucleotide polymorphisms with high sensitivity. Li H, Wang HY, Cui X, Luo M, Hu G, Greenawalt DM, Tereshchenko IV, Li JY, Chu Y, Gao R. Methods Mol Biol. 2007;396 - PubMed PMID:

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18025699. (3) A method comprising multiplexing of an average of 9 assays for sequencing is described in: Nested Patch PCR enables highly multiplexed mutation discovery in candidate genes. Varley KE, Mitra RD. Genome Res. 2008 Nov;18(11):1844-50. Epub 2008 Oct 10. Note that the methods disclosed herein allow multiplexing of orders of magnitude more than in the above references.

Targeted PCR Variants - Nesting

There are many workflows that are possible when conducting PCR; some workflows typical to the methods disclosed herein are described. The steps outlined herein are not meant to exclude other possible steps nor does it imply that any of the steps described herein are required for the method to work properly. A large number of parameter variations or other modifications are known in the literature, and may be made without affecting the essence of the invention. One particular generalized workflow is given below followed by a number of possible variants. The variants typically refer to possible secondary PCR reactions, for example different types of nesting that may be done (step 3). It is important to note that variants may be done at different times, or in different orders than explicitly described herein. Examples that use polymorphic loci for illustration can be readily adapted for the amplification of nonpolymorphic loci if desired.

1. The DNA in the sample may have ligation adapters, often referred to as library tags or ligation adaptor tags (LTs), appended, where the ligation adapters contain a universal priming sequence, followed by a universal amplification. In an embodiment, this may be done using a standard protocol designed to create sequencing libraries after fragmentation. In an embodiment, the DNA sample can be blunt ended, and then an A can be added at the 3' end. A Y-adaptor with a T-overhang can be added and ligated. In some embodiments, other sticky ends can be used other than an A or T overhang. In some embodiments, other adaptors can be added, for example looped ligation adaptors. In some embodiments, the adaptors may have tag designed for PCR amplification.

2. Specific Target Amplification (STA): Pre-amplification of hundreds to thousands to tens of thousands and even hundreds of thousands of targets may be multiplexed in one reaction volume. STA is typically run from 10 to 30 cycles, though it may be run from 5 to 40 cycles, from 2 to 50 cycles, and even from 1 to 100 cycles. Primers may be tailed, for example for a simpler workflow or to avoid sequencing of a large proportion of dimers. Note that

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typically, dimers of both primers carrying the same tag will not be amplified or sequenced efficiently. In some embodiments, between 1 and 10 cycles of PCR may be carried out; in some embodiments between 10 and 20 cycles of PCR may be carried out; in some embodiments between 20 and 30 cycles of PCR may be carried out; in some embodiments between 30 and 40 cycles of PCR may be carried out; in some embodiments more than 40 cycles of PCR may be carried out. The amplification may be a linear amplification. The number of PCR cycles may be optimized to result in an optimal depth of read (DOR) profile. Different DOR profiles may be desirable for different purposes. In some embodiments, a more even distribution of reads between all assays is desirable; if the DOR is too small for some assays, the stochastic noise can be too high for the data to be too useful, while if the depth of read is too high, the marginal usefulness of each additional read is relatively small.

Primer tails may improve the detection of fragmented DNA from universally tagged libraries. If the library tag and the primer-tails contain a homologous sequence, hybridization can be improved (for example, melting temperature (T_M) is lowered) and primers can be extended if only a portion of the primer target sequence is in the sample DNA fragment. In some embodiments, 13 or more target specific base pairs may be used. In some embodiments, 10 to 12 target specific base pairs may be used. In some embodiments, 8 to 9 target specific base pairs may be used. In some embodiments, 6 to 7 target specific base pairs may be used. In some embodiments, STA may be performed on pre-amplified DNA, *e.g.* MDA, RCA, other whole genome amplifications, or adaptor-mediated universal PCR. In some embodiments, STA may be performed on samples that are enriched or depleted of certain sequences and populations, *e.g.* by size selection, target capture, directed degradation.

3. In some embodiments, it is possible to perform secondary multiplex PCRs or primer extension reactions to increase specificity and reduce undesirable products. For example, full nesting, semi-nesting, hemi-nesting, and/or subdividing into parallel reactions of smaller assay pools are all techniques that may be used to increase specificity. Experiments have shown that splitting a sample into three 400-plex reactions resulted in product DNA with greater specificity than one 1,200-plex reaction with exactly the same primers. Similarly, experiments have shown that splitting a sample into four 2,400-plex reactions resulted in product DNA with greater specificity than one 9,600-plex reaction with exactly the same primers. In an embodiment, it is possible to use target-specific and tag specific primers of the same and opposing directionality.

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4. In some embodiments, it is possible to amplify a DNA sample (dilution, purified or otherwise) produced by an STA reaction using tag-specific primers and "universal amplification", *i.e.* to amplify many or all pre-amplified and tagged targets. Primers may contain additional functional sequences, *e.g.* barcodes, or a full adaptor sequence necessary for sequencing on a high throughput sequencing platform.

These methods may be used for analysis of any sample of DNA, and are especially useful when the sample of DNA is particularly small, or when it is a sample of DNA where the DNA originates from more than one individual, such as in the case of maternal plasma. These methods may be used on DNA samples such as a single or small number of cells, genomic DNA, plasma DNA, amplified plasma libraries, amplified apoptotic supernatant libraries, or other samples of mixed DNA. In an embodiment, these methods may be used in the case where cells of different genetic constitution may be present in a single individual, such as with cancer or transplants.

Protocol variants (variants and/or additions to the workflow above)

Direct multiplexed mini-PCR: Specific target amplification (STA) of a plurality of target sequences with tagged primers is shown in FIG. 1. 101 denotes double stranded DNA with a polymorphic locus of interest at X. 102 denotes the double stranded DNA with ligation adaptors added for universal amplification. 103 denotes the single stranded DNA that has been universally amplified with PCR primers hybridized. 104 denotes the final PCR product. In some embodiments, STA may be done on more than 100, more than 200, more than 500, more than 1,000, more than 2,000, more than 5,000, more than 10,000, more than 20,000, more than 50,000, more than 100,000 or more than 200,000 targets. In a subsequent reaction, tag-specific primers amplify all target sequences and lengthen the tags to include all necessary sequences for sequencing, including sample indexes. In an embodiment, primers may not be tagged or only certain primers may be tagged. Sequencing adaptors may be added by conventional adaptor ligation. In an embodiment, the initial primers may carry the tags.

In an embodiment, primers are designed so that the length of DNA amplified is unexpectedly short. Prior art demonstrates that ordinary people skilled in the art typically design 100+ bp amplicons. In an embodiment, the amplicons may be designed to be less than 80 bp. In an embodiment, the amplicons may be designed to be less than 70 bp. In an embodiment, the amplicons may be designed to be less than 60 bp. In an embodiment, the

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amplicons may be designed to be less than 50 bp. In an embodiment, the amplicons may be designed to be less than 45 bp. In an embodiment, the amplicons may be designed to be less than 40 bp. In an embodiment, the amplicons may be designed to be less than 35 bp. In an embodiment, the amplicons may be designed to be between 40 and 65 bp.

An experiment was performed using this protocol using 1200-plex amplification. Both genomic DNA and pregnancy plasma were used; about 70% of sequence reads mapped to targeted sequences. Details are given elsewhere in this document. Sequencing of a 1042-plex without design and selection of assays resulted in >99% of sequences being primer dimer products.

Sequential PCR: After STA1 multiple aliquots of the product may be amplified in parallel with pools of reduced complexity with the same primers. The first amplification can give enough material to split. This method is especially good for small samples, for example those that are about 6-100 pg, about 100 pg to 1 ng, about 1 ng to 10 ng, or about 10 ng to 100 ng. The protocol was performed with 1200-plex into three 400-plexes. Mapping of sequencing reads increased from around 60 to 70 % in the 1200-plex alone to over 95%.

Semi-nested mini-PCR: (see FIG. 2) After STA 1 a second STA is performed comprising a multiplex set of internal nested Forward primers (103 B, 105 b) and one (or few) tag-specific Reverse primers (103 A). 101 denotes double stranded DNA with a polymorphic locus of interest at X. 102 denotes the double stranded DNA with ligation adaptors added for universal amplification. 103 denotes the single stranded DNA that has been universally amplified with Forward primer B and Reverse Primer A hybridized. 104 denotes the PCR product from 103. 105 denotes the product from 104 with nested Forward primer b hybridized, and Reverse tag A already part of the molecule from the PCR that occurred between 103 and 104. 106 denotes the final PCR product. With this workflow usually greater than 95% of sequences map to the intended targets. The nested primer may overlap with the outer Forward primer sequence but introduces additional 3'-end bases. In some embodiments it is possible to use between one and 20 extra 3' bases. Experiments have shown that using 9 or more extra 3' bases in a 1200-plex designs works well.

Fully nested mini-PCR: (see FIG. 3) After STA step 1, it is possible to perform a second multiplex PCR (or parallel m.p. PCRs of reduced complexity) with two nested primers carrying tags (A, a, B, b). 101 denotes double stranded DNA with a polymorphic locus of interest at X. 102 denotes the double stranded DNA with ligation adaptors added for universal

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amplification. 103 denotes the single stranded DNA that has been universally amplified with Forward primer B and Reverse Primer A hybridized. 104 denotes the PCR product from 103. 105 denotes the product from 104 with nested Forward primer b and nested Reverse primer a hybridized. 106 denotes the final PCR product. In some embodiments, it is possible to use two full sets of primers. Experiments using a fully nested mini-PCR protocol were used to perform 146-plex amplification on single and three cells without step 102 of appending universal ligation adaptors and amplifying.

Hemi-nested mini-PCR: (see FIG. 4) It is possible to use target DNA that has and adaptors at the fragment ends. STA is performed comprising a multiplex set of Forward primers (B) and one (or few) tag-specific Reverse primers (A). A second STA can be performed using a universal tag-specific Forward primer and target specific Reverse primer. 101 denotes double stranded DNA with a polymorphic locus of interest at X. 102 denotes the double stranded DNA with ligation adaptors added for universal amplification. 103 denotes the single stranded DNA that has been universally amplified with Reverse Primer A hybridized. 104 denotes the PCR product from 103 that was amplified using Reverse primer A and ligation adaptor tag primer LT. 105 denotes the product from 104 with Forward primer B hybridized. 106 denotes the final PCR product. In this workflow, target specific Forward and Reverse primers are used in separate reactions, thereby reducing the complexity of the reaction and preventing dimer formation of forward and reverse primers. Note that in this example, primers A and B may be considered to be first primers, and primers 'a' and 'b' may be considered to be inner primers. This method is a big improvement on direct PCR as it is as good as direct PCR, but it avoids primer dimers. After first round of hemi nested protocol one typically sees ~99% non-targeted DNA, however, after second round there is typically a big improvement.

Triply hemi-nested mini-PCR: (see FIG. 5) It is possible to use target DNA that has and adaptor at the fragment ends. STA is performed comprising a multiplex set of Forward primers (B) and one (or few) tag-specific Reverse primers (A) and (a). A second STA can be performed using a universal tag-specific Forward primer and target specific Reverse primer. 101 denotes double stranded DNA with a polymorphic locus of interest at X. 102 denotes the double stranded DNA with ligation adaptors added for universal amplification. 103 denotes the single stranded DNA that has been universally amplified with Reverse Primer A hybridized. 104 denotes the PCR product from 103 that was amplified using Reverse primer A and ligation adaptor tag primer LT. 105 denotes the product from 104 with Forward primer B hybridized.

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106 denotes the PCR product from 105 that was amplified using Reverse primer A and Forward primer B. 107 denotes the product from 106 with Reverse primer 'a' hybridized. 108 denotes the final PCR product. Note that in this example, primers 'a' and B may be considered to be inner primers, and A may be considered to be a first primer. Optionally, both A and B may be considered to be first primers, and 'a' may be considered to be an inner primer. The designation of reverse and forward primers may be switched. In this workflow, target specific Forward and Reverse primers are used in separate reactions, thereby reducing the complexity of the reaction and preventing dimer formation of forward and reverse primers. This method is a big improvement on direct PCR as it is as good as direct PCR, but it avoids primer dimers. After first round of hemi nested protocol one typically sees ~99% non-targeted DNA, however, after second round there is typically a big improvement.

One-sided nested mini-PCR: (see FIG. 6) It is possible to use target DNA that has an adaptor at the fragment ends. STA may also be performed with a multiplex set of nested Forward primers and using the ligation adapter tag as the Reverse primer. A second STA may then be performed using a set of nested Forward primers and a universal Reverse primer. 101 denotes double stranded DNA with a polymorphic locus of interest at X. 102 denotes the double stranded DNA with ligation adaptors added for universal amplification. 103 denotes the single stranded DNA that has been universally amplified with Forward Primer A hybridized. 104 denotes the PCR product from 103 that was amplified using Forward primer A and ligation adaptor tag Reverse primer LT. 105 denotes the product from 104 with nested Forward primer a hybridized. 106 denotes the final PCR product. This method can detect shorter target sequences than standard PCR by using overlapping primers in the first and second STAs. The method is typically performed off a sample of DNA that has already undergone STA step 1 above – appending of universal tags and amplification; the two nested primers are only on one side, other side uses the library tag. The method was performed on libraries of apoptotic supernatants and pregnancy plasma. With this workflow around 60% of sequences mapped to the intended targets. Note that reads that contained the reverse adaptor sequence were not mapped, so this number is expected to be higher if those reads that contain the reverse adaptor sequence are mapped

One-sided mini-PCR: It is possible to use target DNA that has an adaptor at the fragment ends (see FIG. 7). STA may be performed with a multiplex set of Forward primers and one (or few) tag-specific Reverse primer. 101 denotes double stranded DNA with a

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polymorphic locus of interest at X. 102 denotes the double stranded DNA with ligation adaptors added for universal amplification. 103 denotes the single stranded DNA with Forward Primer A hybridized. 104 denotes the PCR product from 103 that was amplified using Forward primer A and ligation adaptor tag Reverse primer LT, and which is the final PCR product. This method can detect shorter target sequences than standard PCR. However it may be relatively unspecific, as only one target specific primer is used. This protocol is effectively half of the one sided nested mini PCR

Reverse semi-nested mini-PCR: It is possible to use target DNA that has an adaptor at the fragment ends (see FIG. 8). STA may be performed with a multiplex set of Forward primers and one (or few) tag-specific Reverse primer. 101 denotes double stranded DNA with a polymorphic locus of interest at X. 102 denotes the double stranded DNA with ligation adaptors added for universal amplification. 103 denotes the single stranded DNA with Reverse Primer B hybridized. 104 denotes the PCR product from 103 that was amplified using Reverse primer B and ligation adaptor tag Forward primer LT. 105 denotes the PCR product 104 with hybridized Forward Primer A, and inner Reverse primer 'b'. 106 denotes the PCR product that has been amplified from 105 using Forward primer A and Reverse primer 'b', and which is the final PCR product. This method can detect shorter target sequences than standard PCR.

There also may be more variants that are simply iterations or combinations of the above methods such as doubly nested PCR, where three sets of primers are used. Another variant is one-and-a-half sided nested mini-PCR, where STA may also be performed with a multiplex set of nested Forward primers and one (or few) tag-specific Reverse primer.

Note that in all of these variants, the identity of the Forward primer and the Reverse primer may be interchanged. Note that in some embodiments, the nested variant can equally well be run without the initial library preparation that comprises appending the adapter tags, and a universal amplification step. Note that in some embodiments, additional rounds of PCR may be included, with additional Forward and/or Reverse primers and amplification steps; these additional steps may be particularly useful if it is desirable to further increase the percent of DNA molecules that correspond to the targeted loci.

Nesting Workflows

There are many ways to perform the amplification, with different degrees of nesting, and with different degrees of multiplexing. In FIG. 9, a flow chart is given with some of the

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possible workflows. Note that the use of 10,000-plex PCR is only meant to be an example; these flow charts would work equally well for other degrees of multiplexing.

Looped ligation adaptors

When adding universal tagged adaptors for example for the purpose of making a library for sequencing, there are a number of ways to ligate adaptors. One way is to blunt end the sample DNA, perform A-tailing, and ligate with adaptors that have a T-overhang. There are a number of other ways to ligate adaptors. There are also a number of adaptors that can be ligated. For example, a Y-adaptor can be used where the adaptor consists of two strands of DNA where one strand has a double strand region, and a region specified by a forward primer region, and where the other strand specified by a double strand region that is complementary to the double strand region on the first strand, and a region with a reverse primer. The double stranded region, when annealed, may contain a T-overhang for the purpose of ligating to double stranded DNA with an A overhang.

In an embodiment, the adaptor can be a loop of DNA where the terminal regions are complementary, and where the loop region contains a forward primer tagged region (LFT), a reverse primer tagged region (LRT), and a cleavage site between the two (See FIG. 10). 101 refers to the double stranded, blunt ended target DNA. 102 refers to the A-tailed target DNA. 103 refers to the looped ligation adaptor with T overhang 'T' and the cleavage site 'Z'. 104 refers to the target DNA with appended looped ligation adaptors. 105 refers to the target DNA with the ligation adaptors appended cleaved at the cleavage site. LFT refers to the ligation adaptor Forward tag, and the LRT refers to the ligation adaptor Reverse tag. The complementary region may end on a T overhang, or other feature that may be used for ligation to the target DNA. The cleavage site may be a series of uracils for cleavage by UNG, or a sequence that may be recognized and cleaved by a restriction enzyme or other method of cleavage or just a basic amplification. These adaptors can be uses for any library preparation, for example, for sequencing. These adaptors can be used in combination with any of the other methods described herein, for example the mini-PCR amplification methods.

Internally Tagged Primers

When using sequencing to determine the allele present at a given polymorphic locus, the sequence read typically begins upstream of the primer binding site (a), and then to the

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polymorphic site (X). Tags are typically configured as shown in FIG. 11, left. 101 refers to the single stranded target DNA with polymorphic locus of interest 'X', and primer 'a' with appended tag 'b'. In order to avoid nonspecific hybridization, the primer binding site (region of target DNA complementary to 'a') is typically 18 to 30 bp in length. Sequence tag 'b' is typically about 20 bp; in theory these can be any length longer than about 15 bp, though many people use the primer sequences that are sold by the sequencing platform company. The distance 'd' between 'a' and 'X' may be at least 2 bp so as to avoid allele bias. When performing multiplexed PCR amplification using the methods disclosed herein or other methods, where careful primer design is necessary to avoid excessive primer primer interaction, the window of allowable distance 'd' between 'a' and 'X' may vary quite a bit: from 2 bp to 10 bp, from 2 bp to 20 bp, from 2 bp to 30 bp, or even from 2 bp to more than 30 bp. Therefore, when using the primer configuration shown in FIG. 11, left, sequence reads must be a minimum of 40 bp to obtain reads long enough to measure the polymorphic locus, and depending on the lengths of 'a' and 'd' the sequence reads may need to be up to 60 or 75 bp. Usually, the longer the sequence reads, the higher the cost and time of sequencing a given number of reads, therefore, minimizing the necessary read length can save both time and money. In addition, since, on average, bases read earlier on the read are read more accurately than those read later on the read, decreasing the necessary sequence read length can also increase the accuracy of the measurements of the polymorphic region.

In an embodiment, termed internally tagged primers, the primer binding site (a) is split in to a plurality of segments (a', a", a'"….), and the sequence tag (b) is on a segment of DNA that is in the middle of two of the primer binding sites, as shown in FIG. 11, 103. This configuration allows the sequencer to make shorter sequence reads. In an embodiment, a' + a" should be at least about 18 bp, and can be as long as 30, 40, 50, 60, 80, 100 or more than 100 bp. In an embodiment, a" should be at least about 6 bp, and in an embodiment is between about 8 and 16 bp. All other factors being equal, using the internally tagged primers can cut the length of the sequence reads needed by at least 6 bp, as much as 8 bp, 10 bp, 12 bp, 15 bp, and even by as many as 20 or 30 bp. This can result in a significant money, time and accuracy advantage. An example of internally tagged primers is given in FIG. 12.

Primers with ligation adaptor binding region

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One issue with fragmented DNA is that since it is short in length, the chance that a polymorphism is close to the end of a DNA strand is higher than for a long strand (*e.g.* 101, FIG. 10). Since PCR capture of a polymorphism requires a primer binding site of suitable length on both sides of the polymorphism, a significant number of strands of DNA with the targeted polymorphism will be missed due to insufficient overlap between the primer and the targeted binding site. In an embodiment, the target DNA 101 can have ligation adaptors appended 102, and the target primer 103 can have a region (cr) that is complementary to the ligation adaptor tag (lt) appended upstream of the designed binding region (a) (see FIG. 13); thus in cases where the binding region (region of 101 that is complementary to a) is shorter than the 18 bp typically required for hybridization, the region (cr) on the primer than is complementary to the library tag is able to increase the binding energy to a point where the PCR can proceed. Note that any specificity that is lost due to a shorter binding region can be made up for by other PCR primers with suitably long target binding regions. Note that this embodiment can be used in combination with direct PCR, or any of the other methods described herein, such as nested PCR, semi nested PCR, hemi nested PCR, one sided nested or semi or hemi nested PCR, or other PCR protocols.

When using the sequencing data to determine ploidy in combination with an analytical method that involves comparing the observed allele data to the expected allele distributions for various hypotheses, each additional read from alleles with a low depth of read will yield more information than a read from an allele with a high depth of read. Therefore, ideally, one would wish to see uniform depth of read (DOR) where each locus will have a similar number of representative sequence reads. Therefore, it is desirable to minimize the DOR variance. In an embodiment, it is possible to decrease the coefficient of variance of the DOR (this may be defined as the standard deviation of the DOR / the average DOR) by increasing the annealing times. In some embodiments the annealing temperatures may be longer than 2 minutes, longer than 4 minutes, longer than ten minutes, longer than 30 minutes, and longer than one hour, or even longer. Since annealing is an equilibrium process, there is no limit to the improvement of DOR variance with increasing annealing times. In an embodiment, increasing the primer concentration may decrease the DOR variance.

Exemplary Amplification Methods

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Improved PCR amplification methods have also been developed that minimize or prevent interference due to the amplification of nearby or adjacent target loci in the same reaction volume (such as part of the sample multiplex PCR reaction that simultaneously amplifies all the target loci) (see, USSN 61/982245, filed April 21, 2014; and USSN 61/987407, filed May 1, 2014, which are each hereby incorporated by reference in its entirety).

These methods can be used to simultaneously amplify nearby or adjacent target loci, which is faster and cheaper than having to separate nearby target loci into different reaction volumes so that they can be amplified separately to avoid interference. In particular embodiments, these methods are used to tile a region such that the amplicons include all the nucleotides in the region (such as an exon or all the exons of a gene such as cystic fibrosis).

In some embodiments, the amplification of target loci is performed using a polymerase (*e.g.*, a DNA polymerase, RNA polymerase, or reverse transcriptase) with low 5′→ 3′ exonuclease and/or low strand displacement activity. In some embodiments, the low level of 5′→ 3′ exonuclease reduces or prevents the degradation of a nearby primer (*e.g.*, an unextended primer or a primer that has had one or more nucleotides added to during primer extension). In some embodiments, the low level of strand displacement activity reduces or prevents the displacement of a nearby primer (*e.g.*, an unextended primer or a primer that has had one or more nucleotides added to it during primer extension). In some embodiments, target loci that are adjacent to each other (*e.g.*, no bases between the target loci) or nearby (*e.g.*, loci are within 50, 40, 30, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 base) are amplified. In some embodiments, the 3′ end of one locus is within 50, 40, 30, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 base of the 5′ end of next downstream locus.

In some embodiments, at least 100, 200, 500, 750, 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci are amplified, such as by the simultaneous amplification in one reaction volume In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the amplified products are target amplicons. In various embodiments, the amount of amplified products that are target amplicons is between 50 to 99.5%, such as between 60 to 99%, 70 to 98%, 80 to 98%, 90 to 99.5%, or 95 to 99.5%, inclusive. In some embodiments, at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target loci are amplified (*e.g*, amplified at least 5, 10, 20, 30, 50, or 100-fold compared to the amount prior to amplification), such as by the simultaneous amplification in one reaction volume. In various embodiments, the amount

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target loci that are amplified (*e.g*, amplified at least 5, 10, 20, 30, 50, or 100-fold compared to the amount prior to amplification) is between 50 to 99.5%, such as between 60 to 99%, 70 to 98%, 80 to 99%, 90 to 99.5%, 95 to 99.9%, or 98 to 99.99% inclusive. In some embodiments, fewer non-target amplicons are produced, such as fewer amplicons formed from a forward primer from a first primer pair and a reverse primer from a second primer pair. Such undesired non-target amplicons can be produced using prior amplification methods if, *e.g.*, the reverse primer from the first primer pair and/or the forward primer from the second primer pair are degraded and/or displaced.

In some embodiments, these methods allows longer extension times to be used since the polymerase bound to a primer being extended is less likely to degrade and/or displace a nearby primer (such as the next downstream primer) given the low 5′→ 3′ exonuclease and/or low strand displacement activity of the polymerase. In various embodiments, reaction conditions (such as the extension time and temperature) are used such that the extension rate of the polymerase allows the number of nucleotides that are added to a primer being extended to be equal to or greater than 80, 90, 95, 100, 110, 120, 130, 140, 150, 175, or 200% of the number of nucleotides between the 3' end of the primer binding site and the 5'end of the next downstream primer binding site on the same strand.

In some embodiments, a DNA polymerase is used produce DNA amplicons using DNA as a template. In some embodiments, a RNA polymerase is used produce RNA amplicons using DNA as a template. In some embodiments, a reverse transcriptase is used produce cDNA amplicons using RNA as a template.

In some embodiments, the low level of 5′→ 3′ exonuclease of the polymerase is less than 80, 70, 60, 50, 40, 30, 20, 10, 5, 1, or 0.1% of the activity of the same amount of *Thermus aquaticus* polymerase ("Taq" polymerase, which is a commonly used DNA polymerase from a thermophilic bacterium, PDB 1BGX, EC 2.7.7.7, Murali *et al*., "Crystal structure of Taq DNA polymerase in complex with an inhibitory Fab: the Fab is directed against an intermediate in the helix-coil dynamics of the enzyme," Proc. Natl. Acad. Sci. USA 95:12562-12567, 1998, which is hereby incorporated by reference in its entirety) under the same conditions. In some embodiments, the low level of strand displacement activity of the polymerase is less than 80, 70, 60, 50, 40, 30, 20, 10, 5, 1, or 0.1% of the activity of the same amount of Taq polymerase under the same conditions.

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In some embodiments, the polymerase is a PUSHION DNA polymerase, such as PHUSION High Fidelity DNA polymerase (M0530S, New England BioLabs, Inc.) or PHUSION Hot Start Flex DNA polymerase (M0535S, New England BioLabs, Inc.; Frey and Suppman *BioChemica*. 2:34-35, 1995; Chester and Marshak *Analytical Biochemistry*. 209:284-290, 1993, which are each hereby incorporated by reference in its entirety). The PHUSION DNA polymerase is a *Pyrococcus*-like enzyme fused with a processivity-enhancing domain. PHUSION DNA polymerase possesses 5′→ 3′ polymerase activity and 3′→ 5′ exonuclease activity, and generates blunt-ended products. PHUSION DNA polymerase lacks 5′→ 3′ exonuclease activity and strand displacement activity.

In some embodiments, the polymerase is a Q5® DNA Polymerase, such as Q5® High-Fidelity DNA Polymerase (M0491S, New England BioLabs, Inc.) or Q5® Hot Start High-Fidelity DNA Polymerase (M0493S, New England BioLabs, Inc.). Q5® High-Fidelity DNA polymerase is a high-fidelity, thermostable, DNA polymerase with 3′→ 5′ exonuclease activity, fused to a processivity-enhancing Sso7d domain. Q5® High-Fidelity DNA polymerase lacks 5′→ 3′ exonuclease activity and strand displacement activity.

In some embodiments, the polymerase is a T4 DNA polymerase (M0203S, New England BioLabs, Inc.; Tabor and Struh. (1989). "DNA-Dependent DNA Polymerases," In Ausebel et al. (Ed.), *Current Protocols in Molecular Biology*. 3.5.10-3.5.12. New York: John Wiley & Sons, Inc., 1989; Sambrook et al. *Molecular Cloning: A Laboratory Manual*. (2nd ed.), 5.44-5.47. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1989, which are each hereby incorporated by reference in its entirety). T4 DNA Polymerase catalyzes the synthesis of DNA in the 5′→ 3′ direction and requires the presence of template and primer. This enzyme has a 3′→ 5′ exonuclease activity which is much more active than that found in DNA Polymerase I. T4 DNA polymerase lacks 5′→ 3′ exonuclease activity and strand displacement activity.

In some embodiments, the polymerase is a *Sulfolobus* DNA Polymerase IV (M0327S, New England BioLabs, Inc.; (Boudsocq,. *et al.* (2001). *Nucleic Acids Res.,* 29:4607-4616, 2001; McDonald, *et al.* (2006). *Nucleic Acids Res.,* 34:1102-1111, 2006, which are each hereby incorporated by reference in its entirety). *Sulfolobus* DNA Polymerase IV is a thermostable Y-family lesion-bypass DNA Polymerase that efficiently synthesizes DNA across a variety of DNA template lesions McDonald, J.P. et al. (2006). *Nucleic Acids Res.,*. 34,

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1102-1111, which is hereby incorporated by reference in its entirety). *Sulfolobus* DNA Polymerase IV lacks 5′→ 3′ exonuclease activity and strand displacement activity.

In some embodiments, if a primer binds a region with a SNP, the primer may bind and amplify the different alleles with different efficiencies or may only bind and amplify one allele. For subjects who are heterozygous, one of the alleles may not be amplified by the primer. In some embodiments, a primer is designed for each allele. For example, if there are two alleles (*e.g.*, a biallelic SNP), then two primers can be used to bind the same location of a target locus (*e.g.*, a forward primer to bind the "A" allele and a forward primer to bind the "B" allele). Standard methods, such as the dbSNP database, can be used to determine the location of known SNPs, such as SNP hot spots that have a high heterozygosity rate.

In some embodiments, the amplicons are similar in size. In some embodiments, the range of the length of the target amplicons is less than 100, 75, 50, 25, 15, 10, or 5 nucleotides. In some embodiments (such as the amplification of target loci in fragmented DNA or RNA), the length of the target amplicons is between 50 and 100 nucleotides, such as between 60 and 80 nucleotides, or 60 and 75 nucleotides, inclusive. In some embodiments (such as the amplification of multiple target loci throughout an exon or gene), the length of the target amplicons is between 100 and 500 nucleotides, such as between 150 and 450 nucleotides, 200 and 400 nucleotides, 200 and 300 nucleotides, or 300 and 400 nucleotides, inclusive.

In some embodiments, multiple target loci are simultaneously amplified using a primer pair that includes a forward and reverse primer for each target locus to be amplified in that reaction volume. In some embodiments, one round of PCR is performed with a single primer per target locus, and then a second round of PCR is performed with a primer pair per target locus. For example, the first round of PCR may be performed with a single primer per target locus such that all the primers bind the same strand (such as using a forward primer for each target locus). This allows the PCR to amplify in a linear manner and reduces or eliminates amplification bias between amplicons due to sequence or length differences. In some embodiments, the amplicons are then amplified using a forward and reverse primer for each target locus.

Exemplary Whole Genome Amplification Methods

In some embodiments, a method of the present disclosure may involve amplifying DNA, such as the use of whole genome application to amplify a nucleic acid sample before

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amplifying just the target loci. Amplification of the DNA, a process which transforms a small amount of genetic material to a larger amount of genetic material that comprises a similar set of genetic data, can be done by a wide variety of methods, including, but not limited to polymerase chain reaction (PCR). One method of amplifying DNA is whole genome amplification (WGA). There are a number of methods available for WGA: ligation-mediated PCR (LM-PCR), degenerate oligonucleotide primer PCR (DOP-PCR), and multiple displacement amplification (MDA). In LM-PCR, short DNA sequences called adapters are ligated to blunt ends of DNA. These adapters contain universal amplification sequences, which are used to amplify the DNA by PCR. In DOP-PCR, random primers that also contain universal amplification sequences are used in a first round of annealing and PCR. Then, a second round of PCR is used to amplify the sequences further with the universal primer sequences. MDA uses the phi-29 polymerase, which is a highly processive and non-specific enzyme that replicates DNA and has been used for single-cell analysis. The major limitations to amplification of material from a single cell are (1) necessity of using extremely dilute DNA concentrations or extremely small volume of reaction mixture, and (2) difficulty of reliably dissociating DNA from proteins across the whole genome. Regardless, single-cell whole genome amplification has been used successfully for a variety of applications for a number of years. There are other methods of amplifying DNA from a sample of DNA. The DNA amplification transforms the initial sample of DNA into a sample of DNA that is similar in the set of sequences, but of much greater quantity. In some cases, amplification may not be required.

In some embodiments, DNA may be amplified using a universal amplification, such as WGA or MDA. In some embodiments, DNA may be amplified by targeted amplification, for example using targeted PCR, or circularizing probes. In some embodiments, the DNA may be preferentially enriched using a targeted amplification method, or a method that results in the full or partial separation of desired from undesired DNA, such as capture by hybridization approaches. In some embodiments, DNA may be amplified by using a combination of a universal amplification method and a preferential enrichment method. A fuller description of some of these methods can be found elsewhere in this document.

Exemplary Enrichment and Sequencing Methods

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In an embodiment, a method disclosed herein uses selective enrichment techniques that preserve the relative allele frequencies that are present in the original sample of DNA at each target loci (*e.g.*, each polymorphic locus) from a set of target loci (*e.g.*, polymorphic loci). While enrichment is particularly advantageous for methods for analyzing polymorphic loci, these enrichment methods can be readily adapted for nonpolymorphic loci if desired. In some embodiments the amplification and/or selective enrichment technique may involve PCR such as ligation mediated PCR, fragment capture by hybridization, Molecular Inversion Probes, or other circularizing probes. In some embodiments, methods for amplification or selective enrichment may involve using probes where, upon correct hybridization to the target sequence, the 3-prime end or 5-prime end of a nucleotide probe is separated from the polymorphic site of the allele by a small number of nucleotides. This separation reduces preferential amplification of one allele, termed allele bias. This is an improvement over methods that involve using probes where the 3-prime end or 5-prime end of a correctly hybridized probe are directly adjacent to or very near to the polymorphic site of an allele. In an embodiment, probes in which the hybridizing region may or certainly contains a polymorphic site are excluded. Polymorphic sites at the site of hybridization can cause unequal hybridization or inhibit hybridization altogether in some alleles, resulting in preferential amplification of certain alleles. These embodiments are improvements over other methods that involve targeted amplification and/or selective enrichment in that they better preserve the original allele frequencies of the sample at each polymorphic locus, whether the sample is pure genomic sample from a single individual or mixture of individuals.

The use of a technique to enrich a sample of DNA at a set of target loci followed by sequencing as part of a method for non-invasive prenatal allele calling or ploidy calling may confer a number of unexpected advantages. In some embodiments of the present disclosure, the method involves measuring genetic data for use with an informatics based method, such as PARENTAL SUPPORT™ (PS). The ultimate outcome of some of the embodiments is the actionable genetic data of an embryo or a fetus. There are many methods that may be used to measure the genetic data of the individual and/or the related individuals as part of embodied methods. In an embodiment, a method for enriching the concentration of a set of targeted alleles is disclosed herein, the method comprising one or more of the following steps: targeted amplification of genetic material, addition of loci specific oligonucleotide probes, ligation of specified DNA strands, isolation of sets of desired DNA, removal of unwanted components of

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a reaction, detection of certain sequences of DNA by hybridization, and detection of the sequence of one or a plurality of strands of DNA by DNA sequencing methods. In some cases the DNA strands may refer to target genetic material, in some cases they may refer to primers, in some cases they may refer to synthesized sequences, or combinations thereof. These steps may be carried out in a number of different orders.

For example, a universal amplification step of the DNA prior to targeted amplification may confer several advantages, such as removing the risk of bottlenecking and reducing allelic bias. The DNA may be mixed an oligonucleotide probe that can hybridize with two neighboring regions of the target sequence, one on either side. After hybridization, the ends of the probe may be connected by adding a polymerase, a means for ligation, and any necessary reagents to allow the circularization of the probe. After circularization, an exonuclease may be added to digest to non-circularized genetic material, followed by detection of the circularized probe. The DNA may be mixed with PCR primers that can hybridize with two neighboring regions of the target sequence, one on either side. After hybridization, the ends of the probe may be connected by adding a polymerase, a means for ligation, and any necessary reagents to complete PCR amplification. Amplified or unamplified DNA may be targeted by hybrid capture probes that target a set of loci; after hybridization, the probe may be localized and separated from the mixture to provide a mixture of DNA that is enriched in target sequences.

The use of a method to target certain loci followed by sequencing as part of a method for allele calling or ploidy calling may confer a number of unexpected advantages. Some methods by which DNA may be targeted, or preferentially enriched, include using circularizing probes, linked inverted probes (LIPs, MIPs), capture by hybridization methods such as SURESELECT, and targeted PCR or ligation-mediated PCR amplification strategies.

In some embodiments, a method of the present disclosure involves measuring genetic data for use with an informatics based method, such as PARENTAL SUPPORT™ (PS), which is described further herein. PARENTAL SUPPORT™ is an informatics based approach to manipulating genetic data, aspects of which are described herein. The ultimate outcome of some of the embodiments is the actionable genetic data of an embryo or a fetus followed by a clinical decision based on the actionable data. The algorithms behind the PS method take the measured genetic data of the target individual, often an embryo or fetus, and the measured genetic data from related individuals, and are able to increase the accuracy with which the genetic state of the target individual is known. In an embodiment, the measured genetic data is

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used in the context of making ploidy determinations during prenatal genetic diagnosis. In an embodiment, the measured genetic data is used in the context of making ploidy determinations or allele calls on embryos during in vitro fertilization. There are many methods that may be used to measure the genetic data of the individual and/or the related individuals in the aforementioned contexts. The different methods comprise a number of steps, those steps often involving amplification of genetic material, addition of oligonucleotide probes, ligation of specified DNA strands, isolation of sets of desired DNA, removal of unwanted components of a reaction, detection of certain sequences of DNA by hybridization, detection of the sequence of one or a plurality of strands of DNA by DNA sequencing methods. In some cases the DNA strands may refer to target genetic material, in some cases they may refer to primers, in some cases they may refer to synthesized sequences, or combinations thereof. These steps may be carried out in a number of different orders.

Note that in theory it is possible to target any number loci in the genome, anywhere from one loci to well over one million loci. If a sample of DNA is subjected to targeting, and then sequenced, the percentage of the alleles that are read by the sequencer will be enriched with respect to their natural abundance in the sample. The degree of enrichment can be anywhere from one percent (or even less) to ten-fold, a hundred-fold, a thousand-fold or even many million-fold. In the human genome there are roughly 3 billion base pairs, and nucleotides, comprising approximately 75 million polymorphic loci. The more loci that are targeted, the smaller the degree of enrichment is possible. The fewer the number of loci that are targeted, the greater degree of enrichment is possible, and the greater depth of read may be achieved at those loci for a given number of sequence reads.

In an embodiment of the present disclosure, the targeting or preferential may focus entirely on SNPs. In an embodiment, the targeting or preferential may focus on any polymorphic site. A number of commercial targeting products are available to enrich exons. Surprisingly, targeting exclusively SNPs, or exclusively polymorphic loci, is particularly advantageous when using a method for NPD that relies on allele distributions. There are also published methods for NPD using sequencing, for example U.S. Patent 7,888,017, involving a read count analysis where the read counting focuses on counting the number of reads that map to a given chromosome, where the analyzed sequence reads do not focused on regions of the genome that are polymorphic. Those types of methodology that do not focus on polymorphic alleles would not benefit as much from targeting or preferential enrichment of a set of alleles.

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In an embodiment of the present disclosure, it is possible to use a targeting method that focuses on SNPs to enrich a genetic sample in polymorphic regions of the genome. In an embodiment, it is possible to focus on a small number of SNPs, for example between 1 and 100 SNPs, or a larger number, for example, between 100 and 1,000, between 1,000 and 10,000, between 10,000 and 100,000 or more than 100,000 SNPs. In an embodiment, it is possible to focus on one or a small number of chromosomes that are correlated with live trisomic births, for example chromosomes 13, 18, 21, X and Y, or some combination thereof. In an embodiment, it is possible to enrich the targeted SNPs by a small factor, for example between 1.01 fold and 100 fold, or by a larger factor, for example between 100 fold and 1,000,000 fold, or even by more than 1,000,000 fold. In an embodiment of the present disclosure, it is possible to use a targeting method to create a sample of DNA that is preferentially enriched in polymorphic regions of the genome. In an embodiment, it is possible to use this method to create a mixture of DNA with any of these characteristics where the mixture of DNA contains maternal DNA and also free floating fetal DNA. In an embodiment, it is possible to use this method to create a mixture of DNA that has any combination of these factors. For example, the method described herein may be used to produce a mixture of DNA that comprises maternal DNA and fetal DNA, and that is preferentially enriched in DNA that corresponds to 200 SNPs, all of which are located on either chromosome 18 or 21, and which are enriched an average of 1000 fold. In another example, it is possible to use the method to create a mixture of DNA that is preferentially enriched in 10,000 SNPs that are all or mostly located on chromosomes 13, 18, 21, X and Y, and the average enrichment per loci is greater than 500 fold. Any of the targeting methods described herein can be used to create mixtures of DNA that are preferentially enriched in certain loci.

In some embodiments, a method of the present disclosure further includes measuring the DNA in the mixed fraction using a high throughput DNA sequencer, where the DNA in the mixed fraction contains a disproportionate number of sequences from one or more chromosomes, wherein the one or more chromosomes are taken from the group comprising chromosome 13, chromosome 18, chromosome 21, chromosome X, chromosome Y and combinations thereof.

Described herein are three methods: multiplex PCR, targeted capture by hybridization, and linked inverted probes (LIPs), which may be used to obtain and analyze measurements from a sufficient number of polymorphic loci from a maternal plasma sample in order to detect

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fetal aneuploidy; this is not meant to exclude other methods of selective enrichment of targeted loci. Other methods may equally well be used without changing the essence of the method. In each case the polymorphism assayed may include single nucleotide polymorphisms (SNPs), small indels, or STRs. A preferred method involves the use of SNPs. Each approach produces allele frequency data; allele frequency data for each targeted locus and/or the joint allele frequency distributions from these loci may be analyzed to determine the ploidy of the fetus. Each approach has its own considerations due to the limited source material and the fact that maternal plasma consists of mixture of maternal and fetal DNA. This method may be combined with other approaches to provide a more accurate determination. In an embodiment, this method may be combined with a sequence counting approach such as that described in US Patent 7,888,017. The approaches described could also be used to detect fetal paternity noninvasively from maternal plasma samples. In addition each approach may be applied to other mixtures of DNA or pure DNA samples to detect the presence or absence of aneuploid chromosomes, to genotype a large number of SNP from degraded DNA samples, to detect segmental copy number variations (CNVs), to detect other genotypic states of interest, or some combination thereof.

Accurately Measuring the Allelic Distributions in a Sample

Current sequencing approaches can be used to estimate the distribution of alleles in a sample. One such method involves randomly sampling sequences from a pool DNA, termed shotgun sequencing. The proportion of a particular allele in the sequencing data is typically very low and can be determined by simple statistics. The human genome contains approximately 3 billion base pairs. So, if the sequencing method used make 100 bp reads, a particular allele will be measured about once in every 30 million sequence reads.

In an embodiment, a method of the present disclosure is used to determine the presence or absence of two or more different haplotypes that contain the same set of loci in a sample of DNA from the measured allele distributions of loci from that chromosome. The different haplotypes could represent two different homologous chromosomes from one individual, three different homologous chromosomes from a trisomic individual, three different homologous haplotypes from a mother and a fetus where one of the haplotypes is shared between the mother and the fetus, three or four haplotypes from a mother and fetus where one or two of the haplotypes are shared between the mother and the fetus, or other combinations. Alleles that are

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polymorphic between the haplotypes tend to be more informative, however any alleles where the mother and father are not both homozygous for the same allele will yield useful information through measured allele distributions beyond the information that is available from simple read count analysis.

Shotgun sequencing of such a sample, however, is extremely inefficient as it results in many sequences for regions that are not polymorphic between the different haplotypes in the sample, or are for chromosomes that are not of interest, and therefore reveal no information about the proportion of the target haplotypes. Described herein are methods that specifically target and/or preferentially enrich segments of DNA in the sample that are more likely to be polymorphic in the genome to increase the yield of allelic information obtained by sequencing. Note that for the measured allele distributions in an enriched sample to be truly representative of the actual amounts present in the target individual, it is critical that there is little or no preferential enrichment of one allele as compared to the other allele at a given loci in the targeted segments. Current methods known in the art to target polymorphic alleles are designed to ensure that at least some of any alleles present are detected. However, these methods were not designed for the purpose of measuring the unbiased allelic distributions of polymorphic alleles present in the original mixture. It is non-obvious that any particular method of target enrichment would be able to produce an enriched sample wherein the measured allele distributions would accurately represent the allele distributions present in the original unamplified sample better than any other method. While many enrichment methods may be expected, in theory, to accomplish such an aim, an ordinary person skilled in the art is well aware that there is a great deal of stochastic or deterministic bias in current amplification, targeting and other preferential enrichment methods. One embodiment of a method described herein allows a plurality of alleles found in a mixture of DNA that correspond to a given locus in the genome to be amplified, or preferentially enriched in a way that the degree of enrichment of each of the alleles is nearly the same. Another way to say this is that the method allows the relative quantity of the alleles present in the mixture as a whole to be increased, while the ratio between the alleles that correspond to each locus remains essentially the same as they were in the original mixture of DNA. For some reported methods, preferential enrichment of loci can result in allelic biases of more than 1%, more than 2%, more than 5% and even more than 10%. This preferential enrichment may be due to capture bias when using a capture by hybridization approach, or amplification bias which may be small for each cycle,

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but can become large when compounded over 20, 30 or 40 cycles. For the purposes of this disclosure, for the ratio to remain essentially the same means that the ratio of the alleles in the original mixture divided by the ratio of the alleles in the resulting mixture is between 0.95 and 1.05, between 0.98 and 1.02, between 0.99 and 1.01, between 0.995 and 1.005, between 0.998 and 1.002, between 0.999 and 1.001, or between 0.9999 and 1.0001. Note that the calculation of the allele ratios presented here may not be used in the determination of the ploidy state of the target individual, and may only a metric to be used to measure allelic bias.

In an embodiment, once a mixture has been preferentially enriched at the set of target loci, it may be sequenced using any one of the previous, current, or next generation of sequencing instruments that sequences a clonal sample (a sample generated from a single molecule; examples include ILLUMINA GAIIx, ILLUMINA HISEQ, LIFE TECHNOLOGIES SOLiD, 5500XL). The ratios can be evaluated by sequencing through the specific alleles within the targeted region. These sequencing reads can be analyzed and counted according the allele type and the rations of different alleles determined accordingly. For variations that are one to a few bases in length, detection of the alleles will be performed by sequencing and it is essential that the sequencing read span the allele in question in order to evaluate the allelic composition of that captured molecule. The total number of captured molecules assayed for the genotype can be increased by increasing the length of the sequencing read. Full sequencing of all molecules would guarantee collection of the maximum amount of data available in the enriched pool. However, sequencing is currently expensive, and a method that can measure allele distributions using a lower number of sequence reads will have great value. In addition, there are technical limitations to the maximum possible length of read as well as accuracy limitations as read lengths increase. The alleles of greatest utility will be of one to a few bases in length, but theoretically any allele shorter than the length of the sequencing read can be used. While allele variations come in all types, the examples provided herein focus on SNPs or variants contained of just a few neighboring base pairs. Larger variants such as segmental copy number variants can be detected by aggregations of these smaller variations in many cases as whole collections of SNP internal to the segment are duplicated. Variants larger than a few bases, such as STRs require special consideration and some targeting approaches work while others will not.

There are multiple targeting approaches that can be used to specifically isolate and enrich a one or a plurality of variant positions in the genome. Typically, these rely on taking

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advantage of the invariant sequence flanking the variant sequence. There are reports by others related to targeting in the context of sequencing where the substrate is maternal plasma (see, *e.g.*, Liao et al., Clin. Chem. 2011; 57(1): pp. 92-101). However, these approaches use targeting probes that target exons, and do not focus on targeting polymorphic regions of the genome. In an embodiment, a method of the present disclosure involves using targeting probes that focus exclusively or almost exclusively on polymorphic regions. In an embodiment, a method of the present disclosure involves using targeting probes that focus exclusively or almost exclusively on SNPs. In some embodiments of the present disclosure, the targeted polymorphic sites consist of at least 10% SNPs, at least 20% SNPs, at least 30% SNPs, at least 40% SNPs, at least 50% SNPs, at least 60% SNPs, at least 70% SNPs, at least 80% SNPs, at least 90% SNPs, at least 95% SNPs, at least 98% SNPs, at least 99% SNPs, at least 99.9% SNPs, or exclusively SNPs.

In an embodiment, a method of the present disclosure can be used to determine genotypes (base composition of the DNA at specific loci) and relative proportions of those genotypes from a mixture of DNA molecules, where those DNA molecules may have originated from one or a number of genetically distinct individuals. In an embodiment, a method of the present disclosure can be used to determine the genotypes at a set of polymorphic loci, and the relative ratios of the amount of different alleles present at those loci. In an embodiment the polymorphic loci may consist entirely of SNPs. In an embodiment, the polymorphic loci can comprise SNPs, single tandem repeats, and other polymorphisms. In an embodiment, a method of the present disclosure can be used to determine the relative distributions of alleles at a set of polymorphic loci in a mixture of DNA, where the mixture of DNA comprises DNA that originates from a mother, and DNA that originates from a fetus. In an embodiment, the joint allele distributions can be determined on a mixture of DNA isolated from blood from a pregnant woman. In an embodiment, the allele distributions at a set of loci can be used to determine the ploidy state of one or more chromosomes on a gestating fetus.

In an embodiment, the mixture of DNA molecules could be derived from DNA extracted from multiple cells of one individual. In an embodiment, the original collection of cells from which the DNA is derived may comprise a mixture of diploid or haploid cells of the same or of different genotypes, if that individual is mosaic (germline or somatic). In an embodiment, the mixture of DNA molecules could also be derived from DNA extracted from single cells. In an embodiment, the mixture of DNA molecules could also be derived from

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DNA extracted from mixture of two or more cells of the same individual, or of different individuals. In an embodiment, the mixture of DNA molecules could be derived from DNA isolated from biological material that has already liberated from cells such as blood plasma, which is known to contain cell free DNA. In an embodiment, the this biological material may be a mixture of DNA from one or more individuals, as is the case during pregnancy where it has been shown that fetal DNA is present in the mixture. In an embodiment, the biological material could be from a mixture of cells that were found in maternal blood, where some of the cells are fetal in origin. In an embodiment, the biological material could be cells from the blood of a pregnant which have been enriched in fetal cells.

Circularizing Probes

Some embodiments of the present disclosure involve the use of "Linked Inverted Probes" (LIPs), which have been previously described in the literature, to amplify the target loci before or after amplification using primers that are not LIPs in the multiplex PCR methods of the invention. LIPs is a generic term meant to encompass technologies that involve the creation of a circular molecule of DNA, where the probes are designed to hybridize to targeted region of DNA on either side of a targeted allele, such that addition of appropriate polymerases and/or ligases, and the appropriate conditions, buffers and other reagents, will complete the complementary, inverted region of DNA across the targeted allele to create a circular loop of DNA that captures the information found in the targeted allele. LIPs may also be called pre-circularized probes, pre-circularizing probes, or circularizing probes. The LIPs probe may be a linear DNA molecule between 50 and 500 nucleotides in length, and in an embodiment between 70 and 100 nucleotides in length; in some embodiments, it may be longer or shorter than described herein. Others embodiments of the present disclosure involve different incarnations, of the LIPs technology, such as Padlock Probes and Molecular Inversion Probes (MIPs).

One method to target specific locations for sequencing is to synthesize probes in which the 3' and 5' ends of the probes anneal to target DNA at locations adjacent to and on either side of the targeted region, in an inverted manner, such that the addition of DNA polymerase and DNA ligase results in extension from the 3' end, adding bases to single stranded probe that are complementary to the target molecule (gap-fill), followed by ligation of the new 3' end to the 5' end of the original probe resulting in a circular DNA molecule that can be subsequently

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isolated from background DNA. The probe ends are designed to flank the targeted region of interest. One aspect of this approach is commonly called MIPS and has been used in conjunction with array technologies to determine the nature of the sequence filled in. One drawback to the use of MIPs in the context of measuring allele ratios is that the hybridization, circularization and amplification steps do not happed at equal rates for different alleles at the same loci. This results in measured allele ratios that are not representative of the actual allele ratios present in the original mixture.

In an embodiment, the circularizing probes are constructed such that the region of the probe that is designed to hybridize upstream of the targeted polymorphic locus and the region of the probe that is designed to hybridize downstream of the targeted polymorphic locus are covalently connected through a non-nucleic acid backbone. This backbone can be any biocompatible molecule or combination of biocompatible molecules. Some examples of possible biocompatible molecules are poly(ethylene glycol), polycarbonates, polyurethanes, polyethylenes, polypropylenes, sulfone polymers, silicone, cellulose, fluoropolymers, acrylic compounds, styrene block copolymers, and other block copolymers.

In an embodiment of the present disclosure, this approach has been modified to be easily amenable to sequencing as a means of interrogating the filled in sequence. In order to retain the original allelic proportions of the original sample at least one key consideration must be taken into account. The variable positions among different alleles in the gap-fill region must not be too close to the probe binding sites as there can be initiation bias by the DNA polymerase resulting in differential of the variants. Another consideration is that additional variations may be present in the probe binding sites that are correlated to the variants in the gap-fill region which can result unequal amplification from different alleles. In an embodiment of the present disclosure, the 3' ends and 5' ends of the pre-circularized probe are designed to hybridize to bases that are one or a few positions away from the variant positions (polymorphic sites) of the targeted allele. The number of bases between the polymorphic site (SNP or otherwise) and the base to which the 3' end and/or 5' of the pre-circularized probe is designed to hybridize may be one base, it may be two bases, it may be three bases, it may be four bases, it may be five bases, it may be six bases, it may be seven to ten bases, it may be eleven to fifteen bases, or it may be sixteen to twenty bases, twenty to thirty bases, or thirty to sixty bases. The forward and reverse primers may be designed to hybridize a different number of bases away from the polymorphic site. Circularizing probes can be generated in large numbers

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with current DNA synthesis technology allowing very large numbers of probes to be generated and potentially pooled, enabling interrogation of many loci simultaneously. It has been reported to work with more than 300,000 probes. Two papers that discuss a method involving circularizing probes that can be used to measure the genomic data of the target individual include: Porreca et al., Nature Methods, 2007 4(11), pp. 931-936.; and also Turner et al., Nature Methods, 2009, 6(5), pp. 315-316. The methods described in these papers may be used in combination with other methods described herein. Certain steps of the method from these two papers may be used in combination with other steps from other methods described herein.

In some embodiments of the methods disclosed herein, the genetic material of the target individual is optionally amplified, followed by hybridization of the pre-circularized probes, performing a gap fill to fill in the bases between the two ends of the hybridized probes, ligating the two ends to form a circularized probe, and amplifying the circularized probe, using, for example, rolling circle amplification. Once the desired target allelic genetic information is captured by circularizing appropriately designed oligonucleotide probes, such as in the LIPs system, the genetic sequence of the circularized probes may be being measured to give the desired sequence data. In an embodiment, the appropriately designed oligonucleotides probes may be circularized directly on unamplified genetic material of the target individual, and amplified afterwards. Note that a number of amplification procedures may be used to amplify the original genetic material, or the circularized LIPs, including rolling circle amplification, MDA, or other amplification protocols. Different methods may be used to measure the genetic information on the target genome, for example using high throughput sequencing, Sanger sequencing, other sequencing methods, capture-by-hybridization, capture-by-circularization, multiplex PCR, other hybridization methods, and combinations thereof.

Once the genetic material of the individual has been measured using one or a combination of the above methods, an informatics based method, such as the PARENTAL SUPPORT™ method, along with the appropriate genetic measurements, can then be used to determination the ploidy state of one or more chromosomes on the individual, and/or the genetic state of one or a set of alleles, specifically those alleles that are correlated with a disease or genetic state of interest. Note that the use of LIPs has been reported for multiplexed capture of genetic sequences, followed by genotyping with sequencing. However, the use of sequencing data resulting from a LIPs-based strategy for the amplification of the genetic

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material found in a single cell, a small number of cells, or extracellular DNA, has not been used for the purpose of determining the ploidy state of a target individual.

Applying an informatics based method to determine the ploidy state of an individual from genetic data as measured by hybridization arrays, such as the ILLUMINA INFINIUM array, or the AFFYMETRIX gene chip has been described in documents references elsewhere in this document. However, the method described herein shows improvements over methods described previously in the literature. For example, the LIPs based approach followed by high throughput sequencing unexpectedly provides better genotypic data due to the approach having better capacity for multiplexing, better capture specificity, better uniformity, and low allelic bias. Greater multiplexing allows more alleles to be targeted, giving more accurate results. Better uniformity results in more of the targeted alleles being measured, giving more accurate results. Lower rates of allelic bias result in lower rates of miscalls, giving more accurate results. More accurate results result in an improvement in clinical outcomes, and better medical care.

It is important to note that LIPs may be used as a method for targeting specific loci in a sample of DNA for genotyping by methods other than sequencing. For example, LIPs may be used to target DNA for genotyping using SNP arrays or other DNA or RNA based microarrays.

Ligation-mediated PCR

Ligation-mediated PCR may be used to amplify the target loci before or after PCR amplification using primers that are not ligated. Ligation-mediated PCR is a method of PCR used to preferentially enrich a sample of DNA by amplifying one or a plurality of loci in a mixture of DNA, the method comprising: obtaining a set of primer pairs, where each primer in the pair contains a target specific sequence and a non-target sequence, where the target specific sequence is preferably designed to anneal to a target region, one upstream and one downstream from the polymorphic site, and which can be separated from the polymorphic site by 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11-20, 21-30, 31-40, 41-50, 51-100, or more than 100; polymerization of the DNA from the 3-prime end of upstream primer to the fill the single strand region between it and the 5-prime end of the downstream primer with nucleotides complementary to the target molecule; ligation of the last polymerized base of the upstream primer to the adjacent 5-prime base of the downstream primer; and amplification of only polymerized and ligated molecules

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using the non-target sequences contained at the 5-prime end of the upstream primer and the 3-prime end of the downstream primer. Pairs of primers to distinct targets may be mixed in the same reaction. The non-target sequences serve as universal sequences such that of all pairs of primers that have been successfully polymerized and ligated may be amplified with a single pair of amplification primers.

Capture by Hybridization

In some embodiments, a method of the present disclosure may involve using any of the following capture by hybridization methods in addition to using multiplex PCR to amplify the target loci. Preferential enrichment of a specific set of sequences in a target genome can be accomplished in a number of ways. Elsewhere in this document is a description of how LIPs can be used to target a specific set of sequences, but in all of those applications, other targeting and/or preferential enrichment methods can be used equally well for the same ends. One example of another targeting method is the capture by hybridization approach. Some examples of commercial capture by hybridization technologies include AGILENT's SURE SELECT and ILLUMINA's TRUSEQ. In capture by hybridization, a set of oligonucleotides that is complimentary or mostly complimentary to the desired targeted sequences is allowed to hybridize to a mixture of DNA, and then physically separated from the mixture. Once the desired sequences have hybridized to the targeting oligonucleotides, the effect of physically removing the targeting oligonucleotides is to also remove the targeted sequences. Once the hybridized oligos are removed, they can be heated to above their melting temperature and they can be amplified. Some ways to physically remove the targeting oligonucleotides is by covalently bonding the targeting oligos to a solid support, for example a magnetic bead, or a chip. Another way to physically remove the targeting oligonucleotides is by covalently bonding them to a molecular moiety with a strong affinity for another molecular moiety. An example of such a molecular pair is biotin and streptavidin, such as is used in SURE SELECT. Thus that targeted sequences could be covalently attached to a biotin molecule, and after hybridization, a solid support with streptavidin affixed can be used to pull down the biotinylated oligonucleotides, to which are hybridized to the targeted sequences.

Hybrid capture involves hybridizing probes that are complementary to the targets of interest to the target molecules. Hybrid capture probes were originally developed to target and enrich large fractions of the genome with relative uniformity between targets. In that

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application, it was important that all targets be amplified with enough uniformity that all regions could be detected by sequencing, however, no regard was paid to retaining the proportion of alleles in original sample. Following capture, the alleles present in the sample can be determined by direct sequencing of the captured molecules. These sequencing reads can be analyzed and counted according the allele type. However, using the current technology, the measured allele distributions the captured sequences are typically not representative of the original allele distributions.

In an embodiment, detection of the alleles is performed by sequencing. In order to capture the allele identity at the polymorphic site, it is essential that the sequencing read span the allele in question in order to evaluate the allelic composition of that captured molecule. Since the capture molecules are often of variable lengths upon sequencing cannot be guaranteed to overlap the variant positions unless the entire molecule is sequenced. However, cost considerations as well as technical limitations as to the maximum possible length and accuracy of sequencing reads make sequencing the entire molecule unfeasible. In an embodiment, the read length can be increased from about 30 to about 50 or about 70 bases can greatly increase the number of reads that overlap the variant positions within the targeted sequences.

Another way to increase the number of reads that interrogate the position of interest is to decrease the length of the probe, as long as it does not result in bias in the underlying enriched alleles. The length of the synthesized probe should be long enough such that two probes designed to hybridize to two different alleles found at one locus will hybridize with near equal affinity to the various alleles in the original sample. Currently, methods known in the art describe probes that are typically longer than 120 bases. In a current embodiment, if the allele is one or a few bases then the capture probes may be less than about 110 bases, less than about 100 bases, less than about 90 bases, less than about 80 bases, less than about 70 bases, less than about 60 bases, less than about 50 bases, less than about 40 bases, less than about 30 bases, and less than about 25 bases, and this is sufficient to ensure equal enrichment from all alleles. When the mixture of DNA that is to be enriched using the hybrid capture technology is a mixture comprising free floating DNA isolated from blood, for example maternal blood, the average length of DNA is quite short, typically less than 200 bases. The use of shorter probes results in a greater chance that the hybrid capture probes will capture desired DNA fragments. Larger variations may require longer probes. In an embodiment, the variations of interest are

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one (a SNP) to a few bases in length. In an embodiment, targeted regions in the genome can be preferentially enriched using hybrid capture probes wherein the hybrid capture probes are of a length below 90 bases, and can be less than 80 bases, less than 70 bases, less than 60 bases, less than 50 bases, less than 40 bases, less than 30 bases, or less than 25 bases. In an embodiment, to increase the chance that the desired allele is sequenced, the length of the probe that is designed to hybridize to the regions flanking the polymorphic allele location can be decreased from above 90 bases, to about 80 bases, or to about 70 bases, or to about 60 bases, or to about 50 bases, or to about 40 bases, or to about 30 bases, or to about 25 bases.

There is a minimum overlap between the synthesized probe and the target molecule in order to enable capture. This synthesized probe can be made as short as possible while still being larger than this minimum required overlap. The effect of using a shorter probe length to target a polymorphic region is that there will be more molecules that overlap the target allele region. The state of fragmentation of the original DNA molecules also affects the number of reads that will overlap the targeted alleles. Some DNA samples such as plasma samples are already fragmented due to biological processes that take place in vivo. However, samples with longer fragments by benefit from fragmentation prior to sequencing library preparation and enrichment. When both probes and fragments are short (~60-80 bp) maximum specificity may be achieved relatively few sequence reads failing to overlap the critical region of interest.

In an embodiment, the hybridization conditions can be adjusted to maximize uniformity in the capture of different alleles present in the original sample. In an embodiment, hybridization temperatures are decreased to minimize differences in hybridization bias between alleles. Methods known in the art avoid using lower temperatures for hybridization because lowering the temperature has the effect of increasing hybridization of probes to unintended targets. However, when the goal is to preserve allele ratios with maximum fidelity, the approach of using lower hybridization temperatures provides optimally accurate allele ratios, despite the fact that the current art teaches away from this approach. Hybridization temperature can also be increased to require greater overlap between the target and the synthesized probe so that only targets with substantial overlap of the targeted region are captured. In some embodiments of the present disclosure, the hybridization temperature is lowered from the normal hybridization temperature to about 40°C, to about 45°C, to about 50°C, to about 55°C, to about 60°C, to about 65, or to about 70°C.

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In an embodiment, the hybrid capture probes can be designed such that the region of the capture probe with DNA that is complementary to the DNA found in regions flanking the polymorphic allele is not immediately adjacent to the polymorphic site. Instead, the capture probe can be designed such that the region of the capture probe that is designed to hybridize to the DNA flanking the polymorphic site of the target is separated from the portion of the capture probe that will be in van der Waals contact with the polymorphic site by a small distance that is equivalent in length to one or a small number of bases. In an embodiment, the hybrid capture probe is designed to hybridize to a region that is flanking the polymorphic allele but does not cross it; this may be termed a flanking capture probe. The length of the flanking capture probe may be less than about 120 bases, less than about 110 bases, less than about 100 bases, less than about 90 bases, and can be less than about 80 bases, less than about 70 bases, less than about 60 bases, less than about 50 bases, less than about 40 bases, less than about 30 bases, or less than about 25 bases. The region of the genome that is targeted by the flanking capture probe may be separated by the polymorphic locus by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11-20, or more than 20 base pairs.

Description of a targeted capture based disease screening test using targeted sequence capture. Custom targeted sequence capture, like those currently offered by AGILENT (SURE SELECT), ROCHE-NIMBLEGEN, or ILLUMINA. Capture probes could be custom designed to ensure capture of various types of mutations. For point mutations, one or more probes that overlap the point mutation should be sufficient to capture and sequence the mutation.

For small insertions or deletions, one or more probes that overlap the mutation may be sufficient to capture and sequence fragments comprising the mutation. Hybridization may be less efficient between the probe-limiting capture efficiency, typically designed to the reference genome sequence. To ensure capture of fragments comprising the mutation one could design two probes, one matching the normal allele and one matching the mutant allele. A longer probe may enhance hybridization. Multiple overlapping probes may enhance capture. Finally, placing a probe immediately adjacent to, but not overlapping, the mutation may permit relatively similar capture efficiency of the normal and mutant alleles.

For Simple Tandem Repeats (STRs), a probe overlapping these highly variable sites is unlikely to capture the fragment well. To enhance capture a probe could be placed adjacent to, but not overlapping the variable site. The fragment could then be sequenced as normal to reveal the length and composition of the STR.

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For large deletions, a series of overlapping probes, a common approach currently used in exon capture systems may work. However, with this approach it may be difficult to determine whether or not an individual is heterozygous. Targeting and evaluating SNPs within the captured region could potentially reveal loss of heterozygosity across the region indicating that an individual is a carrier. In an embodiment, it is possible to place non-overlapping or singleton probes across the potentially deleted region and use the number of fragments captured as a measure of heterozygosity. In the case where an individual caries a large deletion, one-half the number of fragments are expected to be available for capture relative to a non-deleted (diploid) reference locus. Consequently, the number of reads obtained from the deleted regions should be roughly half that obtained from a normal diploid locus. Aggregating and averaging the sequencing read depth from multiple singleton probes across the potentially deleted region may enhance the signal and improve confidence of the diagnosis. The two approaches, targeting SNPs to identify loss of heterozygosity and using multiple singleton probes to obtain a quantitative measure of the quantity of underlying fragments from that locus can also be combined. Either or both of these strategies may be combined with other strategies to better obtain the same end.

If during testing cfDNA detection of a male fetus, as indicated by the presence of the Y-chromosome fragments, captured and sequenced in the same test, and either an X-linked dominant mutation where mother and father are unaffected, or a dominant mutation where mother is not affected would indicated heighted risk to the fetus. Detection of two mutant recessive alleles within the same gene in an unaffected mother would imply the fetus had inherited a mutant allele from father and potentially a second mutant allele from mother. In all cases, follow-up testing by amniocentesis or chorionic villus sampling may be indicated.

A targeted capture based disease screening test could be combined with a targeted capture based non-invasive prenatal diagnostic test for aneuploidy.

There are a number of ways to decrease depth of read (DOR) variability: for example, one could increase primer concentrations, one could use longer targeted amplification probes, or one could run more STA cycles (such as more than 25, more than 30, more than 35, or even more than 40)

Exemplary Methods of Determining the Number of DNA Molecules in a Sample.

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A method is described herein to determine the number of DNA molecules in a sample by generating a uniquely identified molecule for each original DNA molecules in the sample during the first round of DNA amplification. Described here is a procedure to accomplish the above end followed by a single molecule or clonal sequencing method.

The approach entails targeting one or more specific loci and generating a tagged copy of the original molecules such manner that most or all of the tagged molecules from each targeted locus will have a unique tag and can be distinguished from one another upon sequencing of this barcode using clonal or single molecule sequencing. Each unique sequenced barcode represents a unique molecule in the original sample. Simultaneously, sequencing data is used to ascertain the locus from which the molecule originates. Using this information one can determine the number of unique molecules in the original sample for each locus.

This method can be used for any application in which quantitative evaluation of the number of molecules in an original sample is required. Furthermore, the number of unique molecules of one or more targets can be related to the number of unique molecules to one or more other targets to determine the relative copy number, allele distribution, or allele ratio. Alternatively, the number of copies detected from various targets can be modeled by a distribution in order to identify the mostly likely number of copies of the original targets. Applications include but are not limited to detection of insertions and deletions such as those found in carriers of Duchenne Muscular Dystrophy; quantitation of deletions or duplications segments of chromosomes such as those observed in copy number variants; chromosome copy number of samples from born individuals; chromosome copy number of samples from unborn individuals such as embryos or fetuses.

The method can be combined with simultaneous evaluation of variations contained in the targeted by sequence. This can be used to determine the number of molecules representing each allele in the original sample. This copy number method can be combined with the evaluation of SNPs or other sequence variations to determine the chromosome copy number of born and unborn individuals; the discrimination and quantification of copies from loci which have short sequence variations, but in which PCR may amplifies from multiple target regions such as in carrier detection of Spinal Muscle Atrophy; determination of copy number of different sources of molecules from samples consisting of mixtures of different individual such as in detection of fetal aneuploidy from free floating DNA obtained from maternal plasma.

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In an embodiment, the method as it pertains to a single target locus may comprise one or more of the following steps: (1) Designing a standard pair of oligomers for PCR amplification of a specific locus. (2) Adding, during synthesis, a sequence of specified bases with no or minimal complimentarity to the target locus or genome to the 5' end of the one of the target specific oligomer. This sequence, termed the tail, is a known sequence, to be used for subsequent amplification, followed by a sequence of random nucleotides. These random nucleotides comprise the random region. The random region comprises a randomly generated sequence of nucleic acids that probabilistically differ between each probe molecule. Consequently, following synthesis, the tailed oligomer pool will consists of a collection of oligomers beginning with a known sequence followed by unknown sequence that differs between molecules, followed by the target specific sequence. (3) Performing one round of amplification (denaturation, annealing, extension) using only the tailed oligomer. (4) adding exonuclease to the reaction, effectively stopping the PCR reaction, and incubating the reaction at the appropriate temperature to remove forward single stranded oligos that did not anneal to temple and extend to form a double stranded product. (5) Incubating the reaction at a high temperature to denature the exonuclease and eliminate its activity. (6) Adding to the reaction a new oligonucleotide that is complementary to tail of the oligomer used in the first reaction along with the other target specific oligomer to enable PCR amplification of the product generated in the first round of PCR. (7) Continuing amplification to generate enough product for downstream clonal sequencing. (8) Measuring the amplified PCR product by a multitude of methods, for example, clonal sequencing, to a sufficient number of bases to span the sequence.

In an embodiment, a method of the present disclosure involves targeting multiple loci in parallel or otherwise. Primers to different target loci can be generated independently and mixed to create multiplex PCR pools. In an embodiment, original samples can be divided into sub-pools and different loci can be targeted in each sub-pool before being recombined and sequenced. In an embodiment, the tagging step and a number of amplification cycles may be performed before the pool is subdivided to ensure efficient targeting of all targets before splitting, and improving subsequent amplification by continuing amplification using smaller sets of primers in subdivided pools.

One example of an application where this technology would be particularly useful is non-invasive prenatal aneuploidy diagnosis where the ratio of alleles at a given locus or a distribution of alleles at a number of loci can be used to help determine the number of copies

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of a chromosome present in a fetus. In this context, it is desirable to amplify the DNA present in the initial sample while maintaining the relative amounts of the various alleles. In some circumstances, especially in cases where there is a very small amount of DNA, for example, fewer than 5,000 copies of the genome, fewer than 1,000 copies of the genome, fewer than 500 copies of the genome, and fewer than 100 copies of the genome, one can encounter a phenomenon called bottlenecking. This is where there are a small number of copies of any given allele in the initial sample, and amplification biases can result in the amplified pool of DNA having significantly different ratios of those alleles than are in the initial mixture of DNA. By applying a unique or nearly unique set of barcodes to each strand of DNA before standard PCR amplification, it is possible to exclude n-1 copies of DNA from a set of n identical molecules of sequenced DNA that originated from the same original molecule.

For example, imagine a heterozygous SNP in the genome of an individual, and a mixture of DNA from the individual where ten molecules of each allele are present in the original sample of DNA. After amplification there may be 100,000 molecules of DNA corresponding to that locus. Due to stochastic processes, the ratio of DNA could be anywhere from 1:2 to 2:1, however, since each of the original molecules was tagged with a unique tag, it would be possible to determine that the DNA in the amplified pool originated from exactly 10 molecules of DNA from each allele. This method would therefore give a more accurate measure of the relative amounts of each allele than a method not using this approach. For methods where it is desirable for the relative amount of allele bias to be minimized, this method will provide more accurate data.

Association of the sequenced fragment to the target locus can be achieved in a number of ways. In an embodiment, a sequence of sufficient length is obtained from the targeted fragment to span the molecule barcode as well a sufficient number of unique bases corresponding to the target sequence to allow unambiguous identification of the target locus. In another embodiment, the molecular bar-coding primer that contains the randomly generated molecular barcode can also contain a locus specific barcode (locus barcode) that identifies the target to which it is to be associated. This locus barcode would be identical among all molecular bar-coding primers for each individual target and hence all resulting amplicons, but different from all other targets. In an embodiment, the tagging method described herein may be combined with a one-sided nesting protocol.

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In an embodiment, the design and generation of molecular barcoding primers may be reduced to practice as follows: the molecular barcoding primers may consist of a sequence that is not complementary to the target sequence followed by random molecular barcode region followed by a target specific sequence. The sequence 5' of molecular barcode may be used for subsequence PCR amplification and may comprise sequences useful in the conversion of the amplicon to a library for sequencing. The random molecular barcode sequence could be generated in a multitude of ways. The preferred method synthesize the molecule tagging primer in such a way as to include all four bases to the reaction during synthesis of the barcode region. All or various combinations of bases may be specified using the IUPAC DNA ambiguity codes. In this manner the synthesized collection of molecules will contain a random mixture of sequences in the molecular barcode region. The length of the barcode region will determine how many primers will contain unique barcodes. The number of unique sequences is related to the length of the barcode region as N^L where N is the number of bases, typically 4, and L is the length of the barcode. A barcode of five bases can yield up to 1024 unique sequences; a barcode of eight bases can yield 65536 unique barcodes. In an embodiment, the DNA can be measured by a sequencing method, where the sequence data represents the sequence of a single molecule. This can include methods in which single molecules are sequenced directly or methods in which single molecules are amplified to form clones detectable by the sequence instrument, but that still represent single molecules, herein called clonal sequencing.

Exemplary Methods and Reagents for Quantification of Amplification Products

Quantitation of specific nucleic acid sequences of interest is typically done by quantitative real-time PCR techniques such as TAQMAN (LIFE TECHNOLOGIES), INVADER probes (THIRD WAVE TECHNOLOGIES), and the like. Such techniques suffer from numerous shortcomings such as limited ability to achieve the simultaneous analysis of multiple sequences in parallel (multiplexation) and the ability to provide accurate quantitative data for only a narrow range of possible amplification cycles (*e.g.*, when the logarithm of PCR amplification production quantity versus the number of cycles is in the linear range). DNA sequencing techniques, particularly high throughput next-generation sequencing techniques (often referred to as massively parallel sequencing techniques) such as those employed in MYSEQ (ILLUMINA), HISEQ (ILLUMINA), ION TORRENT (LIFE TECHNOLOGIES),

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GENOME ANALYZER ILX (ILLUMINA), GS FLEX+ (ROCHE 454) *etc.*, can be used for by quantitative measurements of the number of copies of sequence of interest present in sample, thereby providing quantitative information about the starting materials, *e.g.*, copy number or transcription levels. High throughput genetic sequencers are amenable to the use of bar coding (*i.e.*, sample tagging with distinctive nucleic acid sequences) so as to identify specific samples from individuals thereby permitting the simultaneous analysis of multiple samples in a single run of the DNA sequencer. The number of times a given region of the genome in a library preparation (or other nucleic preparation of interest) is sequenced (number of reads) will be proportional to the number of copies of that sequence in the genome of interest (or expression level in the case of cDNA containing preparations). However, the preparation and sequencing of genetic libraries (and similar genome derived preparations) can introduce numerous biases that interfere with obtaining an accurate quantitative reading for the nucleic acid sequence of interest. For example, different nucleic acid sequences can amplify with different efficiencies during nucleic amplification steps that take place during the genetic library preparation or sample preparation.

The problem with differential amplification efficiencies can be mitigated by using certain embodiments of the subject invention. The subject invention includes various methods and compositions that relate to the use of standards for inclusion in amplification processes that can be used to improve the accuracy of quantitation. The invention is of use in, among other areas, the detection of aneuploidy in a fetus by analyzing free floating fetal DNA in maternal blood, as described herein and as described, among other places, U.S. Patent No. 8,008,018; U.S. Patent No. 7,332,277; PCT Published Application WO 2012/078792A2; and PCT Published Application WO 2011/146632 A1, which are each herein incorporated by reference in its entirety Embodiments of the invention are also of use in the detection of aneuploidy in an *in vitro* generated embryos. Commercially significant aneuploidies that may be detected include aneuploidy of the human chromosomes 13, 18, 21, X and Y.

Embodiments of the invention may be used with either human or non-human nucleic acids, and may be applied to both animal and plant derived nucleic acids. Embodiments of the invention may also be used to detect and/or quantitate alleles for other genetic disorders characterized by deletions or insertions. The deletion containing alleles can be detected in suspected carriers of the allele of interest.

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One embodiment of the subject invention includes standards that are present in a known quantity (relative or absolute). For example, consider a genetic library made from a genetic source that is diploid for chromosome 8 (containing locus A) and triploid for chromosome 21 (containing locus B). A genetic library can be produced from this sample that will contain sequences in quantities that are a function of the number of chromosomes present in the sample, *e.g.*, 200 copies of locus A and 300 copies of locus B. However, if locus A amplifies much more efficiently than locus B, after PCR there may be 60,000 copies of the A amplicon and 30,000 copies of the B amplicon, thus obscuring the true chromosomal copy number of the initial genomic sample when analysis by high throughput DNA sequencing (or other quantitative nucleic acid detection techniques). To mitigate this problem a standard sequence for locus A is employed, wherein the standard sequence amplifies with essentially the same efficiency as locus A. Similarly, a standard sequence for locus B is created, wherein the standard sequence amplifies with the essentially the same efficiency as locus B. A standard sequence of locus A and a standard sequence for locus B are added to the mixture prior to PCR (or other amplification techniques). These standard sequences are present in known quantities, either relative quantities or absolute quantities. Thus if a 1:1 mixture of standard sequence A and standard sequence B were added (prior to amplification) to the mixture in the previous example, 3000 copies of the standard A amplicon would be produced and 1000 copies of the standard B amplicon would be produced, showing that locus A is amplified 3 times more efficiently than locus B, under the same set of conditions.

In various embodiments one or more selected regions of a genome containing a SNP (or other polymorphism) of interest can be specifically amplified and subsequently sequenced. This target specific amplification can take place during the formation of a genetic library for sequencing. The library can contain numerous targeted regions for amplification. In some embodiments at least 10; 100, 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 regions of interest. Examples of such libraries are described herein and can be found in U.S. Patent Application No. 2012/0270212, filed November 18, 2011, which is herein incorporated by reference in its entirety.

Many high throughput DNA sequencing techniques require the modification of the genetic starting material, *e.g.*, the litigation of universal priming sites and/or barcodes, so as to form libraries to facilitate the clonal amplification of small nucleic acid fragments prior to

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performing subsequent sequencing reactions. In some embodiments, one or more standard sequences are added during genetic library formation or added to a precursor component of a genetic library prior to amplification of the library. The standard sequences can be selected so as to mimic (yet be distinguishable based on nucleotide base sequence) target genomic fragments to be prepared for sequencing by a high throughput genetic sequencing technique. In one embodiment, the standard sequence can be identical to the target genomic fragment excepting one, two, three, four to ten, or eleven to twenty nucleotides. In some embodiments, when the target genetic sequence contains a SNP, the standard sequence can be identical to the SNP excepting the nucleotide at the polymorphic base, which may be chosen to be one of the four nucleotides that is not observed at that location in nature. The standard sequences can be used in a highly multiplexed analysis of multiple target loci (such as polymorphic loci). Standard sequences can be added during the process of library formation (prior to amplification) in known quantities (relative or absolute) so as to provide a standard metric for greater accuracy in determining the amount of target sequence of interest in the sample of analysis. The combination of knowledge of the known quantities of the standard sequences used in conjunction with the knowledge of the ploidy level formation of library for sequencing formed from a genome of previously characterized ploidy level, *e.g.*, known to be diploid for all autosomal chromosomes, can be used to calibrate the amplification properties of each standard sequence with respect to its corresponding target sequence and account for variations between batches of mixtures comprising multiple standard sequences. Given that it is often necessary to simultaneously analyze a large number of loci, it is useful to produce a mixture comprising a large set standard sequences. Embodiments of the invention include mixtures comprising multiple standard sequences. Ideally the amount of each standard sequence in the mixture is known with high precision. However, it is extremely difficult to achieve this ideal because as a practical matter there is a significant amount of variation in the quantity of each standard sequence in the mixture, particularly for mixtures comprising a large number of different synthetic oligonucleotides. This variation has numerous sources, *e.g.*, variations in *in vitro* oligonucleotide synthesis reaction efficiencies between batch, inaccuracies in volume measurement, variations in pipetting, Furthermore, this variation can occur between different batches of that theoretically contain the exact same set of standard sequences in the exact same amounts. Accordingly, it is of interest to calibrate each batch of standard sequences independently. Batches of standard sequences can be calibrated against reference genomes of

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known chromosomal composition. Batched of standard sequences can be calibrated by sequencing the batch of standard sequences with minimal or no amplifications steps included in the sequencing protocol. Embodiments of the invention include calibrated mixtures of different standard sequences. Other embodiments of the invention include methods of calibrating mixtures of different standard sequences and calibrated mixtures of different standard sequences made by the subject methods.

Various embodiments of the subject mixtures of standard sequences and methods for using them can comprise at least 10; 100, 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 or more standards sequences, as well as various intermediate amounts. The number of the standard sequences can be the same as the number of target sequences selected for analysis during the generation of a targeted library for DNA sequencing. However, in some embodiments, it may be advantageous to use a lower number of standard sequences than the number of targeted regions in the library being constructed. It may be advantageous to use the lower number so as avoid coming up against the limits of the sequencing capacity of the high throughput DNA sequencer being employed. The number of standard sequences can be 50% or less than the number of targeted regions, 40% or less than the number of targeted regions, be 30% or less than the number of targeted regions, 20% or less than the number of targeted regions, be 10% or less than the number of targeted regions, 5% or less than the number of targeted regions, 1% or less than the number of targeted regions, as well as various intermediate values. For example, if a genetic library is created using 15,000 pairs of primers targeted to specific SNP containing loci, a suitable a mixture containing 1500 standard sequences corresponding to 1500 of the 15,000 targeted loci can be added prior to the amplification step of library constructions.

The amount of standard sequences added during library construction can vary considerably among different embodiments. In some embodiments, the amount of each standard sequence can be approximately the same as the predicted amount of the target sequence present in the genomic material sample used for library preparation. In other embodiments, the amount of each standard sequence can be greater or less than the predicted amount of the target sequence present in the genomic material sample used for library preparation. While the initial relative amounts of the target sequence and the standard sequence are not critical for the function of the invention, it is preferable that the amount be

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within the range 100 times greater to 100 times less than the amount of the target sequence present in the genomic material sample used for library preparation. Excessive amounts of standard may use too much sequencing capacity of the DNA sequencer in a given run of the instrument. Using too low an amount of standard sequences will produce insufficient data to aid in the analysis of variation in amplification efficiency.

The standard sequences may be selected to be very similar in nucleotide base sequence to the amplified regions of interest; preferably the standard sequence has the exact same primer-binding sites as the analyzed genomic region, *i.e.*, the "target sequence." The standard sequence must be distinguishable from the corresponding target sequence at a given locus. For the sake of convenience, this distinguishable region of the standard sequence will be referred to as a "marker sequence." In some embodiments, the marker sequence region of the target sequences contains the polymorphic region, *e.g.*, a SNP, and can be flanked on both sides by primer binding regions. The standard sequence may be selected to closely match the GC content of the corresponding target sequence. In some embodiments, the primer binding regions of the standard sequence are flanked by universal priming sites. These universal priming sites are selected to match universal priming sites used in a genomic library for analysis. In other embodiments, the standard sequences do not have universal priming sites and the universal priming sites are added during the creation of a library. Standard sequences are typically provided in single stranded form. A standard sequence is defined with respect to a corresponding target sequence and the sequence specific reagents used to amplify the target sequence. In some embodiments, the target sequence contains the polymorphism of interest, *e.g.*, a SNP, a deletion, or insertion, present in the nucleic acid sample for analysis. The standard sequence is a synthetic polynucleotide that is similar in nucleotide base sequence to the target sequence, but is nonetheless distinguishable from the target sequence by virtue of at least one nucleotide base difference, thereby providing a mechanism for distinguishing amplicon sequences derived from the standard sequence form amplicon sequences derived from the target sequence. Standard sequences are selected so as to have essentially the same amplification properties as the corresponding target sequence when amplified with the same set of amplification reagents, *e.g.*, PCR primers. In some embodiments, the standard sequences can have the same primer sequence binding sites than the corresponding target sequences. In other embodiments, the standard sequences can have a different primer sequence binding sites than the corresponding target sequences. In some embodiments, the

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standard sequences can be selected to produce amplicons that have the same length as the length of amplicons produced from the corresponding target sequences. In other embodiments, the standard sequences can be selected to produce amplicons that have the slightly different lengths than the length of amplicons produced from the corresponding target sequences.

After the amplification reactions have been completed, the library is sequenced on a high throughput DNA sequencer where individual molecule are clonally amplified and sequenced. The number of sequence reads for each allele of the target sequence is counted, also counted are the number of sequence reads for the standard sequence corresponding to the target sequence. The process is also carried out for at least one other pair of target sequences and corresponding standard sequences. Consider for example, locus A, X_{A1} reads for allele 1 of locus A are produced; X_{A2} reads for allele 2 of locus A are produced, and X_{AC} reads for standard sequence A are produced. The ratio of (X_{A1} plus X_{A2}) to X_{AC} is determined for each locus of interest. As discussed earlier, the process can be performed on a reference genome, *e.g.*, a genome that is known to be diploid for all chromosomes. The process can be repeated many times in order to provide a large number of read values so as to determine a mean number of reads and the standard deviation in the number of reads. The process is performed with a mixture comprising a large number of different standard sequences corresponding to different loci. By assuming that (1) X_{A1} plus X_{A2} corresponds to the known number of chromosome, *e.g.*, 2 for the normal human female genome and (2) the standard sequences have similar amplification (and detectability) properties as their corresponding natural loci, the relative amounts of the different standard sequences in the multiplex standard mixture can be determined. The calibrated multiplex standard sequence mixture can then be used to adjust for the variability in amplification efficiency between the different loci in a multiplex amplification reaction.

Other embodiments of the invention include methods and compositions for measuring the copy number of specific genes of interest, including duplications and mutant genes characterized by large deletions that would interfere with quantitation by sequencing. Sequencing would have problems detecting alleles having such deletions. Standard sequences included the amplification process can be used to reduce this problem.

In one embodiment of the invention the target sequence for analysis is a gene having a wild type (*i.e.* functional) form and a mutant form characterized by a deletion. Exemplary of such genes is SMN1, an allele having deletion being responsible for the genetic disease spinal

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muscular atrophy (SMA). It is of interest to detect an individual carrying the mutant form of the gene by means of high throughput genetic sequencing techniques. The application of such techniques to the detection of deletion mutations can be problematic because, among other reasons, the lack of sequences observed in sequencing (as opposed to detecting a simple point mutation or SNP). Such embodiments employ (1) a pair of amplification primers specific for the gene of interest, where in the amplification primers will amplify the gene of interest (or a portion thereof) and will not significantly amplify the mutant allele, (2) a standard sequence corresponding to the wild type allele of the gene of interest(*i.e.*, a target sequence), but differing by at least one detectable nucleotide base, (3) a pair of amplification primers specific for a second target sequence that serves as a reference sequence, and (4) a standard sequence corresponding to the reference sequence.

In one embodiment of the invention is provided a method for measuring the number of copies of the gene of interest, where in the gene of interest has one meaning allele that comprises a deletion. The method can employ amplification reagent specific for the gene of interest, *e.g.*, PCR primers, that are specific for the gene of interest by amplifying at least a portion of the gene of interest, or the entire gene of interest, or a region adjacent to the gene of interest, while not amplifying the deletion comprising allele of the gene of interest. Additionally the subject method employs a standard sequence corresponding to the gene of interest, wherein the standard sequence differs by at least one nucleotide base from the gene of interest (so that the sequence of the standard sequence can be readily distinguished from the naturally occurring gene of interest). Typically, the standard sequence will contain the same primer binding sites as the gene of interest so as to minimize any amplification discrimination between the gene of interest and the standard sequence corresponding to the gene of interest. The reaction will also comprises amplification reagents specific for a reference sequence. The reference sequence is a sequence of known (or at least assumed to be known) copy number in the genome to be analyzed. The reaction further comprises a standard sequence corresponding to the reference sequence. Typically, the standard sequence corresponding to the reference sequence will contain the same primer binding sites as the reference sequence so as to minimize any amplification discrimination between the reference sequence and the standard sequence corresponding to the reference sequence.

Exemplary PCR Conditions

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If desired, any of the PCR conditions disclosed herein or any standard PCR conditions can be used to test a primer library to determine, *e.g.*, the percent of primer dimers, percent of target amplicons, and percent of target loci that are amplified. If desired, standard methods can be used to optimize the reaction conditions to improve the performance of a primer library.

In some embodiments, the reaction volume includes ethylenediaminetetraacetic acid (EDTA), magnesium, tetramethyl ammonium chloride (TMAC), or any combination thereof. In some embodiments, the concentration of TMAC is between 20 and 80 mM, such as between 25 and 70 mM, 30 and 60 mM, 30 and 40 mM, 40 and 50 mM, 50 and 60 mM, or 60 and 70 mM, inclusive. While not meant to be bound to any particular theory, it is believed thatTMAC binds to DNA, stabilizes duplexes, increases primer specificity, and/or equalizes the melting temperatures of different primers. In some embodiments, TMAC increases the uniformity in the amount of amplified products for the different targets. In some embodiments, the concentration of magnesium (such as magnesium from magnesium chloride) is between 1 and 10 mM, such as between 1 and 8 mM, 1 and 5 mM, 1 and 3 mM, 3 and 5 mM, or 5 and 8 mM, inclusive. In some embodiments, EDTA is used to decrease the amount of magnesium available as a cofactor for the polymerase since high concentrations of magnesium can result in PCR errors, such as amplification of non-target loci. In some embodiments, the concentration of EDTA reduces the amount of available magnesium to between 1 and 5 mM (such as between 3 and 5 mM). In some embodiments, the pH is between 7.5 and 8.5, such as between 7.5 and 8, 8 and 8.3, or 8.3 and 8.5, inclusive. In some embodiments, a combination of KCl and $(NH_4)_2SO_4$ is used, such as between 50 and 150 mM KCl and between 10 and 90 mM $(NH_4)_2SO_4$, inclusive. In some embodiments, the concentration of KCl is between 50 and 100 mM or between 100 and 150 mM, inclusive. In some embodiments, the concentration of $(NH_4)_2SO_4$ is between 10 and 50 mM or between 50 and 90 mM, inclusive. In some embodiments, 100 mM KCl, 50 mM $(NH_4)_2SO_4$, 3 mM $MgCl_2$, 7.5 nM of each primer in the library, 50 mM TMAC, and 7 ul DNA template in a 20 ul final volume at pH 8.1 is used.

In some embodiments QIAGEN Multiplex PCR Kit is used (QIAGEN catalog No. 206143; *see, e.g.*, information available at the world wide web at qiagen.com/products/catalog/assay-technologies/end-point-pcr-and-rt-pcr-reagents/qiagen-multiplex-pcr-kit, is which is hereby incorporated by reference in its entirety). For 100 x 50 µl multiplex PCR reactions, the kit includes 2x QIAGEN Multiplex PCR Master Mix (providing a final concentration of 3 mM $MgCl_2$, 3 x 0.85 ml), 5x Q-Solution (1 x 2.0 ml), and RNase-

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Free Water (2 x 1.7 ml).. The QIAGEN Multiplex PCR Master Mix (MM) contains a combination of KCl and $(NH_4)_2SO_4$ as well as the PCR additive, Factor MP, which increases the local concentration of primers at the template. Factor MP stabilizes specifically bound primers, allowing efficient primer extension by HotStarTaq DNA Polymerase. HotStarTaq DNA Polymerase is a modified form of *Taq* DNA polymerase and has no polymerase activity at ambient temperatures. In some embodiments, HotStarTaq DNA Polymerase is activated by a 15-minute incubation at 95°C which can be incorporated into any existing thermal-cycler program.

In some embodiments, 1x QIAGEN MM final concentration (the recommended concentration), 7.5 nM of each primer in the library, 50 mM TMAC, and 7 ul DNA template in a 20 ul final volume is used. In some embodiments, the PCR thermocycling conditions include 95°C for 10 minutes (hot start); 20 cycles of 96°C for 30 seconds; 65°C for 15 minutes; and 72°C for 30 seconds; followed by 72°C for 2 minutes (final extension); and then a 4°C hold.

In some embodiments, 2x QIAGEN MM final concentration (twice the recommended concentration), 2 nM of each primer in the library, 70 mM TMAC, and 7 ul DNA template in a 20 ul total volume is used. In some embodiments, up to 4 mM EDTA is also included. In some embodiments, the PCR thermocycling conditions include 95°C for 10 minutes (hot start); 25 cycles of 96°C for 30 seconds; 65°C for 20 minutes; and 72°C for 30 seconds); followed by 72°C for 2 minutes (final extension); and then a 4°C hold.

Another exemplary set of PCR thermocyling conditions includes 95°C for 10 minutes, 15 cycles of 95°C for 30 seconds, 65°C for 1 minute, 60°C for 5 minutes, 65°C for 5 minutes and 72°C for 30 seconds; and then 72°C for 2 minutes. In some embodiments, this set of PCR thermocyling conditions is used with the following reaction conditions: 100 mM KCl, 50 mM $(NH_4)_2SO_4$, 3 mM $MgCl_2$, 7.5 nM of each primer in the library, 50 mM TMAC, and 7 ul DNA template in a 20 ul final volume at pH 8.1.

Another exemplary set of conditions includes a semi-nested PCR approach. The first PCR reaction uses 20 ul a reaction volume with 2x QIAGEN MM final concentration, 1.875 nM of each primer in the library (outer forward and reverse primers), and DNA template. Thermocycling parameters include 95°C for 10 minutes; 25 cycles of 96°C for 30 seconds, 65°C for 1 minute, 58°C for 6 minutes, 60°C for 8 minutes, 65°C for 4 minutes, and 72°C for 30 seconds; and then 72°C for 2 minutes, and then a 4°C hold. Next, 2 ul of the resulting

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product, diluted 1:200, is as input in a second PCR reaction. This reaction uses a 10 ul reaction volume with 1x QIAGEN MM final concentration, 20 nM of each inner forward primer, and 1 uM of reverse primer tag. Thermocycling parameters include 95°C for 10 minutes; 15 cycles of 9°5C for 30 seconds, 65°C for 1 minute, 60°C for 5 minutes, 65°C for 5 minutes, and 72°C for 30 seconds; and then 72°C for 2 minutes, and then a 4°C hold.

Any of the methods disclosed herein or any standard methods can be used to test a primer library to determine, *e.g.*, the percent of primer dimers, percent of target amplicons, and percent of target loci that are amplified. In some embodiments, the PCR products are sequenced as described in Experiment 15 or using standard sequencing methods. In some embodiments, the percentage of primer dimers can be determined by measuring the number of sequencing reads from primer dimers, the percentage of amplified products that are target amplicons can be determined by measuring the number of sequencing reads that map to target loci; the percent of target loci that are amplified can be determined by measuring the number of target loci for which there are sequencing reads that map to the target loci; the number of copies of a particular amplified target loci can be determined based on the number of sequencing reads that map to that target loci (such as by comparing the number of sequencing reads compared to the sequences reads from a standard of known concentration or amount).

Exemplary Nucleic Acid Samples

In some embodiments, the genetic sample may be prepared and/or purified. There are a number of standard procedures known in the art to accomplish such an end. In some embodiments, the sample may be centrifuged to separate various layers. In some embodiments, the DNA may be isolated using filtration. In some embodiments, the preparation of the DNA may involve amplification, separation, purification by chromatography, liquid liquid separation, isolation, preferential enrichment, preferential amplification, targeted amplification, or any of a number of other techniques either known in the art or described herein.

In some embodiments, a method disclosed herein could be used in situations where there is a very small amount of DNA present, such as in *in vitro* fertilization, or in forensic situations, where one or a few cells are available (typically less than ten cells, less than twenty cells or less than 40 cells.) In these embodiments, a method disclosed herein serves to make ploidy calls from a small amount of DNA that is not contaminated by other DNA, but where the ploidy calling very difficult the small amount of DNA. In some embodiments, a method

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disclosed herein could be used in situations where the target DNA is contaminated with DNA of another individual, for example in maternal blood in the context of prenatal diagnosis, paternity testing, or products of conception testing. Some other situations where these methods would be particularly advantageous would be in the case of cancer testing where only one or a small number of cells were present among a larger amount of normal cells. The genetic measurements used as part of these methods could be made on any sample comprising DNA or RNA, for example but not limited to: blood, plasma, body fluids, urine, hair, tears, saliva, tissue, skin, fingernails, blastomeres, embryos, fetal cells, amniotic fluid, chorionic villus samples, feces, bile, lymph, cervical mucus, semen, or other cells or materials comprising nucleic acids. In an embodiment, a method disclosed herein could be run with nucleic acid detection methods such as sequencing, microarrays, qPCR, digital PCR, or other methods used to measure nucleic acids. If for some reason it were found to be desirable, the ratios of the allele count probabilities at a locus could be calculated, and the allele ratios could be used to determine ploidy state in combination with some of the methods described herein, provided the methods are compatible. In some embodiments, a method disclosed herein involves calculating, on a computer, allele ratios at the plurality of polymorphic loci from the DNA measurements made on the processed samples. In some embodiments, a method disclosed herein involves calculating, on a computer, allele ratios at the plurality of polymorphic loci from the DNA measurements made on the processed samples along with any combination of other improvements described in this disclosure. Exemplary methods for isolating fetal cells, such as a single fetal cell are disclosed in USSN 61/978648, filed April 11, 2014 and USSN 61/984546, filed April 25, 2014. Fetal cells or fetal nucleic acids can be isolated from a pregnant mother using invasive (such as CVS or amniocentesis) or noninvasive methods (such as from a maternal blood sample).

In some embodiments, this method may be used to genotype a single cell, a small number of cells, two to five cells, six to ten cells, ten to twenty cells, twenty to fifty cell, fifty to one hundred cells, one hundred to one thousand cells, or a small amount of extracellular DNA, for example from one to ten picograms, from ten to one hundred pictograms, from one hundred pictograms to one nanogram, from one to ten nanograms, from ten to one hundred nanograms, from 30 to 500 nanograms, or from one hundred nanograms to one microgram. In some embodiments, nucleic acids (such as DNA and/or RNA) from less than 100, 75, 50, 40, 30, 20, 10, 8, 6, 4, 2, or 1 cell is amplified with any of the methods of the invention. In some

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embodiments, the nucleic acid sample includes less than 80, 60, 40, 20, or 10% of the nucleic acids (such as DNA and/or RNA) from a single cell. In some embodiments, in which a small number of cells (such as one cell) or a small amount of nucleic acids is used, nested PCR such as hemi-nested or semi-nested PCR is used and/or the number of PCR cycles is increased compare to that used for samples with a larger amount of cells or nucleic acids. In some embodiments, a large amount of cells or nucleic acids are used (such as in cases in which a larger amount is desired to improve performance of any of the methods of the invention. In some embodiments, a sample with at least 2, 5, 10, 15, 20, 30, 50, 100, or more cells (or DNA or RNA from such cells) is used in any of the methods of the invention. In some embodiments, at least 0.5, 1, 10, 25, 50, 100, 500, 1,000; or 5,000 ng of DNA or RNA is used.

In some embodiments, the cells in the sample are lysed prior to PCR. In some embodiments, the:Arcturus PicoPure DNA extraction kit from Applied Biosystems is used. (Applied Biosystems cat. No. KIT0103, *see, e.g.*, information available at the world wide web at lifetechnologies.com/order/catalog/product/KIT0103?ICID=search-product, which is hereby incorporated by reference in its entirety). This kit contains Arcturus reconstitution buffer and Protease K. In some embodiments, the following cell lysis thermocycling protocol is used: 56°C for 1 hour, 95°C for 10 minutes, 25°C for 15 minutes, and then a 4°C hold.

Exemplary Nucleic Acid Studies

The multiplex PCR methods of the invention can be used to increase the number of target loci that can be evaluated to measure the amount of one or more specific nucleic acid molecules of interest or of one or more types of nucleic acids. In some embodiments, there is a change in the total amount or concentration of one or more types of DNA (such as cfDNA cf mDNA, cf nDNA, cellular DNA, or mitochondrial DNA) or RNA (cfRNA, cellular RNA, cytoplasmic RNA, coding cytoplasmic RNA, non-coding cytoplasmic RNA, mRNA, miRNA, mitochondrial RNA, rRNA, or tRNA). In some embodiments, there is a change in the amount or concentration of one or more specific DNA (such as cfDNA cf mDNA, cf nDNA, cellular DNA, or mitochondrial DNA) or RNA (cfRNA, cellular RNA, cytoplasmic RNA, coding cytoplasmic RNA, non-coding cytoplasmic RNA, mRNA, miRNA, mitochondrial RNA, rRNA, or tRNA) molecules. In some embodiments, one allele is expressed more than another allele of a locus of interest. Exemplary miRNAs are short 20–22 nucleotide RNA molecules

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that regulate the expression of a gene. In some embodiments, there is a change in the transcriptome, such as a change in the identity or amount of one or more RNA molecules.

In some embodiments, an increase in the total amount or concentration of cfDNA or cfRNA is associated with a disease such as cancer, or an increased risk for a disease such as cancer. In some embodiments, the total concentration of a type of DNA (such as cfDNA cf mDNA, cf nDNA, cellular DNA, or mitochondrial DNA) or RNA (cfRNA, cellular RNA, cytoplasmic RNA, coding cytoplasmic RNA, non-coding cytoplasmic RNA, mRNA, miRNA, mitochondrial RNA, rRNA, or tRNA) increases by at least 2, 3, 4, 5, 6, 7, 8, 9, 10-fold, or more compared to the total concentration of that type of DNA or RNA in healthy (such as non-cancerous) subjects. In some embodiments, a total concentration of cfDNA between 75 to 100 ng/mL, 100 to 150 ng/mL, 150 to 200 ng/mL, 200 to 300 ng/mL, 300 to 400 ng/mgL, 400 to 600 ng/mL, 600 to 800 ng/mL, 800 to 1,000 ng/mL, inclusive, or a total concentration of cfDNA of more than 100 ng, mL, such as more than 200, 300, 400, 500, 600, 700, 800, 900, or 1,000 ng/mL is indicative of cancer, an increased risk for cancer, an increased risk of a tumor being malignant rather than benign, a decreased probably of the cancer going into remission, or a worse prognosis for the cancer. In some embodiments, the amount of a type of DNA (such as cfDNA cf mDNA, cf nDNA, cellular DNA, or mitochondrial DNA) or RNA (cfRNA, cellular RNA, cytoplasmic RNA, coding cytoplasmic RNA, non-coding cytoplasmic RNA, mRNA, miRNA, mitochondrial RNA, rRNA, or tRNA) having one or more polymorphisms/mutations (such as deletions or duplications) associated with a disease such as cancer or an increased risk for a disease such as cancer is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 18, 20, or 25% of the total amount of that type of DNA or RNA. In some embodiments, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 16, 18, 20, or 25% of the total amount of a type of DNA (such as cfDNA cf mDNA, cf nDNA, cellular DNA, or mitochondrial DNA) or RNA (cfRNA, cellular RNA, cytoplasmic RNA, coding cytoplasmic RNA, non-coding cytoplasmic RNA, mRNA, miRNA, mitochondrial RNA, rRNA, or tRNA) has a particular polymorphism or mutation (such as a deletion or duplication) associated with a disease such as cancer or an increased risk for a disease such as cancer.

Exemplary RNA Expression Studies

The multiplex PCR methods of the invention can be used to increase the number of target loci that can be evaluated during gene expression profiling experiments. For example,

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the expression levels of thousands of genes can be simultaneously monitored to determine whether a person has a sequence (such as a polymorphism or other mutation) associated with a disease (such as cancer) or an increased risk of a disease. These methods can be used to identify sequences (such as polymorphisms or other mutations) associated with an increased or decreased risk for a disease such as cancer by comparing gene expression (such as the expression of particular mRNA alleles) in samples from patients with and without the disease. Additionally, the effect of particular treatments, diseases, or developmental stages on gene expression can be determined. Similarly, these methods can be used to identify genes whose expression is changed in response to pathogens or other organisms by comparing gene expression in infected and uninfected cells or tissues. In these methods the number of sequencing reads can be adjusted based on the frequency of the polymorphisms that are being analyzed such that sufficient reads are performed for the polymorphisms to be detected if they are present.

In some embodiments, a sample containing RNA (such as mRNA) is amplified using a reverse transcriptase (RT) and the resulting DNA (such as cDNA) is then amplified using a DNA polymerase (PCR). The RT and PCR steps may be carried out sequentially in the same reaction volume or separately. Any of the primer libraries of the invention can be used in this reverse transcription polymerase chain reaction (RT-PCR) method. In various embodiments, the reverse transcription is performed using oligo-dT, random primers, a mixture of oligo-dT and random primers, or primers specific to the target loci. To avoid amplification of contaminating genomic DNA, primers for RT-PCR can be designed so that part of one primer hybridizes to the 3' end of one exon and the other part of the primer hybridizes to the 5' end of the adjacent exon. Such primers anneal to cDNA synthesized from spliced mRNAs, but not to genomic DNA. To detect amplification of contaminating DNA, RT-PCR primer pairs may be designed to flank a region that contains at least one intron. Products amplified from cDNA (no introns) are smaller than those amplified from genomic DNA (containing introns). Size difference in products is used to detect the presence of contaminating DNA. In some embodiments when only the mRNA sequence is known, primer annealing sites are chosen that are at least 300–400 base pairs apart since it is likely that fragments of this size from eukaryotic DNA contain splice junctions. Alternatively, the sample can be treated with DNase to degrade contaminating DNA.

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Exemplary Methods for Paternity Testing

The multiplex PCR methods of the invention can be used to improve the accuracy of paternity testing since so many target loci can be analyzed at once (see, *e.g*, U.S. Publication No. 2012/0122701, filed December 22, 2011, is which is hereby incorporated by reference in its entirety). For example, the multiplex PCR method can allow thousands of polymorphic loci (such as SNPs) to be analyzed for use in the PARENTAL SUPPORT algorithm described herein to determine whether an alleged father in is the biological father of a fetus. In some embodiments the method involves (i) simultaneously amplifying a plurality of polymorphic loci that includes at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different polymorphic loci on genetic material from the alleged father to produce a first set of amplified products; (ii) simultaneously amplifying the corresponding plurality of polymorphic loci on a mixed sample of DNA originating from a blood sample from the pregnant mother to produce a second set of amplified products; wherein the mixed sample of DNA comprises fetal DNA and maternal DNA; (iii) determining on a computer the probability that the alleged father is the biological father of the fetus using genotypic measurements based on the first and second sets of amplified products; and (iv) establishing whether the alleged father is the biological father of the fetus using the determined probability that the alleged father is the biological father of the fetus. In various embodiments, the method further includes simultaneously amplifying the corresponding plurality of polymorphic loci on genetic material from the mother to produce a third set of amplified products; wherein the probability that the alleged father is the biological father of the fetus is determined using genotypic measurements based on the first, second, and third sets of amplified products.

Exemplary Methods for Embryo Characterization and Selection

The multiplex PCR methods of the invention can be used to improve the selection of embryos for *in vitro* fertilization by allowing thousands of target loci to be analyzed at once (*see, e.g*, U.S. Pub. No. 2011/0092763, filed May 27, 2008, filed December 22, 2011, is which is hereby incorporated by reference in its entirety). For example, the multiplex PCR method can allow thousands of polymorphic loci (such as SNPs) to be analyzed for use in the PARENTAL SUPPORT algorithm described herein to select an embryo out of a set of embryos for *in vitro* fertilization

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In some embodiments, the invention provides methods of estimating relative likelihoods that each embryo from a set of embryos will develop as desired. In some embodiments, the method involves contacting a sample from each embryo with a library of primers that simultaneously hybridize to at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci to produce a reaction mixture for each embryo, wherein the samples are each derived from one or more cells from an embryo. In some embodiments, each reaction mixture is subjected to primer extension reaction conditions to produce amplified products. In some embodiments, the method includes determining on a computer one or more characteristics of at least one cell from each embryo based on the amplified products; and estimating on a computer the relative likelihoods that each embryo will develop as desired, based on the one or more characteristics of the at least one cell for each embryo. In some embodiments, the method includes using an informatics based method to determine the at least one characteristic, such as the PARENTAL SUPPORT algorithm described herein. In some embodiments, the characteristic includes a ploidy state. In some embodiments, the characteristic is selected from the group consisting of aneuploid, euploid, mosaic, nullsomy, monosomy, uniparental disomy, trisomy, tetrasomy, a type of aneuploidy, unmatched copy error trisomy, matched copy error trisomy, maternal origin of aneuploidy, paternal origin of aneuploidy, a presence or absence of a disease-linked gene, a chromosomal identity of any aneuploid chromosome, an abnormal genetic condition, a deletion or duplication, a likelihood of a characteristic, and combinations thereof. The characteristic may be associated with a chromosome taken from the group consisting of chromosome one, chromosome two, chromosome three, chromosome four, chromosome five, chromosome six, chromosome seven, chromosome eight, chromosome nine, chromosome ten, chromosome eleven, chromosome twelve, chromosome thirteen, chromosome fourteen, chromosome fifteen, chromosome sixteen, chromosome seventeen, chromosome eighteen, chromosome nineteen, chromosome twenty, chromosome twenty-one, chromosome twenty-two, X chromosome or Y chromosome, and combinations thereof.

Exemplary Prenatal Diagnostic Methods

The multiplex PCR methods of the present invention can be used to improve prenatal diagnostic methods, such as the determination of the ploidy status of fetal chromosomes.

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Given that the large number of target loci that can be simultaneously amplified, more accurate determinations can be made.

In an embodiment, the present disclosure provides *ex vivo* methods for determining the ploidy status of a chromosome in a gestating fetus from genotypic data measured from a mixed sample of DNA (*i.e.*, DNA from the mother of the fetus, and DNA from the fetus) and optionally from genotypic data measured from a sample of genetic material from the mother and possibly also from the father, wherein the determining is done by using a joint distribution model to create a set of expected allele distributions for different possible fetal ploidy states given the parental genotypic data, and comparing the expected allelic distributions to the actual allelic distributions measured in the mixed sample, and choosing the ploidy state whose expected allelic distribution pattern most closely matches the observed allelic distribution pattern. In an embodiment, the mixed sample is derived from maternal blood, or maternal serum or plasma. In an embodiment, the mixed sample of DNA may be preferentially enriched at a target loci (*e.g.*, plurality of polymorphic loci). In an embodiment, the preferential enrichment is done in a way that minimizes the allelic bias. In an embodiment, the present disclosure relates to a composition of DNA that has been preferentially enriched at a plurality of loci such that the allelic bias is low. In an embodiment, the allelic distribution(s) are measured by sequencing the DNA from the mixed sample. In an embodiment, the joint distribution model assumes that the alleles will be distributed in a binomial fashion. In an embodiment, the set of expected joint allele distributions are created for genetically linked loci while considering the extant recombination frequencies from various sources, for example, using data from the International HapMap Consortium.

In an embodiment, the present disclosure provides methods for non-invasive prenatal diagnosis (NPD), specifically, determining the aneuploidy status of a fetus by observing allele measurements at a plurality of polymorphic loci in genotypic data measured on DNA mixtures, where certain allele measurements are indicative of an aneuploid fetus, while other allele measurements are indicative of a euploid fetus. In an embodiment, the genotypic data is measured by sequencing DNA mixtures that were derived from maternal plasma. In an embodiment, the DNA sample may be preferentially enriched in molecules of DNA that correspond to the plurality of loci whose allele distributions are being calculated. In an embodiment a sample of DNA comprising only or almost only genetic material from the mother and possibly also a sample of DNA comprising only or almost only genetic material

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from the father are measured. In an embodiment, the genetic measurements of one or both parents along with the estimated fetal fraction are used to create a plurality of expected allele distributions corresponding to different possible underlying genetic states of the fetus; the expected allele distributions may be termed hypotheses. In an embodiment, the maternal genetic data is not determined by measuring genetic material that is exclusively or almost exclusively maternal in nature, rather, it is estimated from the genetic measurements made on maternal plasma that comprises a mixture of maternal and fetal DNA. In some embodiments the hypotheses may comprise the ploidy of the fetus at one or more chromosomes, which segments of which chromosomes in the fetus were inherited from which parents, and combinations thereof. In some embodiments, the ploidy state of the fetus is determined by comparing the observed allele measurements to the different hypotheses where at least some of the hypotheses correspond to different ploidy states, and selecting the ploidy state that corresponds to the hypothesis that is most likely to be true given the observed allele measurements. In an embodiment, this method involves using allele measurement data from some or all measured SNPs, regardless of whether the loci are homozygous or heterozygous, and therefore does not involve using alleles at loci that are only heterozygous. This method may not be appropriate for situations where the genetic data pertains to only one polymorphic locus. This method is particularly advantageous when the genetic data comprises data for more than ten polymorphic loci for a target chromosome or more than twenty polymorphic loci. This method is especially advantageous when the genetic data comprises data for more than 50 polymorphic loci for a target chromosome, more than 100 polymorphic loci or more than 200 polymorphic loci for a target chromosome. In some embodiments, the genetic data may comprise data for more than 500 polymorphic loci for a target chromosome, more than 1,000 polymorphic loci, more than 2,000 polymorphic loci, or more than 5,000 polymorphic loci for a target chromosome.

In an embodiment, a method disclosed herein yields a quantitative measure of the number of independent observations of each allele at a polymorphic locus. This is unlike most methods such as microarrays or qualitative PCR which provide information about the ratio of two alleles but do not quantify the number of independent observations of either allele. With methods that provide quantitative information regarding the number of independent observations, only the ratio is utilized in ploidy calculations, while the quantitative information by itself is not useful. To illustrate the importance of retaining information about the number of

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independent observations consider the sample locus with two alleles, A and B. In a first experiment twenty A alleles and twenty B alleles are observed, in a second experiment 200 A alleles and 200 B alleles are observed. In both experiments the ratio (A/(A+B)) is equal to 0.5, however the second experiment conveys more information than the first about the certainty of the frequency of the A or B allele. Some methods by others involve averaging or summing allele ratios (channel ratios) (*i.e.* x_i/y_i) from individual allele and analyzes this ratio, either comparing it to a reference chromosome or using a rule pertaining to how this ratio is expected to behave in particular situations. No allele weighting is implied in such methods, where it is assumed that one can ensure about the same amount of PCR product for each allele and that all the alleles should behave the same way. Such a method has a number of disadvantages, and more importantly, precludes the use a number of improvements that are described elsewhere in this disclosure.

In an embodiment, a method disclosed herein explicitly models the allele frequency distributions expected in disomy as well as a plurality of allele frequency distributions that may be expected in cases of trisomy resulting from nondisjunction during meiosis I, nondisjunction during meiosis II, and/or nondisjunction during mitosis early in fetal development. To illustrate why this is important, imagine a case where there were no crossovers: nondisjunction during meiosis I would result a trisomy in which two different homologs were inherited from one parent; in contrast, nondisjunction during meiosis II or during mitosis early in fetal development would result in two copies of the same homolog from one parent. Each scenario would result in different expected allele frequencies at each polymorphic locus and also at all loci considered jointly, due to genetic linkage. Crossovers, which result in the exchange of genetic material between homologs, make the inheritance pattern more complex; in an embodiment, the instant method accommodates for this by using recombination rate information in addition to the physical distance between loci. In an embodiment, to enable improved distinction between meiosis I nondisjunction and meiosis II or mitotic nondisjunction the instant method incorporate into the model an increasing probability of crossover as the distance from the centromere increases. Meiosis II and mitotic nondisjunction can distinguished by the fact that mitotic nondisjunction typically results in identical or nearly identical copies of one homolog while the two homologs present following a meiosis II nondisjunction event often differ due to one or more crossovers during gametogenesis.

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In some embodiments, a method disclosed herein involves comparing the observed allele measurements to theoretical hypotheses corresponding to possible fetal genetic aneuploidy, and does not involve a step of quantitating a ratio of alleles at a heterozygous locus. Where the number of loci is lower than about 20, the ploidy determination made using a method comprising quantitating a ratio of alleles at a heterozygous locus and a ploidy determination made using a method comprising comparing the observed allele measurements to theoretical allele distribution hypotheses corresponding to possible fetal genetic states may give a similar result. However, where the number of loci is above 50 these two methods is likely to give significantly different results; where the number of loci is above 400, above, 1,000 or above 2,000 these two methods are very likely to give results that are increasingly significantly different. These differences are due to the fact that a method that comprises quantitating a ratio of alleles at a heterozygous locus without measuring the magnitude of each allele independently and aggregating or averaging the ratios precludes the use of techniques including using a joint distribution model, performing a linkage analysis, using a binomial distribution model, and/or other advanced statistical techniques, whereas using a method comprising comparing the observed allele measurements to theoretical allele distribution hypotheses corresponding to possible fetal genetic states may use these techniques which can substantially increase the accuracy of the determination.

In an embodiment, a method disclosed herein involves determining whether the distribution of observed allele measurements is indicative of a euploid or an aneuploid fetus using a joint distribution model. The use of a joint distribution model is a different from and a significant improvement over methods that determine heterozygosity rates by treating polymorphic loci independently in that the resultant determinations are of significantly higher accuracy. Without being bound by any particular theory, it is believed that one reason they are of higher accuracy is that the joint distribution model takes into account the linkage between SNPs, and likelihood of crossovers having occurred during the meiosis that gave rise to the gametes that formed the embryo that grew into the fetus. The purpose of using the concept of linkage when creating the expected distribution of allele measurements for one or more hypotheses is that it allows the creation of expected allele measurements distributions that correspond to reality considerably better than when linkage is not used. For example, imagine that there are two SNPs, 1 and 2 located nearby one another, and the mother is A at SNP 1 and A at SNP 2 on one homolog, and B at SNP 1 and B at SNP 2 on homolog two. If the father is

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A for both SNPs on both homologs, and a B is measured for the fetus SNP 1, this indicates that homolog two has been inherited by the fetus, and therefore that there is a much higher likelihood of a B being present on the fetus at SNP 2. A model that takes into account linkage would predict this, while a model that does not take linkage into account would not. Alternately, if a mother was AB at SNP 1 and AB at nearby SNP 2, then two hypotheses corresponding to maternal trisomy at that location could be used – one involving a matching copy error (nondisjunction in meiosis II or mitosis in early fetal development), and one involving an unmatching copy error (nondisjunction in meiosis I). In the case of a matching copy error trisomy, if the fetus inherited an AA from the mother at SNP 1, then the fetus is much more likely to inherit either an AA or BB from the mother at SNP 2, but not AB. In the case of an unmatching copy error, the fetus would inherit an AB from the mother at both SNPs. The allele distribution hypotheses made by a ploidy calling method that takes into account linkage would make these predictions, and therefore correspond to the actual allele measurements to a considerably greater extent than a ploidy calling method that did not take into account linkage. Note that a linkage approach is not possible when using a method that relies on calculating allele ratios and aggregating those allele ratios.

One reason that it is believed that ploidy determinations that use a method that comprises comparing the observed allele measurements to theoretical hypotheses corresponding to possible fetal genetic states are of higher accuracy is that when sequencing is used to measure the alleles, this method can glean more information from data from alleles where the total number of reads is low than other methods; for example, a method that relies on calculating and aggregating allele ratios would produce disproportionately weighted stochastic noise. For example, imagine a case that involved measuring the alleles using sequencing, and where there was a set of loci where only five sequence reads were detected for each locus. In an embodiment, for each of the alleles, the data may be compared to the hypothesized allele distribution, and weighted according to the number of sequence reads; therefore the data from these measurements would be appropriately weighted and incorporated into the overall determination. This is in contrast to a method that involved quantitating a ratio of alleles at a heterozygous locus, as this method could only calculate ratios of 0%, 20%, 40%, 60%, 80% or 100% as the possible allele ratios; none of these may be close to expected allele ratios. In this latter case, the calculated allele rations would either have to be discarded due to insufficient reads or else would have disproportionate weighting and introduce stochastic noise

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into the determination, thereby decreasing the accuracy of the determination. In an embodiment, the individual allele measurements may be treated as independent measurements, where the relationship between measurements made on alleles at the same locus is no different from the relationship between measurements made on alleles at different loci.

In an embodiment, a method disclosed herein involves determining whether the distribution of observed allele measurements is indicative of a euploid or an aneuploid fetus without comparing any metrics to observed allele measurements on a reference chromosome that is expected to be disomic (termed the RC method). This is a significant improvement over methods, such as methods using shotgun sequencing which detect aneuploidy by evaluating the proportion of randomly sequenced fragments from a suspect chromosomes relative to one or more presumed disomic reference chromosome. This RC method yields incorrect results if the presumed disomic reference chromosome is not actually disomic. This can occur in cases where aneuploidy is more substantial than trisomy of a single chromosome or where the fetus is triploid and all autosomes are trisomic. In the case of a female triploid (69, XXX) fetus there are in fact no disomic chromosomes at all. The method described herein does not require a reference chromosome and would be able to correctly identify trisomic chromosomes in a female triploid fetus. For each chromosome, hypothesis, child fraction and noise level, a joint distribution model may be fit, without any of: reference chromosome data, an overall child fraction estimate, or a fixed reference hypothesis.

In an embodiment, a method disclosed herein demonstrates how observing allele distributions at polymorphic loci can be used to determine the ploidy state of a fetus with greater accuracy than methods in the prior art. In an embodiment, the method uses the targeted sequencing to obtain mixed maternal-fetal genotypes and optionally mother and/or father genotypes at a plurality of SNPs to first establish the various expected allele frequency distributions under the different hypotheses, and then observing the quantitative allele information obtained on the maternal-fetal mixture and evaluating which hypothesis fits the data best, where the genetic state corresponding to the hypothesis with the best fit to the data is called as the correct genetic state. In an embodiment, a method disclosed herein also uses the degree of fit to generate a confidence that the called genetic state is the correct genetic state. In an embodiment, a method disclosed herein involves using algorithms that analyze the distribution of alleles found for loci that have different parental contexts, and comparing the observed allele distributions to the expected allele distributions for different ploidy states for

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the different parental contexts (different parental genotypic patterns). This is different from and an improvement over methods that do not use methods that enable the estimation of the number of independent instances of each allele at each locus in a mixed maternal-fetal sample. In an embodiment, a method disclosed herein involves determining whether the distribution of observed allele measurements is indicative of a euploid or an aneuploid fetus using observed allelic distributions measured at loci where the mother is heterozygous. This is different from and an improvement over methods that do not use observed allelic distributions at loci where the mother is heterozygous because, in cases where the DNA is not preferentially enriched or is preferentially enriched for loci that are not known to be highly informative for that particular target individual, it allows the use of about twice as much genetic measurement data from a set of sequence data in the ploidy determination, resulting in a more accurate determination.

In an embodiment, a method disclosed herein uses a joint distribution model that assumes that the allele frequencies at each locus are multinomial (and thus binomial when SNPs are biallelic) in nature. In some embodiments the joint distribution model uses beta-binomial distributions. When using a measuring technique, such as sequencing, provides a quantitative measure for each allele present at each locus, binomal model can be applied to each locus and the degree underlying allele frequencies and the confidence in that frequency can be ascertained. With methods known in the art that generate ploidy calls from allele ratios, or methods in which quantitative allele information is discarded, the certainty in the observed ratio cannot be ascertained. The instant method is different from and an improvement over methods that calculate allele ratios and aggregate those ratios to make a ploidy call, since any method that involves calculating an allele ratio at a particular locus, and then aggregating those ratios, necessarily assumes that the measured intensities or counts that are indicative of the amount of DNA from any given allele or locus will be distributed in a Gaussian fashion. The method disclosed herein does not involve calculating allele ratios. In some embodiments, a method disclosed herein may involve incorporating the number of observations of each allele at a plurality of loci into a model. In some embodiments, a method disclosed herein may involve calculating the expected distributions themselves, allowing the use of a joint binomial distribution model which may be more accurate than any model that assumes a Gaussian distribution of allele measurements. The likelihood that the binomial distribution model is significantly more accurate than the Gaussian distribution increases as the number of loci increases. For example, when fewer than 20 loci are interrogated, the likelihood that the

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binomial distribution model is significantly better is low. However, when more than 100, or especially more than 400, or especially more than 1,000, or especially more than 2,000 loci are used, the binomial distribution model will have a very high likelihood of being significantly more accurate than the Gaussian distribution model, thereby resulting in a more accurate ploidy determination. The likelihood that the binomial distribution model is significantly more accurate than the Gaussian distribution also increases as the number of observations at each locus increases. For example, when fewer than 10 distinct sequences are observed at each locus are observed, the likelihood that the binomial distribution model is significantly better is low. However, when more than 50 sequence reads, or especially more than 100 sequence reads, or especially more than 200 sequence reads, or especially more than 300 sequence reads are used for each locus, the binomial distribution model will have a very high likelihood of being significantly more accurate than the Gaussian distribution model, thereby resulting in a more accurate ploidy determination.

In an embodiment, a method disclosed herein uses sequencing to measure the number of instances of each allele at each locus in a DNA sample. Each sequencing read may be mapped to a specific locus and treated as a binary sequence read; alternately, the probability of the identity of the read and/or the mapping may be incorporated as part of the sequence read, resulting in a probabilistic sequence read, that is, the probable whole or fractional number of sequence reads that map to a given loci. Using the binary counts or probability of counts it is possible to use a binomial distribution for each set of measurements, allowing a confidence interval to be calculated around the number of counts. This ability to use the binomial distribution allows for more accurate ploidy estimations and more precise confidence intervals to be calculated. This is different from and an improvement over methods that use intensities to measure the amount of an allele present, for example methods that use microarrays, or methods that make measurements using fluorescence readers to measure the intensity of fluorescently tagged DNA in electrophoretic bands.

In an embodiment, a method disclosed herein uses aspects of the present set of data to determine parameters for the estimated allele frequency distribution for that set of data. This is an improvement over methods that utilize training set of data or prior sets of data to set parameters for the present expected allele frequency distributions, or possibly expected allele ratios. This is because there are different sets of conditions involved in the collection and measurement of every genetic sample, and thus a method that uses data from the instant set of

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data to determine the parameters for the joint distribution model that is to be used in the ploidy determination for that sample will tend to be more accurate.

In an embodiment, a method disclosed herein involves determining whether the distribution of observed allele measurements is indicative of a euploid or an aneuploid fetus using a maximum likelihood technique. The use of a maximum likelihood technique is different from and a significant improvement over methods that use single hypothesis rejection technique in that the resultant determinations will be made with significantly higher accuracy. One reason is that single hypothesis rejection techniques set cut off thresholds based on only one measurement distribution rather than two, meaning that the thresholds are usually not optimal. Another reason is that the maximum likelihood technique allows the optimization of the cut off threshold for each individual sample instead of determining a cut off threshold to be used for all samples regardless of the particular characteristics of each individual sample. Another reason is that the use of a maximum likelihood technique allows the calculation of a confidence for each ploidy call. The ability to make a confidence calculation for each call allows a practitioner to know which calls are accurate, and which are more likely to be wrong. In some embodiments, a wide variety of methods may be combined with a maximum likelihood estimation technique to enhance the accuracy of the ploidy calls. In an embodiment, the maximum likelihood technique may be used in combination with the method described in US Patent 7,888,017. In an embodiment, the maximum likelihood technique may be used in combination with the method of using targeted PCR amplification to amplify the DNA in the mixed sample followed by sequencing and analysis using a read counting method such as used by TANDEM DIAGNOSTICS, as presented at the International Congress of Human Genetics 2011, in Montreal in October 2011. In an embodiment, a method disclosed herein involves estimating the fetal fraction of DNA in the mixed sample and using that estimation to calculate both the ploidy call and the confidence of the ploidy call. Note that this is both different and distinct from methods that use estimated fetal fraction as a screen for sufficient fetal fraction, followed by a ploidy call made using a single hypothesis rejection technique that does not take into account the fetal fraction nor does it produce a confidence calculation for the call.

In an embodiment, a method disclosed herein takes into account the tendency for the data to be noisy and contain errors by attaching a probability to each measurement. The use of maximum likelihood techniques to choose the correct hypothesis from the set of hypotheses that were made using the measurement data with attached probabilistic estimates makes it

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more likely that the incorrect measurements will be discounted, and the correct measurements will be used in the calculations that lead to the ploidy call. To be more precise, this method systematically reduces the influence of data that is incorrectly measured on the ploidy determination. This is an improvement over methods where all data is assumed to be equally correct or methods where outlying data is arbitrarily excluded from calculations leading to a ploidy call. Existing methods using channel ratio measurements claim to extend the method to multiple SNPs by averaging individual SNP channel ratios. Not weighting individual SNPs by expected measurement variance based on the SNP quality and observed depth of read reduces the accuracy of the resulting statistic, resulting in a reduction of the accuracy of the ploidy call significantly, especially in borderline cases.

In an embodiment, a method disclosed herein does not presuppose the knowledge of which SNPs or other polymorphic loci are heterozygous on the fetus. This method allows a ploidy call to be made in cases where paternal genotypic information is not available. This is an improvement over methods where the knowledge of which SNPs are heterozygous must be known ahead of time in order to appropriately select loci to target, or to interpret the genetic measurements made on the mixed fetal/maternal DNA sample.

The methods described herein are particularly advantageous when used on samples where a small amount of DNA is available, or where the percent of fetal DNA is low. This is due to the correspondingly higher allele dropout rate that occurs when only a small amount of DNA is available and/or the correspondingly higher fetal allele dropout rate when the percent of fetal DNA is low in a mixed sample of fetal and maternal DNA. A high allele dropout rate, meaning that a large percentage of the alleles were not measured for the target individual, results in poorly accurate fetal fractions calculations, and poorly accurate ploidy determinations. Since methods disclosed herein may use a joint distribution model that takes into account the linkage in inheritance patterns between SNPs, significantly more accurate ploidy determinations may be made. The methods described herein allow for an accurate ploidy determination to be made when the percent of molecules of DNA that are fetal in the mixture is less than 40%, less than 30%, less than 20%, less than 10%, less than 8%, and even less than 6%.

In an embodiment, it is possible to determine the ploidy state of an individual based on measurements when that individual's DNA is mixed with DNA of a related individual. In an embodiment, the mixture of DNA is the free floating DNA found in maternal plasma, which

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may include DNA from the mother, with known karyotype and known genotype, and which may be mixed with DNA of the fetus, with unknown karyotype and unknown genotype. It is possible to use the known genotypic information from one or both parents to predict a plurality of potential genetic states of the DNA in the mixed sample for different ploidy states, different chromosome contributions from each parent to the fetus, and optionally, different fetal DNA fractions in the mixture. Each potential composition may be referred to as a hypothesis. The ploidy state of the fetus can then be determined by looking at the actual measurements, and determining which potential compositions are most likely given the observed data.

Further discussion of the points above may be found elsewhere in this document.

Non-Invasive Prenatal Diagnosis (NPD)

The process of non-invasive prenatal diagnosis involves a number of steps. Some of the steps may include: (1) obtaining the genetic material from the fetus; (2) enriching the genetic material of the fetus that may be in a mixed sample, *ex vivo*; (3) amplifying the genetic material, *ex vivo*; (4) preferentially enriching specific loci in the genetic material, *ex vivo*; (5) measuring the genetic material, *ex vivo*; and (6) analyzing the genotypic data, on a computer, and *ex vivo*. Methods to reduce to practice these six and other relevant steps are described herein. At least some of the method steps are not directly applied on the body. In an embodiment, the present disclosure relates to methods of treatment and diagnosis applied to tissue and other biological materials isolated and separated from the body. At least some of the method steps are executed on a computer.

Some embodiments of the present disclosure allow a clinician to determine the genetic state of a fetus that is gestating in a mother in a non-invasive manner such that the health of the baby is not put at risk by the collection of the genetic material of the fetus, and that the mother is not required to undergo an invasive procedure. Moreover, in certain aspects, the present disclosure allows the fetal genetic state to be determined with high accuracy, significantly greater accuracy than, for example, the non-invasive maternal serum analyte based screens, such as the triple test, that are in wide use in prenatal care.

The high accuracy of the methods disclosed herein is a result of an informatics approach to analysis of the genotype data, as described herein. Modern technological advances have resulted in the ability to measure large amounts of genetic information from a genetic sample using such methods as high throughput sequencing and genotyping arrays. The

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methods disclosed herein allow a clinician to take greater advantage of the large amounts of data available, and make a more accurate diagnosis of the fetal genetic state. The details of a number of embodiments are given below. Different embodiments may involve different combinations of the aforementioned steps. Various combinations of the different embodiments of the different steps may be used interchangeably.

In an embodiment, a blood sample is taken from a pregnant mother, and the free floating DNA in the plasma of the mother's blood, which contains a mixture of both DNA of maternal origin, and DNA of fetal origin, is isolated and used to determine the ploidy status of the fetus. In an embodiment, a method disclosed herein involves preferential enrichment of those DNA sequences in a mixture of DNA that correspond to polymorphic alleles in a way that the allele ratios and/or allele distributions remain mostly consistent upon enrichment. In an embodiment, a method disclosed herein involves the highly efficient targeted PCR based amplification such that a very high percentage of the resulting molecules correspond to targeted loci. In an embodiment, a method disclosed herein involves sequencing a mixture of DNA that contains both DNA of maternal origin, and DNA of fetal origin. In an embodiment, a method disclosed herein involves using measured allele distributions to determine the ploidy state of a fetus that is gestating in a mother. In an embodiment, a method disclosed herein involves reporting the determined ploidy state to a clinician. In an embodiment, a method disclosed herein involves taking a clinical action, for example, performing follow up invasive testing such as chorionic villus sampling or amniocentesis, preparing for the birth of a trisomic individual or an elective termination of a trisomic fetus.

This application makes reference to U.S. Utility Application Serial No. 11/603,406, filed November 28, 2006 (US Publication No.: 20070184467); U.S. Utility Application Serial No. 12/076,348, filed March 17, 2008 (US Publication No.: 20080243398); PCT Application Serial No. PCT/US09/52730, filed August 4, 2009 (PCT Publication No.: WO/2010/017214); PCT Application Serial No. PCT/US10/050824, filed September 30, 2010 (PCT Publication No.: WO/2011/041485), U.S. Utility Application Serial No. 13/110,685, filed May 18, 2011, and PCT Application Serial No. PCT/12/58578, filed October 3, 2012, which are each herein incorporated by reference in its entirety. Some of the vocabulary used in this filing may have its antecedents in these references. Some of the concepts described herein may be better understood in light of the concepts found in these references.

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Screening Maternal Blood Comprising Free Floating Fetal DNA

The methods described herein may be used to help determine the genotype of a child, fetus, or other target individual where the genetic material of the target is found in the presence of a quantity of other genetic material. In some embodiments the genotype may refer to the ploidy state of one or a plurality of chromosomes, it may refer to one or a plurality of disease linked alleles, or some combination thereof. In this disclosure, the discussion focuses on determining the genetic state of a fetus where the fetal DNA is found in maternal blood, but this example is not meant to limit to possible contexts that this method may be applied to. In addition, the method may be applicable in cases where the amount of target DNA is in any proportion with the non-target DNA; for example, the target DNA could make up anywhere between 0.000001 and 99.999999% of the DNA present. In addition, the non-target DNA does not necessarily need to be from one individual, or even from a related individual, as long as genetic data from some or all of the relevant non-target individual(s) is known. In an embodiment, a method disclosed herein can be used to determine genotypic data of a fetus from maternal blood that contains fetal DNA. It may also be used in a case where there are multiple fetuses in the uterus of a pregnant woman, or where other contaminating DNA may be present in the sample, for example from other already born siblings.

This technique may make use of the phenomenon of fetal blood cells gaining access to maternal circulation through the placental villi. Ordinarily, only a very small number of fetal cells enter the maternal circulation in this fashion (not enough to produce a positive Kleihauer-Betke test for fetal-maternal hemorrhage). The fetal cells can be sorted out and analyzed by a variety of techniques to look for particular DNA sequences, but without the risks that invasive procedures inherently have. This technique may also make use of the phenomenon of free floating fetal DNA gaining access to maternal circulation by DNA release following apoptosis of placental tissue where the placental tissue in question contains DNA of the same genotype as the fetus. The free floating DNA found in maternal plasma has been shown to contain fetal DNA in proportions as high as 30-40% fetal DNA.

In an embodiment, blood may be drawn from a pregnant woman. Research has shown that maternal blood may contain a small amount of free floating DNA from the fetus, in addition to free floating DNA of maternal origin. In addition, there also may be enucleated fetal blood cells comprising DNA of fetal origin, in addition to many blood cells of maternal origin, which typically do not contain nuclear DNA. There are many methods know in the art

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to isolate fetal DNA, or create fractions enriched in fetal DNA. For example, chromatography has been show to create certain fractions that are enriched in fetal DNA.

Once the sample of maternal blood, plasma, or other fluid, drawn in a relatively non-invasive manner, and that contains an amount of fetal DNA, either cellular or free floating, either enriched in its proportion to the maternal DNA, or in its original ratio, is in hand, one may genotype the DNA found in said sample. In some embodiments, the blood may be drawn using a needle to withdraw blood from a vein, for example, the basilica vein. The method described herein can be used to determine genotypic data of the fetus. For example, it can be used to determine the ploidy state at one or more chromosomes, it can be used to determine the identity of one or a set of SNPs, including insertions, deletions, and translocations. It can be used to determine one or more haplotypes, including the parent of origin of one or more genotypic features.

Note that this method will work with any nucleic acids that can be used for any genotyping and/or sequencing methods, such as the ILLUMINA INFINIUM ARRAY platform, AFFYMETRIX GENECHIP, ILLUMINA GENOME ANALYZER, or LIFE TECHNOLGIES' SOLID SYSTEM. This includes extracted free-floating DNA from plasma or amplifications (*e.g.* whole genome amplification, PCR) of the same; genomic DNA from other cell types (*e.g.* human lymphocytes from whole blood) or amplifications of the same. For preparation of the DNA, any extraction or purification method that generates genomic DNA suitable for the one of these platforms will work as well. This method could work equally well with samples of RNA. In an embodiment, storage of the samples may be done in a way that will minimize degradation (*e.g.* below freezing, at about -20 C, or at a lower temperature).

Parental Support

Some embodiments may be used in combination with the PARENTAL SUPPORT™ (PS) method, embodiments of which are described in U.S. Application No. 11/603,406 (US Publication No.: 20070184467), U.S. Application No. 12/076,348 (US Publication No.: 20080243398), U.S. Application 13/110,685, PCT Application PCT/US09/52730 (PCT Publication No.: WO/2010/017214), and PCT Application No. PCT/US10/050824 (PCT Publication No.: WO/2011/041485) which are incorporated herein by reference in their entirety. PARENTAL SUPPORT™ is an informatics based approach that can be used to analyze genetic data. In some embodiments, the methods disclosed herein may be considered

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as part of the PARENTAL SUPPORT™ method. In some embodiments, The PARENTAL SUPPORT ™ method is a collection of methods that may be used to determine the genetic data of a target individual, with high accuracy, of one or a small number of cells from that individual, or of a mixture of DNA consisting of DNA from the target individual and DNA from one or a plurality of other individuals, specifically to determine disease-related alleles, other alleles of interest, and/or the ploidy state of one or a plurality of chromosomes in the target individual. PARENTAL SUPPORT™ may refer to any of these methods. PARENTAL SUPPORT™ is an example of an informatics based method. Exemplary embodiments of the PARENTAL SUPPORT™ method are illustrated in FIGs. 29-31G and described in Experiment 19.

The PARENTAL SUPPORT™ method makes use of known parental genetic data, *i.e.* haplotypic and/or diploid genetic data of the mother and/or the father, together with the knowledge of the mechanism of meiosis and the imperfect measurement of the target DNA, and possibly of one or more related individuals, along with population based crossover frequencies, in order to reconstruct, *in silico*, the genotype at a plurality of alleles, and/or the ploidy state of an embryo or of any target cell(s), and the target DNA at the location of key loci with a high degree of confidence. The PARENTAL SUPPORT™ method can reconstruct not only single nucleotide polymorphisms (SNPs) that were measured poorly, but also insertions and deletions, and SNPs or whole regions of DNA that were not measured at all. Furthermore, the PARENTAL SUPPORT™ method can both measure multiple disease-linked loci as well as screen for aneuploidy, from a single cell. In some embodiments, the PARENTAL SUPPORT™ method may be used to characterize one or more cells from embryos biopsied during an IVF cycle to determine the genetic condition of the one or more cells.

The PARENTAL SUPPORT™ method allows the cleaning of noisy genetic data. This may be done by inferring the correct genetic alleles in the target genome (embryo) using the genotype of related individuals (parents) as a reference. PARENTAL SUPPORT™ may be particularly relevant where only a small quantity of genetic material is available (*e.g.* PGD) and where direct measurements of the genotypes are inherently noisy due to the limited amounts of genetic material. PARENTAL SUPPORT™ may be particularly relevant where only a small fraction of the genetic material available is from the target individual (*e.g.* NPD) and where direct measurements of the genotypes are inherently noisy due to the contaminating DNA signal from another individual. The PARENTAL SUPPORT™ method is able to

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reconstruct highly accurate ordered diploid allele sequences on the embryo, together with copy number of chromosomes segments, even though the conventional, unordered diploid measurements may be characterized by high rates of allele dropouts, drop-ins, variable amplification biases and other errors. The method may employ both an underlying genetic model and an underlying model of measurement error. The genetic model may determine both allele probabilities at each SNP and crossover probabilities between SNPs. Allele probabilities may be modeled at each SNP based on data obtained from the parents and model crossover probabilities between SNPs based on data obtained from the HapMap database, as developed by the International HapMap Project. Given the proper underlying genetic model and measurement error model, *maximum a posteriori* (MAP) estimation may be used, with modifications for computationally efficiency, to estimate the correct, ordered allele values at each SNP in the embryo.

The techniques outlined above, in some cases, are able to determine the genotype of an individual given a very small amount of DNA originating from that individual. This could be the DNA from one or a small number of cells, or it could be from the small amount of fetal DNA found in maternal blood.

Hypotheses

In the context of this disclosure, a hypothesis refers to a possible genetic state. It may refer to a possible ploidy state. It may refer to a possible allelic state. A set of hypotheses may refer to a set of possible genetic states, a set of possible allelic states, a set of possible ploidy states, or combinations thereof. In some embodiments, a set of hypotheses may be designed such that one hypothesis from the set will correspond to the actual genetic state of any given individual. In some embodiments, a set of hypotheses may be designed such that every possible genetic state may be described by at least one hypothesis from the set. In some embodiments of the present disclosure, one aspect of a method is to determine which hypothesis corresponds to the actual genetic state of the individual in question.

In another embodiment of the present disclosure, one step involves creating a hypothesis. In some embodiments it may be a copy number hypothesis. In some embodiments it may involve a hypothesis concerning which segments of a chromosome from each of the related individuals correspond genetically to which segments, if any, of the other related individuals. Creating a hypothesis may refer to the act of setting the limits of the variables such

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that the entire set of possible genetic states that are under consideration are encompassed by those variables.

A "copy number hypothesis," also called a "ploidy hypothesis," or a "ploidy state hypothesis," may refer to a hypothesis concerning a possible ploidy state for a given chromosome copy, chromosome type, or section of a chromosome, in the target individual. It may also refer to the ploidy state at more than one of the chromosome types in the individual. A set of copy number hypotheses may refer to a set of hypotheses where each hypothesis corresponds to a different possible ploidy state in an individual. A set of hypotheses may concern a set of possible ploidy states, a set of possible parental haplotypes contributions, a set of possible fetal DNA percentages in the mixed sample, or combinations thereof.

A normal individual contains one of each chromosome type from each parent. However, due to errors in meiosis and mitosis, it is possible for an individual to have 0, 1, 2, or more of a given chromosome type from each parent. In practice, it is rare to see more that two of a given chromosomes from a parent. In this disclosure, some embodiments only consider the possible hypotheses where 0, 1, or 2 copies of a given chromosome come from a parent; it is a trivial extension to consider more or less possible copies originating from a parent. In some embodiments, for a given chromosome, there are nine possible hypotheses: the three possible hypothesis concerning 0, 1, or 2 chromosomes of maternal origin, multiplied by the three possible hypotheses concerning 0, 1, or 2 chromosomes of paternal origin. Let (m,f) refer to the hypothesis where m is the number of a given chromosome inherited from the mother, and f is the number of a given chromosome inherited from the father. Therefore, the nine hypotheses are (0,0), (0,1), (0,2), (1,0), (1,1), (1,2), (2,0), (2,1), and (2,2). These may also be written as H_{00}, H_{01}, H_{02}, H_{10}, H_{12}, H_{20}, H_{21}, and H_{22}. The different hypotheses correspond to different ploidy states. For example, (1,1) refers to a normal disomic chromosome; (2,1) refers to a maternal trisomy, and (0,1) refers to a paternal monosomy. In some embodiments, the case where two chromosomes are inherited from one parent and one chromosome is inherited from the other parent may be further differentiated into two cases: one where the two chromosomes are identical (matched copy error), and one where the two chromosomes are homologous but not identical (unmatched copy error). In these embodiments, there are sixteen possible hypotheses. It should be understood that it is possible to use other sets of hypotheses, and a different number of hypotheses.

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In some embodiments of the present disclosure, the ploidy hypothesis refers to a hypothesis concerning which chromosome from other related individuals correspond to a chromosome found in the target individual's genome. In some embodiments, a key to the method is the fact that related individuals can be expected to share haplotype blocks, and using measured genetic data from related individuals, along with a knowledge of which haplotype blocks match between the target individual and the related individual, it is possible to infer the correct genetic data for a target individual with higher confidence than using the target individual's genetic measurements alone. As such, in some embodiments, the ploidy hypothesis may concern not only the number of chromosomes, but also which chromosomes in related individuals are identical, or nearly identical, with one or more chromosomes in the target individual.

Once the set of hypotheses have been defined, when the algorithms operate on the input genetic data, they may output a determined statistical probability for each of the hypotheses under consideration. The probabilities of the various hypotheses may be determined by mathematically calculating, for each of the various hypotheses, the value that the probability equals, as stated by one or more of the expert techniques, algorithms, and/or methods described elsewhere in this disclosure, using the relevant genetic data as input.

Once the probabilities of the different hypotheses are estimated, as determined by a plurality of techniques, they may be combined. This may entail, for each hypothesis, multiplying the probabilities as determined by each technique. The product of the probabilities of the hypotheses may be normalized. Note that one ploidy hypothesis refers to one possible ploidy state for a chromosome.

The process of "combining probabilities," also called "combining hypotheses," or combining the results of expert techniques, is a concept that should be familiar to one skilled in the art of linear algebra. One possible way to combine probabilities is as follows: When an expert technique is used to evaluate a set of hypotheses given a set of genetic data, the output of the method is a set of probabilities that are associated, in a one-to-one fashion, with each hypothesis in the set of hypotheses. When a set of probabilities that were determined by a first expert technique, each of which are associated with one of the hypotheses in the set, are combined with a set of probabilities that were determined by a second expert technique, each of which are associated with the same set of hypotheses, then the two sets of probabilities are multiplied. This means that, for each hypothesis in the set, the two probabilities that are

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associated with that hypothesis, as determined by the two expert methods, are multiplied together, and the corresponding product is the output probability. This process may be expanded to any number of expert techniques. If only one expert technique is used, then the output probabilities are the same as the input probabilities. If more than two expert techniques are used, then the relevant probabilities may be multiplied at the same time. The products may be normalized so that the probabilities of the hypotheses in the set of hypotheses sum to 100%.

In some embodiments, if the combined probabilities for a given hypothesis are greater than the combined probabilities for any of the other hypotheses, then it may be considered that that hypothesis is determined to be the most likely. In some embodiments, a hypothesis may be determined to be the most likely, and the ploidy state, or other genetic state, may be called if the normalized probability is greater than a threshold. In an embodiment, this may mean that the number and identity of the chromosomes that are associated with that hypothesis may be called as the ploidy state. In an embodiment, this may mean that the identity of the alleles that are associated with that hypothesis may be called as the allelic state. In some embodiments, the threshold may be between about 50% and about 80%. In some embodiments the threshold may be between about 80% and about 90%. In some embodiments the threshold may be between about 90% and about 95%. In some embodiments the threshold may be between about 95% and about 99%. In some embodiments the threshold may be between about 99% and about 99.9%. In some embodiments the threshold may be above about 99.9%.

Parental Contexts

The parental context refers to the genetic state of a given allele, on each of the two relevant chromosomes for one or both of the two parents of the target. Note that in an embodiment, the parental context does not refer to the allelic state of the target, rather, it refers to the allelic state of the parents. The parental context for a given SNP may consist of four base pairs, two paternal and two maternal; they may be the same or different from one another. It is typically written as "$m_1m_2|f_1f_2$," where m_1 and m_2 are the genetic state of the given SNP on the two maternal chromosomes, and f_1 and f_2 are the genetic state of the given SNP on the two paternal chromosomes. In some embodiments, the parental context may be written as "$f_1f_2|m_1m_2$." Note that subscripts "1" and "2" refer to the genotype, at the given allele, of the first and second chromosome; also note that the choice of which chromosome is labeled "1" and which is labeled "2" is arbitrary.

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Note that in this disclosure, A and B are often used to generically represent base pair identities; A or B could equally well represent C (cytosine), G (guanine), A (adenine) or T (thymine). For example, if, at a given SNP based allele, the mother's genotype was T at that SNP on one chromosome, and G at that SNP on the homologous chromosome, and the father's genotype at that allele is G at that SNP on both of the homologous chromosomes, one may say that the target individual's allele has the parental context of AB|BB; it could also be said that the allele has the parental context of AB|AA. Note that, in theory, any of the four possible nucleotides could occur at a given allele, and thus it is possible, for example, for the mother to have a genotype of AT, and the father to have a genotype of GC at a given allele. However, empirical data indicate that in most cases only two of the four possible base pairs are observed at a given allele. It is possible, for example when using single tandem repeats, to have more than two parental, more than four and even more than ten contexts. In this disclosure the discussion assumes that only two possible base pairs will be observed at a given allele, although the embodiments disclosed herein could be modified to take into account the cases where this assumption does not hold.

A "parental context" may refer to a set or subset of target SNPs that have the same parental context. For example, if one were to measure 1000 alleles on a given chromosome on a target individual, then the context AA|BB could refer to the set of all alleles in the group of 1,000 alleles where the genotype of the mother of the target was homozygous, and the genotype of the father of the target is homozygous, but where the maternal genotype and the paternal genotype are dissimilar at that locus. If the parental data is not phased, and thus AB = BA, then there are nine possible parental contexts: AA|AA, AA|AB, AA|BB, AB|AA, AB|AB, AB|BB, BB|AA, BB|AB, and BB|BB. If the parental data is phased, and thus AB $\neq$ BA, then there are sixteen different possible parental contexts: AA|AA, AA|AB, AA|BA, AA|BB, AB|AA, AB|AB, AB|BA, AB|BB, BA|AA, BA|AB, BA|BA, BA|BB, BB|AA, BB|AB, BB|BA, and BB|BB. Every SNP allele on a chromosome, excluding some SNPs on the sex chromosomes, has one of these parental contexts. The set of SNPs wherein the parental context for one parent is heterozygous may be referred to as the heterozygous context.

Use of Parental Contexts in NPD

Non-invasive prenatal diagnosis is an important technique that can be used to determine the genetic state of a fetus from genetic material that is obtained in a non-invasive

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manner, for example from a blood draw on the pregnant mother. The blood could be separated and the plasma isolated, followed by isolation of the plasma DNA. Size selection could be used to isolate the DNA of the appropriate length. The DNA may be preferentially enriched at a set of loci. This DNA can then be measured by a number of means, such as by hybridizing to a genotyping array and measuring the fluorescence, or by sequencing on a high throughput sequencer.

When sequencing is used for ploidy calling of a fetus in the context of non-invasive prenatal diagnosis, there are a number of ways to use the sequence data. The most common way one could use the sequence data is to simply count the number of reads that map to a given chromosome. For example, imagine if you are trying to determine the ploidy state of chromosome 21 on the fetus. Further imagine that the DNA in the sample is comprised of 10% DNA of fetal origin, and 90% DNA of maternal origin. In this case, you could look at the average number of reads on a chromosome which can be expected to be disomic, for example chromosome 3, and compare that to the number of read on chromosome 21, where the reads are adjusted for the number of base pairs on that chromosome that are part of a unique sequence. If the fetus were euploid, one would expect the amount of DNA per unit of genome to be about equal at all locations (subject to stochastic variations). On the other hand, if the fetus were trisomic at chromosome 21, then one would expect there to be more slightly more DNA per genetic unit from chromosome 21 than the other locations on the genome. Specifically one would expect there to be about 5% more DNA from chromosome 21 in the mixture. When sequencing is used to measure the DNA, one would expect about 5% more uniquely mappable reads from chromosome 21 per unique segment than from the other chromosomes. One could use the observation of an amount of DNA from a particular chromosome that is higher than a certain threshold, when adjusted for the number of sequences that are uniquely mappable to that chromosome, as the basis for an aneuploidy diagnosis. Another method that may be used to detect aneuploidy is similar to that above, except that parental contexts could be taken into account.

When considering which alleles to target, one may consider the likelihood that some parental contexts are likely to be more informative than others. For example, AA|BB and the symmetric context BB|AA are the most informative contexts, because the fetus is known to carry an allele that is different from the mother. For reasons of symmetry, both AA|BB and BB|AA contexts may be referred to as AA|BB. Another set of informative parental contexts are

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AA|AB and BB|AB, because in these cases the fetus has a 50% chance of carrying an allele that the mother does not have. For reasons of symmetry, both AA|AB and BB|AB contexts may be referred to as AA|AB. A third set of informative parental contexts are AB|AA and AB|BB, because in these cases the fetus is carrying a known paternal allele, and that allele is also present in the maternal genome. For reasons of symmetry, both AB|AA and AB|BB contexts may be referred to as AB|AA. A fourth parental context is AB|AB where the fetus has an unknown allelic state, and whatever the allelic state, it is one in which the mother has the same alleles. The fifth parental context is AA|AA, where the mother and father are heterozygous.

Different Implementations of the Presently Disclosed Embodiments

Methods are disclosed herein for determining the ploidy state of a target individual. The target individual may be a blastomere, an embryo, or a fetus. In some embodiments of the present disclosure, a method for determining the ploidy state of one or more chromosome in a target individual may include any of the steps described in this document, and combinations thereof:

In some embodiments the source of the genetic material to be used in determining the genetic state of the fetus may be fetal cells, such as nucleated fetal red blood cells, isolated from the maternal blood. The method may involve obtaining a blood sample from the pregnant mother. The method may involve isolating a fetal red blood cell using visual techniques, based on the idea that a certain combination of colors are uniquely associated with nucleated red blood cell, and a similar combination of colors is not associated with any other present cell in the maternal blood. The combination of colors associated with the nucleated red blood cells may include the red color of the hemoglobin around the nucleus, which color may be made more distinct by staining, and the color of the nuclear material which can be stained, for example, blue. By isolating the cells from maternal blood and spreading them over a slide, and then identifying those points at which one sees both red (from the Hemoglobin) and blue (from the nuclear material) one may be able to identify the location of nucleated red blood cells. One may then extract those nucleated red blood cells using a micromanipulator, use genotyping and/or sequencing techniques to measure aspects of the genotype of the genetic material in those cells.

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In an embodiment, one may stain the nucleated red blood cell with a die that only fluoresces in the presence of fetal hemoglobin and not maternal hemoglobin, and so remove the ambiguity between whether a nucleated red blood cell is derived from the mother or the fetus. Some embodiments of the present disclosure may involve staining or otherwise marking nuclear material. Some embodiments of the present disclosure may involve specifically marking fetal nuclear material using fetal cell specific antibodies.

There are many other ways to isolate fetal cells from maternal blood, or fetal DNA from maternal blood, or to enrich samples of fetal genetic material in the presence of maternal genetic material. Some of these methods are listed here, but this is not intended to be an exhaustive list. Some appropriate techniques are listed here for convenience: using fluorescently or otherwise tagged antibodies, size exclusion chromatography, magnetically or otherwise labeled affinity tags, epigenetic differences, such as differential methylation between the maternal and fetal cells at specific alleles, density gradient centrifugation succeeded by CD45/14 depletion and CD71-positive selection from CD45/14 negative-cells, single or double Percoll gradients with different osmolalities, or galactose specific lectin method.

In an embodiment of the present disclosure, the target individual is a fetus, and the different genotype measurements are made on a plurality of DNA samples from the fetus. In some embodiments of the present disclosure, the fetal DNA samples are from isolated fetal cells where the fetal cells may be mixed with maternal cells. In some embodiments of the present disclosure, the fetal DNA samples are from free floating fetal DNA, where the fetal DNA may be mixed with free floating maternal DNA. In some embodiments, the fetal DNA samples may be derived from maternal plasma or maternal blood that contains a mixture of maternal DNA and fetal DNA. In some embodiments, the fetal DNA may be mixed with maternal DNA in maternal:fetal ratios ranging from 99.9:0.1% to 99:1%; 99:1% to 90:10%; 90:10% to 80:20%; 80:20% to 70:30%; 70:30% to 50:50%; 50:50% to 10:90%; or 10:90% to 1:99%; 1:99% to 0.1:99.9%.

The genetic data of the target individual and/or of the related individual can be transformed from a molecular state to an electronic state by measuring the appropriate genetic material using tools and or techniques taken from a group including, but not limited to: genotyping microarrays, and high throughput sequencing. Some high throughput sequencing methods include Sanger DNA sequencing, pyrosequencing, the ILLUMINA SOLEXA platform, ILLUMINA's GENOME ANALYZER, or APPLIED BIOSYSTEM's 454

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sequencing platform, HELICOS's TRUE SINGLE MOLECULE SEQUENCING platform, HALCYON MOLECULAR's electron microscope sequencing method, or any other sequencing method,. All of these methods physically transform the genetic data stored in a sample of DNA into a set of genetic data that is typically stored in a memory device en route to being processed.

A relevant individual's genetic data may be measured by analyzing substances taken from a group including, but not limited to: the individual's bulk diploid tissue, one or more diploid cells from the individual, one or more haploid cells from the individual, one or more blastomeres from the target individual, extra-cellular genetic material found on the individual, extra-cellular genetic material from the individual found in maternal blood, cells from the individual found in maternal blood, one or more embryos created from (a) gamete(s) from the related individual, one or more blastomeres taken from such an embryo, extra-cellular genetic material found on the related individual, genetic material known to have originated from the related individual, and combinations thereof.

In some embodiments, a set of at least one ploidy state hypothesis may be created for each of the chromosomes types of interest of the target individual. Each of the ploidy state hypotheses may refer to one possible ploidy state of the chromosome or chromosome segment of the target individual. The set of hypotheses may include some or all of the possible ploidy states that the chromosome of the target individual may be expected to have. Some of the possible ploidy states may include nullsomy, monosomy, disomy, uniparental disomy, euploidy, trisomy, matching trisomy, unmatching trisomy, maternal trisomy, paternal trisomy, tetrasomy, balanced (2:2) tetrasomy, unbalanced (3:1) tetrasomy, pentasomy, hexasomy, other aneuploidy, and combinations thereof. Any of these aneuploidy states may be mixed or partial aneuploidy such as unbalanced translocations, balanced translocations, Robertsonian translocations, recombinations, deletions, insertions, crossovers, and combinations thereof.

In some embodiments, the knowledge of the determined ploidy state may be used to make a clinical decision. This knowledge, typically stored as a physical arrangement of matter in a memory device, may then be transformed into a report. The report may then be acted upon. For example, the clinical decision may be to terminate the pregnancy; alternately, the clinical decision may be to continue the pregnancy. In some embodiments the clinical decision may involve an intervention designed to decrease the severity of the phenotypic presentation of a genetic disorder, or a decision to take relevant steps to prepare for a special needs child.

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In an embodiment of the present disclosure, any of the methods described herein may be modified to allow for multiple targets to come from same target individual, for example, multiple blood draws from the same pregnant mother. This may improve the accuracy of the model, as multiple genetic measurements may provide more data with which the target genotype may be determined. In an embodiment, one set of target genetic data served as the primary data which was reported, and the other served as data to double-check the primary target genetic data. In an embodiment, a plurality of sets of genetic data, each measured from genetic material taken from the target individual, are considered in parallel, and thus both sets of target genetic data serve to help determine which sections of parental genetic data, measured with high accuracy, composes the fetal genome.

In an embodiment, the method may be used for the purpose of paternity testing. For example, given the SNP-based genotypic information from the mother, and from a man who may or may not be the genetic father, and the measured genotypic information from the mixed sample, it is possible to determine if the genotypic information of the male indeed represents that actual genetic father of the gestating fetus. A simple way to do this is to simply look at the contexts where the mother is AA, and the possible father is AB or BB. In these cases, one may expect to see the father contribution half (AA|AB) or all (AA|BB) of the time, respectively. Taking into account the expected ADO, it is straightforward to determine whether or not the fetal SNPs that are observed are correlated with those of the possible father.

One embodiment of the present disclosure could be as follows: a pregnant woman wants to know if her fetus is afflicted with Down Syndrome, and/or if it will suffer from Cystic Fibrosis, and she does not wish to bear a child that is afflicted with either of these conditions. A doctor takes her blood, and stains the hemoglobin with one marker so that it appears clearly red, and stains nuclear material with another marker so that it appears clearly blue. Knowing that maternal red blood cells are typically anuclear, while a high proportion of fetal cells contain a nucleus, the doctor is able to visually isolate a number of nucleated red blood cells by identifying those cells that show both a red and blue color. The doctor picks up these cells off the slide with a micromanipulator and sends them to a lab which amplifies and genotypes ten individual cells. By using the genetic measurements, the PARENTAL SUPPORT^{TM} method is able to determine that six of the ten cells are maternal blood cells, and four of the ten cells are fetal cells. If a child has already been born to a pregnant mother, PARENTAL SUPPORT^{TM} can also be used to determine that the fetal cells are distinct from

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the cells of the born child by making reliable allele calls on the fetal cells and showing that they are dissimilar to those of the born child. Note that this method is similar in concept to the paternal testing embodiment of the present disclosure. The genetic data measured from the fetal cells may be of very poor quality, comprising many allele drop outs, due to the difficulty of genotyping single cells. The clinician is able to use the measured fetal DNA along with the reliable DNA measurements of the parents to infer aspects of the genome of the fetus with high accuracy using PARENTAL SUPPORT™, thereby transforming the genetic data contained on genetic material from the fetus into the predicted genetic state of the fetus, stored on a computer. The clinician is able to determine both the ploidy state of the fetus, and the presence or absence of a plurality of disease-linked genes of interest. It turns out that the fetus is euploid, and is not a carrier for cystic fibrosis, and the mother decides to continue the pregnancy.

In an embodiment of the present disclosure, a pregnant mother would like to determine if her fetus is afflicted with any whole chromosomal abnormalities. She goes to her doctor, and gives a sample of her blood, and she and her husband gives samples of their own DNA from cheek swabs. A laboratory researcher genotypes the parental DNA using the MDA protocol to amplify the parental DNA, and ILLUMINA INFINIUM arrays to measure the genetic data of the parents at a large number of SNPs. The researcher then spins down the blood, takes the plasma, and isolates a sample of free-floating DNA using size exclusion chromatography. Alternately, the researcher uses one or more fluorescent antibodies, such as one that is specific to fetal hemoglobin to isolate a nucleated fetal red blood cell. The researcher then takes the isolated or enriched fetal genetic material and amplifies it using a library of 70-mer oligonucleotides appropriately designed such that two ends of each oligonucleotide corresponded to the flanking sequences on either side of a target allele. Upon addition of a polymerase, ligase, and the appropriate reagents, the oligonucleotides underwent gap-filling circularization, capturing the desired allele. An exonuclease was added, heat-inactivated, and the products were used directly as a template for PCR amplification. The PCR products were sequenced on an ILLUMINA GENOME ANALYZER. The sequence reads were used as input for the PARENTAL SUPPORT™ method, which then predicted the ploidy state of the fetus.

In another embodiment, a couple - where the mother, who is pregnant, and is of advanced maternal age - wants to know whether the gestating fetus has Down syndrome,

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Turner Syndrome, Prader Willi syndrome, or some other whole chromosomal abnormality. The obstetrician takes a blood draw from the mother and father. The blood is sent to a laboratory, where a technician centrifuges the maternal sample to isolate the plasma and the buffy coat. The DNA in the buffy coat and the paternal blood sample are transformed through amplification and the genetic data encoded in the amplified genetic material is further transformed from molecularly stored genetic data into electronically stored genetic data by running the genetic material on a high throughput sequencer to measure the parental genotypes. The plasma sample is preferentially enriched at a set of loci using a 5,000-plex hemi-nested targeted PCR method. The mixture of DNA fragments is prepared into a DNA library suitable for sequencing. The DNA is then sequenced using a high throughput sequencing method, for example, the ILLUMINA GAIIx GENOME ANALYZER. The sequencing transforms the information that is encoded molecularly in the DNA into information that is encoded electronically in computer hardware. An informatics based technique that includes the presently disclosed embodiments, such as PARENTAL SUPPORT™, may be used to determine the ploidy state of the fetus. This may involve calculating, on a computer, allele count probabilities at the plurality of polymorphic loci from the DNA measurements made on the prepared sample; creating, on a computer, a plurality of ploidy hypotheses each pertaining to a different possible ploidy state of the chromosome; building, on a computer, a joint distribution model for the expected allele counts at the plurality of polymorphic loci on the chromosome for each ploidy hypothesis; determining, on a computer, a relative probability of each of the ploidy hypotheses using the joint distribution model and the allele counts measured on the prepared sample; and calling the ploidy state of the fetus by selecting the ploidy state corresponding to the hypothesis with the greatest probability. It is determined that the fetus has Down syndrome. A report is printed out, or sent electronically to the pregnant woman's obstetrician, who transmits the diagnosis to the woman. The woman, her husband, and the doctor sit down and discuss their options. The couple decides to terminate the pregnancy based on the knowledge that the fetus is afflicted with a trisomic condition.

In an embodiment, a company may decide to offer a diagnostic technology designed to detect aneuploidy in a gestating fetus from a maternal blood draw. Their product may involve a mother presenting to her obstetrician, who may draw her blood. The obstetrician may also collect a genetic sample from the father of the fetus. A clinician may isolate the plasma from

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the maternal blood, and purify the DNA from the plasma. A clinician may also isolate the buffy coat layer from the maternal blood, and prepare the DNA from the buffy coat. A clinician may also prepare the DNA from the paternal genetic sample. The clinician may use molecular biology techniques described in this disclosure to append universal amplification tags to the DNA in the DNA derived from the plasma sample. The clinician may amplify the universally tagged DNA. The clinician may preferentially enrich the DNA by a number of techniques including capture by hybridization and targeted PCR. The targeted PCR may involve nesting, hemi-nesting or semi-nesting, or any other approach to result in efficient enrichment of the plasma derived DNA. The targeted PCR may be massively multiplexed, for example with 10,000 primers in one reaction volume, where the primers target SNPs on chromosomes 13, 18, 21, X and those loci that are common to both X and Y, and optionally other chromosomes as well. The selective enrichment and/or amplification may involve tagging each individual molecule with different tags, molecular barcodes, tags for amplification, and/or tags for sequencing. The clinician may then sequence the plasma sample, and also possibly also the prepared maternal and/or paternal DNA. The molecular biology steps may be executed either wholly or partly by a diagnostic box. The sequence data may be fed into a single computer, or to another type of computing platform such as may be found in 'the cloud'. The computing platform may calculate allele counts at the targeted polymorphic loci from the measurements made by the sequencer. The computing platform may create a plurality of ploidy hypotheses pertaining to nullsomy, monosomy, disomy, matched trisomy, and unmatched trisomy for each of chromosomes 13, 18, 21, X and Y. The computing platform may build a joint distribution model for the expected allele counts at the targeted loci on the chromosome for each ploidy hypothesis for each of the five chromosomes being interrogated. The computing platform may determine a probability that each of the ploidy hypotheses is true using the joint distribution model and the allele counts measured on the preferentially enriched DNA derived from the plasma sample. The computing platform may call the ploidy state of the fetus, for each of chromosome 13, 18, 21, X and Y by selecting the ploidy state corresponding to the germane hypothesis with the greatest probability. A report may be generated comprising the called ploidy states, and it may be sent to the obstetrician electronically, displayed on an output device, or a printed hard copy of the report may be delivered to the obstetrician. The obstetrician may inform the patient and optionally the father

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of the fetus, and they may decide which clinical options are open to them, and which is most desirable.

In another embodiment, a pregnant woman, hereafter referred to as "the mother" may decide that she wants to know whether or not her fetus(es) are carrying any genetic abnormalities or other conditions. She may want to ensure that there are not any gross abnormalities before she is confident to continue the pregnancy. She may go to her obstetrician, who may take a sample of her blood. He may also take a genetic sample, such as a buccal swab, from her cheek. He may also take a genetic sample from the father of the fetus, such as a buccal swab, a sperm sample, or a blood sample. He may send the samples to a clinician. The clinician may enrich the fraction of free floating fetal DNA in the maternal blood sample. The clinician may enrich the fraction of enucleated fetal blood cells in the maternal blood sample. The clinician may use various aspects of the methods described herein to determine genetic data of the fetus. That genetic data may include the ploidy state of the fetus, and/or the identity of one or a number of disease linked alleles in the fetus. A report may be generated summarizing the results of the prenatal diagnosis. The report may be transmitted or mailed to the doctor, who may tell the mother the genetic state of the fetus. The mother may decide to discontinue the pregnancy based on the fact that the fetus has one or more chromosomal, or genetic abnormalities, or undesirable conditions. She may also decide to continue the pregnancy based on the fact that the fetus does not have any gross chromosomal or genetic abnormalities, or any genetic conditions of interest.

Another example may involve a pregnant woman who has been artificially inseminated by a sperm donor, and is pregnant. She wants to minimize the risk that the fetus she is carrying has a genetic disease. She has blood drawn at a phlebotomist, and techniques described in this disclosure are used to isolate three nucleated fetal red blood cells, and a tissue sample is also collected from the mother and genetic father. The genetic material from the fetus and from the mother and father are amplified as appropriate and genotyped using the ILLUMINA INFINIUM BEADARRAY, and the methods described herein clean and phase the parental and fetal genotype with high accuracy, as well as to make ploidy calls for the fetus. The fetus is found to be euploid, and phenotypic susceptibilities are predicted from the reconstructed fetal genotype, and a report is generated and sent to the mother's physician so that they can decide what clinical decisions may be best.

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In an embodiment, the raw genetic material of the mother and the father is transformed by way of amplification to an amount of DNA that is similar in sequence, but larger in quantity. Then, by way of a genotyping method, the genotypic data that is encoded by nucleic acids is transformed into genetic measurements that may be stored physically and/or electronically on a memory device, such as those described above. The relevant algorithms that makeup the PARENTAL SUPPORT™ algorithm, relevant parts of which are discussed in detail herein, are translated into a computer program, using a programming language. Then, through the execution of the computer program on the computer hardware, instead of being physically encoded bits and bytes, arranged in a pattern that represents raw measurement data, they become transformed into a pattern that represents a high confidence determination of the ploidy state of the fetus. The details of this transformation will rely on the data itself and the computer language and hardware system used to execute the method described herein. Then, the data that is physically configured to represent a high quality ploidy determination of the fetus is transformed into a report which may be sent to a health care practitioner. This transformation may be carried out using a printer or a computer display. The report may be a printed copy, on paper or other suitable medium, or else it may be electronic. In the case of an electronic report, it may be transmitted, it may be physically stored on a memory device at a location on the computer accessible by the health care practitioner; it also may be displayed on a screen so that it may be read. In the case of a screen display, the data may be transformed to a readable format by causing the physical transformation of pixels on the display device. The transformation may be accomplished by way of physically firing electrons at a phosphorescent screen, by way of altering an electric charge that physically changes the transparency of a specific set of pixels on a screen that may lie in front of a substrate that emits or absorbs photons. This transformation may be accomplished by way of changing the nanoscale orientation of the molecules in a liquid crystal, for example, from nematic to cholesteric or smectic phase, at a specific set of pixels. This transformation may be accomplished by way of an electric current causing photons to be emitted from a specific set of pixels made from a plurality of light emitting diodes arranged in a meaningful pattern. This transformation may be accomplished by any other way used to display information, such as a computer screen, or some other output device or way of transmitting information. The health care practitioner may then act on the report, such that the data in the report is transformed into an action. The action may be to continue or discontinue the pregnancy, in which case a gestating fetus with a genetic

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abnormality is transformed into non-living fetus. The transformations listed herein may be aggregated, such that, for example, one may transform the genetic material of a pregnant mother and the father, through a number of steps outlined in this disclosure, into a medical decision consisting of aborting a fetus with genetic abnormalities, or consisting of continuing the pregnancy. Alternately, one may transform a set of genotypic measurements into a report that helps a physician treat his pregnant patient.

In an embodiment of the present disclosure, the method described herein can be used to determine the ploidy state of a fetus even when the host mother, *i.e.* the woman who is pregnant, is not the biological mother of the fetus she is carrying. In an embodiment of the present disclosure, the method described herein can be used to determine the ploidy state of a fetus using only the maternal blood sample, and without the need for a paternal genetic sample.

Some of the math in the presently disclosed embodiments makes hypotheses concerning a limited number of states of aneuploidy. In some cases, for example, only zero, one or two chromosomes are expected to originate from each parent. In some embodiments of the present disclosure, the mathematical derivations can be expanded to take into account other forms of aneuploidy, such as quadrosomy, where three chromosomes originate from one parent, pentasomy, hexasomy etc., without changing the fundamental concepts of the present disclosure. At the same time, it is possible to focus on a smaller number of ploidy states, for example, only trisomy and disomy. Note that ploidy determinations that indicate a non-whole number of chromosomes may indicate mosaicism in a sample of genetic material.

In some embodiments, the genetic abnormality is a type of aneuploidy, such as Down syndrome (or trisomy 21), Edwards syndrome (trisomy 18), Patau syndrome (trisomy 13), Turner Syndrome (45X), Klinefelter's syndrome (a male with 2 X chromosomes), Prader-Willi syndrome, and DiGeorge syndrome (UPD 15). Congenital disorders, such as those listed in the prior sentence, are commonly undesirable, and the knowledge that a fetus is afflicted with one or more phenotypic abnormalities may provide the basis for a decision to terminate the pregnancy, to take necessary precautions to prepare for the birth of a special needs child, or to take some therapeutic approach meant to lessen the severity of a chromosomal abnormality.

In some embodiments, the methods described herein can be used at a very early gestational age, for example as early as four week, as early as five weeks, as early as six weeks, as early as seven weeks, as early as eight weeks, as early as nine weeks, as early as ten weeks, as early as eleven weeks, and as early as twelve weeks.

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In some embodiments, a method disclosed herein is used in the context of pre-implantation genetic diagnosis (PGD) for embryo selection during in vitro fertilization, where the target individual is an embryo, and the parental genotypic data can be used to make ploidy determinations about the embryo from sequencing data from a single or two cell biopsy from a day 3 embryo or a trophectoderm biopsy from a day 5 or day 6 embryo. In a PGD setting, only the child DNA is measured, and only a small number of cells are tested, generally one to five but as many as ten, twenty or fifty. The total number of starting copies of the A and B alleles (at a SNP) are then trivially determined by the child genotype and the number of cells. In NPD, the number of starting copies is very high and so the allele ratio after PCR is expected to accurately reflect the starting ratio. However, the small number of starting copies in PGD means that contamination and imperfect PCR efficiency have a non-trivial effect on the allele ratio following PCR. This effect may be more important than depth of read in predicting the variance in the allele ratio measured after sequencing. The distribution of measured allele ratio given a known child genotype may be created by Monte Carlo simulation of the PCR process based on the PCR probe efficiency and probability of contamination. Given an allele ratio distribution for each possible child genotype, the likelihoods of various hypotheses can be calculated as described for NIPD.

Maximum Likelihood Estimates

Most methods known in the art for detecting the presence or absence of biological phenomenon or medical condition involve the use of a single hypothesis rejection test, where a metric that is correlated with the condition is measured, and if the metric is on one side of a given threshold, the condition is present, while of the metric falls on the other side of the threshold, the condition is absent. A single-hypothesis rejection test only looks at the null distribution when deciding between the null and alternate hypotheses. Without taking into account the alternate distribution, one cannot estimate the likelihood of each hypothesis given the observed data and therefore cannot calculate a confidence on the call. Hence with a single-hypothesis rejection test, one gets a yes or no answer without a feeling for the confidence associated with the specific case.

In some embodiments, the method disclosed herein is able to detect the presence or absence of biological phenomenon or medical condition using a maximum likelihood method. This is a substantial improvement over a method using a single hypothesis rejection technique

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as the threshold for calling absence or presence of the condition can be adjusted as appropriate for each case. This is particularly relevant for diagnostic techniques that aim to determine the presence or absence of aneuploidy in a gestating fetus from genetic data available from the mixture of fetal and maternal DNA present in the free floating DNA found in maternal plasma. This is because as the fraction of fetal DNA in the plasma derived fraction changes, the optimal threshold for calling aneuploidy vs. euploidy changes. As the fetal fraction drops, the distribution of data that is associated with an aneuploidy becomes increasingly similar to the distribution of data that is associated with a euploidy.

The maximum likelihood estimation method uses the distributions associated with each hypothesis to estimate the likelihood of the data conditioned on each hypothesis. These conditional probabilities can then be converted to a hypothesis call and confidence. Similarly, maximum a posteriori estimation method uses the same conditional probabilities as the maximum likelihood estimate, but also incorporates population priors when choosing the best hypothesis and determining confidence.

Therefore, the use of a maximum likelihood estimate (MLE) technique, or the closely related maximum a posteriori (MAP) technique give two advantages, first it increases the chance of a correct call, and it also allows a confidence to be calculated for each call. In an embodiment, selecting the ploidy state corresponding to the hypothesis with the greatest probability is carried out using maximum likelihood estimates or maximum a posteriori estimates. In an embodiment, a method is disclosed for determining the ploidy state of a gestating fetus that involves taking any method currently known in the art that uses a single hypothesis rejection technique and reformulating it such that it uses a MLE or MAP technique. Some examples of methods that can be significantly improved by applying these techniques can be found in US Pat 8,008,018, US Patent 7,888,017, or US Patent 7,332,277.

In an embodiment, a method is described for determining presence or absence of fetal aneuploidy in a maternal plasma sample comprising fetal and maternal genomic DNA, the method comprising: obtaining a maternal plasma sample; measuring the DNA fragments found in the plasma sample with a high throughput sequencer; mapping the sequences to the chromosome and determining the number of sequence reads that map to each chromosome; calculating the fraction of fetal DNA in the plasma sample; calculating an expected distribution of the amount of a target chromosome that would be expected to be present if that if the second target chromosome were euploid and one or a plurality of expected distributions

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that would be expected if that chromosome were aneuploid, using the fetal fraction and the number of sequence reads that map to one or a plurality of reference chromosomes expected to be euploid; and using a MLE or MAP determine which of the distributions is most likely to be correct, thereby indicating the presence or absence of a fetal aneuploidy. In an embodiment, the measuring the DNA from the plasma may involve conducting massively parallel shotgun sequencing. In an embodiment, the measuring the DNA from the plasma sample may involve sequencing DNA that has been preferentially enriched, for example through targeted amplification, at a plurality of polymorphic or non-polymorphic loci. The plurality of loci may be designed to target one or a small number of suspected aneuploid chromosomes and one or a small number of reference chromosomes. The purpose of the preferential enrichment is to increase the number of sequence reads that are informative for the ploidy determination.

Ploidy Calling Informatics Methods

Described herein is a method for determining the ploidy state of a fetus given sequence data. In some embodiments, this sequence data may be measured on a high throughput sequencer. In some embodiments, the sequence data may be measured on DNA that originated from free floating DNA isolated from maternal blood, wherein the free floating DNA comprises some DNA of maternal origin, and some DNA of fetal / placental origin. This section will describe one embodiment of the present disclosure in which the ploidy state of the fetus is determined assuming that fraction of fetal DNA in the mixture that has been analyzed is not known and will be estimated from the data. It will also describe an embodiment in which the fraction of fetal DNA ("fetal fraction") or the percentage of fetal DNA in the mixture can be measured by another method, and is assumed to be known in determining the ploidy state of the fetus. In some embodiments the fetal fraction can be calculated using only the genotyping measurements made on the maternal blood sample itself, which is a mixture of fetal and maternal DNA. In some embodiments the fraction may be calculated also using the measured or otherwise known genotype of the mother and/or the measured or otherwise known genotype of the father. In another embodiment ploidy state of the fetus can be determined solely based on the calculated fraction of fetal DNA for the chromosome in question compared to the calculated fraction of fetal DNA for the reference chromosome assumed disomic.

In the preferred embodiment, suppose that, for a particular chromosome, we observe and analyze N SNPs, for which we have:

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- Set of NR free floating DNA sequence measurements $S=(s_1,\ldots,s_{NR})$. Since this method utilizes the SNP measurements, all sequence data that corresponds to non-polymorphic loci can be disregarded. In a simplified version, where we have (A,B) counts on each SNP, where A and B correspond to the two alleles present at a given locus, S can be written as $S=((a_1,b_1),\ldots,(a_N, b_N))$, where a_i is the A count on SNP i, b_i is the B count on SNP i, and $\sum_{i=1:N}(a_i + b_i) = NR$
- Parent data consisting of
    - genotypes from a SNP microarray or other intensity based genotyping platform: mother $M=(m_1,\ldots,m_N)$, father $F=(f_1, \ldots, f_N)$, where m_i, $f_i \in$(AA,AB, BB).
    - AND/OR sequence data measurements: NRM mother measurements $SM=(sm_1,\ldots,sm_{nrm})$, NRF father measurements $SF=(sf_1,\ldots,sf_{nrf})$. Similar to the above simplification, if we have (A,B) counts on each SNP $SM=((am_1,bm_1),\ldots,(am_N, bm_N))$, $SF=((af_1,bf_1),\ldots,(af_N, bf_N))$

Collectively, the mother, father child data are denoted as D = (M,F,SM,SF,S). Note that the parent data is desired and increases the accuracy of the algorithm, but is NOT necessary, especially the father data. This means that even in the absence of mother and/or father data, it is possible to get very accurate copy number results.

It is possible to derive the best copy number estimate (H^*) by maximizing the data log likelihood $LIK(D|H)$ over all hypotheses (H) considered. In particular it is possible to determine the relative probability of each of the ploidy hypotheses using the joint distribution model and the allele counts measured on the prepared sample, and using those relative probabilities to determine the hypothesis most likely to be correct as follows:

$$H^* = \underset{H}{\operatorname{argmax}}\, LIK(D|H)$$

Similarly the a posteriori hypothesis likelihood given the data may be written as:

$$H^* = \underset{H}{\operatorname{argmax}}\, LIK(D|H) * priorprob(H)$$

Where priorprob(H) is the prior probability assigned to each hypothesis H, based on model design and prior knowledge.

It is also possible to use priors to find the maximum a posteriori estimate:

$$H_{MA} = \underset{H}{\operatorname{argmax}}\, LIK(D|H)$$

In an embodiment, the copy number hypotheses that may be considered are:

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- Monosomy:
  - maternal H10 (one copy from mother)
  - paternal H01 (one copy from father)
- Disomy: H11 (one copy each mother and father)
- Simple trisomy, no crossovers considered:
  - Maternal: H21_matched (two identical copies from mother, one copy from father), H21_unmatched (BOTH copies from mother, one copy from father)
  - Paternal: H12_matched (one copy from mother , two identical copies from father), H12_unmatched (one copy from mother , both copies from father)
- Composite trisomy, allowing for crossovers (using a joint distribution model):
  - maternal H21 (two copies from mother, one from father),
  - paternal H12 (one copy from mother, two copies from father)

In other embodiments, other ploidy states, such as nullsomy (H00), uniparental disomy (H20 and H02), and tetrasomy (H04, H13, H22, H31 and H40), may be considered.

If there are no crossovers, each trisomy, whether the origin was mitosis, meiosis I, or meiosis II, would be one of the matched or unmatched trisomies. Due to crossovers, true trisomy is usually a combination of the two. First, a method to derive hypothesis likelihoods for simple hypotheses is described. Then a method to derive hypothesis likelihoods for composite hypotheses is described, combining individual SNP likelihood with crossovers.

LIK(D|H) for a Simple Hypothesis

In an embodiment, LIK(D|H) may be determined for simple hypotheses, as follows. For simple hypotheses H, LIK(H), the log likelihood of hypothesis H on a whole chromosome, may be calculated as the sum of log likelihoods of individual SNPs, assuming known or derived child fraction cf. In an embodiment it is possible to derive cf from the data.

$$\mathrm{LIK}(\mathrm{D}|\mathrm{H}) = \sum_{\mathrm{i}} \mathrm{LIK}(\mathrm{D}|\mathrm{H}, \mathrm{cf}, \mathrm{i})$$

This hypothesis does not assume any linkage between SNPs, and therefore does not utilize a joint distribution model.

In some embodiments, the Log Likelihood may be determined on a per SNP basis. On a particular SNP i, assuming fetal ploidy hypothesis H and percent fetal DNA cf, log likelihood of observed data D is defined as:

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$$\mathrm{LIK(D|H,i)} = \log \mathrm{P(D|H,cf,i)} = \log\left(\sum_{\mathrm{m,f,c}} \mathrm{P(D|m,f,c,H,cf,i)P(c|m,f,H)P(m|i)P(f|i)}\right)$$

where m are possible true mother genotypes , f are possible true father genotypes, where m,f ∈ {AA,AB,BB}, and c are possible child genotypes given the hypothesis H. In particular, for monosomy $c \in \{A, B\}$, for disomy $c \in \{AA, AB, BB\}$, for trisomy $c \in \{AAA, AAB, ABB, BBB\}$.

Genotype prior frequency: p(m|i) is the general prior probability of mother genotype m on SNP i, based on the known population frequency at SNP I, denoted pA_i. In particular

$$p(AA|pA_i) = (pA_i)^2, p(AB|pA_i) = 2(pA_i) * (1 - pA_i), p(BB|pA_i) = (1 - pA_i)^2$$

Father genotype probability, p(f|i), may be determined in an analogous fashion.

True child probability: $p(c|m,f,H)$ is the probability of getting true child genotype = c, given parents m, f, and assuming hypothesis H, which can be easily calculated. For example, for H11, H21 matched and H21 unmatched, p(c|m,f,H) is given below.

p(c|m,f,H)												
		H11			H21 matched				H21 unmatched			
m	f	AA	AB	BB	AAA	AAB	ABB	BBB	AAA	AAB	ABB	BBB
AA	AA	1	0	0	1	0	0	0	1	0	0	0
AB	AA	0.5	0.5	0	0.5	0	0.5	0	0	1	0	0
BB	AA	0	1	0	0	0	1	0	0	0	1	0
AA	AB	0.5	0.5	0	0.5	0.5	0	0	0.5	0.5	0	0
AB	AB	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0	0.5	0.5	0
BB	AB	0	0.5	0.5	0	0	0.5	0.5	0	0	0.5	0.5
AA	BB	0	1	0	0	1	0	0	0	1	0	0
AB	BB	0	0.5	0.5	0	0.5	0	0.5	0	0	1	0
BB	BB	0	0	1	0	0	0	1	0	0	0	1

Data likelihood: $\mathrm{P(D|m,f,c,H,i,cf)}$ is the probability of given data D on SNP i, given true mother genotype m, true father genotype f, true child genotype c, hypothesis H and child fraction cf. It can be broken down into the probability of mother, father and child data as follows:

$$\mathrm{P(D|m,f,c,H,cf,i)} = \mathrm{P(SM|m,i)P(M|m,i)P(SF|f,i)P(F|f,i)P(S|m,c,H,cf,i)}$$

Mother SNP array data likelihood: Probability of mother SNP array genotype data m_i at SNP i compared to true genotype m, assuming SNP array genotypes are correct, is simply

$$\mathrm{P(M|m,i)} = \begin{cases} 1 & \mathrm{m_i = m} \\ 0 & \mathrm{m_i \neq m} \end{cases}$$

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Mother sequence data likelihood: the probability of the mother sequence data at SNP i, in the case of counts $S_i=(am_i,bm_i)$, with no extra noise or bias involved, is the binomial probability defined as $P(SM|m,i)=P_{X|m}(am_i)$ where $X|m\sim Binom(p_m(A), am_i+bm_i)$ with $p_m(A)$ defined as

m	AA	AB	BB	A	B	nocall
p(A)	1	0.5	0	1	0	0.5

Father data likelihood: a similar equation applies for father data likelihood.

Note that it is possible to determine the child genotype without the parent data, especially father data. For example if no father genotype data F is available, one may just use $P(F|f,i) = 1$. If no father sequence data SF is available, one may just use $P(SF|f,i)=1$.

In some embodiments, the method involves building a joint distribution model for the expected allele counts at a plurality of polymorphic loci on the chromosome for each ploidy hypothesis; one method to accomplish such an end is described here. Free fetal DNA data likelihood: $P(S|m,c,H,cf,i)$ is the probability of free fetal DNA sequence data on SNP i, given true mother genotype m, true child genotype c, child copy number hypothesis H, and assuming child fraction cf. It is in fact the probability of sequence data S on SNP I, given the true probability of A content on SNP i $\mu(m,c,cf,H)$

$$P(S|m,c,H,cf,i) = P(S|\mu(m,c,cf,H),i)$$

For counts, where $S_i=(a_i,b_i)$, with no extra noise or bias in data involved,

$$P(S|\mu(m,c,cf,H),i) = P_x(a_i)$$

where $X\sim Binom(p(A), a_i+b_i)$ with $p(A)= \mu(m,c,cf,H)$. In a more complex case where the exact alignment and (A,B) counts per SNP are not known, $P(S|\mu(m,c,cf,H),i)$ is a combination of integrated binomials.

True A content probability: $\mu(m,c,cf,H)$, the true probability of A content on SNP i in this mother/child mixture, assuming that true mother genotype = m, true child genotype = c, and overall child fraction = cf, is defined as

$$\mu(m,c,cf,H) = \frac{\#A(m)*(1-cf)+\#A(c)*cf}{n_m*(1-cf)+n_c*cf}$$

where #A(g) = number of A's in genotype g, $n_m = 2$ is somy of mother and n_c is ploidy of the child under hypothesis H (1 for monosomy, 2 for disomy, 3 for trisomy).

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Using A Joint Distribution Model: LIK(D|H) for a Composite Hypothesis

In some embodiments, the method involves building a joint distribution model for the expected allele counts at the plurality of polymorphic loci on the chromosome for each ploidy hypothesis; one method to accomplish such an end is described here. In many cases, trisomy is usually not purely matched or unmatched, due to crossovers, so in this section results for composite hypotheses H21 (maternal trisomy) and H12 (paternal trisomy) are derived, which combine matched and unmatched trisomy, accounting for possible crossovers.

In the case of trisomy, if there were no crossovers, trisomy would be simply matched or unmatched trisomy. Matched trisomy is where child inherits two copies of the identical chromosome segment from one parent. Unmatched trisomy is where child inherits one copy of each homologous chromosome segment from the parent. Due to crossovers, some segments of a chromosome may have matched trisomy, and other parts may have unmatched trisomy. Described in this section is how to build a joint distribution model for the heterozygosity rates for a set of alleles; that is, for the expected allele counts at a number of loci for one or more hypotheses.

Suppose that on SNP i, $\mathrm{LIK(D|Hm,i)}$ is the fit for matched hypothesis H_m, and $\mathrm{LIK(D|Hu,i)}$ is the fit for unmatched hypothesis H_u, and pc(i) = probability of crossover between SNPs i-1 andi. One may then calculate the full likelihood as:

$$\mathrm{LIK(D|H)} = \sum_{\mathrm{E}} \mathrm{LIK(D|E,1{:}N)}$$

where $\mathrm{LIK(D|E,1{:}N)}$ is the likelihood of ending in hypothesis E, for SNPs 1:N. E = hypothesis of the last SNP, $\mathrm{E} \in (Hm, Hu)$. Recursively, one may calculate:

$$\mathrm{LIK(D|E,1{:}i)} = \mathrm{LIK(D|E,i)} + \log(\exp(\mathrm{LIK(D|E,1{:}i-1)}) * \big(1 - \mathrm{pc(i)}\big) + \exp(\mathrm{LIK(D|{\sim}E,1{:}i-1)}) * \mathrm{pc(i)})$$

where ~E is the hypothesis other than E (not E), where hypotheses considered are H_m and H_u. In particular, one may calculate the likelihood of 1:i SNPs, based on likelihood of 1 to (i-1) SNPs with either the same hypothesis and no crossover, or the opposite hypothesis and a crossover, multiplied by the likelihood of the SNP i

For SNP 1, i=1, $\mathrm{LIK(D|E,1{:}1)} = \mathrm{LIK(D|E,1)}$.

For SNP 2, i=2, $\mathrm{LIK(D|E,1{:}2)} = \mathrm{LIK(D|E,2)} + \log(\exp(\mathrm{LIK(D|E,1)}) * \big(1 - \mathrm{pc(2)}\big) + \exp(\mathrm{LIK(D|{\sim}E,1)}) * \mathrm{pc(2)})$,

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and so on for i=3:N.

In some embodiments, the child fraction may be determined. The child fraction may refer to the proportion of sequences in a mixture of DNA that originate from the child. In the context of non-invasive prenatal diagnosis, the child fraction may refer to the proportion of sequences in the maternal plasma that originate from the fetus or the portion of the placenta with fetal genotype. It may refer to the child fraction in a sample of DNA that has been prepared from the maternal plasma, and may be enriched in fetal DNA. One purpose of determining the child fraction in a sample of DNA is for use in an algorithm that can make ploidy calls on the fetus, therefore, the child fraction could refer to whatever sample of DNA was analyzed by sequencing for the purpose of non-invasive prenatal diagnosis.

Some of the algorithms presented in this disclosure that are part of a method of non-invasive prenatal aneuploidy diagnosis assume a known child fraction, which may not always the case. In an embodiment, it is possible to find the most likely child fraction by maximizing the likelihood for disomy on selected chromosomes, with or without the presence of the parental data

In particular, suppose that LIK(D| H11, cf, chr) = log likelihood as described above, for the disomy hypothesis, and for child fraction cf on chromosome chr. For selected chromosomes in Cset (usually 1:16), assumed to be euploid, the full likelihood is:

$$\mathrm{LIK(cf)} = \sum_{\mathrm{chr} \in \mathrm{Cset}} \mathrm{Lik(D|\,H11, cf, chr)}$$

The most likely child fraction (cf^*)is derived as $cf^* = \mathrm{argmax}_{cf}\,\mathrm{LIK(cf)}$.

It is possible to use any set of chromosomes. It is also possible to derive child fraction without assuming euploidy on the reference chromosomes. Using this method it is possible to determine the child fraction for any of the following situations: (1) one has array data on the parents and shotgun sequencing data on the maternal plasma; (2) one has array data on the parents and targeted sequencing data on the maternal plasma; (3) one has targeted sequencing data on both the parents and maternal plasma; (4) one has targeted sequencing data on both the mother and the maternal plasma fraction; (5) one has targeted sequencing data on the maternal plasma fraction; (6) other combinations of parental and child fraction measurements.

In some embodiments the informatics method may incorporate data dropouts; this may result in ploidy determinations of higher accuracy. Elsewhere in this disclosure it has been assumed that the probability of getting an A is a direct function of the true mother genotype,

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the true child genotype, the fraction of the child in the mixture, and the child copy number. It is also possible that mother or child alleles can drop out, for example instead of measuring true child AB in the mixture, it may be the case that only sequences mapping to allele A are measured. One may denote the parent dropout rate for genomic illumina data d_{pg}, parent dropout rate for sequence data d_{ps} and child dropout rate for sequence data d_{cs}. In some embodiments, the mother dropout rate may be assumed to be zero, and child dropout rates are relatively low; in this case, the results are not severely affected by dropouts. In some embodiments the possibility of allele dropouts may be sufficiently large that they result in a significant effect of the predicted ploidy call. For such a case, allele dropouts have been incorporated into the algorithm here:

Parent SNP array data dropouts: For mother genomic data M, suppose that the genotype after the dropout is m_d, then

$$P(M|m,i) = \sum_{m_d} P(M|m_d,i)P(m_d|m)$$

where $P(M|m_d,i) = \begin{cases} 1 & m_i = m_d \\ 0 & m_i \neq m_d \end{cases}$ as before, and $P(m_d|m)$ is the likelihood of genotype m_d after the possible dropout given the true genotype m, defined as below, for dropout rate d

	md					
m	AA	AB	BB	A	B	nocall
AA	(1-d)^2	0	0	2d(1-d)	0	d^2
AB	0	(1-d)^2	0	d(1-d)	d(1-d)	d^2
BB	0	0	(1-d)^2	0	2d(1-d)	d^2

A similar equation applies for father SNP array data.

Parent sequence data dropouts: For mother sequence data SM

$$P(SM|m,i) = \sum_{m_d} P_{X|m_d}(am_i)P(m_d|m)$$

where $P(m_d|m)$ is defined as in previous section and $P_{X|m_d}(am_i)$ probability from a binomial distribution is defined as before in the parent data likelihood section. A similar equation applies to the paternal sequence data.

Free floating DNA sequence data dropout:

$$P(S|m,c,H,cf,i) = \sum_{m_d,c_d} P(S|\mu(m_d,c_d,cf,H),i)P(m_d|m)P(c_d|c)$$

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where $P(S|\mu(m_d, c_d, cf, H), i)$ is as defined in the section on free floating data likelihood.

In an embodiment, $p(m_d|m)$ is the probability of observed mother genotype m_d, given true mother genotype m, assuming dropout rate d_{ps}, and $p(c_d|c)$is the probability of observed child genotype c_d, given true child genotype c, assuming dropout rate d_{cs}. If nA_T = number of A alleles in true genotype c, nA_D = number of A alleles in observed genotype c_d, where $nA_T \geq nA_D$, and similarly nB_T = number of B alleles in true genotype c, nB_D = number of B alleles in observed genotype c_d, where $nB_T \geq nB_D$ and d = dropout rate, then

$$\boldsymbol{p(c_d|c) = \binom{nA_T}{nA_D} * d^{nA_T - nA_D} * (1-d)^{nA_D} * \binom{nB_T}{nB_D} * d^{nB_T - nB_D} * (1-d)^{nB_D}}$$

In an embodiment, the informatics method may incorporate random and consistent bias. In an ideal word there is no per SNP consistent sampling bias or random noise (in addition to the binomial distribution variation) in the number of sequence counts. In particular, on SNP i, for mother genotype m, true child genotype c and child fraction cf, and X = the number of A's in the set of (A+B) reads on SNP i, X acts like a X~Binomial(p, A+B), where p = $\mu(m, c, cf, H)$ = true probability of A content.

In an embodiment, the informatics method may incorporate random bias. As is often the case, suppose that there is a bias in the measurements, so that the probability of getting an A on this SNP is equal to q, which is a bit different than p as defined above. How much different p is from q depends on the accuracy of the measurement process and number of other factors and can be quantified by standard deviations of q away from p. In an embodiment, it is possible to model q as having a beta distribution, with parameters α, β depending on the mean of that distribution being centered at p, and some specified standard deviation s. In particular, this gives $X|q \sim Bin(q, D_i)$, where $q \sim Beta(\alpha, \beta)$. If we let $E(q) = p, V(q) = s^2$, and parameters α, β can be derived as $\alpha = pN, \beta = (1-p)N$, where $N = \frac{p(1-p)}{s^2} - 1$.

This is the definition of a beta-binomial distribution, where one is sampling from a binomial distribution with variable parameter q, where q follows a beta distribution with mean p. So, in a setup with no bias, on SNP i, the parent sequence data (SM) probability assuming true mother genotype (m), given mother sequence A count on SNP i (am_i) and mother sequence B count on SNP i (bm_i) may be calculated as:

$$P(SM|m,i) = P_{X|m}(am_i) \text{ where } X|m \sim Binom(p_m(A), am_i + bm_i)$$

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Now, including random bias with standard deviation s, this becomes:

$$X|m \sim BetaBinom(p_m(A),\ am_i+bm_i,s)$$

In the case with no bias, the maternal plasma DNA sequence data (S) probability assuming true mother genotype (m), true child genotype (c), child fraction (cf), assuming child hypothesis H, given free floating DNA sequence A count on SNP i (a_i) and free floating sequence B count on SNP i (b_i) may be calculated as

$$P(S|m, c, cf, H, i) = P_x(a_i)$$

where $X \sim Binom(p(A), a_i+b_i)$ with $p(A)= \mu(m, c, cf, H)$.

In an embodiment, including random bias with standard deviation s, this becomes $X \sim BetaBinom(p(A),a_i+b_i,s)$, where the amount of extra variation is specified by the deviation parameter s, or equivalently N. The smaller the value of s (or the larger the value of N) the closer this distribution is to the regular binomial distribution. It is possible to estimate the amount of bias, *i.e.* estimate N above, from unambiguous contexts AA|AA, BB|BB, AA|BB, BB|AA and use estimated $\widehat{N}$ in the above probability. Depending on the behavior of the data, N may be made to be a constant irrespective of the depth of read a_i+b_i, or a function of a_i+b_i, making bias smaller for larger depths of read.

In an embodiment, the informatics method may incorporate consistent per-SNP bias. Due to artifacts of the sequencing process, some SNPs may have consistently lower or higher counts irrespective of the true amount of A content. Suppose that SNP i consistently adds a bias of w_i percent to the number of A counts. In some embodiments, this bias can be estimated from the set of training data derived under same conditions, and added back in to the parent sequence data estimate as:

$$P(SM|m,i)=P_{X|m}(am_i) \text{ where } X|m \sim BetaBinom(p_m(A)+\ w_i,\ am_i+bm_i,s)$$

and with the free floating DNA sequence data probability estimate as:

$$P(S|m, c, cf, H, i) = P_x(a_i) \text{ where } X \sim BetaBinom(p(A)+\ w_i,a_i+b_i,s),$$

In some embodiments, the method may be written to specifically take into account additional noise, differential sample quality, differential SNP quality, and random sampling bias. An example of this is given here. This method has been shown to be particularly useful in the context of data generated using the massively multiplexed mini-PCR protocol, and was used in Experiments 7 through 13. The method involves several steps that each introduce different kind of noise and/or bias to the final model:

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(1) Suppose the first sample that comprises a mixture of maternal and fetal DNA contains an original amount of DNA of size=N_0 molecules, usually in the range 1,000-40,000, where p = true %refs

(2) In the amplification using the universal ligation adaptors, assume that N_1 molecules are sampled; usually $N_1 \sim N_0/2$ molecules and random sampling bias is introduced due to sampling. The amplified sample may contain a number of molecules N_2 where $N_2 >> N_1$. Let X_1 represent the amount of reference loci (on per SNP basis) out of N_1 sampled molecules, with a variation in $p_1 = X_1/N_1$ that introduces random sampling bias throughout the rest of protocol. This sampling bias is included in the model by using a Beta-Binomial (BB) distribution instead of using a simple Binomial distribution model. Parameter N of the Beta-Binomial distribution may be estimated later on per sample basis from training data after adjusting for leakage and amplification bias, on SNPs with $0<p<1$. Leakage is the tendency for a SNP to be read incorrectly.

(3) The amplification step will amplify any allelic bias, thus amplification bias introduced due to possible uneven amplification. Suppose that one allele at a locus is amplified f times another allele at that locus is amplified g times, where $f=ge^b$, where b=0 indicates no bias. The bias parameter, b, is centered at 0, and indicates how much more or less the A allele get amplified as opposed to the B allele on a particular SNP. The parameter b may differ from SNP to SNP. Bias parameter b may be estimated on per SNP basis, for example from training data.

(4) The sequencing step involves sequencing a sample of amplified molecules. In this step there may be leakage, where leakage is the situation where a SNP is read incorrectly. Leakage may result from any number of problems, and may result in a SNP being read not as the correct allele A, but as another allele B found at that locus or as an allele C or D not typically found at that locus. Suppose the sequencing measures the sequence data of a number of DNA molecules from an amplified sample of size N_3, where $N_3 < N_2$. In some embodiments, N_3 may be in the range of 20,000 to 100,000; 100,000 to 500,000; 500,000 to 4,000,000; 4,000,000 to 20,000,000; or 20,000,000 to 100,000,000. Each molecule sampled has a probability p_g of being read correctly, in which case it will show up correctly as allele A. The sample will be incorrectly read as an allele unrelated to the original molecule with probability $1-p_g$, and will look like allele A with probability p_r, allele B with probability p_m or allele C or allele D with

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probability p_o, where $p_r+p_m+p_o=1$. Parameters p_g, p_r, p_m, p_o are estimated on per SNP basis from the training data.

Different protocols may involve similar steps with variations in the molecular biology steps resulting in different amounts of random sampling, different levels of amplification and different leakage bias. The following model may be equally well applied to each of these cases. The model for the amount of DNA sampled, on per SNP basis, is given by:

$$X_3 \sim \text{BetaBinomial}(L(F(p,b),p_r,p_g),\ N*H(p,b))$$

where p = the true amount of reference DNA, b = per SNP bias, and as described above, p_g is the probability of a correct read, p_r is the probability of read being read incorrectly but serendipitously looking like the correct allele, in case of a bad read, as described above, and:

$$F(p,b)= pe^b/(pe^b+(1-p)),\ H(p,b) = (e^bp+(1-p))^2/e^b,\ L(p,p_r,p_g)=p*p_g+p_r*(1-p_g).$$

In some embodiments, the method uses a Beta-Binomial distribution instead of a simple binomial distribution; this takes care of the random sampling bias. Parameter N of the Beta-Binomial distribution is estimated on per sample basis on an as needed basis. Using bias correction F(p,b), H(p,b), instead of just p, takes care of the amplification bias. Parameter b of the bias is estimated on per SNP basis from training data ahead of time.

In some embodiments the method uses leakage correction $L(p,p_r,p_g)$, instead of just p; this takes care of the leakage bias, *i.e.* varying SNP and sample quality. In some embodiments, parameters p_g, p_r, p_o are estimated on per SNP basis from the training data ahead of time. In some embodiments, the parameters p_g, p_r, p_o may be updated with the current sample on the go, to account for varying sample quality.

The model described herein is quite general and can account for both differential sample quality and differential SNP quality. Different samples and SNPs are treated differently, as exemplified by the fact that some embodiments use Beta-Binomial distributions whose mean and variance are a function of the original amount of DNA, as well as sample and SNP quality.

Platform modeling

Consider a single SNP where the expected allele ratio present in the plasma is r (based on the maternal and fetal genotypes). The expected allele ratio is defined as the expected fraction of A alleles in the combined maternal and fetal DNA. For maternal genotype g_m and

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child genotype g_c, the expected allele ratio is given by equation 1, assuming that the genotypes are represented as allele ratios as well.

$$r = fg_c + (1 - f)g_m \qquad (1)$$

The observation at the SNP consists of the number of mapped reads with each allele present, n_a and n_b, which sum to the depth of read d. Assume that thresholds have already been applied to the mapping probabilities and phred scores such that the mappings and allele observations can be considered correct. A phred score is a numerical measure that relates to the probability that a particular measurement at a particular base is wrong. In an embodiment, where the base has been measured by sequencing, the phred score may be calculated from the ratio of the dye intensity corresponding to the called base to the dye intensity of the other bases. The simplest model for the observation likelihood is a binomial distribution which assumes that each of the d reads is drawn independently from a large pool that has allele ratio r. Equation 2 describes this model.

$$P(n_a,n_b|r) = p_{bino}(n_a; n_a + n_b, r) = \binom{n_a + n_b}{n_a} r^{n_a}(1-r)^{n_b} \qquad (2)$$

The binomial model can be extended in a number of ways. When the maternal and fetal genotypes are either all A or all B, the expected allele ratio in plasma will be 0 or 1, and the binomial probability will not be well-defined. In practice, unexpected alleles are sometimes observed in practice. In an embodiment, it is possible to use a corrected allele ratio $\hat{r} = 1/(n_a + n_b)$ to allow a small number of the unexpected allele. In an embodiment, it is possible to use training data to model the rate of the unexpected allele appearing on each SNP, and use this model to correct the expected allele ratio. When the expected allele ratio is not 0 or 1, the observed allele ratio may not converge with a sufficiently high depth of read to the expected allele ratio due to amplification bias or other phenomena. The allele ratio can then be modeled as a beta distribution centered at the expected allele ratio, leading to a beta-binomial distribution for $P(n_a, n_b|r)$ which has higher variance than the binomial.

The platform model for the response at a single SNP will be defined as $F(a, b, g_c, g_m, f)$ (3), or the probability of observing $n_a = a$ and $n_b = b$ given the maternal and fetal genotypes, which also depends on the fetal fraction through equation 1. The functional form of F may be a binomial distribution, beta-binomial distribution, or similar functions as discussed above.

$$F(a, b, g_c, g_m, f) = P(n_a = a, n_b = b|g_c, g_m, f) = P(n_a = a, n_b = b|r(g_c, g_m, f)) \qquad (3)$$

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In an embodiment, the child fraction may be determined as follows. A maximum likelihood estimate of the fetal fraction f for a prenatal test may be derived without the use of paternal information. This may be relevant where the paternal genetic data is not available, for example where the father of record is not actually the genetic father of the fetus. The fetal fraction is estimated from the set of SNPs where the maternal genotype is 0 or 1, resulting in a set of only two possible fetal genotypes. Define S_0 as the set of SNPs with maternal genotype 0 and S_1 as the set of SNPs with maternal genotype 1. The possible fetal genotypes on S_0 are 0 and 0.5, resulting in a set of possible allele ratios $R_0(f) = \{0, f/2\}$. Similarly, $R_1(f) = \{1-f/2, 1\}$. This method can be trivially extended to include SNPs where maternal genotype is 0.5, but these SNPs will be less informative due to the larger set of possible allele ratios.

Define N_{a0} and N_{b0} as the vectors formed by n_{as} and n_{bs} for SNPs s in S_0, and N_{a1} and N_{b1} similarly for S_1. The maximum likelihood estimate $\hat{f}$ of f is defined by equation 4.

$$\hat{f} = \arg\max_f P(N_{a0}, N_{b0}|f)\ P(N_{a1}, N_{b1}|f) \qquad (4)$$

Assuming that the allele counts at each SNP are independent conditioned on the SNP's plasma allele ratio, the probabilities can be expressed as products over the SNPs in each set (5).

$$P(N_{a0}, N_{b0}|f) = \prod_{s\epsilon S_0} P(n_{as}, n_{bs}|f) \qquad (5)$$
$$P(N_{a1}, N_{b1}|f) = \prod_{s\epsilon S_1} P(n_{as}, n_{bs}|f)$$

The dependence on f is through the sets of possible allele ratios $R_0(f)$ and $R_1(f)$. The SNP probability $P(n_{as}, n_{bs}|f)$ can be approximated by assuming the maximum likelihood genotype conditioned on f. At reasonably high fetal fraction and depth of read, the selection of the maximum likelihood genotype will be high confidence. For example, at fetal fraction of 10 percent and depth of read of 1000, consider a SNP where the mother has genotype zero. The expected allele ratios are 0 and 5 percent, which will be easily distinguishable at sufficiently high depth of read. Substitution of the estimated child genotype into equation 5 results in the complete equation (6) for the fetal fraction estimate.

$$\hat{f} = \arg\max_f \left[\prod_{s\epsilon S_0}\left(\max_{r_s\epsilon R_0(f)} P(n_{as}, n_{bs}|r_s)\right) \prod_{s\epsilon S_1}\left(\max_{r_s\epsilon R_1(f)} P(n_{as}, n_{bs}|r_s)\right)\right] \quad (6)$$

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The fetal fraction must be in the range [0, 1] and so the optimization can be easily implemented by a constrained one-dimensional search.

In the presence of low depth of read or high noise level, it may be preferable not to assume the maximum likelihood genotype, which may result in artificially high confidences. Another method would be to sum over the possible genotypes at each SNP, resulting in the following expression (7) for $P(n_a, n_b|f)$ for a SNP in S_0. The prior probability P(r) could be assumed uniform over $R_0(f)$, or could be based on population frequencies. The extension to group S_1 is trivial.

$$P(n_a, n_b|f) = \sum_{r\epsilon R_0(f)} P(n_a, n_a|r)P(r) \qquad (7)$$

In some embodiments the probabilities may be derived as follows. A confidence can be calculated from the data likelihoods of the two hypotheses H_t and H_f. The likelihood of each hypothesis is derived based on the response model, the estimated fetal fraction, the mother genotypes, allele population frequencies, and the plasma allele counts.

Define the following notation:

G_m, G_c	true maternal and child genotypes
G_{af}, G_{tf}	true genotypes of alleged father and of true father
$G(g_c, g_m, g_{tf}) = P(G_c = g_c \mid G_m = g_m, G_{tf} = g_{tf})$	inheritance probabilities
$P(g) = P(G_{tf} = g)$	population frequency of genotype g at particular SNP

Assuming that the observation at each SNP is independent conditioned on the plasma allele ratio, the likelihood of a paternity hypothesis is the product of the likelihoods on the SNPs. The following equations derive the likelihood for a single SNP. Equation 8 is a general expression for the likelihood of any hypothesis h, which will then be broken down into the specific cases of H_t and H_f.

$$\begin{aligned} P(n_a, n_b|h, G_m, G_{tf}, f) &= \sum_{g_c\epsilon(0,0.5,1)} P(n_a, n_b|G_c = g_c, G_m, G_{tf}, h, f)P(G_c = g_c, G_m, G_{tf}, h, f) \\ &= \sum_{g_c\epsilon(0,0.5,1)} P(n_a, n_b|G_c = g_c, G_m, f)P(G_c = g_c|G_m, G_{tf}, h) \\ &= \sum_{g_c\epsilon(0,0.5,1)} F(n_a, n_b, g_c, g_m, f)P(G_c = g_c|G_m, G_{tf}, h) \qquad (8) \end{aligned}$$

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In the case of H_t, the alleged father is the true father and the fetal genotypes are inherited from the maternal genotypes and alleged father genotypes according to equation 9.

$$P(n_a, n_b|H_t,G_m,G_{tf},f) = \sum_{g_c\epsilon(0,0.5,1)} F(n_a,n_b,g_c,g_m,f)P(G_c = g_c|G_m,G_{tf},H_t) = \sum_{g_c\epsilon(0,0.5,1)} F(n_a,n_b,g_c,g_m,f)G(g_c,G_m,G_{tf}) \quad (9)$$

In the case of H_f, the alleged father is not the true father. The best estimate of the true father genotypes are given by the population frequencies at each SNP. Thus, the probabilities of child genotypes are determined by the known mother genotypes and the population frequencies, as in equation 10.

$$P(n_a, n_b|H_t,G_m,G_{tf},f) = \sum_{g_c\epsilon(0,0.5,1)} F(n_a,n_b,g_c,g_m,f)P(G_c = g_c|G_m,G_{tf},H_f) = \sum_{g_c\epsilon(0,0.5,1)} F(n_a,n_b,g_c,g_m,f)P(G_c = g_c|G_m)$$

$$= \sum_{g_c\epsilon(0,0.5,1)} \sum_{g_{tf}\epsilon(0,0.5,1)} F(n_a,n_b,g_c,g_m,f)P(G_c = g_c|G_m,G_{tf} = g_{tf})P(G_{tf} = g_{tf})$$

$$= \sum_{g_c\epsilon(0,0.5,1)} \sum_{g_{tf}\epsilon(0,0.5,1)} F(n_a,n_b,g_c,g_m,f)G(g_c,G_m,g_{tf})P(g_{tf})$$

The confidence C_p on correct paternity is calculated from the product over SNPs of the two likelihoods using Bayes rule (11).

$$Cp = \frac{\prod_s P(n_{as},n_{bs}|H_t,G_{ms},G_{tf},f)}{\prod_s P(n_{as},n_{bs}|H_t,G_{ms},G_{tf},f) + \prod_s P(n_{as},n_{bs}|H_f,G_{ms},G_{tf},f)} \quad (11)$$

Maximum Likelihood Model using Percent Fetal Fraction

Determining the ploidy status of a fetus by measuring the free floating DNA contained in maternal serum, or by measuring the genotypic material in any mixed sample, is a non-trivial exercise. There are a number of methods, for example, performing a read count analysis where the presumption is that if the fetus is trisomic at a particular chromosome, then the overall amount of DNA from that chromosome found in the maternal blood will be elevated with respect to a reference chromosome. One way to detect trisomy in such fetuses is to normalize the amount of DNA expected for each chromosome, for example, according to the number of SNPs in the analysis set that correspond to a given chromosome, or according to the

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number of uniquely mappable portions of the chromosome. Once the measurements have been normalized, any chromosomes for which the amount of DNA measured exceeds a certain threshold are determined to be trisomic. This approach is described in Fan, et al. PNAS, 2008; 105(42); pp. 16266–16271, and also in Chiu et al. BMJ 2011;342:c7401. In the Chiu et al. paper, the normalization was accomplished by calculating a Z score as follows:

> Z score for percentage chromosome 21 in test case = ((percentage chromosome 21 in test case) − (mean percentage chromosome 21 in reference controls)) / (standard deviation of percentage chromosome 21 in reference controls).

These methods determine the ploidy status of the fetus using a single hypothesis rejection method. However, they suffer from some significant shortcomings. Since these methods for determining ploidy in the fetus are invariant according to the percentage of fetal DNA in the sample, they use one cut off value; the result of this is that the accuracies of the determinations are not optimal, and those cases where the percentage of fetal DNA in the mixture are relatively low will suffer the worst accuracies.

In an embodiment, a method of the present disclosure is used to determine the ploidy state of the fetus involves taking into account the fraction of fetal DNA in the sample. In another embodiment of the present disclosure, the method involves the use of maximum likelihood estimations. In an embodiment, a method of the present disclosure involves calculating the percent of DNA in a sample that is fetal or placental in origin. In an embodiment, the threshold for calling aneuploidy is adaptively adjusted based on the calculated percent fetal DNA. In some embodiments, the method for estimating the percentage of DNA that is of fetal origin in a mixture of DNA, comprises obtaining a mixed sample that comprises genetic material from the mother, and genetic material from the fetus, obtaining a genetic sample from the father of the fetus, measuring the DNA in the mixed sample, measuring the DNA in the father sample, and calculating the percentage of DNA that is of fetal origin in the mixed sample using the DNA measurements of the mixed sample, and of the father sample.

In an embodiment of the present disclosure, the fraction of fetal DNA, or the percentage of fetal DNA in the mixture can be measured. In some embodiments the fraction can be calculated using only the genotyping measurements made on the maternal plasma

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sample itself, which is a mixture of fetal and maternal DNA. In some embodiments the fraction may be calculated also using the measured or otherwise known genotype of the mother and/or the measured or otherwise known genotype of the father. In some embodiments the percent fetal DNA may be calculated using the measurements made on the mixture of maternal and fetal DNA along with the knowledge of the parental contexts. In an embodiment, the fraction of fetal DNA may be calculated using population frequencies to adjust the model on the probability on particular allele measurements.

In an embodiment of the present disclosure, a confidence may be calculated on the accuracy of the determination of the ploidy state of the fetus. In an embodiment, the confidence of the hypothesis of greatest likelihood (H_{major}) may be calculated as (1- H_{major}) / Σ(all H). It is possible to determine the confidence of a hypothesis if the distributions of all of the hypotheses are known. It is possible to determine the distribution of all of the hypotheses if the parental genotype information is known. It is possible to calculate a confidence of the ploidy determination if the knowledge of the expected distribution of data for the euploid fetus and the expected distribution of data for the aneuploid fetus are known. It is possible to calculate these expected distributions if the parental genotype data are known. In an embodiment one may use the knowledge of the distribution of a test statistic around a normal hypothesis and around an abnormal hypothesis to determine both the reliability of the call as well as refine the threshold to make a more reliable call. This is particularly useful when the amount and/or percent of fetal DNA in the mixture is low. It will help to avoid the situation where a fetus that is actually aneuploid is found to be euploid because a test statistic, such as the Z statistic does not exceed a threshold that is made based on a threshold that is optimized for the case where there is a higher percent fetal DNA.

In an embodiment, a method disclosed herein can be used to determine a fetal aneuploidy by determining the number of copies of maternal and fetal target chromosomes in a mixture of maternal and fetal genetic material. This method may entail obtaining maternal tissue comprising both maternal and fetal genetic material; in some embodiments this maternal tissue may be maternal plasma or a tissue isolated from maternal blood. This method may also entail obtaining a mixture of maternal and fetal genetic material from said maternal tissue by processing the aforementioned maternal tissue. This method may entail distributing the genetic material obtained into a plurality of reaction samples, to randomly provide individual reaction samples that comprise a target sequence from a target chromosome and individual reaction

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samples that do not comprise a target sequence from a target chromosome, for example, performing high throughput sequencing on the sample. This method may entail analyzing the target sequences of genetic material present or absent in said individual reaction samples to provide a first number of binary results representing presence or absence of a presumably euploid fetal chromosome in the reaction samples and a second number of binary results representing presence or absence of a possibly aneuploid fetal chromosome in the reaction samples. Either of the number of binary results may be calculated, for example, by way of an informatics technique that counts sequence reads that map to a particular chromosome, to a particular region of a chromosome, to a particular locus or set of loci. This method may involve normalizing the number of binary events based on the chromosome length, the length of the region of the chromosome, or the number of loci in the set. This method may entail calculating an expected distribution of the number of binary results for a presumably euploid fetal chromosome in the reaction samples using the first number. This method may entail calculating an expected distribution of the number of binary results for a presumably aneuploid fetal chromosome in the reaction samples using the first number and an estimated fraction of fetal DNA found in the mixture, for example, by multiplying the expected read count distribution of the number of binary results for a presumably euploid fetal chromosome by (1 + n/2) where n is the estimated fetal fraction. In some embodiments, the sequence reads may be treated at probabilistic mappings rather than binary results; this method would yield higher accuracies, but require more computing power. The fetal fraction may be estimated by a plurality of methods, some of which are described elsewhere in this disclosure. This method may involve using a maximum likelihood approach to determine whether the second number corresponds to the possibly aneuploid fetal chromosome being euploid or being aneuploid. This method may involve calling the ploidy status of the fetus to be the ploidy state that corresponds to the hypothesis with the maximum likelihood of being correct given the measured data.

Note that the use of a maximum likelihood model may be used to increase the accuracy of any method that determines the ploidy state of a fetus. Similarly, a confidence maybe calculated for any method that determines the ploidy state of the fetus. The use of a maximum likelihood model would result in an improvement of the accuracy of any method where the ploidy determination is made using a single hypothesis rejection technique. A maximum likelihood model may be used for any method where a likelihood distribution can be calculated

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for both the normal and abnormal cases. The use of a maximum likelihood model implies the ability to calculate a confidence for a ploidy call.

Further Discussion of the Method

In an embodiment, a method disclosed herein utilizes a quantitative measure of the number of independent observations of each allele at a polymorphic locus, where this does not involve calculating the ratio of the alleles. This is different from methods, such as some microarray based methods, which provide information about the ratio of two alleles at a locus but do not quantify the number of independent observations of either allele. Some methods known in the art can provide quantitative information regarding the number of independent observations, but the calculations leading to the ploidy determination utilize only the allele ratios, and do not utilize the quantitative information. To illustrate the importance of retaining information about the number of independent observations consider the sample locus with two alleles, A and B. In a first experiment twenty A alleles and twenty B alleles are observed, in a second experiment 200 A alleles and 200 B alleles are observed. In both experiments the ratio (A/(A+B)) is equal to 0.5, however the second experiment conveys more information than the first about the certainty of the frequency of the A or B allele. The instant method, rather than utilizing the allele ratios, uses the quantitative data to more accurately model the most likely allele frequencies at each polymorphic locus.

In an embodiment, the instant methods build a genetic model for aggregating the measurements from multiple polymorphic loci to better distinguish trisomy from disomy and also to determine the type of trisomy. Additionally, the instant method incorporates genetic linkage information to enhance the accuracy of the method. This is in contrast to some methods known in the art where allele ratios are averaged across all polymorphic loci on a chromosome. The method disclosed herein explicitly models the allele frequency distributions expected in disomy as well as and trisomy resulting from nondisjunction during meiosis I, nondisjunction during meiosis II, and nondisjunction during mitosis early in fetal development. To illustrate why this is important, if there were no crossovers nondisjunction during meiosis I would result a trisomy in which two different homologs were inherited from one parent; nondisjunction during meiosis II or during mitosis early in fetal development would result in two copies of the same homolog from one parent. Each scenario results in different expected allele frequencies at each polymorphic locus and also at all physically linked loci (*i.e.* loci on

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the same chromosome) considered jointly. Crossovers, which result in the exchange of genetic material between homologs, make the inheritance pattern more complex, but the instant method accommodates for this by using genetic linkage information, *i.e.* recombination rate information and the physical distance between loci. To better distinguish between meiosis I nondisjunction and meiosis II or mitotic nondisjunction the instant method incorporates into the model an increasing probability of crossover as the distance from the centromere increases. Meiosis II and mitotic nondisjunction can distinguished by the fact that mitotic nondisjunction typically results in identical or nearly identical copies of one homolog while the two homologs present following a meiosis II nondisjunction event often differ due to one or more crossovers during gametogenesis.

In an embodiment, a method of the present disclosure may not determine the haplotypes of the parents if disomy is assumed. In an embodiment, in case of trisomy, the instant method can make a determination about the haplotypes of one or both parents by using the fact that plasma takes two copies from one parent, and parent phase information can be determined by noting which two copies have been inherited from the parent in question. In particular, a child can inherit either two of the same copies of the parent (matched trisomy) or both copies of the parent (unmatched trisomy). At each SNP one can calculate the likelihood of the matched trisomy and of the unmatched trisomy. A ploidy calling method that does not use the linkage model accounting for crossovers would calculate the overall likelihood of the trisomy as a simple weighted average of the matched and unmatched trisomies over all chromosomes. However, due to the biological mechanisms that result in disjunction error and crossing over, trisomy can change from matched to unmatched (and vice versa) on a chromosome only if a crossover occurs. The instant method probabilistically takes into account the likelihood of crossover, resulting in ploidy calls that are of greater accuracy than those methods that do not.

In an embodiment, a reference chromosome is used to determine the child fraction and noise level amount or probability distribution. In an embodiment, the child fraction, noise level, and/or probability distribution is determined using only the genetic information available from the chromosome whose ploidy state is being determined. The instant method works without the reference chromosome, as well as without fixing the particular child fraction or noise level. This is a significant improvement and point of differentiation from methods known

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in the art where genetic data from a reference chromosome is necessary to calibrate the child fraction and chromosome behavior.

In an embodiment where a reference chromosome is not needed to determine the fetal fraction, determining the hypothesis is done as follows:

$$H^* = \text{argmax}_H \, LIK(D|H)*priorprob(H)$$

With the algorithm with reference chromosome, one typically assumes that the reference chromosome is a disomy, and then one may either (a) fix the most likely child fraction and random noise level N based on this assumption and reference chromosome data :

$$[cfr^*, N^*] = \underset{cfr,N}{\text{argmax}} \, LIK(D(ref.chrom)|H11, cfr, N)$$

And then reduce

$$LIK(D|H) = LIK(D|H, cfr^*, N^*)$$

or (b) estimate the child fraction and noise level distribution based on this assumption and reference chromosome data. In particular, one would not fix just one value for cfr and N, but assign probability p(cfr, N) for the wider range of possible cfr, N values:

$$p(cfr, N) \sim LIK(D(ref.chrom)|H11, cfr, N) * priorprob(cfr, N)$$

where priorprob(cfr, N) is the prior probability of particular child fraction and noise level, determined by prior knowledge and experiments. If desired, just uniform over the range of cfr, N. One may then write:

$$LIK(D|H) = \sum_{cfr,N} LIK(D|H, cfr, N) * p(cfr, N)$$

Both methods above give good results.

Note that in some instances using a reference chromosome is not desirable, possible or feasible. In such a case, it is possible to derive the best ploidy call for each chromosome separately. In particular:

$$LIK(D|H) = \sum_{cfr,N} LIK(D|H, cfr, N) * p(cfr, N|H)$$

$p(cfr, N|H)$ may be determined as above, for each chromosome separately, assuming hypothesis H, not just for the reference chromosome assuming disomy. It is possible, using this method, to keep both noise and child fraction parameters fixed, fix either of the parameters, or keep both parameters in probabilistic form for each chromosome and each hypothesis.

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Measurements of DNA are noisy and/or error prone, especially measurements where the amount of DNA is small, or where the DNA is mixed with contaminating DNA. This noise results in less accurate genotypic data, and less accurate ploidy calls. In some embodiments, platform modeling or some other method of noise modeling may be used to counter the deleterious effects of noise on the ploidy determination. The instant method uses a joint model of both channels, which accounts for the random noise due to the amount of input DNA, DNA quality, and/or protocol quality.

This is in contrast to some methods known in the art where the ploidy determinations are made using the ratio of allele intensities at a locus. This method precludes accurate SNP noise modeling. In particular, errors in the measurements typically do not specifically depend on the measured channel intensity ratio, which reduces the model to using one-dimensional information. Accurate modeling of noise, channel quality and channel interaction requires a two-dimensional joint model, which can *not* be modeled using allele ratios.

In particular, projecting two channel information to the ratio r where f(x,y) is r = x/y, does not lend itself to accurate channel noise and bias modeling. Noise on a particular SNP is not a function of the ratio, *i.e.* noise(x,y) $\neq$ f(x,y) but is in fact a joint function of both channels. For example, in the binomial model, noise of the measured ratio has a variance of r(1-r)/(x+y) which is not a function purely of r. In such a model, where any channel bias or noise is included, suppose that on SNP i, the observed channel X value is $x=a_iX+b_i$, where X is the true channel value, b_i is the extra channel bias and random noise. Similarly, suppose that $y=c_iY+d_i$. The observed ratio r=x/y can not accurately predict the true ratio X/Y or model the leftover noise, since (aiX+bi)/(ciY+di) is not a function of X/Y.

The method disclosed herein describes an effective way to model noise and bias using joint binomial distributions of all of the measurement channels individually. Relevant equations may be found elsewhere in the document in sections which speaks of per SNP consistent bias, P(good) and P(ref|bad), P(mut|bad) which effectively adjust SNP behavior. In an embodiment, a method of the present disclosure uses a BetaBinomial distribution, which avoids the limiting practice of relying on the allele ratios only, but instead models the behavior based on both channel counts.

In an embodiment, a method disclosed herein can call the ploidy of a gestating fetus from genetic data found in maternal plasma by using all available measurements. In an embodiment, a method disclosed herein can call the ploidy of a gestating fetus from genetic

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data found in maternal plasma by using the measurements from only a subset of parental contexts. Some methods known in the art only use measured genetic data where the parental context is from the AA|BB context, that is, where the parents are both homozygous at a given locus, but for a different allele. One problem with this method is that a small proportion of polymorphic loci are from the AA|BB context, typically less than 10%. In an embodiment of a method disclosed herein, the method does not use genetic measurements of the maternal plasma made at loci where the parental context is AA|BB. In an embodiment, the instant method uses plasma measurements for only those polymorphic loci with the AA|AB, AB|AA, and AB|AB parental context.

Some methods known in the art involve averaging allele ratios from SNPs in the AA|BB context, where both parent genotypes are present, and claim to determine the ploidy calls from the average allele ratio on these SNPs. This method suffers from significant inaccuracy due differential SNP behavior. Note that this method assumes that have both parent genotypes are known. In contrast, in some embodiments, the instant method uses a joint channel distribution model that does not assume the presence of either of the parents, and does not assume the uniform SNP behavior. In some embodiments, the instant method accounts for the different SNP behavior/weighing. In some embodiments, the instant method does not require the knowledge of one or both parental genotypes. An example of how the instant method may accomplish this follows:

In some embodiments, the log likelihood of a hypothesis may be determined on a per SNP basis. On a particular SNP i, assuming fetal ploidy hypothesis H and percent fetal DNA cf, the log likelihood of observed data D is defined as:

$$\mathrm{LIK}(\mathrm{D}|\mathrm{H},\mathrm{i}) = \log \mathrm{P}(\mathrm{D}|\mathrm{H},\mathrm{cf},\mathrm{i}) = \log\left(\sum_{\mathrm{m,f,c}} \mathrm{P}(\mathrm{D}|\mathrm{m},\mathrm{f},\mathrm{c},\mathrm{H},\mathrm{cf},\mathrm{i})\mathrm{P}(\mathrm{c}|\mathrm{m},\mathrm{f},\mathrm{H})\mathrm{P}(\mathrm{m}|\mathrm{i})\mathrm{P}(\mathrm{f}|\mathrm{i})\right)$$

where m are possible true mother genotypes, f are possible true father genotypes, where m,f $\in$ {AA,AB,BB}, and where c are possible child genotypes given the hypothesis H. In particular, for monosomy c $\{A,B\}$, for disomy c $\in \{AA,AB,BB\}$, for trisomy c $\in \{AAA,AAB,ABB,BBB\}$. Note that including parental genotypic data typically results in more accurate ploidy determinations, however, parental genotypic data is not necessary for the instant method to work well.

Some methods known in the art involve averaging allele ratios from SNPs where the mother is homozygous but a different allele is measured in the plasma (either AA|AB or

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AA|BB contexts), and claim to determine the ploidy calls from the average allele ratio on these SNPs. This method is intended for cases where the paternal genotype is not available. Note that it is questionable how accurately one can claim that plasma is heterozygous on a particular SNP without the presence of homozygous and opposite father BB: for cases with low child fraction, what looks like presence of B allele could be just presence of noise; additionally, what looks like no B present could be simple allele drop out of the fetal measurements. Even in a case where one can actually determine heterozygosity of the plasma, this method will not be able to distinguish paternal trisomies. In particular, for SNPs where mother is AA, and where some B is measured in the plasma, if the father is GG, the resulting child genotype is AGG, resulting in an average ratio of 33% A (for child fraction=100%). But in the case where the father is AG, the resulting child genotype could be AGG for matched trisomy, contributing to the 33% A ratio, or AAG for unmatched trisomy, drawing the average ratio more toward 66% A. Given that many trisomies are on chromosomes with crossovers, the overall chromosome can have anywhere between no unmatched trisomy and all unmatched trisomy, this ratio can vary anywhere between 33-66%. For a plain disomy, the ratio should be around 50%. Without the use of a linkage model or an accurate error model of the average, this method would miss many cases of paternal trisomy. In contrast, the method disclosed herein assigns parental genotype probabilities for each parental genotypic candidate, based on available genotypic information and population frequency, and does not explicitly require parental genotypes. Additionally, the method disclosed herein is able to detect trisomy even in the absence or presence of parent genotypic data, and can compensate by identifying the points of possible crossovers from matched to unmatched trisomy using a linkage model.

Some methods known in the art claim a method for averaging allele ratios from SNPs where neither the maternal or paternal genotype is known, and for determining the ploidy calls from average ratio on these SNPs. However, a method to accomplish these ends is not disclosed. The method disclosed herein is able to make accurate ploidy calls in such a situation, and the reduction to practice is disclosed elsewhere in this document, using a joint probability maximum likelihood method and optionally utilizes SNP noise and bias models, as well as a linkage model.

Some methods known in the art involve averaging allele ratios and claim to determine the ploidy calls from the average allele ratio at one or a few SNPs. However, such methods do

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not utilize the concept of linkage. The methods disclosed herein do not suffer from these drawbacks.

Using Sequence Length as a Prior to Determine the Origin of DNA

It has been reported that the distribution of length of sequences differ for maternal and fetal DNA, with fetal generally being shorter. In an embodiment of the present disclosure, it is possible to use previous knowledge in the form of empirical data, and construct prior distribution for expected length of both mother(P(X| maternal)) and fetal DNA (P(X| fetal)). Given new unidentified DNA sequence of length x, it is possible to assign a probability that a given sequence of DNA is either maternal or fetal DNA, based on prior likelihood of x given either maternal or fetal. In particular if P(x|maternal) > P(x|fetal), then the DNA sequence can be classified as maternal, with P(x|maternal) = P(x|maternal)/[(P(x|maternal) + P(x| fetal)], and if p(x|maternal) < p(x|fetal), then the DNA sequence can be classified as fetal, P(x| fetal) = P(x| fetal)/[(P(x|maternal) + P(x| fetal)]. In an embodiment of the present disclosure, a distributions of maternal and fetal sequence lengths can be determined that is specific for that sample by considering the sequences that can be assigned as maternal or fetal with high probability, and then that sample specific distribution can be used as the expected size distribution for that sample.

Variable Read Depth to Minimize Sequencing Cost

In many clinical trials concerning a diagnostic, for example, in Chiu et al. BMJ 2011;342:c7401, a protocol with a number of parameters is set, and then the same protocol is executed with the same parameters for each of the patients in the trial. In the case of determining the ploidy status of a fetus gestating in a mother using sequencing as a method to measure genetic material one pertinent parameter is the number of reads. The number of reads may refer to the number of actual reads, the number of intended reads, fractional lanes, full lanes, or full flow cells on a sequencer. In these studies, the number of reads is typically set at a level that will ensure that all or nearly all of the samples achieve the desired level of accuracy. Sequencing is currently an expensive technology, a cost of roughly $200 per 5 mappable million reads, and while the price is dropping, any method which allows a sequencing based diagnostic to operate at a similar level of accuracy but with fewer reads will necessarily save a considerable amount of money.

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The accuracy of a ploidy determination is typically dependent on a number of factors, including the number of reads and the fraction of fetal DNA in the mixture. The accuracy is typically higher when the fraction of fetal DNA in the mixture is higher. At the same time, the accuracy is typically higher if the number of reads is greater. It is possible to have a situation with two cases where the ploidy state is determined with comparable accuracies wherein the first case has a lower fraction of fetal DNA in the mixture than the second, and more reads were sequenced in the first case than the second. It is possible to use the estimated fraction of fetal DNA in the mixture as a guide in determining the number of reads necessary to achieve a given level of accuracy.

In an embodiment of the present disclosure, a set of samples can be run where different samples in the set are sequenced to different reads depths, wherein the number of reads run on each of the samples is chosen to achieve a given level of accuracy given the calculated fraction of fetal DNA in each mixture. In an embodiment of the present disclosure, this may entail making a measurement of the mixed sample to determine the fraction of fetal DNA in the mixture; this estimation of the fetal fraction may be done with sequencing, it may be done with TAQMAN, it may be done with qPCR, it may be done with SNP arrays, it may be done with any method that can distinguish different alleles at a given loci. The need for a fetal fraction estimate may be eliminated by including hypotheses that cover all or a selected set of fetal fractions in the set of hypotheses that are considered when comparing to the actual measured data. After the fraction fetal DNA in the mixture has been determined, the number of sequences to be read for each sample may be determined.

In an embodiment of the present disclosure, 100 pregnant women visit their respective OB's, and their blood is drawn into blood tubes with an anti-lysant and/or something to inactivate DNAase. They each take home a kit for the father of their gestating fetus who gives a saliva sample. Both sets of genetic materials for all 100 couples are sent back to the laboratory, where the mother blood is spun down and the buffy coat is isolated, as well as the plasma. The plasma comprises a mixture of maternal DNA as well as placentally derived DNA. The maternal buffy coat and the paternal blood is genotyped using a SNP array, and the DNA in the maternal plasma samples are targeted with SURESELECT hybridization probes. The DNA that was pulled down with the probes is used to generate 100 tagged libraries, one for each of the maternal samples, where each sample is tagged with a different tag. A fraction from each library is withdrawn, each of those fractions are mixed together and added to two

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lanes of a ILLUMINA HISEQ DNA sequencer in a multiplexed fashion, wherein each lane resulted in approximately 50 million mappable reads, resulting in approximately 100 million mappable reads on the 100 multiplexed mixtures, or approximately 1 million reads per sample. The sequence reads were used to determine the fraction of fetal DNA in each mixture. 50 of the samples had more than 15% fetal DNA in the mixture, and the 1 million reads were sufficient to determine the ploidy status of the fetuses with a 99.9% confidence.

Of the remaining mixtures, 25 had between 10 and 15% fetal DNA; a fraction of each of the relevant libraries prepped from these mixtures were multiplexed and run down one lane of the HISEQ generating an additional 2 million reads for each sample. The two sets of sequence data for each of the mixture with between 10 and 15% fetal DNA were added together, and the resulting 3 million reads per sample which were sufficient to determine the ploidy state of those fetuses with 99.9% confidence.

Of the remaining mixtures, 13 had between 6 and 10% fetal DNA; a fraction of each of the relevant libraries prepped from these mixtures were multiplexed and run down one lane of the HISEQ generating an additional 4 million reads for each sample. The two sets of sequence data for each of the mixture with between 6 and 10% fetal DNA were added together, and the resulting 5 million total reads per mixture which were sufficient to determine the ploidy state of those fetuses with 99.9% confidence.

Of the remaining mixtures, 8 had between 4 and 6% fetal DNA; a fraction of each of the relevant libraries prepped from these mixtures were multiplexed and run down one lane of the HISEQ generating an additional 6 million reads for each sample. The two sets of sequence data for each of the mixture with between 4 and 6% fetal DNA were added together, and the resulting 7 million total reads per mixture which were sufficient to determine the ploidy state of those fetuses with 99.9% confidence.

Of the remaining four mixtures, all of them had between 2 and 4% fetal DNA; a fraction of each of the relevant libraries prepped from these mixtures were multiplexed and run down one lane of the HISEQ generating an additional 12 million reads for each sample. The two sets of sequence data for each of the mixture with between 2 and 4% fetal DNA were added together, and the resulting 13 million total reads per mixture which were sufficient to determine the ploidy state of those fetuses with 99.9% confidence.

This method required six lanes of sequencing on a HISEQ machine to achieve 99.9% accuracy over 100 samples. If the same number of runs had been required for every sample, to

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ensure that every ploidy determination was made with a 99.9% accuracy, it would have taken 25 lanes of sequencing, and if a no-call rate or error rate of 4% was tolerated, it could have been achieved with 14 lanes of sequencing.

Using Raw Genotyping Data

There are a number of methods that can accomplish NPD using fetal genetic information measured on fetal DNA found in maternal blood. Some of these methods involve making measurements of the fetal DNA using SNP arrays, some methods involve untargeted sequencing, and some methods involve targeted sequencing. The targeted sequencing may target SNPs, it may target STRs, it may target other polymorphic loci, it may target non-polymorphic loci, or some combination thereof. Some of these methods may involve using a commercial or proprietary allele caller that calls the identity of the alleles from the intensity data that comes from the sensors in the machine doing the measuring. For example, the ILLUMINA INFINIUM system or the AFFYMETRIX GENECHIP microarray system involves beads or microchips with attached DNA sequences that can hybridize to complementary segments of DNA; upon hybridization, there is a change in the fluorescent properties of the sensor molecule that can be detected. There are also sequencing methods, for example the ILLUMINA SOLEXA GENOME SEQUENCER or the ABI SOLID GENOME SEQUENCER, wherein the genetic sequence of fragments of DNA are sequenced; upon extension of the strand of DNA complementary to the strand being sequenced, the identity of the extended nucleotide is typically detected via a fluorescent or radio tag appended to the complementary nucleotide. In all of these methods the genotypic or sequencing data is typically determined on the basis of fluorescent or other signals, or the lack thereof. These systems are typically combined with low level software packages that make specific allele calls (secondary genetic data) from the analog output of the fluorescent or other detection device (primary genetic data). For example, in the case of a given allele on a SNP array, the software will make a call, for example, that a certain SNP is present or not present if the fluorescent intensity is measure above or below a certain threshold. Similarly, the output of a sequencer is a chromatogram that indicates the level of fluorescence detected for each of the dyes, and the software will make a call that a certain base pair is A or T or C or G. High throughput sequencers typically make a series of such measurements, called a read, that represents the most likely structure of the DNA sequence that was sequenced. The direct

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analog output of the chromatogram is defined here to be the primary genetic data, and the base pair / SNP calls made by the software are considered here to be the secondary genetic data. In an embodiment, primary data refers to the raw intensity data that is the unprocessed output of a genotyping platform, where the genotyping platform may refer to a SNP array, or to a sequencing platform. The secondary genetic data refers to the processed genetic data, where an allele call has been made, or the sequence data has been assigned base pairs, and/or the sequence reads have been mapped to the genome.

Many higher level applications take advantage of these allele calls, SNP calls and sequence reads, that is, the secondary genetic data, that the genotyping software produces. For example, DNA NEXUS, ELAND or MAQ will take the sequencing reads and map them to the genome. For example, in the context of non-invasive prenatal diagnosis, complex informatics, such as PARENTAL SUPPORT™, may leverage a large number of SNP calls to determine the genotype of an individual. Also, in the context of preimplantation genetic diagnosis, it is possible to take a set of sequence reads that are mapped to the genome, and by taking a normalized count of the reads that are mapped to each chromosome, or section of a chromosome, it may be possible to determine the ploidy state of an individual. In the context of non-invasive prenatal diagnosis it may be possible to take a set of sequence reads that have been measured on DNA present in maternal plasma, and map them to the genome. One may then take a normalized count of the reads that are mapped to each chromosome, or section of a chromosome, and use that data to determine the ploidy state of an individual. For example, it may be possible to conclude that those chromosomes that have a disproportionately large number of reads are trisomic in the fetus that is gestating in the mother from which the blood was drawn.

However, in reality, the initial output of the measuring instruments is an analog signal. When a certain base pair is called by the software that is associated with the sequencing software, for example the software may call the base pair a T, in reality the call is the call that the software believes to be most likely. In some cases, however, the call may be of low confidence, for example, the analog signal may indicate that the particular base pair is only 90% likely to be a T, and 10% likely to be an A. In another example, the genotype calling software that is associated with a SNP array reader may call a certain allele to be G. However, in reality, the underlying analog signal may indicate that it is only 70% likely that the allele is G, and 30% likely that the allele is T. In these cases, when the higher level applications use

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the genotype calls and sequence calls made by the lower level software, they are losing some information. That is, the primary genetic data, as measured directly by the genotyping platform, may be messier than the secondary genetic data that is determined by the attached software packages, but it contains more information. In mapping the secondary genetic data sequences to the genome, many reads are thrown out because some bases are not read with enough clarity and or mapping is not clear. When the primary genetic data sequence reads are used, all or many of those reads that may have been thrown out when first converted to secondary genetic data sequence read can be used by treating the reads in a probabilistic manner.

In an embodiment of the present disclosure, the higher level software does not rely on the allele calls, SNP calls, or sequence reads that are determined by the lower level software. Instead, the higher level software bases its calculations on the analog signals directly measured from the genotyping platform. In an embodiment of the present disclosure, an informatics based method such as PARENTAL SUPPORT™ is modified so that its ability to reconstruct the genetic data of the embryo / fetus / child is engineered to directly use the primary genetic data as measured by the genotyping platform. In an embodiment of the present disclosure, an informatics based method such as PARENTAL SUPPORT™ is able to make allele calls, and/or chromosome copy number calls using primary genetic data, and not using the secondary genetic data. In an embodiment of the present disclosure, all genetic calls, SNPs calls, sequence reads, sequence mapping is treated in a probabilistic manner by using the raw intensity data as measured directly by the genotyping platform, rather than converting the primary genetic data to secondary genetic calls. In an embodiment, the DNA measurements from the prepared sample used in calculating allele count probabilities and determining the relative probability of each hypothesis comprise primary genetic data.

In some embodiments, the method can increase the accuracy of genetic data of a target individual which incorporates genetic data of at least one related individual, the method comprising obtaining primary genetic data specific to a target individual's genome and genetic data specific to the genome(s) of the related individual(s), creating a set of one or more hypotheses concerning possibly which segments of which chromosomes from the related individual(s) correspond to those segments in the target individual's genome, determining the probability of each of the hypotheses given the target individual's primary genetic data and the related individual(s)'s genetic data, and using the probabilities associated with each hypothesis

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to determine the most likely state of the actual genetic material of the target individual. In some embodiments, the method can determining the number of copies of a segment of a chromosome in the genome of a target individual, the method comprising creating a set of copy number hypotheses about how many copies of the chromosome segment are present in the genome of a target individual, incorporating primary genetic data from the target individual and genetic information from one or more related individuals into a data set, estimating the characteristics of the platform response associated with the data set, where the platform response may vary from one experiment to another, computing the conditional probabilities of each copy number hypothesis, given the data set and the platform response characteristics, and determining the copy number of the chromosome segment based on the most probable copy number hypothesis. In an embodiment, a method of the present disclosure can determine a ploidy state of at least one chromosome in a target individual, the method comprising obtaining primary genetic data from the target individual and from one or more related individuals, creating a set of at least one ploidy state hypothesis for each of the chromosomes of the target individual, using one or more expert techniques to determine a statistical probability for each ploidy state hypothesis in the set, for each expert technique used, given the obtained genetic data, combining, for each ploidy state hypothesis, the statistical probabilities as determined by the one or more expert techniques, and determining the ploidy state for each of the chromosomes in the target individual based on the combined statistical probabilities of each of the ploidy state hypotheses. In an embodiment, a method of the present disclosure can determine an allelic state in a set of alleles, in a target individual, and from one or both parents of the target individual, and optionally from one or more related individuals, the method comprising obtaining primary genetic data from the target individual, and from the one or both parents, and from any related individuals, creating a set of at least one allelic hypothesis for the target individual, and for the one or both parents, and optionally for the one or more related individuals, where the hypotheses describe possible allelic states in the set of alleles, determining a statistical probability for each allelic hypothesis in the set of hypotheses given the obtained genetic data, and determining the allelic state for each of the alleles in the set of alleles for the target individual, and for the one or both parents, and optionally for the one or more related individuals, based on the statistical probabilities of each of the allelic hypotheses.

In some embodiments, the genetic data of the mixed sample may comprise sequence data wherein the sequence data may not uniquely map to the human genome. In some

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embodiments, the genetic data of the mixed sample may comprise sequence data wherein the sequence data maps to a plurality of locations in the genome, wherein each possible mapping is associated with a probability that the given mapping is correct. In some embodiments, the sequence reads are not assumed to be associated with a particular position in the genome. In some embodiments, the sequence reads are associated with a plurality of positions in the genome, and an associated probability belonging to that position.

Counting Method to Determine Chromosome Copy Number

In one aspect, the invention features methods of testing for an abnormal distribution of a fetal chromosome by comparing the number of sequence tags that align to different chromosomes (*see, e.g.*, U.S. Pat. No. 8,296,076, filed April 20, 2012, which is hereby incorporated by reference in its entirety). As is known in the art, the term "sequence tag" refers to a relatively short (*e.g.*, 15-100) nucleic acid sequence that can be used to identify a certain larger sequence, *e.g.*, be mapped to a chromosome or genomic region or gene. In some embodiments, the method involves (i) contacting a sample that includes a mixture of maternal and fetal DNA with a library of primers that simultaneously hybridize to at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci to produce a reaction mixture; wherein the target loci are from a plurality of different chromosomes; and wherein the plurality of different chromosomes comprise at least one first chromosome suspected of having an abnormal distribution in the sample and at least one second chromosome presumed to be normally distributed in the sample; (ii) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products; (iii) sequencing the amplified products to obtain a plurality of sequence tags aligning to the target loci; wherein the sequence tags are of sufficient length to be assigned to a specific target locus; (iv) assigning on a computer the plurality of sequence tags to their corresponding target loci; (v) determining on a computer a number of sequence tags aligning to the target loci of the first chromosome and a number of sequence tags aligning to the target loci of the second chromosome; and (vi) comparing the numbers from step (v) to determine the presence or absence of an abnormal distribution of the first chromosome.

In one aspect, the invention provides methods for detecting the presence or absence of a fetal aneuploidy by comparing the relative frequency of target amplicons between

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chromosomes (*see, e.g.,* PCT Publ. No. WO 2012/103031, filed Jan. 23, 2012, which is hereby incorporated by reference in its entirety). In some embodiments, the method involves (i) contacting a sample that includes a mixture of maternal and fetal DNA with a library of primers that simultaneously hybridize to at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different non-polymorphic target loci to produce a reaction mixture; wherein the target loci are from a plurality of different chromosomes; (ii) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products that includes target amplicons; (iii) quantifying on a computer a relative frequency of the target amplicons from the first and second chromosomes of interest; (iv) comparing on a computer the relative frequency of the target amplicons from the first and second chromosomes of interest; and (v) identifying the presence or absence of an aneuploidy based on the compared relative frequencies of the first and second chromosome of interest. In some embodiments, the first chromosome is a chromosome suspected of being euploid. In some embodiments, the second chromosome is a chromosome suspected of being aneuploidy

Combining Methods of Prenatal Diagnosis

There are many methods that may be used for prenatal diagnosis or prenatal screening of aneuploidy or other genetic defects. Described elsewhere in this document, and in U.S. Utility Application Serial No. 11/603,406, filed November 28, 2006; U.S. Utility Application Serial No. 12/076,348, filed March 17, 2008, and PCT Application Serial No. PCT/S09/52730 is one such method that uses the genetic data of related individuals to increase the accuracy with which genetic data of a target individual, such as a fetus, is known, or estimated. Other methods used for prenatal diagnosis involve measuring the levels of certain hormones in maternal blood, where those hormones are correlated with various genetic abnormalities. An example of this is called the triple test, a test wherein the levels of several (commonly two, three, four or five) different hormones are measured in maternal blood. In a case where multiple methods are used to determine the likelihood of a given outcome, where none of the methods are definitive in and of themselves, it is possible to combine the information given by those methods to make a prediction that is more accurate than any of the individual methods. In the triple test, combining the information given by the three different hormones can result in a prediction of genetic abnormalities that is more accurate than the individual hormone levels

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may predict.

Disclosed herein is a method for making more accurate predictions about the genetic state of a fetus, specifically the possibility of genetic abnormalities in a fetus that comprises combining predictions of genetic abnormalities in a fetus where those predictions were made using a variety of methods. A "more accurate" method may refer to a method for diagnosing an abnormality that has a lower false negative rate at a given false positive rate. In a favored embodiment of the present disclosure, one or more of the predictions are made based on the genetic data known about the fetus, where the genetic knowledge was determined using the PARENTAL SUPPORT™ method, that is, using genetic data of individual related to the fetus to determine the genetic data of the fetus with greater accuracy. In some embodiments the genetic data may include ploidy states of the fetus. In some embodiments, the genetic data may refer to a set of allele calls on the genome of the fetus. In some embodiments some of the predictions may have been made using the triple test. In some embodiments, some of the predictions may have been made using measurements of other hormone levels in maternal blood. In some embodiments, predictions made by methods considered diagnoses may be combined with predictions made by methods considered screening. In some embodiments, the method involves measuring maternal blood levels of alpha-fetoprotein (AFP). In some embodiments, the method involves measuring maternal blood levels of unconjugated estriol (UE_3). In some embodiments, the method involves measuring maternal blood levels of beta human chorionic gonadotropin (beta-hCG). In some embodiments, the method involves measuring maternal blood levels of invasive trophoblast antigen (ITA). In some embodiments, the method involves measuring maternal blood levels of inhibin. In some embodiments, the method involves measuring maternal blood levels of pregnancy-associated plasma protein A (PAPP-A). In some embodiments, the method involves measuring maternal blood levels of other hormones or maternal serum markers. In some embodiments, some of the predictions may have been made using other methods. In some embodiments, some of the predictions may have been made using a fully integrated test such as one that combines ultrasound and blood test at around 12 weeks of pregnancy and a second blood test at around 16 weeks. In some embodiments, the method involves measuring the fetal nuchal translucency (NT). In some embodiments, the method involves using the measured levels of the aforementioned hormones for making predictions. In some embodiments the method involves a combination of the aforementioned methods.

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There are many ways to combine the predictions, for example, one could convert the hormone measurements into a multiple of the median (MoM) and then into likelihood ratios (LR). Similarly, other measurements could be transformed into LRs using the mixture model of NT distributions. The LRs for NT and the biochemical markers could be multiplied by the age and gestation-related risk to derive the risk for various conditions, such as trisomy 21. Detection rates (DRs) and false-positive rates (FPRs) could be calculated by taking the proportions with risks above a given risk threshold.

In an embodiment, a method to call the ploidy state involves combining the relative probabilities of each of the ploidy hypotheses determined using the joint distribution model and the allele count probabilities with relative probabilities of each of the ploidy hypotheses that are calculated using statistical techniques taken from other methods that determine a risk score for a fetus being trisomic, including but not limited to: a read count analysis, comparing heterozygosity rates, a statistic that is only available when parental genetic information is used, the probability of normalized genotype signals for certain parent contexts, a statistic that is calculated using an estimated fetal fraction of the first sample or the prepared sample, and combinations thereof.

Another method could involve a situation with four measured hormone levels, where the probability distribution around those hormones is known: $p(x_1, x_2, x_3, x_4|e)$ for the euploid case and $p(x_1, x_2, x_3, x_4|a)$ for the aneuploid case. Then one could measure the probability distribution for the DNA measurements, $g(y|e)$ and $g(y|a)$ for the euploid and aneuploid cases respectively. Assuming they are independent given the assumption of euploid/aneuploid, one could combine as $p(x_1, x_2, x_3, x_4|a)g(y|a)$ and $p(x_1, x_2, x_3, x_4|e)g(y|e)$ and then multiply each by the prior $p(a)$ and $p(e)$ given the maternal age. One could then choose the one that is highest.

In an embodiment, it is possible to evoke central limit theorem to assume distribution on $g(y|a$ or $e)$ is Gaussian, and measure mean and standard deviation by looking at multiple samples. In another embodiment, one could assume they are not independent given the outcome and collect enough samples to estimate the joint distribution $p(x_1, x_2, x_3, x_4|a$ or $e)$.

In an embodiment, the ploidy state for the target individual is determined to be the ploidy state that is associated with the hypothesis whose probability is the greatest. In some cases, one hypothesis will have a normalized, combined probability greater than 90%. Each hypothesis is associated with one, or a set of, ploidy states, and the ploidy state associated with the hypothesis whose normalized, combined probability is greater than 90%, or some other

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threshold value, such as 50%, 80%, 95%, 98%, 99%, or 99.9%, may be chosen as the threshold required for a hypothesis to be called as the determined ploidy state.

DNA from Children from Previous Pregnancies in Maternal Blood

One difficulty to non-invasive prenatal diagnosis is differentiating fetal cells from the current pregnancy from fetal cells from previous pregnancies. Some believe that genetic matter from prior pregnancies will go away after some time, but conclusive evidence has not been shown. In an embodiment of the present disclosure, it is possible to determine fetal DNA present in the maternal blood of paternal origin (that is, DNA that the fetus inherited from the father) using the PARENTAL SUPPORT™ (PS) method, and the knowledge of the paternal genome. This method may utilize phased parental genetic information. It is possible to phase the parental genotype from unphased genotypic information using grandparental genetic data (such as measured genetic data from a sperm from the grandfather), or genetic data from other born children, or a sample of a miscarriage. One could also phase unphased genetic information by way of a HapMap-based phasing, or a haplotyping of paternal cells. Successful haplotyping has been demonstrated by arresting cells at phase of mitosis when chromosomes are tight bundles and using microfluidics to put separate chromosomes in separate wells. In another embodiment it is possible to use the phased parental haplotypic data to detect the presence of more than one homolog from the father, implying that the genetic material from more than one child is present in the blood. By focusing on chromosomes that are expected to be euploid in a fetus, one could rule out the possibility that the fetus was afflicted with a trisomy. Also, it is possible to determine if the fetal DNA is not from the current father, in which case one could use other methods such as the triple test to predict genetic abnormalities.

There may be other sources of fetal genetic material available via methods other than a blood draw. In the case of the fetal genetic material available in maternal blood, there are two main categories: (1) whole fetal cells, for example, nucleated fetal red blood cells or erythroblats, and (2) free floating fetal DNA. In the case of whole fetal cells, there is some evidence that fetal cells can persist in maternal blood for an extended period of time such that it is possible to isolate a cell from a pregnant woman that contains the DNA from a child or fetus from a prior pregnancy. There is also evidence that the free floating fetal DNA is cleared from the system in a matter of weeks. One challenge is how to determine the identity of the individual whose genetic material is contained in the cell, namely to ensure that the measured

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genetic material is not from a fetus from a prior pregnancy. In an embodiment of the present disclosure, the knowledge of the maternal genetic material can be used to ensure that the genetic material in question is not maternal genetic material. There are a number of methods to accomplish this end, including informatics based methods such as PARENTAL SUPPORT™, as described in this document or any of the patents referenced in this document.

In an embodiment of the present disclosure, the blood drawn from the pregnant mother may be separated into a fraction comprising free floating fetal DNA, and a fraction comprising nucleated red blood cells. The free floating DNA may optionally be enriched, and the genotypic information of the DNA may be measured. From the measured genotypic information from the free floating DNA, the knowledge of the maternal genotype may be used to determine aspects of the fetal genotype. These aspects may refer to ploidy state, and/or a set of allele identities. Then, individual nucleated red blood cells may be genotyped using methods described elsewhere in this document, and other referent patents, especially those mentioned in the first section of this document. The knowledge of the maternal genome would allow one to determine whether or not any given single blood cell is genetically maternal. And the aspects of the fetal genotype that were determined as described above would allow one to determine if the single blood cell is genetically derived from the fetus that is currently gestating. In essence, this aspect of the present disclosure allows one to use the genetic knowledge of the mother, and possibly the genetic information from other related individuals, such as the father, along with the measured genetic information from the free floating DNA found in maternal blood to determine whether an isolated nucleated cell found in maternal blood is either (a) genetically maternal, (b) genetically from the fetus currently gestating, or (c) genetically from a fetus from a prior pregnancy.

Prenatal Sex Chromosome Aneuploidy Determination

In methods known in the art, people attempting to determine the sex of a gestating fetus from the blood of the mother have used the fact that fetal free floating DNA (fffDNA) is present in the plasma of the mother. If one is able to detect Y-specific loci in the maternal plasma, this implies that the gestating fetus is a male. However, the lack of detection of Y-specific loci in the plasma does not always guarantee that the gestating fetus is a female when using methods known in the art, as in some cases the amount of fffDNA is too low to ensure that the Y-specific loci would be detected in the case of a male fetus.

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Presented here is a novel method that does not require the measurement of Y-specific nucleic acids, that is, DNA that is from loci that are exclusively paternally derived. The Parental Support method, disclosed previously, uses crossover frequency data, parental genotypic data, and informatics techniques, to determine the ploidy state of a gestating fetus. The sex of a fetus is simply the ploidy state of the fetus at the sex chromosomes. A child that is XX is female, and XY is male. The method described herein is also able to determine the ploidy state of the fetus. Note that sexing is effectively synonymous with ploidy determination of the sex chromosomes; in the case of sexing, an assumption is often made that the child is euploid, therefore there are fewer possible hypotheses.

The method disclosed herein involves looking at loci that are common to both the X and Y chromosome to create a baseline in terms of expected amount of fetal DNA present for a fetus. Then, those regions that are specific only to the X chromosome can be interrogated to determine if the fetus is female or male. In the case of a male, we expect to see less fetal DNA from loci that are specific to the X chromosome than from loci that are specific to both the X and the Y. In contrast, in female fetuses, we expect the amount of DNA for each of these groups to be the same. The DNA in question can be measured by any technique that can quantitate the amount of DNA present on a sample, for example, qPCR, SNP arrays, genotyping arrays, or sequencing. For DNA that is exclusively from an individual we would expect to see the following:

	DNA specific to X	DNA specific to X and Y	DNA specific to Y
Male (XY)	A	2A	A
Female (XX)	2A	2A	0

In the case of DNA from a fetus that is mixed with DNA from the mother, and where the fraction of fetal DNA in the mixture is F, and where the fraction of maternal DNA in the mixture is M, such that F+M = 100%, we would expect to see the following:

	DNA specific to X	DNA specific to X and Y	DNA specific to Y
Male fetus (XY)	M + ½ F	M + F	½ F
Female fetus (XX)	M + F	M + F	0

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In the case where F and M are known, the expected ratios can be computed, and the observed data can be compared to the expected data. In the case where M and F are not known, a threshold can be selected based on historical data. In both cases, the measured amount of DNA at loci specific to both X and Y can be used as a baseline, and the test for the sex of the fetus can be based on the amount of DNA observed on loci specific to only the X chromosome. If that amount is lower than the baseline by an amount roughly equal to ½ F, or by an amount that causes it to fall below a predefined threshold, the fetus is determined to be male, and if that amount is about equal to the baseline, or if is not lower by an amount that causes it to fall below a predefined threshold, the fetus is determined to be female.

In another embodiment, one can look only at those loci that are common to both the X and the Y chromosomes, often termed the Z chromosome. A subset of the loci on the Z chromosome are typically always A on the X chromosome, and B on the Y chromosome. If SNPs from the Z chromosome are found to have the B genotype, then the fetus is called a male; if the SNPs from the Z chromosome are found to only have A genotype, then the fetus is called a female. In another embodiment, one can look at the loci that are found only on the X chromosome. Contexts such as AA|B are particularly informative as the presence of a B indicates that the fetus has an X chromosome from the father. Contexts such as AB|B are also informative, as we expect to see B present only half as often in the case of a female fetus as compared to a male fetus. In another embodiment, one can look at the SNPs on the Z chromosome where both A and B alleles are present on both the X and the Y chromosome, and where the it is known which SNPs are from the paternal Y chromosome, and which are from the paternal X chromosome.

In an embodiment, it is possible to amplify single nucleotide positions known to varying between the homologous non-recombining (HNR) region shared by chromosome Y and chromosome X. The sequence within this HNR region is largely identical between the X and Y chromosomes. Within this identical region are single nucleotide positions that, while invariant among X chromosomes and among Y chromosomes in the population, are different between the X and Y chromosomes. Each PCR assay could amplify a sequence from loci that are present on both the X and Y chromosomes. Within each amplified sequence would be a single base that can be detected using sequencing or some other method (*see*, for example, U.S. Publication No. 2011/0178719, filed February 3, 2011, which is hereby incorporated by reference in its entirety).

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In an embodiment, the sex of the fetus could be determined from the fetal free floating DNA found in maternal plasma, the method comprising some or all of the following steps: 1) Design PCR (either regular or mini-PCR, plus multiplexing if desired) primers amplify X/Y variant single nucleotide positions within HNR region, 2) obtain maternal plasma, 3) PCR Amplify targets from maternal plasma using HNR X/Y PCR assays, 4) sequence the amplicons, 5) Examine sequence data for presence of Y-allele within one or more of the amplified sequences. The presence of one or more would indicate a male fetus. Absence of all Y-alleles from all amplicons indicates a female fetus.

In an embodiment, one could use targeted sequencing to measure the DNA in the maternal plasma and/or the parental genotypes. In an embodiment, one could ignore all sequences that clearly originate from paternally sourced DNA. For example, in the context AA|AB, one could count the number of A sequences and ignore all the B sequences. In order to determine a heterozygosity rate for the above algorithm, one could compare the number of observed A sequences to the expected number of total sequences for the given probe. There are many ways one could calculate an expected number of sequences for each probe on a per sample basis. In an embodiment, it is possible to use historical data to determine what fraction of all sequence reads belongs to each specific probe and then use this empirical fraction, combined with the total number of sequence reads, to estimate the number of sequences at each probe. Another approach could be to target some known homozygous alleles and then use historical data to relate the number of reads at each probe with the number of reads at the known homozygous alleles. For each sample, one could then measure the number of reads at the homozygous alleles and then use this measurement, along with the empirically derived relationships, to estimate the number of sequence reads at each probe.

In some embodiments, it is possible to determine the sex of the fetus by combining the predictions made by a plurality of methods. In some embodiments the plurality of methods are taken from methods described in this disclosure. In some embodiments, at least one of the plurality of methods are taken from methods described in this disclosure.

In some embodiments the method described herein can be used to determine the ploidy state of the gestating fetus. In an embodiment, the ploidy calling method uses loci that are specific to the X chromosome, or common to both the X and Y chromosome, but does not make use of any Y-specific loci. In an embodiment, the ploidy calling method uses one or more of the following: loci that are specific to the X chromosome, loci that are common to

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both the X and Y chromosome, and loci that are specific to the Y chromosome. In an embodiment, where the ratios of sex chromosomes are similar, for example 45,X (Turner Syndrome), 46,XX (normal female) and 47,XXX (trisomy X), the differentiation can be accomplished by comparing the allele distributions to expected allele distributions according to the various hypotheses. In another embodiment, this can be accomplished by comparing the relative number of sequence reads for the sex chromosomes to one or a plurality of reference chromosomes that are assumed to be euploid. Also note that these methods can be expanded to include aneuploid cases.

Single Gene Disease Screening

In an embodiment, a method for determining the ploidy state of the fetus may be extended to enable simultaneous testing for single gene disorders. Single-gene disease diagnosis leverages the same targeted approach used for aneuploidy testing, and requires additional specific targets. In an embodiment, the single gene NPD diagnosis is through linkage analysis. In many cases, direct testing of the cfDNA sample is not reliable, as the presence of maternal DNA makes it virtually impossible to determine if the fetus has inherited the mother's mutation. Detection of a unique paternally-derived allele is less challenging, but is only fully informative if the disease is dominant and carried by the father, limiting the utility of the approach. In an embodiment, the method involves PCR or related amplification approaches.

In some embodiments, the method involves phasing the abnormal allele with surrounding very tightly linked SNPs in the parents using information from first-degree relatives. Then Parental Support may be run on the targeted sequencing data obtained from these SNPs to determine which homologs, normal or abnormal, were inherited by the fetus from both parents. As long as the SNPs are sufficiently linked, the inheritance of the genotype of the fetus can be determined very reliably. In some embodiments, the method comprises (a) adding a set of SNP loci to densely flank a specified set of common diseases to our multiplex pool for aneuploidy testing; (b) reliably phasing the alleles from these added SNPs with the normal and abnormal alleles based on genetic data from various relatives; and (c) reconstructing the fetal haplotype, or set of phased SNP alleles on the inherited maternal and paternal homologs in the region surrounding the disease locus to determine fetal genotype. In

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some embodiments additional probes that are closely linked to a disease linked locus are added to the set of polymorphic locus being used for aneuploidy testing.

Reconstructing fetal diplotype is challenging because the sample is a mixture of maternal and fetal DNA. In some embodiments, the method incorporates relative information to phase the SNPs and disease alleles, then take into account physical distance of the SNPs and recombination data from location specific recombination likelihoods and the data observed from the genetic measurements of the maternal plasma to obtain the most likely genotype of the fetus.

In an embodiment, a number of additional probes per disease linked locus are included in the set of targeted polymorphic loci; the number of additional probes per disease linked locus may be between 4 and 10, between 11 and 20, between 21 and 40, between 41 and 60, between 61 and 80, or combinations thereof.

Phasing the diploid data from the parents can be challenging, and there are a number of ways this can be accomplished. Some are discussed in this disclosure, others are described in greater detail in other disclosures (*see*, *e.g.*, PCT Publ. No. WO2009105531, filed February 9, 2009, and PCT Publ. No. WO2010017214, filed August 4, 2009, which are each hereby incorporated by reference in its entirety). In one embodiment, a parent can be phased by inference by measuring tissue from the parent that is haploid, for example by measuring one or more sperm or eggs. In one embodiment the parent can be phased by inference using the measured genotypic data of a first degree relative such as the parent's parent(s) or siblings. In one embodiment, the parent can be phased by dilution where the DNA is diluted, in one or a plurality of wells, to the point where there is expected to be no more than approximately one copy of each haplotype in each well, and then measuring the DNA in the one or more wells. In one embodiment, the parent genotype can be phased by using computer programs that use population based haplotype frequencies to infer the most likely phase. In one embodiment, the parent can be phased if the phased haplotypic data is known for the other parent, along with the unphased genetic data of one or more genetic offspring of the parents. In some embodiments, the genetic offspring of the parents may be one or more embryos, fetuses, and/or born children. Some of these methods and other methods for phasing one or both parents are disclosed in greater detail in, *e.g.*, U.S. Publ. No. 2011/0033862, filed August 19, 2010; U.S. Publ. No. 2011/0178719, filed Feb. 3, 2011; U.S. Publ. No. 2007/0184467, filed Nov. 22, 2006; U.S.

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Publ. No. 2008/0243398, filed March 17, 2008, which are each hereby incorporated by reference in its entirety.

Fetal Genome Reconstruction

In one aspect, the invention features methods for determining a haplotype of a fetus. In various embodiments, this method allows one to determine which polymorphic loci (such as SNPs) were inherited by the fetus and to reconstruct which homologs (including recombination events) are present in the fetus (and thereby interpolate the sequence between the polymorphic loci). If desired, essentially the entire genome of the fetus can be reconstructed. If there is some remaining ambiguity in the genome of the fetus (such as in intervals with a crossover), this ambiguity can be minimized if desired by analyzing additional polymorphic loci. In various embodiments, the polymorphic loci are chosen to cover one or more of the chromosomes at a density to reduce any ambiguity to a desired level. This method has important applications for the detection of polymorphisms or other mutations of interest in a fetus since it enables their detection based on linkage (such as the presence of linked polymorphic loci in the fetal genome) rather than by directing detecting the polymorphism or other mutation of interest in the fetal genome. For example, if a parent is a carrier for a mutation associated with cystic fibrosis (CF), a nucleic acid sample that includes maternal DNA from the mother of the fetus and fetal DNA from the fetus can be analyzed to determine whether the fetal DNA include the haplotype containing the CF mutation. In particular, polymorphic loci can be analyzed to determine whether the fetal DNA includes the haplotype containing the CF mutation without having to detect the CF mutation itself in the fetal DNA.

In some embodiments, the method involves determining a parental haplotype (*e.g.*, a haplotype of the mother or father of the fetus). In some embodiments, this determination is made without using data from a relative of the mother or father. In some embodiments, a parental haplotype is determined using a dilution approach followed by SNP genotyping or sequencing as described herein and elsewhere (*see*, *e.g.*, U.S. Publ. No. 2011/0033862, filed August 19, 2010, which is hereby incorporated by reference in its entirety). Because the DNA is diluted, it is unlikely that more than one haplotype is in the same fraction (or tube). Thus, there may be effectively a single molecule of DNA in the tube, which allows the haplotype on a single DNA molecule to be determined. In some embodiments, the method includes dividing a DNA sample into a plurality of fractions such that at least one of the fractions includes one

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chromosome or one chromosome segment from a pair of chromosomes, and genotyping (*e.g.*, determining the presence of two or more polymorphic loci) the DNA sample in at least one of the fractions, thereby determining a parental haplotype. In some embodiments, the genotyping involves sequencing (such as shotgun sequencing). In some embodiments, the genotyping involves use of a SNP array to detect polymorphic loci, such as at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different polymorphic loci. In some embodiments, the genotyping involves the use of multiplex PCR. In some embodiments, the method involves contacting the sample in a fraction with a library of primers that simultaneously hybridize to at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different polymorphic loci (such as SNPs) to produce a reaction mixture; and subjecting the reaction mixture to primer extension reaction conditions to produce amplified products that are measured with a high throughput sequencer to produce sequencing data.

In some embodiments, a haplotype of the mother is determined by any of the methods described herein using data from a relative of the mother. In some embodiments, a haplotype of the father is determined by any of the methods described herein using data from a relative of the father. In some embodiments, a haplotype is determined for both the father and the mother. In some embodiments, a SNP array is used to determine the presence of at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different polymorphic loci in a DNA sample from the mother (or father) and a relative of the mother (or father). In some embodiments, the method involves contacting a DNA sample from the mother (or father) and/or a relative of the mother (or father) with a library of primers that simultaneously hybridize to at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different polymorphic loci (such as SNPs) to produce a reaction mixture; and subjecting the reaction mixture to primer extension reaction conditions to produce amplified products that are measured with a high throughput sequencer to produce sequencing data. The parental haplotype may be determined based on the SNP array or sequencing data. In some embodiments, parental data may be phased by methods described or referred to elsewhere in this document.

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This parental haplotype data can be used to determine if the fetus inherited the parental haplotype. In some embodiments, a nucleic acid sample that includes maternal DNA from the mother of the fetus and fetal DNA from the fetus is analyzed using a SNP array to detect at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different polymorphic loci. In some embodiments, a nucleic acid sample that includes maternal DNA from the mother of the fetus and fetal DNA from the fetus is analyzed by contacting the sample with a library of primers that simultaneously hybridize to at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different polymorphic loci (such as SNPs) to produce a reaction mixture. In some embodiments, the reaction mixture is subjected to primer extension reaction conditions to produce amplified products. In some embodiments, the amplified products are measured with a high throughput sequencer to produce sequencing data. In various embodiments, the SNP array or sequencing data is used to determine a parental haplotype by using data about the probability of chromosomes crossing over at different locations in a chromosome (such as by using recombination data such as may be found in the HapMap database to create a recombination risk score for any interval) to model dependence between polymorphic alleles on the chromosome. In some embodiments, allele counts at the polymorphic loci are calculated on a computer based on the sequencing data. In some embodiments, a plurality of ploidy hypotheses each pertaining to a different possible ploidy state of the chromosome are created on a computer; a model (such as a joint distribution model) for the expected allele counts at the polymorphic loci on the chromosome is built on a computer for each ploidy hypothesis; a relative probability of each of the ploidy hypotheses is determined on a computer using the joint distribution model and the allele counts; and the ploidy state of the fetus is called by selecting the ploidy state corresponding to the hypothesis with the greatest probability. In some embodiments, building a joint distribution model for allele counts and the step of determining the relative probability of each hypothesis are done using a method that does not require the use of a reference chromosome.

In some embodiments, a fetal haplotype is determined for one or more chromosomes taken from the group consisting of chromosomes 13, 18, 21, X, and Y. In some embodiments, a fetal haplotype is determined for all of the fetal chromosomes. In various embodiments, the method determines essentially the entire genome of the fetus. In some embodiments, the

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haplotype is determined for at least 30, 40, 50, 60, 70, 80, 90, or 95% of the genome of the fetus. In some embodiments, the haplotype determination of the fetus includes information about which allele is present for at least 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different polymorphic loci.

Compositions of DNA

When performing an informatics analysis on sequencing data measured on a mixture of fetal and maternal blood to determine genomic information pertaining to the fetus, for example the ploidy state of the fetus, it may be advantageous to measure the allele distributions at a set of alleles. Unfortunately, in many cases, such as when attempting to determine the ploidy state of a fetus from the DNA mixture found in the plasma of a maternal blood sample, the amount of DNA available is not sufficient to directly measure the allele distributions with good fidelity in the mixture. In these cases, amplification of the DNA mixture will provide sufficient numbers of DNA molecules that the desired allele distributions may be measured with good fidelity. However, current methods of amplification typically used in the amplification of DNA for sequencing are often very biased, meaning that they do not amplify both alleles at a polymorphic locus by the same amount. A biased amplification can result in allele distributions that are quite different from the allele distributions in the original mixture. For most purposes, highly accurate measurements of the relative amounts of alleles present at polymorphic loci are not needed. In contrast, in an embodiment of the present disclosure, amplification or enrichment methods that specifically enrich polymorphic alleles and preserve allelic ratios is advantageous.

A number of methods are described herein that may be used to preferentially enrich a sample of DNA at a plurality of loci in a way that minimizes allelic bias. Some examples are using circularizing probes to target a plurality of loci where the 3' ends and 5' ends of the pre-circularized probe are designed to hybridize to bases that are one or a few positions away from the polymorphic sites of the targeted allele. Another is to use PCR probes where the 3' end PCR probe is designed to hybridize to bases that are one or a few positions away from the polymorphic sites of the targeted allele. Another is to use a split and pool approach to create mixtures of DNA where the preferentially enriched loci are enriched with low allelic bias without the drawbacks of direct multiplexing. Another is to use a hybrid capture approach

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where the capture probes are designed such that the region of the capture probe that is designed to hybridize to the DNA flanking the polymorphic site of the target is separated from the polymorphic site by one or a small number of bases.

In the case where measured allele distributions at a set of polymorphic loci are used to determine the ploidy state of an individual, it is desirable to preserve the relative amounts of alleles in a sample of DNA as it is prepared for genetic measurements. This preparation may involve WGA amplification, targeted amplification, selective enrichment techniques, hybrid capture techniques, circularizing probes or other methods meant to amplify the amount of DNA and/or selectively enhance the presence of molecules of DNA that correspond to certain alleles.

In some embodiments of the present disclosure, there is a set of DNA probes designed to target loci where the loci have maximal minor allele frequencies. In some embodiments of the present disclosure, there is a set of probes that are designed to target where the loci have the maximum likelihood of the fetus having a highly informative SNP at those loci. In some embodiments of the present disclosure, there is a set of probes that are designed to target loci where the probes are optimized for a given population subgroup. In some embodiments of the present disclosure, there is a set of probes that are designed to target loci where the probes are optimized for a given mix of population subgroups. In some embodiments of the present disclosure, there is a set of probes that are designed to target loci where the probes are optimized for a given pair of parents which are from different population subgroups that have different minor allele frequency profiles. In some embodiments of the present disclosure, there is a circularized strand of DNA that comprises at least one base pair that annealed to a piece of DNA that is of fetal origin. In some embodiments of the present disclosure, there is a circularized strand of DNA that comprises at least one base pair that annealed to a piece of DNA that is of placental origin. In some embodiments of the present disclosure, there is a circularized strand of DNA that circularized while at least some of the nucleotides were annealed to DNA that was of fetal origin. In some embodiments of the present disclosure, there is a circularized strand of DNA that circularized while at least some of the nucleotides were annealed to DNA that was of placental origin. In some embodiments of the present disclosure, there is a set of probes wherein some of the probes target single tandem repeats, and some of the probes target single nucleotide polymorphisms. In some embodiments, the loci are selected for the purpose of non-invasive prenatal diagnosis. In some embodiments, the probes are used

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for the purpose of non-invasive prenatal diagnosis. In some embodiments, the loci are targeted using a method that could include circularizing probes, MIPs, capture by hybridization probes, probes on a SNP array, or combinations thereof. In some embodiments, the probes are used as circularizing probes, MIPs, capture by hybridization probes, probes on a SNP array, or combinations thereof. In some embodiments, the loci are sequenced for the purpose of non-invasive prenatal diagnosis.

In the case where the relative informativeness of a sequence is greater when combined with relevant parent contexts, it follows that maximizing the number of sequence reads that contain a SNP for which the parental context is known may maximize the informativeness of the set of sequencing reads on the mixed sample. In an embodiment, the number of sequence reads that contain a SNP for which the parent contexts are known may be enhanced by using qPCR to preferentially amplify specific sequences. In an embodiment, the number of sequence reads that contain a SNP for which the parent contexts are known may be enhanced by using circularizing probes (for example, MIPs) to preferentially amplify specific sequences. In an embodiment, the number of sequence reads that contain a SNP for which the parent contexts are known may be enhanced by using a capture by hybridization method (for example SURESELECT) to preferentially amplify specific sequences. Different methods may be used to enhance the number of sequence reads that contain a SNP for which the parent contexts are known. In an embodiment, the targeting may be accomplished by extension ligation, ligation without extension, capture by hybridization, or PCR.

In a sample of fragmented genomic DNA, a fraction of the DNA sequences map uniquely to individual chromosomes; other DNA sequences may be found on different chromosomes. Note that DNA found in plasma, whether maternal or fetal in origin is typically fragmented, often at lengths under 500 bp. In a typical genomic sample, roughly 3.3% of the mappable sequences will map to chromosome 13; 2.2% of the mappable sequences will map to chromosome 18; 1.35% of the mappable sequences will map to chromosome 21; 4.5% of the mappable sequences will map to chromosome X in a female; 2.25% of the mappable sequences will map to chromosome X (in a male); and 0.73% of the mappable sequences will map to chromosome Y (in a male). These are the chromosomes that are most likely to be aneuploid in a fetus. Also, among short sequences, approximately 1 in 20 sequences will contain a SNP, using the SNPs contained on dbSNP. The proportion may well be higher given that there may be many SNPs that have not been discovered.

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In an embodiment of the present disclosure, targeting methods may be used to enhance the fraction of DNA in a sample of DNA that map to a given chromosome such that the fraction significantly exceeds the percentages listed above that are typical for genomic samples. In an embodiment of the present disclosure, targeting methods may be used to enhance the fraction of DNA in a sample of DNA such that the percentage of sequences that contain a SNP are significantly greater than what may be found in typical for genomic samples. In an embodiment of the present disclosure, targeting methods may be used to target DNA from a chromosome or from a set of SNPs in a mixture of maternal and fetal DNA for the purposes of prenatal diagnosis.

Note that a method has been reported (U.S. Patent 7,888,017) for determining fetal aneuploidy by counting the number of reads that map to a suspect chromosome and comparing it to the number of reads that map to a reference chromosome, and using the assumption that an overabundance of reads on the suspect chromosome corresponds to a triploidy in the fetus at that chromosome. Those methods for prenatal diagnosis would not make use of targeting of any sort, nor do they describe the use of targeting for prenatal diagnosis.

By making use of targeting approaches in sequencing the mixed sample, it may be possible to achieve a certain level of accuracy with fewer sequence reads. The accuracy may refer to sensitivity, it may refer to specificity, or it may refer to some combination thereof. The desired level of accuracy may be between 90% and 95%; it may be between 95% and 98%; it may be between 98% and 99%; it may be between 99% and 99.5%; it may be between 99.5% and 99.9%; it may be between 99.9% and 99.99%; it may be between 99.99% and 99.999%, it may be between 99.999% and 100%. Levels of accuracy above 95% may be referred to as high accuracy.

There are a number of published methods in the prior art that demonstrate how one may determine the ploidy state of a fetus from a mixed sample of maternal and fetal DNA, for example: G.J. W. Liao et al. Clinical Chemistry 2011; 57(1) pp. 92-101. These methods focus on thousands of locations along each chromosome. The number of locations along a chromosome that may be targeted while still resulting in a high accuracy ploidy determination on a fetus, for a given number of sequence reads, from a mixed sample of DNA is unexpectedly low. In an embodiment of the present disclosure, an accurate ploidy determination may be made by using targeted sequencing, using any method of targeting, for example qPCR, ligand mediated PCR, other PCR methods, capture by hybridization, or

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circularizing probes, wherein the number of loci along a chromosome that need to be targeted may be between 5,000 and 2,000 loci; it may be between 2,000 and 1,000 loci; it may be between 1,000 and 500 loci; it may be between 500 and 300 loci; it may be between 300 and 200 loci; it may be between 200 and 150 loci; it may be between 150 and 100 loci; it may be between 100 and 50 loci; it may be between 50 and 20 loci; it may be between 20 and 10 loci. Optimally, it may be between 100 and 500 loci. The high level of accuracy may be achieved by targeting a small number of loci and executing an unexpectedly small number of sequence reads. The number of reads may be between 100 million and 50 million reads; the number of reads may be between 50 million and 20 million reads; the number of reads may be between 20 million and 10 million reads; the number of reads may be between 10 million and 5 million reads; the number of reads may be between 5 million and 2 million reads; the number of reads may be between 2 million and 1 million; the number of reads may be between 1 million and 500,000; the number of reads may be between 500,000 and 200,000; the number of reads may be between 200,000 and 100,000; the number of reads may be between 100,000 and 50,000; the number of reads may be between 50,000 and 20,000; the number of reads may be between 20,000 and 10,000; the number of reads may be below 10,000. Fewer number of read are necessary for larger amounts of input DNA.

In some embodiments, there is a composition comprising a mixture of DNA of fetal origin, and DNA of maternal origin, wherein the percent of sequences that uniquely map to chromosome 13 is greater than 4%, greater than 5%, greater than 6%, greater than 7%, greater than 8%, greater than 9%, greater than 10%, greater than 12%, greater than 15%, greater than 20%, greater than 25%, or greater than 30%. In some embodiments of the present disclosure, there is a composition comprising a mixture of DNA of fetal origin, and DNA of maternal origin, wherein the percent of sequences that uniquely map to chromosome 18 is greater than 3%, greater than 4%, greater than 5%, greater than 6%, greater than 7%, greater than 8%, greater than 9%, greater than 10%, greater than 12%, greater than 15%, greater than 20%, greater than 25%, or greater than 30%. In some embodiments of the present disclosure, there is a composition comprising a mixture of DNA of fetal origin, and DNA of maternal origin, wherein the percent of sequences that uniquely map to chromosome 21 is greater than 2%, greater than 3%, greater than 4%, greater than 5%, greater than 6%, greater than 7%, greater than 8%, greater than 9%, greater than 10%, greater than 12%, greater than 15%, greater than 20%, greater than 25%, or greater than 30%. In some embodiments of the present disclosure,

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there is a composition comprising a mixture of DNA of fetal origin, and DNA of maternal origin, wherein the percent of sequences that uniquely map to chromosome X is greater than 6%, greater than 7%, greater than 8%, greater than 9%, greater than 10%, greater than 12%, greater than 15%, greater than 20%, greater than 25%, or greater than 30%. In some embodiments of the present disclosure, there is a composition comprising a mixture of DNA of fetal origin, and DNA of maternal origin, wherein the percent of sequences that uniquely map to chromosome Y is greater than 1%, greater than 2%, greater than 3%, greater than 4%, greater than 5%, greater than 6%, greater than 7%, greater than 8%, greater than 9%, greater than 10%, greater than 12%, greater than 15%, greater than 20%, greater than 25%, or greater than 30%.

In some embodiments, a composition is described comprising a mixture of DNA of fetal origin, and DNA of maternal origin, wherein the percent of sequences that uniquely map to a chromosome, and that contains at least one single nucleotide polymorphism is greater than 0.2%, greater than 0.3%, greater than 0.4%, greater than 0.5%, greater than 0.6%, greater than 0.7%, greater than 0.8%, greater than 0.9%, greater than 1%, greater than 1.2%, greater than 1.4%, greater than 1.6%, greater than 1.8%, greater than 2%, greater than 2.5%, greater than 3%, greater than 4%, greater than 5%, greater than 6%, greater than 7%, greater than 8%, greater than 9%, greater than 10%, greater than 12%, greater than 15%, or greater than 20%, and where the chromosome is taken from the group 13, 18, 21, X, or Y. In some embodiments of the present disclosure, there is a composition comprising a mixture of DNA of fetal origin, and DNA of maternal origin, wherein the percent of sequences that uniquely map to a chromosome and that contain at least one single nucleotide polymorphism from a set of single nucleotide polymorphisms is greater than 0.15%, greater than 0.2%, greater than 0.3%, greater than 0.4%, greater than 0.5%, greater than 0.6%, greater than 0.7%, greater than 0.8%, greater than 0.9%, greater than 1%, greater than 1.2%, greater than 1.4%, greater than 1.6%, greater than 1.8%, greater than 2%, greater than 2.5%, greater than 3%, greater than 4%, greater than 5%, greater than 6%, greater than 7%, greater than 8%, greater than 9%, greater than 10%, greater than 12%, greater than 15%, or greater than 20%, where the chromosome is taken from the set of chromosome 13, 18, 21, X and Y, and where the number of single nucleotide polymorphisms in the set of single nucleotide polymorphisms is between 1 and 10, between 10 and 20, between 20 and 50, between 50 and 100, between 100 and 200, between 200 and 500, between 500 and 1,000, between 1,000 and 2,000, between 2,000 and 5,000, between 5,000

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and 10,000, between 10,000 and 20,000, between 20,000 and 50,000, and between 50,000 and 100,000.

In theory, each cycle in the amplification doubles the amount of DNA present; however, in reality, the degree of amplification is slightly lower than two. In theory, amplification, including targeted amplification, will result in bias free amplification of a DNA mixture; in reality, however, different alleles tend to be amplified to a different extent than other alleles. When DNA is amplified, the degree of allelic bias typically increases with the number of amplification steps. In some embodiments, the methods described herein involve amplifying DNA with a low level of allelic bias. Since the allelic bias compounds with each additional cycle, one can determine the per cycle allelic bias by calculating the nth root of the overall bias where n is the base 2 logarithm of degree of enrichment. In some embodiments, there is a composition comprising a second mixture of DNA, where the second mixture of DNA has been preferentially enriched at a plurality of polymorphic loci from a first mixture of DNA where the degree of enrichment is at least 10, at least 100, at least 1,000, at least 10,000, at least 100,000 or at least 1,000,000, and where the ratio of the alleles in the second mixture of DNA at each locus differs from the ratio of the alleles at that locus in the first mixture of DNA by a factor that is, on average, less than 1,000%, 500%, 200%, 100%, 50%, 20%, 10%, 5%, 2%, 1%, 0.5%, 0.2%, 0.1%, 0.05%, 0.02%, or 0.01%. In some embodiments, there is a composition comprising a second mixture of DNA, where the second mixture of DNA has been preferentially enriched at a plurality of polymorphic loci from a first mixture of DNA where the per cycle allelic bias for the plurality of polymorphic loci is, on average, less than 10%, 5%, 2%, 1%, 0.5%, 0.2%, 0.1%, 0.05%, or 0.02%. In some embodiments, the plurality of polymorphic loci comprises at least 10 loci, at least 20 loci, at least 50 loci, at least 100 loci, at least 200 loci, at least 500 loci, at least 1,000 loci, at least 2,000 loci, at least 5,000 loci, at least 10,000 loci, at least 20,000 loci, or at least 50,000 loci.

Some Embodiments

In some embodiments, a method is disclosed herein for generating a report disclosing the determined ploidy status of a chromosome in a gestating fetus, the method comprising: obtaining a first sample that contains DNA from the mother of the fetus and DNA from the fetus; obtaining genotypic data from one or both parents of the fetus; preparing the first sample by isolating the DNA so as to obtain a prepared sample; measuring the DNA in the prepared

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sample at a plurality of polymorphic loci; calculating, on a computer, allele counts or allele count probabilities at the plurality of polymorphic loci from the DNA measurements made on the prepared sample; creating, on a computer, a plurality of ploidy hypotheses concerning expected allele count probabilities at the plurality of polymorphic loci on the chromosome for different possible ploidy states of the chromosome; building, on a computer, a joint distribution model for allele count probability of each polymorphic locus on the chromosome for each ploidy hypothesis using genotypic data from the one or both parents of the fetus; determining, on a computer, a relative probability of each of the ploidy hypotheses using the joint distribution model and the allele count probabilities calculated for the prepared sample; calling the ploidy state of the fetus by selecting the ploidy state corresponding to the hypothesis with the greatest probability; and generating a report disclosing the determined ploidy status.

In some embodiments, the method is used to determine the ploidy state of a plurality of gestating fetuses in a plurality of respective mothers, the method further comprising: determining the percent of DNA that is of fetal origin in each of the prepared samples; and wherein the step of measuring the DNA in the prepared sample is done by sequencing a number of DNA molecules in each of the prepared samples, where more molecules of DNA are sequenced from those prepared samples that have a smaller fraction of fetal DNA than those prepared samples that have a larger fraction of fetal DNA.

In some embodiments, the method is used to determine the ploidy state of a plurality of gestating fetuses in a plurality of respective mothers, and where the measuring the DNA in the prepared sample is done, for each of the fetuses, by sequencing a first fraction of the prepared sample of DNA to give a first set of measurements, the method further comprising: making a first relative probability determination for each of the ploidy hypotheses for each of the fetuses, given the first set of DNA measurements; resequencing a second fraction of the prepared sample from those fetuses where the first relative probability determination for each of the ploidy hypotheses indicates that a ploidy hypothesis corresponding to an aneuploid fetus has a significant but not conclusive probability, to give a second set of measurements; making a second relative probability determination for ploidy hypotheses for the fetuses using the second set of measurements and optionally also the first set of measurements; and calling the ploidy states of the fetuses whose second sample was resequenced by selecting the ploidy state

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corresponding to the hypothesis with the greatest probability as determined by the second relative probability determination.

In some embodiments, a composition of matter is disclosed, the composition of matter comprising: a sample of preferentially enriched DNA, wherein the sample of preferentially enriched DNA has been preferentially enriched at a plurality of polymorphic loci from a first sample of DNA, wherein the first sample of DNA consisted of a mixture of maternal DNA and fetal DNA derived from maternal plasma, where the degree of enrichment is at least a factor of 2, and wherein the allelic bias between the first sample and the preferentially enriched sample is, on average, selected from the group consisting of less than 2%, less than 1%, less than 0.5%, less than 0.2%, less than 0.1%, less than 0.05%, less than 0.02%, and less than 0.01%. In some embodiments, a method is disclosed to create a sample of such preferentially enriched DNA.

In some embodiment, a method is disclosed for determining the presence or absence of a fetal aneuploidy in a maternal tissue sample comprising fetal and maternal genomic DNA, wherein the method comprises: (a) obtaining a mixture of fetal and maternal genomic DNA from said maternal tissue sample; (b) selectively enriching the mixture of fetal and maternal DNA at a plurality of polymorphic alleles; (c) distributing selectively enriched fragments from the mixture of fetal and maternal genomic DNA of step a to provide reaction samples comprising a single genomic DNA molecule or amplification products of a single genomic DNA molecule; (d) conducting massively parallel DNA sequencing of the selectively enriched fragments of genomic DNA in the reaction samples of step c) to determine the sequence of said selectively enriched fragments; (e) identifying the chromosomes to which the sequences obtained in step d) belong; (f) analyzing the data of step d) to determine i) the number of fragments of genomic DNA from step d) that belong to at least one first target chromosome that is presumed to be diploid in both the mother and the fetus, and ii) the number of fragments of genomic DNA from step d) that belong to a second target chromosome, wherein said second chromosome is suspected to be aneuploid in the fetus; (g) calculating an expected distribution of the number of fragments of genomic DNA from step d) for the second target chromosome if the second target chromosome is euploid, using the number determined in step f) part i); (h) calculating an expected distribution of the number of fragments of genomic DNA from step d) for the second target chromosome if the second target chromosome is aneuploid, using the first number is step f) part i) and an estimated fraction of fetal DNA found in the mixture of step b);

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and (i) using a maximum likelihood or maximum a posteriori approach to determine whether the number of fragments of genomic DNA determined in step f) part ii) is more likely to be part of the distribution calculated in step g) or the distribution calculated in step h); thereby indicating the presence or absence of a fetal aneuploidy.

Exemplary Cancer Diagnostic Methods

Note that it has been demonstrated that DNA that originated from cancer that is living in a host can be found in the blood of the host. In the same way that genetic diagnoses can be made from the measurement of mixed DNA found in maternal blood, genetic diagnoses can equally well be made from the measurement of mixed DNA found in host blood. The genetic diagnoses may include aneuploidy states, or gene mutations. Any claim in the instant disclosure that reads on determining the ploidy state or genetic state of a fetus from the measurements made on maternal blood can equally well read on determining the ploidy state or genetic state of a cancer from the measurements on host blood.

In some embodiments, a method of the present disclosure allows one to determine the ploidy status of a cancer, the method including obtaining a mixed sample that contains genetic material from the host, and genetic material from the cancer; measuring the DNA in the mixed sample; calculating the fraction of DNA that is of cancer origin in the mixed sample; and determining the ploidy status of the cancer using the measurements made on the mixed sample and the calculated fraction. In some embodiments, the method may further include administering a cancer therapeutic based on the determination of the ploidy state of the cancer. In some embodiments, the method may further include administering a cancer therapeutic based on the determination of the ploidy state of the cancer, wherein the cancer therapeutic is taken from the group comprising a pharmaceutical, a biologic therapeutic, and antibody based therapy and combination thereof.

Exemplary Implementation Methods

Any of the embodiments disclosed herein may be implemented in digital electronic circuitry, integrated circuitry, specially designed ASICs (application-specific integrated circuits), computer hardware, firmware, software, or in combinations thereof. Apparatus of the presently disclosed embodiments can be implemented in a computer program product tangibly embodied in a machine-readable storage device for execution by a programmable processor;

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and method steps of the presently disclosed embodiments can be performed by a programmable processor executing a program of instructions to perform functions of the presently disclosed embodiments by operating on input data and generating output. The presently disclosed embodiments can be implemented advantageously in one or more computer programs that are executable and/or interpretable on a programmable system including at least one programmable processor, which may be special or general purpose, coupled to receive data and instructions from, and to transmit data and instructions to, a storage system, at least one input device, and at least one output device. Each computer program can be implemented in a high-level procedural or object-oriented programming language or in assembly or machine language if desired; and in any case, the language can be a compiled or interpreted language. A computer program may be deployed in any form, including as a stand-alone program, or as a module, component, subroutine, or other unit suitable for use in a computing environment. A computer program may be deployed to be executed or interpreted on one computer or on multiple computers at one site, or distributed across multiple sites and interconnected by a communication network.

Computer readable storage media, as used herein, refers to physical or tangible storage (as opposed to signals) and includes without limitation volatile and non-volatile, removable and non-removable media implemented in any method or technology for the tangible storage of information such as computer-readable instructions, data structures, program modules or other data. Computer readable storage media includes, but is not limited to, RAM, ROM, EPROM, EEPROM, flash memory or other solid state memory technology, CD-ROM, DVD, or other optical storage, magnetic cassettes, magnetic tape, magnetic disk storage or other magnetic storage devices, or any other physical or material medium which can be used to tangibly store the desired information or data or instructions and which can be accessed by a computer or processor.

Any of the methods described herein may include the output of data in a physical format, such as on a computer screen, or on a paper printout. In explanations of any embodiments elsewhere in this document, it should be understood that the described methods may be combined with the output of the actionable data in a format that can be acted upon by a physician. In addition, the described methods may be combined with the actual execution of a clinical decision that results in a clinical treatment, or the execution of a clinical decision to make no action. Some of the embodiments described in the document for determining genetic

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data pertaining to a target individual may be combined with the decision to select one or more embryos for transfer in the context of IVF, optionally combined with the process of transferring the embryo to the womb of the prospective mother. Some of the embodiments described in the document for determining genetic data pertaining to a target individual may be combined with the notification of a potential chromosomal abnormality, or lack thereof, with a medical professional, optionally combined with the decision to abort, or to not abort, a fetus in the context of prenatal diagnosis. Some of the embodiments described herein may be combined with the output of the actionable data, and the execution of a clinical decision that results in a clinical treatment, or the execution of a clinical decision to make no action.

Exemplary Diagnostic Boxes

In an embodiment, the present disclosure comprises a diagnostic box that is capable of partly or completely carrying out any of the methods described in this disclosure. In an embodiment, the diagnostic box may be located at a physician's office, a hospital laboratory, or any suitable location reasonably proximal to the point of patient care. The box may be able to run the entire method in a wholly automated fashion, or the box may require one or a number of steps to be completed manually by a technician. In an embodiment, the box may be able to analyze at least the genotypic data measured on the maternal plasma. In an embodiment, the box may be linked to means to transmit the genotypic data measured on the diagnostic box to an external computation facility which may then analyze the genotypic data, and possibly also generate a report. The diagnostic box may include a robotic unit that is capable of transferring aqueous or liquid samples from one container to another. It may comprise a number of reagents, both solid and liquid. It may comprise a high throughput sequencer. It may comprise a computer.

Experimental Section

The presently disclosed embodiments are described in the following Examples, which are set forth to aid in the understanding of the disclosure, and should not be construed to limit in any way the scope of the disclosure as defined in the claims which follow thereafter. The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to use the described embodiments, and are not intended to limit the scope of the disclosure nor are they intended to represent that the

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experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.* amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by volume, and temperature is in degrees Centigrade. It should be understood that variations in the methods as described may be made without changing the fundamental aspects that the experiments are meant to illustrate.

Experiment 1

The objective was to show that a Bayesian maximum likelihood estimation (MLE) algorithm that uses parent genotypes to calculate fetal fraction improves accuracy of non-invasive prenatal trisomy diagnosis compared to published methods.

Simulated sequencing data for maternal cfDNA was created by sampling reads obtained on trisomy-21 and respective mother cell lines. The rate of correct disomy and trisomy calls were determined from 500 simulations at various fetal fractions for a published method (Chiu et al. BMJ 2011;342:c7401) and our MLE-based algorithm. We validated the simulations by obtaining 5 million shotgun reads from four pregnant mothers and respective fathers collected under an IRB-approved protocol. Parental genotypes were obtained on a 290K SNP array. (See FIG. 14)

In simulations, the MLE-based approach achieved 99.0% accuracy for fetal fractions as low as 9% and reported confidences that corresponded well to overall accuracy. We validated these results using four real samples wherein we obtained all correct calls with a computed confidence exceeding 99%. In contrast, our implementation of the published algorithm for Chiu et al. required 18% fetal fraction to achieve 99.0% accuracy, and achieved only 87.8% accuracy at 9% fetal DNA.

Fetal fraction determination from parental genotypes in conjunction with a MLE-based approach achieves greater accuracy than published algorithms at the fetal fractions expected during the 1st and early 2nd trimester. Furthermore, the method disclosed herein produces a confidence metric that is crucial in determining the reliability of the result, especially at low fetal fractions where ploidy detection is more difficult. Published methods use a less accurate threshold method for calling ploidy based on large sets of disomy training data, an approach that predefines a false positive rate. In addition, without a confidence metric, published methods are at risk of reporting false negative results when there is insufficient fetal cfDNA to

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make a call. In some embodiments, a confidence estimate is calculated for the called ploidy state.

Experiment 2

The objective was to improve non-invasive detection of fetal trisomy 18, 21, and X particularly in samples consisting of low fetal fraction by using a targeted sequencing approach combined with parent genotypes and Hapmap data in a Bayesian Maximum Likelihood Estimation (MLE) algorithm.

Maternal samples from four euploid and two trisomy-positive pregnancies and respective paternal samples were obtained under an IRB-approved protocol from patients where fetal karyotype was known. Maternal cfDNA was extracted from plasma and roughly 10 million sequence reads were obtained following preferential enrichment that targeted specific SNPs. Parent samples were similarly sequenced to obtain genotypes.

The described algorithm correctly called chromosome 18 and 21 disomy for all euploid samples and normal chromosomes of aneuploid samples. Trisomy 18 and 21 calls were correct, as were chromosome X copy numbers in male and female fetuses. The confidence produced by the algorithm was in excess of 98% in all cases.

The method described accurately reported the ploidy of all tested chromosomes from six samples, including samples comprised of less than 12% fetal DNA, which account for roughly 30% of 1st and early 2nd-trimester samples. The crucial difference between the instant MLE algorithm and published methods is that it leverages parent genotypes and Hapmap data to improve accuracy and generate a confidence metric. At low fetal fractions, all methods become less accurate; it is important to correctly identify samples without sufficient fetal cfDNA to make a reliable call. Others have used chromosome Y specific probes to estimate fetal fraction of male fetuses, but concurrent parental genotyping enables estimation of fetal fraction for both sexes. Another inherent limitation of published methods using untargeted shotgun sequencing is that accuracy of ploidy calling varies among chromosomes due to differences in factors such as GC richness. The instant targeted sequencing approach is largely independent of such chromosome-scale variations and yields more consistent performance between chromosomes.

Experiment 3

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The objective was to determine if trisomy is detectable with high confidence on a triploid fetus, using novel informatics to analyze SNP loci of free floating fetal DNA in maternal plasma.

20mL of blood was drawn from a pregnant patient following abnormal ultrasound. After centrifugation, maternal DNA was extracted from the buffy coat (DNEASY, QIAGEN); cell-free DNA was extracted from plasma (QIAAMP QIAGEN). Targeted sequencing was applied to SNP loci on chromosomes 2, 21, and X in both DNA samples. Maximum-Likelihood Bayesian estimation selected the most likely hypothesis from the set of all possible ploidy states. The method determines fetal DNA fraction, ploidy state and explicit confidences in the ploidy determination. No assumptions are made about the ploidy of a reference chromosome. The diagnostic uses a test statistic that is independent of sequence read counts, which is the recent state of the art.

The instant method accurately diagnosed trisomy of chromosomes 2 and 21. Child fraction was estimated at 11.9% [CI 11.7-12.1]. The fetus was found to have one maternal and two paternal copies of chromosomes 2 and 21 with confidence of effectively 1 (error probability$<10^{-30}$). This was achieved with 92,600 and 258,100 reads on chromosomes 2 and 21 respectively.

This is the first demonstration of non-invasive prenatal diagnosis of trisomic chromosomes from maternal blood where the fetus was triploid, as confirmed by metaphase karyotype. Extant methods of non-invasive diagnosis would not detect aneuploidy in this sample. Current methods rely on a surplus of sequence reads on a trisomic chromosome relative to disomic reference chromosomes; but a triploid fetus has no disomic reference. Furthermore, extant methods would not achieve similarly high-confidence ploidy determination with this fraction of fetal DNA and number of sequence reads. It is straightforward to extend the approach to all 24 chromosomes.

Experiment 4

The following protocol was used for 800-plex amplification of DNA isolated from maternal plasma from a euploid pregnancy and also genomic DNA from a triploidy 21 cell line using standard PCR (meaning no nesting was used). Library preparation and amplification involved single tube blunt ending followed by A-tailing. Adaptor ligation was run using the ligation kit found in the AGILENT SURESELECT kit, and PCR was run for 7 cycles. Then,

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15 cycles of STA (95˚C for 30s; 72˚C for 1 min; 60˚C for 4 min; 65˚C for 1 min; 72˚C for 30s) using 800 different primer pairs targeting SNPs on chromosomes 2, 21 and X. The reaction was run with 12.5 nM primer concentration. The DNA was then sequenced with an ILLUMINA IIGAX sequencer. The sequencer output 1.9 million reads, of which 92% mapped to the genome; of those reads that mapped to the genome, more than 99% mapped to one of the regions targeted by the targeted primers. The numbers were essentially the same for both the plasma DNA and the genomic DNA. FIG. 15 shows the ratio of the two alleles for the ~780 SNPs that were detected by the sequencer in the genomic DNA that was taken from a cell line with known trisomy at chromosome 21. Note that the allele ratios are plotted here for ease of visualization, because the allele distributions are not straightforward to read visually. The circles represent SNPs on disomic chromosomes, while the stars represent SNPs on a trisomic chromosome. FIG. 16 is another representation of the same data as in FIG. X, where the Y-axis is the relative number of A and B measured for each SNP, and where the X-axis is the SNP number where the SNPs are separated by chromosome. In FIG. 16, SNP 1 to 312 are found on chromosome 2, from SNP 313 to 605 are found on chromosome 21 which is trisomic, and from SNP 606 to 800 are on chromosome X. The data from chromosomes 2 and X show a disomic chromosome, as the relative sequence counts lie in three clusters: AA at the top of the graph, BB at the bottom of the graph, and AB in the middle of the graph. The data from chromosome 21, which is trisomic, shows four clusters: AAA at the top of the graph, AAB around the 0.65 line (2/3), ABB around the .35 line (1/3), and BBB at the bottom of the graph.

FIGs. 17A-D show data for the same 800-plex protocol, but measured on DNA that was amplified from four plasma samples from pregnant women. For these four samples, we expect to see seven clusters of dots: (1) along the top of the graph are those loci where both the mother and the fetus are AA, (2) slightly below the top of the graph are those loci where the mother is AA and the fetus is AB, (3) slightly above the 0.5 line are those loci where the mother is AB and the fetus is AA, (4) along the 0.5 line are those loci where the mother and the fetus are both AB, (5) slightly below the 0.5 line are those loci where the mother is AB and the fetus is BB, (6) slightly above the bottom of the graph are those loci where the mother is BB and the fetus is AB, (1) along the bottom of the graph are those loci where both the mother and the fetus are BB. The smaller the fetal fraction, the less the separation between clusters (1) and (2), between clusters (3), (4) and (5), and between clusters (6) and (7). The separation is

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expected to be half of the fraction of DNA that is of fetal origin. For example if the DNA is 20% fetal, and 80% maternal, we expect (1) through (7) to be centered at 1.0, 0.9, 0.6, 0.5, 0.4, 0.1 and 0.0 respectively; see for example FIG. 17D, POOL1_BC5_ref_rate. If, instead the DNA is 8% fetal, and 92% maternal, we expect (1) through (7) to be centered at 1.00, 0.96, 0.54, 0.50, 0.46, 0.04 and 0.00 respectively; see for example FIG. 17B, POOL1_BC2_ref_rate. If there is not fetal DNA detected, we do not expect to see (2), (3), (5), or (6); alternately we could say that the separation is zero, and therefore (1) and (2) are on top of each other, as are (3), (4) and (5), and also (6) and (7); see *e.g.* FIG. 17C, POOL1_BC7_ref_rate. Note that the fetal fraction for FIG. 17A, POOL1_BC1_ref_rate is about 25%.

Experiment 5

Most methods of DNA amplification and measurement will produce some allele bias, wherein the two alleles that are typically found at a locus are detected with intensities or counts that are not representative of the actual amounts of alleles in the sample of DNA. For example, for a single individual, at a heterozygous locus we expect to see a 1:1 ratio of the two alleles, which is the theoretical ratio expected for a heterozygous locus; however due to allele bias, we may see 55:45, or even 60:40. Also note that in the context of sequencing, if the depth of read is low, then simple stochastic noise could result in significant allele bias. In an embodiment, it is possible to model the behavior of each SNP such that if a consistent bias is observed for particular alleles, this bias can be corrected for. FIG. 18 shows the fraction of data that can be explained by binomial variance, before and after bias correction. In FIG. 18, the stars represent the observed allele bias on raw sequence data for the 800-plex experiment; the circles represent the allele bias after correction. Note that if there were no allele bias at all, we would expect the data to fall along the x=y line. A similar set of data that was produced by amplifying DNA using a 150-plex targeted amplification produced data that fell very closely on the 1:1 line after bias correction.

Experiment 6

Universal amplification of DNA using ligated adaptors with primers specific to the adaptor tags, where the primer annealing and extension times are limited to a few minutes has the effect of enriching the proportion of shorter DNA strands. Most library protocols designed for creating DNA libraries suitable for sequencing contain such a step, and example protocols

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are published and well known to those in the art. In some embodiments of the invention, adaptors with a universal tag are ligated to the plasma DNA, and amplified using primers specific to the adaptor tag. In some embodiments, the universal tag can be the same tag as used for sequencing, it can be a universal tag only for PCR amplification, or it can be a set of tags. Since the fetal DNA is typically short in nature, while the maternal DNA can be both short and long in nature, this method has the effect of enriching the proportion of fetal DNA in the mixture. The free floating DNA, thought to be DNA from apoptotic cells, and which contains both fetal and maternal DNA, is short – mostly under 200 bp. Cellular DNA released by cell lysis, a common phenomenon after phlebotomy, is typically almost exclusively maternal, and is also quite long – mostly above 500 bp. Therefore, blood samples that have sat around for more than a few minutes will contain a mixture of short (fetal + maternal) and longer (maternal) DNA. Performing a universal amplification with relatively short extension times on maternal plasma followed by targeted amplification will tend to increase the relative proportion of fetal DNA when compared to the plasma that has been amplified using targeted amplification alone. This can be seen in FIG. 19 which shows the measured fetal percent when the input is plasma DNA (vertical axis) vs. the measured fetal percent when the input DNA is plasma DNA that has had a library prepared using the ILLUMINA GAIIx library preparation protocol. All the dots fall below the line, indicating that the library preparation step enriches the fraction of DNA that is of fetal origin. Two samples of plasma that were red, indicating hemolysis and therefore that there would be an increased amount of long maternal DNA present from cell lysis, show a particularly significant enrichment of fetal fraction when the library preparation is performed prior to targeted amplification. The method disclosed herein is particularly useful in cases where there is hemolysis or some other situation has occurred where cells comprising relatively long strands of contaminating DNA have lysed, contaminating the mixed sample of short DNA with the long DNA. Typically the relatively short annealing and extension times are between 30 seconds and 2 minutes, though they could be as short as 5 or 10 seconds or less, or as long as 5 or 10 minutes.

Experiment 7

The following protocol was used for 1,200-plex amplification of DNA isolated from maternal plasma from a euploid pregnancy and also genomic DNA from a triploidy 21 cell line using a direct PCR protocol, and also a semi-nested approach. Library preparation and

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amplification involved single tube blunt ending followed by A-tailing. Adaptor ligation was run using a modification of the ligation kit found in the AGILENT SURESELECT kit, and PCR was run for 7 cycles. In the targeted primer pool, there were 550 assays for SNPs from chromosome 21, and 325 assays for SNPs from each of chromosomes 1 and X. Both protocols involved 15 cycles of STA (95˚C for 30s; 72˚C for 1 min; 60˚C for 4 min; 65˚C for 30s; 72˚C for 30s) using 16 nM primer concentration. The semi-nested PCR protocol involved a second amplification of 15 cycles of STA (95˚C for 30s; 72˚C for 1 min; 60˚C for 4 min; 65˚C for 30s; 72˚C for 30s) using an inner forward tag concentration of 29 nM, and a reverse tag concentration of 1 uM or 0.1 uM. The DNA was then sequenced with an ILLUMINA IIGAX sequencer. For the direct PCR protocol, 73% of the reads map to the genome; for the semi-nested protocol, 97.2% of the sequence reads map to the genome. Therefore, the semi-nested protocol result in approximately 30% more information, presumably mostly due to the elimination of primers that are most likely to cause primer dimers.

The depth of read variability tends to be higher when using the semi-nested protocol than when the direct PCR protocol is used (see FIG. 20) where the diamonds refer to the depth of read for loci run with the semi-nested protocol, and the squares refer to the depth of read for loci run with no nesting. The SNPs are arranged by depth of read for the diamonds, so the diamonds all fall on a curved line, while the squares appear to be loosely correlated; the arrangements of the SNPs is arbitrary, and it is the height of the dot that denotes depth of read rather than its location left to right.

In some embodiments, the methods described herein can achieve excellent depth of read (DOR) variances. For example, in one version of this experiment (FIG. 21) using a 1,200-plex direct PCR amplification of genomic DNA, of the 1,200 assays: 1186 assays had a DOR greater than 10; the average depth of read was 400; 1063 assays (88.6%) had a depth of read of between 200 and 800, and ideal window where the number of reads for each allele is high enough to give meaningful data, while the number of reads for each allele is not so high that the marginal use of those reads was particularly small. Only 12 alleles had higher depth of read with the highest at 1035 reads. The standard deviation of the DOR was 290, the average DOR was 453, the coefficient of variance of the DOR was 64%, there were 950,000 total reads, and 63.1% of the reads mapped to the genome. In another experiment (FIG. 22) using a 1,200-plex semi-nested protocol, the DOR was higher. The standard deviation of the DOR was 583, the average DOR was 630, the coefficient of variance of the DOR was 93%, there were 870,000

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total reads, and 96.3% of the reads mapped to the genome. Note, in both these cases, the SNPs are arranged by the depth of read for the mother, so the curved line represents the maternal depth of read. The differentiation between child and father is not significant; it is only the trend that is significant for the purpose of this explanation.

Experiment 8

In an experiment, the semi-nested 1,200-plex PCR protocol was used to amplify DNA from one cell and from three cells. This experiment is relevant to prenatal aneuploidy testing using fetal cells isolated from maternal blood, or for preimplantation genetic diagnosis using biopsied blastomeres or trophectoderm samples. There were 3 replicates of 1 and 3 cells from 2 individuals (46 XY and 47 XX+21) per condition. Assays targeted chromosomes 1, 21 and X. Three different lysis methods were used: ARCTURUS, MPERv2 and Alkaline lysis. Sequencing was run multiplexing 48 samples in one sequencing lane. The algorithm returned correct ploidy calls for each of the three chromosomes, and for each of the replicates.

Experiment 9

In one experiment, four maternal plasma samples were prepared and amplified using a hemi-nested 9,600-plex protocol. The samples were prepared in the following way: Up to 40 mL of maternal blood were centrifuged to isolate the buffy coat and the plasma. The genomic DNA in the maternal sample was prepared from the buffy coat and paternal DNA was prepared from a blood sample or saliva sample. Cell-free DNA in the maternal plasma was isolated using the QIAGEN CIRCULATING NUCLEIC ACID kit and eluted in 45 uL TE buffer according to manufacturer's instructions. Universal ligation adapters were appended to the end of each molecule of 35 uL of purified plasma DNA and libraries were amplified for 7 cycles using adaptor specific primers. Libraries were purified with AGENCOURT AMPURE beads and eluted in 50 ul water.

3 ul of the DNA was amplified with 15 cycles of STA (95˚C for 10 min for initial polymerase activation, then 15 cycles of 95˚C for 30s; 72˚C for 10 s; 65˚C for 1 min; 60˚C for 8 min; 65˚C for 3 min and 72˚C for 30s; and a final extension at 72˚C for 2 min) using 14.5 nM primer concentration of 9600 target-specific tagged reverse primers and one library adaptor specific forward primer at 500 nM.

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The hemi-nested PCR protocol involved a second amplification of a dilution of the first STAs product for 15 cycles of STA (95˚C for 10 min for initial polymerase activation, then 15 cycles of 95˚C for 30s; 65˚C for 1 min; 60˚C for 5 min; 65˚C for 5 min and 72˚C for 30s; and a final extension at 72˚C for 2 min) using reverse tag concentration of 1000 nM, and a concentration of 16.6 u nM for each of 9600 target-specific forward primers.

An aliquot of the STA products was then amplified by standard PCR for 10 cycles with 1 uM of tag-specific forward and barcoded reverse primers to generate barcoded sequencing libraries. An aliquot of each library was mixed with libraries of different barcodes and purified using a spin column.

In this way, 9,600 primers were used in the single-well reactions; the primers were designed to target SNPs found on chromosomes 1, 2, 13, 18, 21, X and Y. The amplicons were then sequenced using an ILLUMINA GAIIX sequencer. Per sample, approximately 3.9 million reads were generated by the sequencer, with 3.7 million reads mapping to the genome (94%), and of those, 2.9 million reads (74%) mapped to targeted SNPs with an average depth of read of 344 and a median depth of read of 255. The fetal fraction for the four samples was found to be 9.9%, 18.9%, 16.3%, and 21.2%

Relevant maternal and paternal genomic DNA samples amplified using a semi-nested 9600-plex protocol and sequenced. The semi-nested protocol is different in that it applies 9,600 outer forward primers and tagged reverse primers at 7.3 nM in the first STA. Thermocycling conditions and composition of the second STA, and the barcoding PCR were the same as for the hemi-nested protocol.

The sequencing data was analyzed using informatics methods disclosed herein and the ploidy state was called at six chromosomes for the fetuses whose DNA was present in the 4 maternal plasma samples. The ploidy calls for all 28 chromosomes in the set were called correctly with confidences above 99.2% except for one chromosome that was called correctly, but with a confidence of 83%.

FIG. 23 shows the depth of read of the 9,600-plex hemi-nesting approach along with the depth of read of the 1,200-plex semi-nested approach described in Experiment 7, though the number of SNPs with a depth of read greater than 100, greater than 200 and greater than 400 was significantly higher than in the 1,200-plex protocol. The number of reads at the 90th percentile can be divided by the number of reads at the 10th percentile to give a dimensionless metric that is indicative of the uniformity of the depth of read; the smaller the number, the

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more uniform (narrow) the depth of read. The average 90th percentile/10th percentile ratio is 11.5 for the method run in Experiment 9, while it is 5.6 for the method run in Experiment 7. A narrower depth of read for a given protocol plexity is better for sequencing efficiency, as fewer sequence reads are necessary to ensure that a certain percentage of reads are above a read number threshold.

Experiment 10

In one experiment, four maternal plasma samples were prepared and amplified using a semi-nested 9,600-plex protocol. Details of Experiment 10 were very similar to Experiment 9, the exception being the nesting protocol, and including the identity of the four samples. The ploidy calls for all 28 chromosomes in the set were called correctly with confidences above 99.7%. 7.6 million (97%) of reads mapped to the genome, and 6.3 million (80%) of the reads mapped to the targeted SNPs. The average depth of read was 751, and the median depth of read was 396.

Experiment 11

In one experiment, three maternal plasma samples were split into five equal portions, and each portion was amplified using either 2,400 multiplexed primers (four portions) or 1,200 multiplexed primers (one portion) and amplified using a semi-nested protocol, for a total of 10,800 primers. After amplification, the portions were pooled together for sequencing. Details of Experiment 11 were very similar to Experiment 9, the exception being the nesting protocol, and the split and pool approach. The ploidy calls for all 21 chromosomes in the set were called correctly with confidences above 99.7%, except for one missed call where the confidence was 83%. 3.4 million reads mapped to targeted SNPs, the average depth of read was 404 and the median depth of read was 258.

Experiment 12

In one experiment, four maternal plasma samples were split into four equal portions, and each portion was amplified using 2,400 multiplexed primers and amplified using a semi-nested protocol, for a total of 9,600 primers. After amplification, the portions were pooled together for sequencing. Details of Experiment 12 were very similar to Experiment 9, the exception being the nesting protocol, and the split and pool approach. The ploidy calls for all

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28 chromosomes in the set were called correctly with confidences above 97%, except for one missed call where the confidence was 78%. 4.5 million reads mapped to targeted SNPs, the average depth of read was 535 and the median depth of read was 412.

Experiment 13

In one experiment, four maternal plasma samples were prepared and amplified using a 9,600-plex triply hemi-nested protocol, for a total of 9,600 primers. Details of Experiment 12 were very similar to Experiment 9, the exception being the nesting protocol which involved three rounds of amplification; the three rounds involved 15, 10 and 15 STA cycles respectively. The ploidy calls for 27 of 28 chromosomes in the set were called correctly with confidences above 99.9%, except for one that was called correctly with 94.6%, and one missed call with a confidence of 80.8%. 3.5 million reads mapped to targeted SNPs, the average depth of read was 414 and the median depth of read was 249.

Experiment 14

In one experiment 45 sets of cells were amplified using a 1,200-plex semi-nested protocol, sequenced, and ploidy determinations were made at three chromosomes. Note that this experiment is meant to simulate the conditions of performing pre-implantation genetic diagnosis on single-cell biopsies from day 3 embryos, or trophectoderm biopsies from day 5 embryos. 15 individual single cells and 30 sets of three cells were placed in 45 individual reaction tubes for a total of 45 reactions where each reaction contained cells from only one cell line, but the different reactions contained cells from different cell lines. The cells were prepared into 5 ul washing buffer and lysed the by adding 5 ul ARCTURUS PICOPURE lysis buffer (APPLIED BIOSYSTEMS) and incubating at 56˚C for 20 min, 95˚C for 10 min..

The DNA of the single/three cells was amplified with 25 cycles of STA (95˚C for 10 min for initial polymerase activation, then 25 cycles of 95˚C for 30s; 72˚C for 10 s; 65˚C for 1 min; 60˚C for 8 min; 65˚C for 3 min and 72˚C for 30s; and a final extension at 72˚C for 2 min) using 50 nM primer concentration of 1200 target-specific forward and tagged reverse primers.

The semi-nested PCR protocol involved three parallel second amplification of a dilution of the first STAs product for 20 cycles of STA (95˚C for 10 min for initial polymerase activation, then 15 cycles of 95˚C for 30s; 65˚C for 1 min; 60˚C for 5 min; 65˚C for 5 min and

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72˚C for 30s; and a final extension at 72˚C for 2 min) using reverse tag specific primer concentration of 1000 nM, and a concentration of 60 nM for each of 400 target-specific nested forward primers. In the three parallel 400-plex reactions the total of 1200 targets amplified in the first STA were thus amplified.

An aliquot of the STA products was then amplified by standard PCR for 15 cycles with 1 uM of tag-specific forward and barcoded reverse primers to generate barcoded sequencing libraries. An aliquot of each library was mixed with libraries of different barcodes and purified using a spin column.

In this way, 1,200 primers were used in the single cell reactions; the primers were designed to target SNPs found on chromosomes 1, 21 and X. The amplicons were then sequenced using an ILLUMINA GAIIX sequencer. Per sample, approximately 3.9 million reads were generated by the sequencer, with 500,000 to 800,000 million reads mapping to the genome (74% to 94% of all reads per sample).

Relevant maternal and paternal genomic DNA samples from cell lines were analyzed using the same semi-nested 1200-plex assay pool with a similar protocol with fewer cycles and 1200-plex second STA, and sequenced.

The sequencing data was analyzed using informatics methods disclosed herein and the ploidy state was called at the three chromosomes for the samples.

FIG. 24 shows normalized depth of read ratios (vertical axis) for six samples at three chromosomes (1 = chrom 1; 2 = chrom 21; 3 = chrom X). The ratios were set to be equal to the number of reads mapping to that chromosome, normalized, and divided by the number of reads mapping to that chromosome averaged over three wells each comprising three 46XY cells. The three sets of data points corresponding to the 46XY reactions are expected to have ratios of 1:1. The three sets of data points corresponding to the 47XX+21 cells are expected to have ratios of 1:1 for chromosome 1, 1.5:1 for chromosome 21, and 2:1 for chromosome X.

FIG. 25A-25C show allele ratios plotted for three chromosomes (1, 21, X) for three reaction. The reaction in the lower left shows a reaction on three 46XY cells (FIG. 25B). The left region are the allele ratios for chromosome 1, the middle region are the allele ratios for chromosome 21, and the right region are the allele ratios for chromosome X. For the 46XY cells, for chromosome 1 we expect to see ratios of 1, 0.5 and 0, corresponding to AA, AB and BB SNP genotypes. For the 46XY cells, for chromosome 21 we expect to see ratios of 1, 0.5 and 0, corresponding to AA, AB and BB SNP genotypes. For the 46XY cells, for chromosome

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X we expect to see ratios of 1 and 0, corresponding to A, and B SNP genotypes. The reaction in the lower right shows a reaction on three 47XX+21 cells (FIG. 25C). The allele ratios are segregated by chromosome as in the lower left graph. For the 47XX+21 cells, for chromosome 1 we expect to see ratios of 1, 0.5 and 0, corresponding to AA, AB and BB SNP genotypes. For the 47XX+21 cells, for chromosome 21 we expect to see ratios of 1, 0.67, 0.33 and 0, corresponding to AAA, AAB, ABB and BBB SNP genotypes. For the 47XX+21 cells, for chromosome X we expect to see ratios of 1, 0.5 and 0, corresponding to AA, AB, and BB SNP genotypes. The plot in the upper right was made on a reaction comprising 1 ng of genomic DNA from the 47XX+21 cell line (FIG. 25A). FIGs. and 26B shows the same graphs as in FIG. 25A-25C, but for reactions performed on only one cell. The left graph was a reaction that contained a 47XX+21 cell (FIG. 26A), and the right graph was for a reaction that contained a 46XX cell (FIG. 26B).

From the graphs shown in FIGs. 25A-25C and FIGs. 26A and 26B, it is visually apparent that there are two clusters of dots for chromosomes where we expect to see ratios of 1 and 0; three clusters of dots for chromosomes where we expect to see ratios of 1, 0.5, and 0, and four clusters of dots for chromosomes where we expect to see ratios of 1, 0.67, 0.33 and 0. The parental support algorithm was able to make correct calls on all of the three chromosomes for all of the 45 reactions.

Experiment 15

In one experiment, maternal plasma samples were prepared and amplified using a hemi-nested 19,488-plex protocol. The samples were prepared in the following way: up to 20 mL of maternal blood were centrifuged to isolate the buffy coat and the plasma. The genomic DNA in the maternal sample was prepared from the buffy coat and paternal DNA was prepared from a blood sample or saliva sample. Cell-free DNA in the maternal plasma was isolated using the QIAGEN CIRCULATING NUCLEIC ACID kit and eluted in 50 uL TE buffer according to manufacturer's instructions. Universal ligation adapters were appended to the end of each molecule of 40 uL of purified plasma DNA and libraries were amplified for 9 cycles using adaptor specific primers. Libraries were purified with AGENCOURT AMPURE beads and eluted in 50 ul DNA suspension buffer.

6 ul of the DNA was amplified with 15 cycles of STAR 1 (95°C for 10 min for initial polymerase activation, then 15 cycles of 96°C for 30s; 65°C for 1 min; 58°C for 6 min; 60°C

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for 8 min; 65˚C for 4 min and 72˚C for 30s; and a final extension at 72˚C for 2 min) using 7.5 nM primer concentration of 19,488 target-specific tagged reverse primers and one library adaptor specific forward primer at 500 nM.

The hemi-nested PCR protocol involved a second amplification of a dilution of the STAR 1 product for 15 cycles (STAR 2) (95˚C for 10 min for initial polymerase activation, then 15 cycles of 95˚C for 30s; 65˚C for 1 min; 60˚C for 5 min; 65˚C for 5 min and 72˚C for 30s; and a final extension at 72˚C for 2 min) using reverse tag concentration of 1000 nM, and a concentration of 20 nM for each of 19,488 target-specific forward primers.

An aliquot of the STAR 2 products was then amplified by standard PCR for 12 cycles with 1 uM of tag-specific forward and barcoded reverse primers to generate barcoded sequencing libraries. An aliquot of each library was mixed with libraries of different barcodes and purified using a spin column.

In this way, 19,488 primers were used in the single-well reactions; the primers were designed to target SNPs found on chromosomes 1, 2, 13, 18, 21, X and Y. The amplicons were then sequenced using an ILLUMINA GAIIX sequencer. For plasma samples, approximately 10 million reads were generated by the sequencer, with 9.4-9.6 million reads mapping to the genome (94-96 %), and of those, 99.95 % mapped to targeted SNPs with a mean depth of read of 460 and a median depth of read of 350. For comparison, a perfectly even distribution would be: 10M reads / 19,488 targets = 513 reads/target. For primer-dimers, 30,000 reads were from sequenced primer-dimers (0.3% of the reads generated by the sequencer). For genomic samples, 99.4-99.7% of the reads mapped to the genome, of those, 99.99% of the mapped to targeted SNPs, and 0.1% of the reads generated by the sequencer were primer-dimers.

For plasma samples with 10 million sequencing reads, typically at least 19,350 of the 19,488 targeted SNPs (99.3 %) are amplified and sequenced. For DNA samples with 2M sequencing reads, typically at least 19,000 targeted SNPs (97.5%) are amplified and sequenced. The lower number may be due to sampling noise since the number of reads is lower and the sequencer misses some of the amplified products. If desired, the number of sequencing reads can be increased to increase the number of targeted SNPs that are amplified and sequenced.

Relevant maternal and paternal genomic DNA samples amplified using a semi-nested 19,488 outer forward primers and tagged reverse primers at 7.5 nM in the STAR 1.

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Thermocycling conditions and composition of STAR 2, and the barcoding PCR were the same as for the hemi-nested protocol.

The average fetal fraction for 407 samples was found to be 14.8%. The sequencing data was analyzed using informatics methods disclosed herein and the ploidy state was called at four chromosomes (13, 18, 21, Y) for the fetuses whose DNA was present in 378 of the 407 maternal plasma samples, and at chromosome X in 375 of the 407 maternal plasma samples. The ploidy calls for all 1,887 chromosomes in the set were called correctly with confidences above 90%. 1882 of the 1887 calls were above 95%; and 1,862 of the 1,887 calls were called with confidences above 99%.

A similar control experiment was performed using water instead of DNA extracted from plasma in the plasma PCR protocol. Based on six such trials of an experiment, 5-6% of the sequenced reads were primer-dimers. Other sequenced reads were due to background noise. This experiment demonstrates that even in the absence of a nucleic acid sample with target loci for the primers to hybridize to (rather than hybridizing to other primers and forming amplified primer dimers) few primer dimers are formed.

Experiment 16

The following experiment illustrates an exemplary method for designing and selecting a library of primers that can be used in any of the multiplexed PCR methods of the invention. The goal is to select primers from an initial library of candidate primers that can be used to simultaneously amplify a large number of target loci (or a subset of target loci) in a single reaction. For an initial set of candidate target loci, primers did not have to be designed or selected for each target locus. Preferably, primers are designed and selected for a large portion of the most desirable target loci.

Step 1

A set of candidate target loci (such as SNPs) were selected based on publically available information about desired parameters for the target loci, such as frequency of the SNPs within a target population or heterozygosity rate of the SNPs (worldwide web at ncbi.nlm.nih.gov/projects/SNP/; Sherry ST, Ward MH, Kholodov M, et al. dbSNP: the NCBI database of genetic variation. Nucleic Acids Res. 2001 Jan 1;29(1):308-11, which are each incorporated by reference in its entirety). For each candidate locus, one or more PCR primer

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pairs were designed using the Primer3 program (the worldwide web at primer3.sourceforge.net; libprimer3 release 2.2.3, which is hereby incorporated by reference in its entirety). If there were no feasible designs for PCR primers for a particular target locus, then that target locus was eliminated from further consideration. If desired, a "target locus score" (higher score representing higher desirability) can be calculated for most or all of the target loci, such as a target locus score calculated based on a weighted average of various desired parameters for the target loci. The parameters may be assigned different weights based on their importance for the particular application that the primers will be used for. Exemplary parameters include the heterozygosity rate of the target locus, the disease prevalence associated with a sequence (*e.g.*, a polymorphism) at the target locus, the disease penetrance associated with a sequence (*e.g.*, a polymorphism) at the target locus, the specificity of the candidate primer(s) used to amplify the target locus, the size of the candidate primer(s) used to amply the target locus, and the size of the target amplicon. In some embodiments, the specificity of the candidate primer for the target locus includes the likelihood that the candidate primer will mis-prime by binding and amplifying a locus other than the target locus it was designed to amplify. In some embodiments, one or more or all the candidate primers that mis-prime are removed from the library.

Step 2

A thermodynamic interaction score was calculated between each primer and all primers for all other target loci from Step 1 (*see, e.g.*, Allawi, H. T. & SantaLucia, J., Jr. (1998), "Thermodynamics of Internal C-T Mismatches in DNA", *Nucleic Acids Res. 26*, 2694-2701; Peyret, N., Seneviratne, P. A., Allawi, H. T. & SantaLucia, J., Jr. (1999), "Nearest-Neighbor Thermodynamics and NMR of DNA Sequences with Internal A-A, C-C, G-G, and T-T Mismatches", *Biochemistry 38*, 3468-3477; Allawi, H. T. & SantaLucia, J., Jr. (1998), "Nearest-Neighbor Thermodynamics of Internal A-C Mismatches in DNA: Sequence Dependence and pH Effects", *Biochemistry 37*, 9435-9444.; Allawi, H. T. & SantaLucia, J., Jr. (1998), "Nearest Neighbor Thermodynamic Parameters for Internal G-A Mismatches in DNA", *Biochemistry 37*, 2170-2179; and Allawi, H. T. & SantaLucia, J., Jr. (1997), "Thermodynamics and NMR of Internal G-T Mismatches in DNA", *Biochemistry 36*, 10581-10594; MultiPLX 2.1 (Kaplinski L, Andreson R, Puurand T, Remm M. MultiPLX: automatic grouping and evaluation of PCR primers. Bioinformatics. 2005 Apr 15;21(8):1701-2, which

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are each hereby incorporated by reference in its entirety). This step resulted in a 2D matrix of interaction scores. The interaction score predicted the likelihood of primer-dimers involving the two interacting primers. The score was calculated as follows:

interaction_score = max(- deltaG_2, 0.8 * (- deltaG_1))

where

deltaG_2 = Gibbs energy (energy required to break the dimer) for a dimer that is extensible by PCR on both ends, *i.e,*. the 3' end of each primer anneals to the other primer; and

deltaG_1 = Gibbs energy for a dimer that is extensible by PCR on at least one end.

Step 3:

For each target locus, if there was more than one primer-pair design, then one design was selected using the following method:

1 For each primer-pair design for the locus, find the worst-case (highest) interaction score for the two primers in that design and all primers from all designs for all other target loci.

2 Pick the design with the best (lowest) worst-case interaction score.

Step 4

A graph was built such that each node represented one locus and its associated primer-pair design (*e.g.*, a Maximal Clique problem). One edge was created between every pair of nodes. A weight was assigned to each edge equal to the worst-case (highest) interaction score between the primers associated with the two nodes connected by the edge.

Step 5

If desired, for every pair of designs for two different target loci where one of the primers from one design and one of the primers from the other design would anneal to overlapping target regions, an additional edge was added between the nodes for the two design. The weight of these edges was set equal to the highest weight assigned in Step 4. Thus, Step 5 prevents the library from having primers that would anneal to overlapping target regions, and thus interfere with each other during a multiplex PCR reaction.

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Step 6

An initial interaction score threshold was calculated as follows:

weight_threshold = max(edge_weight) - 0.05 * (max(edge_weight) - min(edge_weight))

where

max(edge_weight) is the maximum edge weight in the graph; and

min(edge_weight) is the minimum edge weight in the graph.

The initial bounds for the threshold were set as follows:

max_weight_threshold = max(edge_weight)

min_weight_threshold = min(edge_weight)

Step 7

A new graph was constructed consisting of the same set of nodes as the graph from Step 5, only including edges with weights that exceed weight_threshold. Thus, step ignores interactions with scores equal to or below weight_threshold.

Step 8

Nodes (and all of the edges connected to the removed nodes) were removed from the graph of Step 7 until there were no edges left. Nodes were removed by applying the following procedure repeatedly:

1 Find the node with the highest degree (highest number of edges). If there is more than one then pick one arbitrarily.

2 Define the set of nodes consisting of the node picked above and all of the nodes connected to it, but excluding any nodes that have degree less than the node picked above.

3 Choose the node from the set that has the lowest target locus score (lower score representing lower desirability) from Step 1. Remove that node from the graph.

Step 9

If the number of nodes remaining in the graph satisfies the required number of target loci for the multiplexed PCR pool (within an acceptable tolerance), then the method was continued at Step 10.

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If there were too many or too few nodes remaining in the graph, then a binary search was performed to determine what threshold values would result in the desired number of nodes remaining in the graphs. If there were too many nodes in the graph then, the weight threshold bounds were adjusted as follows:

max_weight_threshold = weight_threshold

Otherwise (if there are two few nodes in the graph), then the weight threshold bounds were adjusted as follows:

min_weight_threshold = weight_threshold

Then, the weight threshold was adjusted follows:

weight_threshold = (max_weight_threshold + min_weight_threshold) / 2

Steps 7-9 were repeated.

Step 10

The primer-pair designs associated with the nodes remaining in the graph were selected for the library of primers. This primer library can be used in any of the methods of the invention.

If desired, this method of designing and selecting primers can be performed for primer libraries in which only one primer (instead of a primer pair) is used for amplification of a target locus. In this case, a node presents one primer per target locus (rather than a primer pair).

Experiment 17

FIG. 27 is a graph comparing two primer libraries designed using the methods of the invention. This graph shows the number of loci with a particular minor allele frequency that are targeted by each primer library. During the selection of the "new pool" library, more primers were retained. This library enables the amplification of more target loci, especially target loci with relatively large minor allele frequencies (which are the more informative alleles for some method of the invention, such as for detecting fetal chromosomal abnormalities).

These primer libraries were used in the following multiplex PCR method. Blood (20-40 mL) was collected from each subject into two to four CELL-FREE™ DNA tubes (Streck). Plasma (a minimum of 7 mL) was isolated from each sample via a double centrifugation

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protocol of 2,000 g for 20 min, followed by 3,220 g for 30 min, with supernatant transfer following the first spin. cfDNA was isolated from 7-20 mL plasma using the QIAGEN QIAamp Circulating Nucleic Acid kit and eluted in 45 uL TE buffer. Pure maternal genomic DNA was isolated from the buffy coat obtained following the first centrifugation, and pure paternal genomic DNA was prepared similarly from a blood, saliva or buccal sample.

Maternal cfDNA, maternal genomic DNA, and paternal genomic DNA samples were pre-amplified for 15 cycles using 11,000 target-specific assays and an aliquot was transferred to a second PCR reaction of 15 cycles using nested primers. Finally, samples were prepared for sequencing by adding barcoded tags in a third 12-cycle round of PCR. Thus, 11,000 targets were amplified in a single reaction; the targets included SNPs found on chromosomes 13, 18, 21, X, and Y. The amplicons were then sequenced using an ILLUMINA GAIIx or HISEQ sequencer. Parental genotypes were sequenced at a lower read depth (~20% of cfDNA read depth) than the fetal genotypes.

Experiment 18

If desired, the size and quantity of the PCR products can be analyzed using standard methods, such as the use of the Agilent Technologies 2100 Bioanalyzer (FIG. 28A-M). For example, direct PCR methods described herein without nesting were used in 2,400-plex (FIGs. 28B-28G) and 19,488-plex experiments (FIGs. 28H to 28M). The amount of primer was 10 nM for FIGs. 28B-28D and 28H to 28J. The amount of primer was 1 nM for FIGs. 28E-28G and 28K to 28M. The amount of input DNA was 24 ng for FIGs. 28B, 28E, 28H, and 28K; 80 ng for FIGs. 28C, 28F, 28I, and 28L; and 250 ng for FIGs. 28D, 28G, 28J, and 28M. More input DNA resulted in a greater proportion of the desired 180 base pair product. The peak at 140 base pairs is a primer dimer product.

Experiment 19

A proof-of-principle study demonstrated the detection of T13, T18, T21, 45,X, and 47,XXY with equally high accuracies across all chromosomes.

Patients

Pregnant couples were enrolled at specific prenatal care centers under protocols approved by an Institutional Review Board pursuant to local laws. Inclusion criteria were at

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least 18 years of age, gestational age of at least nine weeks, singleton pregnancies, and signed informed consent. Blood samples were drawn from pregnant mothers, and a blood or buccal sample was collected from the father. Samples from 2 pregnancies with T13 (Patau Syndrome), 2 with T18 (Edwards Syndrome), 2 with T21 (Down's Syndrome), 2 with 45,X, 2 with 47,XXY, and 90 normal pregnancies were selected prior to testing from a cohort of ~500 women to test which chromosomal abnormalities the method detects. Normal fetal karyotype was confirmed by molecular karyotyping for the samples where post-birth child tissue was available. Euploid sample were drawn prior to invasive testing from low-risk women. Aneuploid samples were drawn at least 7 days after invasive testing and aneuploidy was confirmed via cytogenetic karyotyping or fluorescence *in situ* hybridization at independent laboratories.

Sample Preparation and Multiplex PCR

For the data in FIGs. 30A-E, 30G, 30H, and 31A-31G, sample preparation and 19,488-plex-PCR were performed as described in Experiment 15. For the data in FIG. 30F, sample preparation and 11,000-plex-PCR were performed as described in Experiment 17.

Methodology and Data Analysis

The algorithm considers parental genotypes and crossover frequency data (such as data from the HapMap database) to calculate expected allele distributions for 19,488 polymorphic loci for a very large number possible fetal ploidy states, and at various fetal cfDNA fractions. (FIGs. 29A-29C). Unlike allele ratio based-methods, it also takes into account linkage disequilibrium, and uses non-Gaussian data models to describe the expected distribution of allele measurements at a SNP given observed platform characteristics and amplification biases. It then compares the various predicted allele distributions to the actual allelic distributions as measured in the cfDNA sample (FIG. 29C), and calculates the likelihood of each hypothesis (monosomy, disomy, or trisomy, for which there are numerous hypotheses based on the various potential crossovers) based on the sequencing data. The algorithm sums the likelihoods of each individual monosomy, disomy, or trisomy hypotheses (FIG. 29D), and calls the ploidy state with the maximum overall likelihood as the copy number and fetal fraction (FIG. 29E). Although laboratory researchers were not blinded to sample karyotype, the algorithm called the ploidy states without human intervention and was blind to the truth.

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Data Interpretation

Graphical Representations of the Generated Data

To determine the ploidy state of a chromosome of interest, the algorithm considers the distribution of sequence counts from each of two possible alleles at 3,000 to 4,000 SNPs per chromosome. It is important to note that the algorithm makes ploidy calls using an approach that does not lend itself to visualization. Thus, for the purposes of illustration, the data is displayed here in a simplified fashion as ratios of the two most likely alleles, labeled as A and B, so that the relevant trends can be more readily visualized. This simplified illustration does not take into account some of the features of the algorithm. For example, two important aspects of the algorithm that are not possible to illustrate with a method of visualization that displays allele ratios are: 1) the ability to leverage linkage disequilibrium, *i.e.* the influence that a measurement at one SNP has on the likely identity of a neighboring SNP, and 2) the use of non-Gaussian data models that describe the expected distribution of allele measurements at a SNP given platform characteristics and amplification biases. Also note that the algorithm only considers the two most common alleles at each SNP, ignoring other possible alleles.

The graphical representations in FIG. 30A-30H include samples for which two, one, or three fetal chromosomes are present. Generally, these indicate euploidy (FIGs. 30A-30C) monosomy (FIG. 30D), and trisomy (FIGs. 30E-30H), respectively. In all plots, each spot represents a single SNP, where the targeted SNPs are plotted sequentially from left to right for one chromosome along the horizontal axes. The vertical axes indicate the number of reads for the A allele as a fraction of the total number of reads for both the A and B alleles for that SNP. Note that the measurements are made on total cfDNA isolated from maternal blood, and the cfDNA includes both maternal and fetal cfDNA; thus, each spot represents the combination of the fetal and maternal DNA contribution for that SNP. Therefore, increasing the proportion of maternal cfDNA from 0% to 100% will gradually shift some spots up or down within the plots, depending on the maternal and fetal genotype. This is described in more detail below with the corresponding plots.

If desired to facilitate visualization, the spots may be color-coded according to maternal genotype, as maternal genotype contributes more to the localization of each spot and the majority of trisomies are maternally-inherited; this assists in visualizing ploidy states. Specifically, SNPs for which the maternal genotype is AA may be indicated in red, those for

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which the maternal genotype is AB may be indicated in green, and those for which the maternal genotype is BB may be indicated in blue.

In all cases, SNPs that are homozygous for the A allele (AA) in both the mother and the fetus are found tightly associated with the upper limit of the plots, as the fraction of A allele reads is high because there should be no B alleles present. Conversely, SNPs that are homozygous for the B allele in both the mother and the fetus are found tightly associated with the lower limit of the plots, as the fraction of A allele reads is low because there should be only B alleles. The spots that are not tightly associated with the upper and lower limits of the plots represent SNPs for which the mother, the fetus, or both are heterozygous; these spots are useful for identifying fetal ploidy, but can also be informative for determining paternal versus maternal inheritance. These spots segregate based on both maternal and fetal genotypes and fetal fraction, and as such the precise position of each individual spot along the y-axis depends on both stoichiometry and fetal fraction. For example, loci where the mother is AA and the fetus is AB are expected to have a different fraction of A allele reads, and thus different positioning along the y-axis, depending on the fetal fraction.

Two Chromosomes Present

FIGs. 30A-30C depict data that indicate the presence of two chromosomes when the sample is entirely maternal (no fetal cfDNA present, FIG. 30A), contains a moderate fetal cfDNA fraction (FIG. 30B), or contains a high fetal cfDNA fraction (FIG. 30C).

FIG. 30A shows data obtained from cfDNA isolated from the blood of a non-pregnant woman. When there is no fetal cfDNA present and the sample contains only maternal cfDNA, the plots represent purely the euploid maternal genotype; the hallmark pattern includes "clusters" of spots: a red cluster tightly associated with the top of the plot (SNPs where the maternal genotype is AA), a blue cluster tightly associated with the bottom of the plot (SNPs where the maternal genotype is BB), and a single, centered green cluster (SNPs where the maternal genotype is AB).

When fetal cfDNA is present, the location of the spots shifts such that the clusters segregate into discrete "bands". Note that for samples with a fetal fraction of 0%, the groupings of spots are referred to as "clusters" (as in FIG. 30A), and for all samples with a fetal fraction of >0%, the groupings of spots are referred to as "bands" (as in FIGs. 30B-30J). If the fetal fraction is high enough, these discrete bands will be readily visible. Specifically,

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FIGs. 30B and 30C demonstrate the characteristic pattern associated with two fetal chromosomes present at moderate and high fetal fractions, respectively. This pattern includes three central green bands that correspond to SNPs that are heterozygous in the mother, and two "peripheral" bands each at both the top (red) and bottom (blue) of the plots that correspond to SNPs that are homozygous in the mother.

FIG. 30B shows data obtained from cfDNA isolated from a plasma sample from a woman carrying a euploid fetus and with a 12% fetal cfDNA fraction. Here, the clusters of spots tightly associated with the top and bottom of the plot segregate into two discrete bands each: one red and one blue external peripheral band that remains tightly associated with the upper or lower limit of the plots, and one red and one blue internal peripheral band that has separated from the limits of the plots. These internal peripheral bands, centered around 0.92 and 0.08, represent SNPs for which the maternal genotype is AA and the fetal genotype is AB (indicated in red), and SNPs for which the maternal genotype is BB and the fetal genotype is AB (indicated in blue), respectively. The center cluster of green spots broadens, but at this fetal fraction the segregation into distinct bands is not readily visible.

At a high fetal cfDNA fraction, the typical pattern that indicates the presence of two chromosomes (a trio of green bands as well as two red and two blue peripheral bands) is readily apparent. FIG. 30C displays data obtained from a plasma sample from a woman carrying a euploid fetus at a fetal cfDNA fraction of 26%. Here, the peripheral bands have separated such that the internal band has shifted towards the center of the plot due to the altered levels of B alleles from the increased fetal cfDNA fraction. Significantly, at higher fetal fractions, the separation of the center green cluster into three distinct bands is now readily apparent. This central trio of bands, in this case clustering around 0.37, 0.50 and 0.63, corresponds to those SNPs where the maternal genotype is AB, and the fetal genotype is AA (top), AB (middle) and BB (bottom).

These hallmark patterns, namely three green bands and four peripheral bands (two red and two blue), indicate the presence of two chromosomes, as in autosomal euploidy or for the X chromosome in a female (XX) fetus.

One Chromosome Present

When the fetus only inherits a single chromosome, and thus only inherits a single allele, heterozygosity of the fetus is not possible. As such, the only possible fetal SNP

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identities are A or B. Thus, maternally-inherited monosomic chromosomes have a characteristic pattern of two central green bands that represent SNPs for which the mother is heterozygous, and only have single peripheral red and blue bands that represent SNPs for which the mother is homozygous, and which remain tightly associated with the upper and lower limits of the plots (1 and 0), respectively (FIG. 30D). Note the absence of internal peripheral bands. This pattern indicates the presence of one chromosome, as in maternally-inherited autosomal monosomy, or for the X chromosome in a male (XY) fetus.

Three Chromosomes Present

Trisomic chromosomes have three characteristic patterns. The first pattern indicates maternally-inherited meiotic trisomy, a meiotic error where the fetus inherited two homologous, non-identical chromosomes from the mother (FIG. 30E); this pattern includes two central green bands with two each of the peripheral red and blue bands. The second pattern indicates paternally-inherited meiotic trisomy, where the fetus inherited two homologous, non-identical chromosomes from the father (FIG. 30F); this pattern includes four central green bands and three each of the peripheral red and blue bands. The third pattern indicates either maternally- (FIG. 30G) or paternally-inherited (FIG. 30H) mitotic trisomy, a mitotic error where the fetus inherited two identical chromosomes from either the mother or the father; this pattern includes four central green bands with two each of the peripheral red and blue bands. Maternally- and paternally-inherited mitotic trisomies can be distinguished by the placement of the flanking red and blue bands, such that the red and blue internal peripheral bands (those not associated with the limits of the plots) are closer to the center in paternally-inherited mitotic trisomy. This is due to the paternal contribution of identical chromosomes. Note that our previous results indicate that at the blastomere stage, 66.7% of maternally-inherited trisomies are meiotic, and that only 10.2% of trisomies are paternally-inherited.

For the Y chromosome, the PS method considers a different set of hypotheses: zero, one, or two chromosomes present. As there is no maternal contribution to the sequence reads at each locus and because heterozygous loci are not possible (cases of two Y chromosomes necessarily involve two identical chromosomes), the bands remain tightly associated with the top (A alleles) or the bottom (B alleles) of the plot (data not shown), and analysis is greatly simplified, relying on quantitative allele count data. Note that since the method interrogates

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SNPs, it uses homologous non-recombinant SNPs from the Y chromosome, thus obtaining data on both X and Y for one probe pair.

Identifying Aneuploidies

Identification of autosomal aneuploidies using this plot-based visualization method is straightforward given a sufficient fetal fraction, and requires only identifying plots for which there are an abnormal number of chromosomes present, as described above. Combining the knowledge of copy number of the X and Y chromosomes identifies whether sex chromosome aneuploidies are present. Specifically, plots representing a fetus with a 47,XXX genotype will have a typical "three-chromosome" pattern, and plots representing a fetus with a 47,XXY genotype will have the typical "two-chromosome" pattern for the X chromosome, but will also have allele reads indicating the presence of one Y chromosome. The method is similarly able to call 47,XYY, where a "one chromosome" pattern indicates the presence of a single X chromosome, and allele reads indicate the presence of two Y chromosomes. A fetus with a 45,X genotype will have the typical "one-chromosome" pattern for the X chromosome, and data indicating zero Y chromosomes.

Effects of Fetal Fraction

As discussed above, the number of sequence reads from the fetus contributes to the precise location of each spot along the y-axis in the plots. As fetal fraction will affect the proportion of reads that originate from the fetus and the mother, it will also affect the positioning of each spot. At a high fraction of fetal cfDNA (generally above ~20%), as in FIGs. 30C-30E and FIGs. 30G and 30H, it is readily apparent that although the spots cluster based mainly on maternal genotype, the presence of fetal DNA from alleles whose genotype is distinct from the maternal genotype shift the clusters into multiple, distinct bands. However, as the fetal fraction decreases (as in FIGs. 30B and 30F), the spots regress towards the poles and center of the plot, resulting in tighter clusters. Specifically, the set of peripheral red bands, where the maternal genotype is AA, regress towards the top of the plot; the set of peripheral blue bands, where the maternal genotype is BB, regress towards the bottom; the set of central green bands, where the mother is heterozygous, condense into a single cluster at the center of the plot (compare FIGs. 30B and 30C). Although aneuploidy is not readily apparent by eye using this visualization technique for low fetal fraction cases, the algorithm is able to identify

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ploidy states with a very low fetal fraction, such as 3% fetal fraction. It is able to do this because the statistical technique compares the observed data to very precise data models that predict the allele distributions for a given sample parameter set (including copy number, parental genotypes, and fetal fraction, for example). Data model precision is critical in low fetal fraction cases, as the differences between the allele distributions for different ploidy states are proportional to the fetal fraction. In addition, the algorithm is able to determine when a data set does not contain enough data to make a confident fetal ploidy determination.

Results

Sequencing reads that mapped to targeted SNPs were deemed to be informative and were used by the algorithm. More than 95% of targeted loci were observed in the sequencing results. The plots for visualizing key ploidy calls are depicted in FIG. 31A-31G. FIG. 31A indicates a euploid sample. Here, chromosomes 13, 18, and 21 have the typical "two chromosome" pattern (as described herein). This includes a trio of center green bands, and two red and two blue peripheral bands. This, together with the two center green bands for the X chromosome and the presence of Y chromosome bands along the plots' peripheries, indicate a euploid XY genotype.

The most prevalent autosomal trisomies, T13, T18, and T21, are indicated by the plots in FIGs. 31B, 31C, and 31D, respectively. Specifically, FIG. 31B depicts a T13 sample. Here, chromosomes 18 and 21 display the typical "two chromosome" pattern, chromosome X displays the typical "one chromosome" pattern, and there are reads from the Y chromosome. Together, this indicates disomy at chromosomes 18 and 21, and identifies a fetal XY genotype. However, chromosome 13 depicts a typical "three chromosome" pattern – specifically. Similarly, FIG. 31C depicts a T18 sample, and FIG. 31D depicts a T21 sample.

The method is also able to detect sex chromosome aneuploidies, including 45,X (FIG. 31E), 47,XXY (FIG. 31F), and 47,XYY (FIG. 31G). Note that the method is calling copy number at chromosomes 13, 18, 21, X, and Y; the overall chromosome number is reported assuming disomy at the remaining chromosomes. The X chromosome regions of the plot depicting a 45,X sample reveals the presence of a single chromosome. However, the lack of reads from the Y chromosome, coupled with the "two chromosome" pattern for chromosomes 13, 18, and 21, indicate a 45,X genotype. Conversely, the 47,XXY samples generate a plot revealing the presence of two X chromosomes. The data also revealed reads for alleles from

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the Y chromosome. Together with the presence of two copies of chromosomes 13, 18, and 21, this indicates a 47,XXY genotype. A 47,XYY genotype is indicated by the presence of a "one chromosome" pattern for the X chromosome, and reads indicating the presence of two Y chromosomes.

Discussion

This method detected T13, T18, T21, 45,X, 47,XXY, and 47,XYY non-invasively from maternal blood. This method interrogates cfDNA from maternal plasma by targeted multiplex PCR amplification and high-throughput sequencing of 19,488 SNPs. This, coupled with the method's sophisticated informatics analyses that take into account parental genotypic information and numerous sample parameters, including fetal fraction and DNA quality, more robustly detects the fetal signal and makes highly accurate ploidy calls at all of the five chromosomes implicated in the seven most common types of at-birth aneuploidy (T13, T18, T21, 45,X, 47,XXX, 47,XXY, and 47,XYY). This method offers a number of clinical advantages over previous methods, including and most significantly greater clinical coverage and sample-specific calculated accuracies (analogous to a personalized risk score).

Increased Clinical Coverage

This method offers approximately a two-fold increase in aneuploidy coverage compared to clinically available NIPT methodologies, given its ability to accurately detect autosomal trisomies and sex chromosome aneuploidies. The method presented here is the only noninvasive test that calls ploidy at the sex chromosomes with high accuracy. Prior DNA mixing experiments and separate plasma samples analyzed in our experimental assays suggest that this method will detect a larger cohort of sex chromosome anomalies, including 47,XXX. The method presented here also detects aneuploidies at chromosomes 13, 18, and 21 with high sensitivities and specificities, and with appropriate primer design is expected to be able to detect copy number at the remaining chromosomes as well.

Sample-Specific Calculated Accuracies

Significantly, this method calculates a sample-specific accuracy for ploidy calls on each chromosome in each sample. Accuracies calculated by this method are expected to significantly lower the rate of incorrect calls by identifying and flagging individual samples

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that have poor quality DNA or low fetal fractions that are likely to result in a poor accuracy test result. By contrast, massively parallel shotgun sequencing (MPSS)-based methods produce a positive or negative call using a single-hypothesis rejection test, and their accuracy estimate is based on a published study cohort rather than on the characteristics of the individual sample, which are assumed to have the same accuracy as the cohort. However, individual accuracies for samples with parameters in the tail of the cohort distribution may differ significantly. This is exacerbated at low fetal fractions, as in early gestational age, or for samples with low DNA quality. These samples are generally not identified and flagged for follow-up, which can result in missed calls. The present method, however, takes into account many parameters, including fetal fraction and a number of DNA quality metrics, to make each chromosome copy number call, calculating a sample-specific accuracy for that call. This allows the method to identify individual samples with low accuracy and flag them for follow-up. This is expected to nearly eliminate missed calls, especially at the early stages of pregnancy when fetal fractions are typically low. The presumption is that a no call is much preferred to a missed call, since a no call simply requires a redraw and reanalysis.

Converting Calculated Accuracies to Traditional Risk Scores

This method can offer an adjusted risk of aneuploidy for high-risk pregnant women, where the adjusted risk takes into account an *a priori* risk (Benn P, Cuckle H, Pergament E. Non-invasive prenatal diagnosis for Down syndrome: the paradigm will shift, but slowly. Ultrasound Obstet Gynecol 2012;39:127-130, which is hereby incorporated by reference in its entirety). Although the present method offers each patient a customized calculated accuracy, for clinical use these accuracies can be converted to traditional risk scores, which also denote the risk of an aneuploid pregnancy but are expressed as fractions. Traditional risk scores take into account various parameters, including maternal age-related risk and serum levels of biochemical markers, to offer a risk score above which a mother is considered high-risk and for whom follow-up invasive diagnostic procedures are recommended. This method significantly refines this risk score, thus reducing both the false positive and false negative rates, and offering a more accurate assessment of individual maternal risk. A calculated accuracy as used here is the likelihood that the ploidy call is correct, and is expressed as a percentage, but the calculated accuracies used in Experiment 19 do not include an age-related risk. Because calculation of a risk score typically includes an age-related risk, the calculated

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accuracies and traditional risk scores are not interchangeable; they must be combined to convert into a traditional risk score. The formula to combine the age-related risk with the calculated accuracy is:

$$\frac{R_1 R_2}{R_1 R_2 + [1 - R_1][1 - R_2]}$$

where R_1 is the risk score as calculated by the present method and R_2 is the risk score as calculated by first trimester screening.

SNP-Based Methods Negate Issues with Amplification Variation

An inherent drawback to the counting methods used by some other methods is that they determine fetal ploidy state by measuring the ratio of the number of reads mapping to the chromosome of interest (*e.g.*, chromosome 21) to those mapping to a reference chromosome. Chromosomes with high or low GC content, including chromosomes 13, X, and Y, amplify with high variability. This can result in signal variation that is comparable in magnitude to the fetal cfDNA signal, which can confound copy number calls by altering the ratio of allele reads from the chromosome-of-interest to those from the reference chromosome. This can result in low accuracy for chromosomes 13, X, and Y. Significantly, this problem is exacerbated at low fetal cfDNA fractions, as tends to be the case at early gestational ages.

In contrast, SNP-based methods do not rely on consistent amplification levels between chromosomes, and are thus expected to provide results that are equally accurate across all chromosomes. Because the present method looks, in part, at relative counts of different alleles at polymorphic loci, which by definition differ only by a single nucleotide, it does not require the use of reference chromosomes, and this obviates the problems with chromosome-to-chromosome amplification variation that are inherent to methods that rely on quantitating read counts. Unlike quantitative methods that require reference chromosomes that are euploid, the present method is expected to be able to detect triploidy as well as copy-number neutral anomalies like uniparental disomy.

The Importance of Early Detection

Significantly, the combined at-birth prevalence of sex chromosome aneuploidies is higher than that of the most common autosomal aneuploidies (FIG. 32). However, there are currently no routine non-invasive screening methods that reliably detect sex chromosome

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abnormalities. Thus, sex chromosome anomalies are generally detected prenatally as a side-effect of routine testing for Down syndrome or other autosomal aneuploidies; a large proportion of cases are missed entirely. Early and accurate detection is crucial for many of these disorders where early therapeutic intervention improves clinical outcomes. For example, Turner syndrome is often not diagnosed until adolescence, although its overall at-birth prevalence is 1 in 2,500 females. Growth hormone therapy is known to prevent short stature that results from the disorder, but treatments are significantly more effective when initiated prior to the age of 4. Additionally, estrogen replacement therapy can stimulate secondary sexual characteristics in patients with Turner syndrome, but again therapy must be initiated in the pre-teen years, before the syndrome is usually detected. Together, this underscores the importance of early, routine, and safe detection of sex chromosome aneuploidies. This method offers the first approach with the potential to serve as a routine screen for sex chromosome anomalies.

Experiment 20

The following experiment illustrates an exemplary method for designing and selecting primers that can be used in any of the multiplexed PCR methods of the invention. In some embodiments, primers from an initial library of candidate primers are selected so that they can be used to simultaneously amplify a large number of target loci (or a subset of target loci) in a single reaction. In some embodiments, primers from an initial library of candidate primers are selected to form multiple primer pools such that each pool can be used to simultaneously amplify a subset of target loci in a single reaction. Preferably, primers are designed and selected for a large portion or all of the most desirable target loci. Preferably, the minimum number of pools needed to amplify the target loci are created.

Step 1

Calculate a first score for each primer pair design using one or more of the following parameters: number of SNPs within the primers, location of SNPs within the primers, distance from an end of the amplicon to the target bases within the amplicon, number of target loci in an amplicon, heterozygosity rate of the target locus, disease prevalence associated with a sequence (*e.g.*, a polymorphism) at the target locus, disease penetrance associated with a sequence (*e.g.*, a polymorphism) at the target locus, specificity of the candidate primer for the

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target locus, size of the candidate primer, melting temperature of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, GC content of the 3' end of the candidate primer, homopolymer length in the candidate primer, amplification efficiency of the target amplicon, and size of the target amplicon.

Step 2

Compare each primer pair to every other primer pair, and calculate a second score for the pair using one or more of the following parameters: likelihood of dimer formation, amplicon overlap, number of primer designs for a particular target locus, and distance between amplicons. In some embodiments, the score is infinite if amplicons overlap so that two different primer pairs that generate overlapping amplicons are not included in the same primer pool.

Step 3

Aggregate the first score and the second score together (such as by using a weighted average of the scores).

Step 4

If desired, order all target loci into one contiguous list based upon their genomic location in ascending order.

Step 5

Build a minimum priority queue data structure that prioritizes the pairs of designs (in which each design is one primer pair so that a pair of designs includes two primer pairs with a total of 4 primers) based on their score (such as the aggregate score from step 3). In some embodiments, the score for a pair of designs is the worse score (such as the worse aggregate score from step 3) out of the scores for all 4 primers in the pair of designs. The pair of designs with the best (most desirable) score is first in the queue, and the pair of designs with the worst (least desirable) score is last in the queue. If desired, pairs of designs with a score above a threshold (least desirable) are removed from the library of candidate primers such that they are not included in the final pool(s) (for example, these primers may be omitted from the queue). In some embodiments, pairs of design with an interaction score above (worse than) 20, 18, 16,

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14, 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 kcal/mol are removed from the library of candidate primers. In some embodiments, pairs of design with a ΔG value below (worse than) -20, -18, -16, -14, 12, -10, -9, -8, -7, -6, -5, -4, -3, -2, or -1 kcal/mol are removed from the library of candidate primers.

Each design pair can be stored as a node of a doubly linked list with initial "next" and "previous" pointers set to NULL.

Step 6

Begin forming all pools simultaneously by doing the following steps. Take the design pair with the best (most desirable) score from the priority queue and add it to "the potential pools." Begin storing designs in N number of doubly linked list data structures with the design pairs. N represents the current number of different primer pools. Initially, N =1, since there is only one primer pool. In some embodiments, a second pool is only created if necessary to include the desired target loci or the desired level of coverage of target loci. Check to see if the design pair removed from the queue is "connected" to any other existing design pair. By "connected" for purposes of this step is meant that a single design in one pair is the same as a single design in another pair. If two pairs are connected, then assign the appropriate next and previous pointers to one another. If two pairs are not connected, then add them to the "potential pools" In some embodiments, a design pair is only placed in a particular pool if it would be connected to at most two other design pairs in that pool (otherwise it can be assigned to a different pool).

Check to see if (i) any linked list spans from the first target to the last target (such that all the desired target loci are included) or (ii) if a pool meets the cutoff for the desired minimum pool level. If it does, that list now forms a pool and can be added to the "final pools" list.

Step 7

If desired, check to see if the desired level of coverage (such as all the bases in the target loci being included in amplicons from 4 different primer pairs) that is desired for each location. Repeat step 6 until achieving the desired level of coverage.

The resulting primer pool(s) can be used in any of the methods of the invention.

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Experiment 21

The following experiment illustrates an exemplary method for designing and selecting primers that can be used in any of the multiplexed PCR methods of the invention. In some embodiments, the primers are divided into different pools (e.g., 2, 3, 4, 5, 6, or more different pools) such that each pool is used to amplify target loci in a different reaction volume. Each pool is used to simultaneously amplify a large number of target loci (or a subset of target loci) in a single reaction volume. Preferably, primers are designed and selected for a large portion of the most desirable target loci or for all of the target loci. A set of candidate target loci can be selected as described in Experiments 16 or 20 based on the particular polymorphisms or mutations of interest. In some embodiments, one or more of the following type of target loci are included: SNPs, short indels, long indels, exons, and combinations thereof. In some embodiments for target loci that are short indels, the PCR primer or primer pair targets a sequence of adjacent base pairs; and the indel is completely covered by one sequencing read. In some embodiments for target loci that are large indels, two primer pairs are used to target a pair of breakpoints at the boundaries of the indel. In this case, the two primer pairs are designed such that when the deletion is present there is a PCR product and the two primer pairs are selected together for inclusion in the same pool (the four primers are treated by the algorithm as a single assay rather than two assays). In some embodiments for target loci that are exons, a set of primers pairs are designed to tile the full exon.

For each candidate locus, one or more PCR primer pairs are designed using the Primer3 program (available at the worldwide web at primer3.sourceforge.net; libprimer3 release 2.2.3, which is hereby incorporated by reference in its entirety). If there are no feasible designs for PCR primers for a particular target locus, then that target locus is eliminated from further consideration. In some embodiments, each target base is covered by at least two independent PCR assays (such as two independent primer pairs that will amplify the target base) and preferably by four assays, although not all of the available assays for a target must be used. In some embodiments, no targets are omitted. Desirably, the algorithm produces as few pools as possible but may produce more than one pool. In some embodiments, two different primer pairs that are in close proximity in the genome (such as within 2 kbases or 1 kbase) and whose forward primers are on the same strand are not be assigned to the same pool. This constraint avoids primer interference in the extension-and-ligation amplification method In

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some embodiments in which the PCR will be performed using a polymerase with low 5′→ 3′ exonuclease and/or low strand displacement activity, different primer pairs that are in close proximity in the genome and whose forward primers are on the same strand can be assigned to the same pool since the with low 5′→ 3′ exonuclease and/or low strand displacement activity of the polymerase will reduce or prevent primer interference and allow nearby or adjacent amplicons to be produced.

Step 1

Build an interaction graph. Each node represents one assay (such as one primer pair). Each edge represents a conflict between two assays. There are three types. Interaction edges represent a potential primer dimer and have a score indicating the interaction strength. Proximity edges represent physical proximity of the primer binding sites which may result in interference. Target edges represent redundant designs associated with the same target (a special case of a proximity edge).

Step 2

Select an initial value for the maximum interaction score (*e.g.*, 95% of the maximum score).

Step 3

Compute a score such as a utility score for each assay as follows using steps 3A and 3B.

Step 3A

Calculate a score for each assay based on one or more of its intrinsic characteristics. For example, favor assays with amplicons close to the optimal length (such as 300 bp); favor assays with a shorter distance from the beginning of the amplicon to the target; and/or penalize assays with primers overlapping known SNPs. Any other parameter, such as the parameters disclosed herein can also be included.

Step 3B

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Multiply the score for each assay by a factor that varies from 0 to 1 according to the current coverage of the assay's target bases. This factor gives lower weight to targets that are already covered by assays. At the beginning of the algorithm this factor is 1 for all assays because none have been covered. Calculate the factor as follows. For each base in the target, compute a coverage score as 1 / (2 ^ c) where c is the number of previously-selected assays (in other pools) that cover that base. For instance, if three assays cover the base then the coverage score is 1/(2^3) = 0.125. The factor for the target is the maximum value of the coverage score for all bases in the target. For instance, if the target contains 10 bases, 3 bases are covered by 1 target, and 7 bases are covered by 3 targets, then the factor is MAX(1/(2^1), 1/(2^3)) = 0.5. The score in step 3A is then multiplied by this factor.

Step 4

Use a single iteration of the algorithm in Experiment 16 to design a pool given the current maximum interaction score: Construct a new graph with the assays that have not been assigned to a pool yet and with the edges that have weights exceeding the maximum interaction score. Remove nodes (assays) according to the algorithm in Experiment 16 until there are no edges left. The assay utility scores come from step 3 in this experiment rather than the calculation used for Experiment 16

Step 5

Save the assays selected in step 4 as a new pool and remove them from consideration. Then repeat steps 3 and 4 with the remaining assays, and iterate until all targets have sufficient coverage.

Step 6

If desired, evaluate the result. If the total number of pools meets the design goal then reduce the maximum interaction score; otherwise increase the maximum interaction score. Then go back to step 3. Iterate, using a binary search strategy to find the lowest maximum interaction score that produces the desired number of pools.

Step 7

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Output the pools from the final iteration. After the selection process, the primers remaining in the pools may be used in any of the methods of the invention.

Experiment 22

The following experiment illustrates an exemplary method for designing and selecting primers that can be used in any of the multiplexed PCR methods of the invention. In some embodiments, the primers are divided into different pools (e.g., 2, 3, 4, 5, 6, or more different pools) such that each pool is used to amplify target loci in a different reaction volume. Any of the embodiments listed in Experiment 21 can be used for this experiment as well.

This method uses a graph coloring algorithm.

Step 1

Select 2, 3 or 4 of the best assays (such as primer pairs) for each target locus from all of the available assays.

Step 2

Select an initial maximum interaction score.

Step 3

Build an interaction graph containing only edges that exceed the maximum interaction score.

Step 4

Color the graph such that no adjacent nodes have the same color (this is a standard problem with many heuristic solutions). Each color represents a different pool.

Step 5

Go back to step 3 and iterate, refining the maximum interaction score until the desired number of pools is achieved. In some embodiments, after the primers are selected in step 1, the algorithm assumes all assays must be included in a pool.

After the primers are divided into different pools, the pools may be used in any of the methods of the invention.

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Additional Applications

Because this method utilizes targeted amplification, it is uniquely poised to detect sub-microscopic anomalies, such as microdeletions and microduplications. Although non-targeted methods like MPSS have been shown to detect the DiGeorge microdeletion syndrome, this required a sufficiently high level of genomic coverage so as to make the approach unfeasible. This is because non-targeted amplification will be several orders of magnitude less efficient on submicroscopic regions, as very small fraction of the sequencing reads will be informative. Additionally, the fact that the currently available methods have trouble accurately identifying ploidy state for the sex chromosomes suggests that they will also encounter variable amplification problems on smaller chromosomal segments.

Similarly, SNP based methods can detect UPD disorders, which are copy number-neutral anomalies that will not be detected by either the current noninvasive methods that rely on counting or the traditional invasive methods like amniocentesis and CVS that rely on cytogenetic karyotyping and/or fluorescence *in situ* hybridization. This is because SNP-based methods are uniquely able to distinguish individual haplotypes, whereas the clinically available MPSS-based and targeted methods amplify non-polymorphic loci and are thus unable to determine, for example, whether the chromosomes-of-interest originate from the same parent. This means that these microdeletion/microduplication and UPD syndromes, including Prader-Willi, Angelman, and Beckwith-Wiedemann syndromes, are generally not diagnosed prenatally, and are often initially misdiagnosed postnatally. This significantly delays therapeutic intervention. Additionally, because this method targets SNPs, this method will also facilitate parental haplotype reconstruction, allowing for detection of fetal inheritance of individual disease-linked loci (Kitzman JO, Snyder MW, Ventura M, *et al.* Noninvasive whole-genome sequencing of a human fetus. Sci Transl Med 2012;4:137ra76, which is hereby incorporated by reference in its entirety).

The results presented here confirm the expanded scope of this method for identifying prenatal aneuploidy. Specifically, by amplifying and sequencing 19,488 SNPs, this method is able to determine copy number at chromosomes 13, 18, 21, X, and Y, and is uniquely expected to detect other chromosomal abnormalities, such as triploidy and UPD, that are not detected by any other clinically available non-invasive method. The increased clinical coverage and

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powerful sample-specific calculated accuracies suggest that this method may offer a viable adjunct to invasive testing for detecting fetal chromosomal aneuploidies.

All patents, patent applications, and published references cited herein are hereby incorporated by reference in their entirety. While the methods of the present disclosure have been described in connection with the specific embodiments thereof, it will be understood that it is capable of further modification. Furthermore, this application is intended to cover any variations, uses, or adaptations of the methods of the present disclosure, including such departures from the present disclosure as come within known or customary practice in the art to which the methods of the present disclosure pertain, and as fall within the scope of the appended claims. For example, any of the methods disclosed herein for DNA can be readily adapted for RNA by including a reverse transcription step to convert the RNA into DNA. Examples that use polymorphic loci for illustration can be readily adapted for the amplification of nonpolymorphic loci if desired.

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CLAIMS

What is claimed is:

1. A method of amplifying target loci in a nucleic acid sample, the method comprising:

(a) contacting the nucleic acid sample with a library of non-immobilized test primers that simultaneously hybridize to at least 1,000 different target loci to produce a reaction mixture;

(b) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products comprising target amplicons; and

(c) sequencing the amplified products.

2. The method of claim 1, wherein at least 100 of the test primers have at least 90% identity to the corresponding region of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

3. The method of claim 1, wherein at least 100 of the test primers comprise the polynucleotide sequence of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

4. The method of claim 1, wherein at least 1,000 different target loci are amplified.

5. The method of claim 1, wherein at least 10,000 different target loci are amplified.

6. The method of claim 1, wherein at least 20,000 different target loci are amplified.

7. The method of claim 1, wherein at least 30,000 different target loci are amplified.

8. The method of claim 1, wherein at least 90% of the amplified products are target amplicons.

9. The method of claim 1, wherein at least 95% of the amplified products are target amplicons.

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10. The method of claim 1, wherein at least 99% of the amplified products are target amplicons.

11. The method of claim 1, wherein at least 90% of the target loci are amplified.

12. The method of claim 1, wherein at least 95% of the target loci are amplified.

13. The method of claim 1, wherein at least 99% of the target loci are amplified.

14. The method of claim 1, wherein less than 20% of the amplified products are test primer dimers.

15. The method of claim 1, wherein less than 10% of the amplified products are test primer dimers.

16. The method of claim 1, wherein less than 1% of the amplified products are test primer dimers.

17. The method of claim 1, wherein less than 0.1% of the amplified products are test primer dimers.

18. The method of claim 1, wherein the test primers are selected from a library of candidate primers based on one or more parameters.

19. The method of claim 18, wherein the test primers are selected from a library of candidate primers based at least in part on the ability of the candidate primers to form primer dimers.

20. The method of claim 19, wherein the selection comprises

(i) calculating on a computer an score for most or all of the possible combinations of two candidate primers from the library, wherein each score is based at least in part on the likelihood of dimer formation between the two candidate primers;

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(ii) removing the candidate primer with the highest score from the library of candidate primers;

(iii) if the candidate primer removed in step (ii) is a member of a primer pair, then removing the other member of the primer pair from the library of candidate primers; and

(iv) optionally repeating steps (ii) and (iii).

21. The method of claim 19, wherein the selection comprises

(i) calculating on a computer an score for most or all of the possible combinations of two candidate primers from the library, wherein each score is based at least in part on the likelihood of dimer formation between the two candidate primers;

(ii) removing from the library of candidate primers the candidate primer that is part of the greatest number of combinations of two candidate primers with an score above a first minimum threshold;

(iii) if the candidate primer removed in step (ii) is a member of a primer pair, then removing the other member of the primer pair from the library of candidate primers; and

(iv) optionally repeating steps (ii) and (iii).

22. The method of claim 21, comprising further reducing the number of candidate primers remaining in the library by decreasing the first minimum threshold used in step (ii) to a lower second minimum threshold and repeating steps (ii) and (iii) until the scores for the candidate primer combinations remaining in the library are all equal to or below the second minimum threshold, or until the number of candidate primers remaining in the library is reduced to a desired number.

23. The method of claim 21, comprising increasing the first minimum threshold used in step (ii) to a higher second minimum threshold and repeating steps (ii) and (iii) until the scores for the candidate primer combinations remaining in the library are all equal to or below the second minimum threshold, or until the number of candidate primers remaining in the library is reduced to a desired number.

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24. The method of claim 20 or 21, wherein a candidate primer is selected out of a group of two or more candidate primers with equal scores for removal from the library based on one or more parameters.

25. The method of claim 1, wherein the concentration of each test primer is less than 100 nM.

26. The method of claim 1, wherein the concentration of each test primer is less than 10 nM.

27. The method of claim 1, wherein the concentration of each test primer is less than 2 nM.

28. The method of claim 1, wherein the library of test primers comprises at least 300 test primer pairs that include a forward test primer and a reverse test primer where each pair of test primers hybridize to a target locus.

29. The method of claim 1, wherein the library of test primers comprises at least 300 individual test primers that each hybridize to a different target locus.

30. The method of claim 1, wherein the GC content of the test primers is between 30% and 80%, inclusive.

31. 10. The method of claim 1, wherein the range of GC content of the test primers is less than 20%.

32. The method of claim 1, wherein the melting temperature of the test primers is between 40 °C and 80 °C, inclusive.

33. The method of claim 1, wherein the range of melting temperatures of the test primers is less than 5 °C.

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34. The method of claim 1, wherein the length of the test primers is between 17 and 35 nucleotides, inclusive

35. The method of claim 1, wherein the test primers comprise a tag that is not target specific.

36. The method of claim 35, wherein the tag forms an internal loop structure.

37. The method of claim 36, wherein the tag is between two DNA binding regions.

38. The method of claim 36, wherein the test primers comprise a 5' region that is specific for a target locus, an internal region that is not specific for the target locus and forms loop structure, and a 3' region that is specific for the target locus.

39. The method of claim 38, wherein the length of the 3' region is at least 7 nucleotides.

40. The method of claim 39, wherein the length of the 3' region is between 7 and 20 nucleotides, inclusive.

41. The method of claim 35, wherein the test primers comprise a 5' region that is not specific for a target locus followed by a region that is specific for a target locus, an internal region that is not specific for the target locus and forms a loop structure, and a 3' region that is specific for the target locus.

42. The method of claim 1, wherein range of the length of the target amplicons is less than 15 nucleotides.

43. The method of claim 1, wherein the primer extension reaction conditions are polymerase chain reaction conditions (PCR).

44. The method of claim 43, wherein the length of the annealing step is greater than 10 minutes.

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45. The method of claim 1, further comprising determining the presence or absence of at least one target amplicon.

46. The method of claim 1, further comprising determining the sequence of at least one target amplicon.

47. The method of claim 1, wherein the target loci are present on the same chromosome of interest.

48. The method of claim 1, wherein at least some of the target loci are present on different chromosomes nucleic acids of interest.

49. The method of claim 1, wherein the nucleic acid sample comprises fragmented or digested nucleic acids.

50. The method of claim 1, wherein nucleic acid sample comprises genomic DNA, cDNA, or mRNA.

51. The method of claim 1, wherein nucleic acid sample comprises DNA from a single cell.

52. The method of claim 1, wherein nucleic acid sample comprises or is derived from blood, plasma, saliva, sperm, cell culture supernatant, mucus secretion, dental plaque, gastrointestinal tract tissue, stool, urine, or forensic sample.

53. The method of claim 1, wherein the target loci are segments of human nucleic acids.

54. The method of claim 1, wherein the target loci comprise single nucleotide polymorphisms.

55. A method of selecting test primers from a library of candidate primers, the method comprising:

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(a) calculating on a computer an score for most or all of the possible combinations of two candidate primers from the library, wherein each score is based at least in part on the likelihood of dimer formation between the two candidate primers;

(b) removing the candidate primer with the highest score from the library of candidate primers;

(c) if the candidate primer removed in step (b) is a member of a primer pair, then removing the other member of the primer pair from the library of candidate primers; and

(d) optionally repeating steps (b) and (c), thereby selecting a library of test primers.

56. A method of selecting test primers from a library of candidate primers, the method comprising:

(a) calculating on a computer an score for most or all of the possible combinations of two candidate primers from the library, wherein each score is based at least in part on the likelihood of dimer formation between the two candidate primers;

(b) removing from the library of candidate primers the candidate primer that is part of the greatest number of combinations of two candidate primers with an score above a first minimum threshold;

(c) if the candidate primer removed in step (b) is a member of a primer pair, then removing the other member of the primer pair from the library of candidate primers; and

(d) optionally repeating steps (b) and (c), thereby selecting a library of test primers.

57. The method of claim 56, comprising further reducing the number of candidate primers remaining in the library by decreasing the first minimum threshold used in step (b) to a lower second minimum threshold and repeating steps (b) and (c) until the scores for the candidate primer combinations remaining in the library are all equal to or below the second minimum threshold, or until the number of candidate primers remaining in the library is reduced to a desired number.

58. The method of claim 56, comprising increasing the first minimum threshold used in step (b) to a higher second minimum threshold and repeating steps (b) and (c) until the scores for the candidate primer combinations remaining in the library are all equal to or below

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the second minimum threshold, or until the number of candidate primers remaining in the library is reduced to a desired number.

59. The method of claim 55 or 56, wherein a candidate primer is selected out of a group of two or more candidate primers with equal scores for removal from the library of candidate primers based on one or more other parameters.

60. The method of claim 55 or 56, wherein the scores are based at least in part on one or more parameters selected from the group consisting of heterozygosity rate of the target locus, disease prevalence associated with a polymorphism or mutation at the target locus, disease penetrance associated with a polymorphism or mutation at the target locus, specificity of the candidate primer for the target locus, size of the candidate primer, melting temperature of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, GC content of the 3' end of the candidate primer, homopolymer length in the candidate primer, amplification efficiency of the target amplicon, and size of the target amplicon.

61. The method of claim 60, wherein the scores are based at least in part on one or more parameters selected from the group consisting of heterozygosity rate of the target locus, specificity of the candidate primer for the target locus, size of the candidate primer, , melting temperature of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, GC content of the 3' end of the candidate primer, homopolymer length in the candidate primer, amplification efficiency of the target amplicon, and size of the target amplicon; and wherein the test primers are used to simultaneously amplify at least 300 target loci in a sample comprising maternal DNA from the pregnant mother of a fetus and fetal DNA to determine the presence or absence of a fetal chromosome abnormality.

62. The method of claim 61, comprising ligating a universal primer binding site to the DNA molecules in the sample; amplifying the ligated DNA molecules using at least 300 specific primers and a universal primer to produce a first set of amplified products; and amplifying the first set of amplified products using at least 300 pairs of specific primers to produce a second set of amplified products.

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63. The method of claim 60, wherein the scores are based at least in part on one or more parameters selected from the group consisting of heterozygosity rate of the target locus, specificity of the candidate primer for the target locus, size of the candidate primer, melting temperature of the candidate primer, melting temperature of the target amplicon, GC content of the target amplicon, GC content of the 3' end of the candidate primer, homopolymer length in the candidate primer, amplification efficiency of the target amplicon, and size of the target amplicon; and wherein the test primers are used to simultaneously amplify at least 300 target loci in sample comprises DNA from an alleged father of a fetus and to simultaneously amplify the target loci in a sample comprising maternal DNA from the pregnant mother of a fetus and fetal DNA to establish whether the alleged father is the biological father of the fetus.

64. The method of claim 60, wherein the scores are based at least in part on one or more parameters selected from the group consisting of heterozygosity rate of the target locus, specificity of the candidate primer for the target locus; size of the candidate primer, and size of the target amplicon; and wherein the test primers are used to simultaneously amplify at least 300 target loci in a forensic nucleic acid sample using an annealing time of at least 5 minutes.

65. The method of claim 60, comprising using the test primers to simultaneously amplify at least 300 target loci in a control nucleic acid sample to produce a first set of target amplicons and to simultaneously amplify the target loci in a test nucleic acid sample to produce a second set of target amplicons; and comparing the first and second sets of target amplicons to determine whether a target locus is present in one sample but absent in the other, or whether a target locus is present at different levels in the control sample and the test sample.

66. The method of claim 65, wherein the test sample is from an individual suspected of having a disease or phenotype of interest, or an increased risk for a disease or phenotype of interest; and wherein one or more of the target loci comprise a polymorphism associated with an increased risk for the disease or phenotype of interest, or associated with the disease or phenotype of interest.

67. The method of claim 60, comprising using the test primers to simultaneously amplify at least 300 target loci in a control sample comprising RNA to produce a first set of

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target amplicons and to simultaneously amplify the target loci in a test sample comprising RNA to produce a second set of target amplicons; and comparing the first and second sets of target amplicons to determine the presence or absence of a difference in the RNA expression levels between the control sample and the test sample.

68. The method of claim 67, wherein the RNA is mRNA.

69. The method of claim 67, wherein the test sample is from an individual suspected of having cancer or an increased risk for cancer; and wherein one or more of the target loci comprises a polymorphism or other mutation associated with an increased risk for cancer or associated with cancer.

70. The method of claim 67, wherein the test sample is from an individual diagnosed with cancer; and wherein a difference in the RNA expression level between the control sample and test sample indicates a target locus comprises a polymorphism or other mutation associated with an increased or decreased risk for cancer.

71. The method of claim 55 or 56, further comprising, prior to step (b), removing a primer pair from the library that produces a target amplicon that overlaps with a target amplicon produced by another primer pair.

72. The method of claim 55 or 56, wherein the candidate primers remaining in the library are capable of simultaneously amplifying at least 300 different target loci.

73. The method of claim 55 or 56, further comprising:

(e) contacting a nucleic acid sample comprising target loci with the candidate primers remaining in the library to produce a reaction mixture; and

(f) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products comprising target amplicons.

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74. The method of claim 55 or 56, wherein at least 100 of the candidate primers have at least 90% identity to the corresponding region of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

75. The method of claim 55 or 56, wherein at least 100 of the candidate primers comprise the polynucleotide sequence of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

76. A library of non-immobilized primers that simultaneously amplifies at least 1,000 different target loci such that less than 30% of the amplified products are test primer dimers.

77. A library of non-immobilized primers that simultaneously amplifies at least 1,000 different target loci such that at least 80% of the amplified products are target amplicons.

78. A library of non-immobilized primers that simultaneously amplifies target loci such that at least 80% of the target loci out of at least 1,000 different target loci are amplified.

79. The library of any one of claims 76-78, wherein at least 100 of the primers have at least 90% identity to the corresponding region of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

80. The library of claim 79, wherein at least 100 of the primers comprise the polynucleotide sequence of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

81. The library of any one of claims 76-78, wherein at least 1,000 different target loci are amplified.

82. The library of any one of claims 76-78, wherein at least 10,000 different target loci are amplified.

83. The library of any one of claims 76-78, wherein at least 20,000 different target loci are amplified.

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84. The library of any one of claims 76-78, wherein at least 30,000 different target loci are amplified.

85. The library of any one of claims 76-78, wherein at least 90% of the amplified products are target amplicons.

86. The library of any one of claims 76-78, wherein at least 95% of the amplified products are target amplicons.

87. The library of any one of claims 76-78, wherein at least 99% of the amplified products are target amplicons.

88. The library of any one of claims 76-78, wherein at least 90% of the target loci are amplified.

89. The library of any one of claims 76-78, wherein at least 95% of the target loci are amplified.

90. The library of any one of claims 76-78, wherein at least 99% of the target loci are amplified.

91. The library of any one of claims 76-78, wherein less than 20% of the amplified products are primer dimers.

92. The library of any one of claims 76-78, wherein less than 10% of the amplified products are primer dimers.

93. The library of any one of claims 76-78, wherein less than 1% of the amplified products are primer dimers.

94. The library of any one of claims 76-78, wherein less than 0.1% of the amplified products are primer dimers.

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95. The library of any one of claims 76-78, comprising at least 300 primer pairs, wherein each primer pair includes a forward primer and a reverse primer that hybridize to a target locus.

96. The library of any one of claims 76-78, comprising at least 300 individual primers that hybridize to different target loci.

97. A library of primers that comprises at least 100 different primers with at least 90% identity to the corresponding region of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

98. The library of claim 97, wherein at least 100 of the primers comprise the polynucleotide sequence of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

99. The library of claim 97, comprising at least 1,000 different primers with at least 90% identity to the corresponding region of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

100. The library of claim 99, wherein at least 1,000 of the primers comprise the polynucleotide sequence of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

101. The library of claim 97, comprising at least 10,000 different primers with at least 90% identity to the corresponding region of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

102. The library of claim 101, wherein at least 10,000 of the primers comprise the polynucleotide sequence of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

103. The library of claim 99, wherein the library simultaneously amplifies at least 300 different target loci.

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104. A kit for amplifying target loci in a nucleic acid sample comprising (i) the library of any one of claims 76-78 and 97 and (iii) instructions for using the library to amplify the target loci.

105. A method for determining a ploidy status of a chromosome in a gestating fetus, the method comprising:

(a) contacting a nucleic acid sample with a library of primers that simultaneously hybridize to at least 300 different polymorphic loci to produce a reaction mixture; wherein the nucleic acid sample comprises maternal DNA from the mother of the fetus and fetal DNA from the fetus; and

(b) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products;

(c) measuring the amplified products with a high throughput sequencer to produce sequencing data;

(d) calculating, on a computer, allele counts at the polymorphic loci based on the sequencing data;

(e) creating on a computer a plurality of ploidy hypotheses each pertaining to a different possible ploidy state of the chromosome;

(f) building on a computer a joint distribution model for the expected allele counts at the polymorphic loci on the chromosome for each ploidy hypothesis;

(g) determining on a computer a relative probability of each of the ploidy hypotheses using the joint distribution model and the allele counts; and

(h) calling the ploidy state of the fetus by selecting the ploidy state corresponding to the hypothesis with the greatest probability.

106. A method of testing for an abnormal distribution of a chromosome in a sample comprising a mixture of maternal and fetal DNA, the method comprising:

(a) contacting the sample with a library of primers that simultaneously hybridize to at least 300 different target loci to produce a reaction mixture; wherein the target loci are from a plurality of different chromosomes; and wherein the plurality of different chromosomes comprise at least one first chromosome suspected of having an abnormal distribution in the

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sample and at least one second chromosome presumed to be normally distributed in the sample;

(b) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products;

(c) sequencing the amplified products to obtain a plurality of sequence tags aligning to the target loci; wherein the sequence tags are of sufficient length to be assigned to a specific target locus;

(d) assigning on a computer the plurality of sequence tags to their corresponding target loci;

(e) determining on a computer a number of sequence tags aligning to the target loci of the first chromosome and a number of sequence tags aligning to the target loci of the second chromosome; and

(f) comparing on a computer the numbers from step (e) to determine the presence or absence of an abnormal distribution of the first chromosome.

107. A method for detecting the presence or absence of a fetal aneuploidy, the method comprising:

(a) contacting a sample comprising a mixture of maternal and fetal DNA with a library of primers that simultaneously hybridize to at least 300 different non-polymorphic target loci to produce a reaction mixture; wherein the target loci are from a plurality of different chromosomes;

(b) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products comprising target amplicons;

(c) quantifying on a computer a relative frequency of the target amplicons from the first and second chromosomes of interest;

(d) comparing on a computer the relative frequency of the target amplicons from the first and second chromosomes of interest; and

(e) identifying the presence or absence of an aneuploidy based on the compared relative frequencies of the first and second chromosome of interest.

108. The method of any one of claims 105-107, wherein the target loci include one or more SNPs in the homologous non-recombining region of chromosome X and/or

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chromosome Y, and wherein the method further comprises determining the number of copies of chromosome X and/or chromosome Y.

109. The method of any one of claims 105-107, wherein at least 100 of the primers have at least 90% identity to the corresponding region of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

110. The method of claim 109, wherein at least 100 of the primers comprise the polynucleotide sequence of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

111. A method for establishing whether an alleged father is the biological father of a fetus that is gestating in a pregnant mother, the method comprising:

(a) simultaneously amplifying a plurality of polymorphic loci comprising at least 300 different polymorphic loci on genetic material from the alleged father to produce a first set of amplified products;

(b) simultaneously amplifying the corresponding plurality of polymorphic loci on a mixed sample of DNA originating from a blood sample from the pregnant mother to produce a second set of amplified products; wherein the mixed sample of DNA comprises fetal DNA and maternal DNA;

(c) determining on a computer the probability that the alleged father is the biological father of the fetus using genotypic measurements based on the first and second sets of amplified products; and

(d) establishing whether the alleged father is the biological father of the fetus using the determined probability that the alleged father is the biological father of the fetus.

112. The method of claim 111, further comprising simultaneously amplifying the corresponding plurality of polymorphic loci on genetic material from the mother to produce a third set of amplified products; wherein the probability that the alleged father is the biological father of the fetus is determined using genotypic measurements based on the first, second, and third sets of amplified products.

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113. The method of claim 112, wherein the PCR is performed using at least 100 primers that have at least 90% identity to the corresponding region of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

114. The method of claim 113, wherein at least 100 of the primers comprise the polynucleotide sequence of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

115. A method of measuring the amount of two or more target loci in a nucleic acid sample, the method comprising:

(a) using PCR to amplify a nucleic acid sample comprising a first standard locus, a second standard locus, a first target locus, and a second target locus to form amplified products; wherein the first standard locus and the first target locus have the same number of nucleotides but have a sequence that differs at one or more nucleotides; and wherein the second standard locus and the second target locus have the same number of nucleotides but have a sequence that differs at one or more nucleotides;

(b) sequencing the amplified products to determine a standard ratio that compares the relative amount of the amplified first standard locus compared to the amplified second standard locus; wherein the standard ratio indicates the difference in PCR efficiency for the amplification of the first standard locus and the second standard locus;

(c) determining a target ratio that compares the relative amount of the amplified first target locus compared to the amplified second target locus; and

(d) adjusting the target ratio from step (c) based on the standard ratio from step (b) to determine the relative amount of the first target locus and the second target locus in the sample.

116. The method of claim 115, further comprising determining the absolute amount of the first target locus and the second target locus in the sample.

117. The method of claim 115, comprising determining the presence or absence of a target locus in the sample.

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118. The method of claim 115, comprising simultaneously amplifying at least 300 different target loci.

119. The method of claim 115, wherein the target loci are present on the same chromosome of interest.

120. The method of claim 115, wherein at least some of the target loci are present on different chromosomes of interest.

121. The method of claim 115, wherein the PCR is performed using at least 100 primers that have at least 90% identity to the corresponding region of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

122. The method of claim 121, wherein at least 100 of the primers comprise the polynucleotide sequence of a primer in FIG. 34, 35, or 36 (SEQ ID NOs. 1-44,609).

123. The method of any one of claims 1, 105-107, 111, and 115, further comprising non-specifically amplifying nucleic acids in the sample prior to specifically amplifying target loci.

124. The method of claim 123, wherein the non-specific amplification comprises universal PCR.

125. The method of claim 123, wherein the non-specific amplification comprises whole genome application.

126. The method of claim 123, wherein the non-specific amplification comprises ligation-mediated PCR, degenerate oligonucleotide primer PCR, or multiple displacement amplification.

127. The method of any one of claims 1-75 or 105-126, wherein the ΔG values for each possible combination of two primers in the library are all equal to or greater than -5 kcal/mol.

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128. The library of any one of claims 76-103, wherein the ΔG values for each possible combination of two primers in the library are all equal to or greater than -5 kcal/mol.

129. The kit of claim 104, wherein the ΔG values for each possible combination of two primers in the library are all equal to or greater than -5 kcal/mol.

130. The method of any one of claims 1-75 or 105-127, wherein the library is used to amplify nucleic acids from a single cell.

131. The library of any one of claims 76-103 or 128, wherein the library is capable of amplifying nucleic acids from a single cell.

132. The kit of claim 104 or 129, wherein the library is capable of amplifying nucleic acids from a single cell.

133. A composition comprising at least 1,000 different amplicons in solution in one reaction volume; wherein the amplicons are produced from the simultaneous PCR amplification of at least 1,000 different target human loci using at least 1,000 different primer pairs in one reaction volume; wherein (i) less than 20% of the amplicons are primer dimers, and (ii) at least 80% of the amplicons comprise one of the target loci.

134. A composition comprising at least 1,000 different primers and at least 1,000 different amplicons in solution in one reaction volume; wherein the amplicons are produced from the simultaneous PCR amplification of at least 1,000 different target human loci with the primers in one reaction volume; wherein (i) less than 20% of the amplicons are primer dimers, and (ii) at least 80% of the amplicons comprise one of the target loci.

135. A composition comprising a library of any one of claims 76-103 or 128 comprising human nucleic acids and a non-human enzyme.

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ABSTRACT OF THE DISCLOSURE

The invention provides methods for simultaneously amplifying multiple nucleic acid regions of interest in one reaction volume as well as methods for selecting a library of primers for use in such amplification methods. The invention also provides library of primers with desirable characteristics, such as minimal formation of amplified primer dimers or other non-target amplicons.

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