

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

INTEGRATED DNA TECHNOLOGIES, INC.,
Petitioner,

v.

TECAN GENOMICS, INC.,
Patent Owner.

Case IPR2024-01506
U.S. Patent 11,725,241

**PETITION FOR *INTER PARTES* REVIEW
OF U.S. PATENT 11,725,241**

TABLE OF CONTENTS

I.	BACKGROUND	3
A.	Technical Background.....	4
1.	Biological Roles of DNA and RNA	4
a.	DNA.....	4
b.	RNA.....	5
2.	Pre-NGS DNA and RNA Research Tools	5
a.	Polymerase Chain Reaction.....	6
b.	Anchored PCR.....	7
c.	Sequencing By Synthesis (“SBS”)	9
3.	Next Generation Sequencing (“NGS”).....	10
4.	Library Preparation And Adaptors for NGS.....	11
5.	Sample Indexes	12
6.	Molecular Tags	14
II.	THE ’241 PATENT.....	18
A.	Prosecution History	19
B.	The Person Of Ordinary Skill In The Art.....	20
C.	Claim Construction	21
1.	“appended”	21
2.	“identifier site”	22
3.	“indexing site”	22
III.	IDENTIFICATION OF THE CHALLENGE (37 C.F.R. §42.104(b)) AND STATEMENT OF THE PRECISE RELIEF REQUESTED (37 C.F.R. §42.22(a))	22

A.	Priority Date	23
B.	The References In the Grounds Are Prior Art	23
IV.	GROUND I: ANTICIPATION OVER KIVIOJA.....	24
A.	Kivioja Disclosed Methods With All Of The Elements Claimed In The '241 Patent.	24
B.	Kivioja Anticipates Claim 1	27
1.	“A method for detecting duplicate sequencing reads, the method comprising”	28
2.	“obtaining amplicons each comprising an amplified fragment of a nucleic acid with an appended adaptor”	29
3.	“wherein each adaptor comprises an identifier site comprising a plurality of nucleotides unique to the amplified fragment”	33
4.	“sequencing the amplicons to generate sequence reads that include identifier and target sequence, and”	34
5.	“identifying sequence reads with identical identifier and target sequences as duplicates.”	34
C.	Claim 2	35
D.	Claim 3	36
E.	Claim 4	37
F.	Claim 5	38
G.	Claim 6	39
H.	Claim 7	39
I.	Claim 8	40
J.	Claim 9	41
K.	Claim 10	41

L.	Claim 11	42
M.	Claim 12	42
N.	Claim 13	43
O.	Claim 14	43
P.	Claim 15	44
Q.	Claim 16	44
V.	GROUND II: OBVIOUSNESS OVER KIVIOJA	45
A.	Claim 4	45
B.	Claim 6	46
C.	Claim 11	47
D.	Claim 16	48
VI.	GROUND III: ANTICIPATION OVER BIELAS	49
A.	Bielas Disclosed Methods With All Of The Elements Claimed In The '241 Patent	49
B.	Bielas Anticipates Claim 1	55
1.	Claim 1	55
a.	“A method for detecting duplicate sequencing reads, the method comprising:”	55
b.	“obtaining amplicons each comprising an amplified fragment of a nucleic acid with an appended adaptor”	56
c.	“wherein each adaptor comprises an identifier site comprising a plurality of nucleotides unique to the amplified fragment”	57

d.	“sequencing the amplicons to generate sequence reads that include identifier and target sequence, and”	58
e.	“identifying sequence reads with identical identifier and target sequences as duplicates.”	58
2.	Claim 2	61
3.	Claim 3	62
4.	Claim 6	62
5.	Claim 7	62
6.	Claim 8	63
7.	Claim 9	63
8.	Claim 10	64
9.	Claim 11	64
10.	Claim 12	65
11.	Claim 13	65
12.	Claim 14	66
13.	Claim 15	66
14.	Claim 16	66
VII.	GROUND IV: OBVIOUSNESS OVER BIELAS	67
A.	Claim 4	67
B.	Claim 5	69
C.	Claim 11	70
D.	Claim 12	71
E.	Claim 15	72

F.	Claim 16	72
VIII.	DISCRETIONARY DENIAL IS NOT APPROPRIATE	73
A.	Discretionary Denial Under §325(d) Is Not Appropriate	73
B.	Discretionary Denial Under § 314(d) Is Not Appropriate.	74
IX.	STANDING (37 C.F.R. § 42.104(a)) AND PROCEDURAL STATEMENTS	75
X.	MANDATORY NOTICES UNDER 37 C.F.R. §42.8.....	76
A.	Real Party-in Interest (37 C.F.R. §42.8(b)(1)).....	76
B.	Related Matters (37 C.F.R. §42.8(b)(2)).....	76
C.	Lead and Backup Counsel and Service Information (37 C.F.R. § 42.8(b)(3) and (b)(4))	77
XI.	CONCLUSION.....	77
	CERTIFICATION UNDER 37 C.F.R. § 42.24(d)	79
	CERTIFICATE OF SERVICE	80

TABLE OF AUTHORITIES

CASES

<i>3M Innovative Properties Co. v. Avery Dennison Corp.</i> , 350 F.3d 1365 (Fed. Cir. 2003)	21
<i>Advanced Bionics, LLC v. MED-EL Elektromedizinische Geräte G.m.b.H.</i> , IPR2019-01469, Paper 6 (PTAB Feb. 13, 2020).....	74
<i>Advanced Display Sys., Inc. v. Kent State Univ.</i> , 212 F.3d 1272 (Fed. Cir. 2000)	24
<i>Apple Inc. v. Fintiv, Inc.</i> , IPR2020-00019, Paper 11 (PTAB Mar. 20, 2020)	73, 74, 75
<i>Arbutus Biopharma Corp. v. ModernaTX, Inc.</i> , 65 F.4th 656 (Fed. Cir. 2023)	24
<i>CommScope Techs. LLC v. Dali Wireless, Inc.</i> , IPR2022-01242, Paper 23 (PTAB Feb. 27, 2023).....	75
<i>In re Brandt</i> , 886 F.3d 1171 (Fed. Cir. 2018)	47
<i>Uber Tech., Inc. v. X One, Inc.</i> , 957 F.3d 1334 (Fed. Cir. 2020)	47, 69
<i>Vicor Corp. v. SynQor, Inc.</i> , 869 F.3d 1309 (Fed. Cir. 2017)	47

EXHIBIT LIST

Exhibit	Short Name	Document
Ex. 1001		Reserved
Ex. 1002		Reserved
Ex. 1003	'399 patent	U.S. Patent No. 9,546,399
Ex. 1004	'357 patent	U.S. Patent No. 11,098,357
Ex. 1005	'241 patent	U.S. Patent No. 11,725,241
Ex. 1006		Reserved
Ex. 1007	Siebert	Siebert et al., <i>An improved PCR method for walking in uncloned genomic DNA</i> , NUCLEIC ACIDS RES. 23(6) 1087–1088 (1995)
Ex. 1008	Caruccio	Caruccio, <i>Preparation of Next-Generation Sequencing Libraries using Nextera™ Technology: Simultaneous DNA Fragmentation and Adaptor Tagging by In Vitro Transposition</i> , HIGH-THROUGHPUT NEXT GENERATION SEQUENCING: METHODS AND APPLICATIONS, METHODS IN MOLECULAR BIOLOGY, Ch. 17 (2011)
Ex. 1009	Bronner	Bronner et al., <i>Improved Protocols for Illumina Sequencing</i> , CURR PROTOC HUM GENET. (2009)
Ex. 1010		Reserved
Ex. 1011	Iafrate	U.S. Patent Application Publication No. 2013/0303461
Ex. 1012	Kivioja	Kivioja et al., <i>Counting absolute numbers of molecules using unique molecular identifiers</i> , NATURE METHODS (2011)

Exhibit	Short Name	Document
Ex. 1013	Bielas	International Publication No. WO 2013/123442 A1
Ex. 1014	Masoudi-Nejad	Masoudi-Nejad et al., <i>Next Generation Sequencing and Sequence Assembly Methodologies and Algorithms</i> (2013)
Ex. 1015	Zheng	Zheng et al., <i>Anchored multiplex PCR for targeted next-generation sequencing</i> , NATURE MEDICINE (2014)
Ex. 1016		Reserved
Ex. 1017		Reserved
Ex. 1018		File History for U.S. Patent No. 9,546,399
Ex. 1019		File History for U.S. Patent No. 11,098,357
Ex. 1020		File History for U.S. Patent No. 11,725,241
Ex. 1021	Miner	Miner et al., <i>Molecular barcodes detect redundancy and contamination in hairpin-bisulfite PCR</i> , NUCLEIC ACID RESEARCH 32:17 (2004)
Ex. 1022	McCloskey	McCloskey et al., <i>Encoding PCR Products with Batch-stamps and Barcodes</i> , BIOCHEM GENET 45:761–767 (2007)
Ex. 1023	Hiatt	Hiatt et al., <i>Parallel, tag-directed assembly of locally derived short sequence reads</i> , NAT METHODS 7(2):119–122 (2010)
Ex. 1024	König	König et al., <i>iCLIP reveals the function hnRNP particles in splicing at individual nucleotide resolution</i> , NAT STRUC MOL BIOL. 17(7):909–915 (2010)

Exhibit	Short Name	Document
Ex. 1025	Kinde	Kinde et al., <i>Detection and quantification of rare mutations with massively parallel sequencing</i> , PNAS 108(23):9350–9535 (2011)
Ex. 1026	Casbon	Casbon et al., <i>A method for counting PCR template molecules with application to next-generation sequencing</i> , NUCLEIC ACIDS RESEARCH 39:12 (2011)
Ex. 1027	Fu	Fu et al., <i>Counting individual DNA molecules by the stochastic attachment of diverse labels</i> , PNAS 108(22):9026–9031 (2011)
Ex. 1028	Jabara	Jabara et al., <i>Accurate sampling and deep sequencing of the HIV-1 protease gene using a Primer ID</i> , PNAS 108(50):20166–20171 (2011)
Ex. 1029	Tollervey	Tollervey et al., <i>Characterising the RNA targets and position-dependent splicing regulation by TDP-43; implications for neurodegenerative diseases</i> , NAT NEUROSCI. 14(4):452–458 (2011)
Ex. 1030	Shiroguchi	Shiroguchi et al., <i>Digital RNA sequencing minimizes sequence-dependent bias and amplification noise with optimized single-molecule barcodes</i> , PNAS 109(4):1347–1352 (2012)
Ex. 1031	Schmitt	Schmitt et al., <i>Detection of ultra-rare mutations by next-generation sequencing</i> , PNAS 109(36):14508–14513 (2012)

Exhibit	Short Name	Document
Ex. 1032	De Luca	De Luca and Dono, <i>The Opportunities and Challenges of Molecular Tagging Next-Generation Sequencing in Liquid Biopsy</i> , MOLECULAR DIAGNOSIS & THERAPY 25:537–547 (2021)
Ex. 1033	Samuels	U.S. Patent Application Publication No. 2012/0220494
Ex. 1034		Reserved
Ex. 1035		Reserved
Ex. 1036		Reserved
Ex. 1037		Reserved
Ex. 1038		Reserved
Ex. 1039		Reserved
Ex. 1040		Reserved
Ex. 1041		Reserved
Ex. 1042		Reserved
Ex. 1043		Reserved
Ex. 1044		Reserved
Ex. 1045		Reserved
Ex. 1046		Reserved
Ex. 1047		Reserved
Ex. 1048		U.S. Provisional Patent Application 61/645,364

Exhibit	Short Name	Document
Ex. 1049		U.S. Provisional Patent Application 61/679,302
Ex. 1050	Moorthie	Moorthie et al., <i>Review of massively parallel DNA sequencing technologies</i> , HUGO JOURNAL 5:1–12 (2011)
Ex. 1051		Reserved
Ex. 1052		Reserved
Ex. 1053	Syed	Syed, <i>Chapter 19: Second-Generation Sequencing Library Preparation: In Vitro Tagmentation via Transposome Insertion</i> , in TAG-BASED NEXT GENERATION SEQUENCING (Matthias Harbers and Günter Kahl, eds., 2011)
Ex. 1054	Loeb	Loeb et al., <i>Transcriptome-wide miR-155 Binding Map Reveals Widespread Noncanonical MicroRNA Targeting</i> , MOLECULAR CELL 760–770 (2012)
Ex. 1055	Kircher	Kircher et al., <i>Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform</i> , NUCLEIC ACIDS RESEARCH 40:1 (2012)
Ex. 1056	Illumina Letter	Illumina Customer Letter (Sept. 7, 2012)
Ex. 1057	Nextera Prep Guide	Nextera® XT DNA Sample Preparation Guide (Oct. 2012)
Ex. 1058	Troutt	Troutt et al., <i>Ligation-anchored PCR: A simple amplification technique with single-sided specificity</i> , PNAS, USA 9823–9825 (1992)

Exhibit	Short Name	Document
Ex. 1059	Hug & Schuler	Hug and Schuler, <i>Measurement of the Number of Molecules of a Single mRNA Species in a Complex mRNA Preparation</i> , J. THEOR. BIOL. 221:615–624 (2003),
Ex. 1060	Meyer & Kircher	Meyer and Kircher, <i>Illumina Sequencing Library Preparation for Highly Multiplexed Target Capture and Sequencing</i> , COLD SPRING HARBOR PROTOCOLS (2010)
Ex. 1061	Kircher Thesis	Kircher Thesis, <i>Understanding and improving high-throughput sequencing data production and analysis</i> , LEIPZIG UNIVERSITY, Germany (2011)
Ex. 1062	Smith Thesis	Smith Thesis, <i>Low-Level Variant Detection in Human Mitochondrial DNA Using The Illumina® MiSeq™ Next-Generation Sequencing (NGS) Platform</i> , WESTERN CAROLINA UNIVERSITY (2013)
Ex. 1063	Won	Won et al., <i>Detecting Somatic Genetic Alterations in Tumor Specimens by Exon Capture and Massively Parallel Sequencing</i> , J. VISUALIZED EXPERIMENTS (2013)
Ex. 1064	Kozich	Kozich et al., <i>Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform</i> , APPLIED AND ENVIRONMENTAL MICROBIOLOGY 79:17, 5112–5120 (2013)
Ex. 1065	Pray	Pray, <i>Major Molecular Events of DNA Replication</i> , NATURE EDUCATION (2008)

Exhibit	Short Name	Document
Ex. 1066	Mackenzie	Mackenzie, <i>DNA vs. RNA – 5 Key Differences and Comparison</i> , TECHNOLOGY NETWORKS (2020)
Ex. 1067	Clancy	Clancy and Brown, <i>Translation: DNA to mRNA to Protein</i> , NATURE EDUCATION (2008)
Ex. 1068	Gauthier Thesis	Gauthier Thesis, <i>Simulation of polymer translocation through small channels</i> , UNIVERSITY OF OTTAWA, Canada (2008)
Ex. 1069	Lohman	Lohman et al., <i>DNA Ligases</i> , CURRENT PROTOCOLS IN MOLECULAR BIOLOGY 3.14.1-3.14.7 (2011)
Ex. 1070	Margulies	Margulies et al., <i>Genome sequencing in microfabricated high-density picolitre reactors</i> , NATURE 376–380 (2005)
Ex. 1071		DNA Sequencing Costs: Data, https://www.genome.gov/about-genomics/fact-sheets/DNA-Sequencing-Costs-Data
Ex. 1072	Craig	Craig et al., <i>Identification of Genetic Variants Using Barcoded Multiplexed Sequencing</i> , NAT. METHODS 5(10) 887–893 (2008)
Ex. 1073	Parameswaran	Parameswaran et al., <i>A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing</i> , NUCLEIC ACIDS RESEARCH 35:19 (2007)

Exhibit	Short Name	Document
Ex. 1074	Lonigro	Lonigro et al., <i>Detection of Somatic Copy Number Alterations in Cancer Using Targeted Exome Capture Sequencing</i> , NEOPLASIA 13(11) 1019–1025 (2011)
Ex. 1075	Bielas Annotated Figure	Annotated Figure
Ex. 1076	Bielas Illumina P5	Annotated Figure
Ex. 1077	Bielas Read 1 Primer	Annotated Figure
Ex. 1078	Bielas i5 Index	Annotated Figure
Ex. 1079	Sims Declaration	Declaration of Peter A. Sims

Integrated DNA Technologies, Inc. (“IDT” or “Petitioner”) requests *inter partes* review of claims 1-16 of U.S. Patent No. 11,725,241 (the “Challenged Claims” of the “’241 patent”). EX1005. Petitioner is a market leader in assay solutions for next-generation DNA sequencing (“NGS”), and sells NGS products and software under the Archer brand name. These products use Petitioner’s anchored multiplex PCR (“AMP”) technology, which is based on chemistry developed at Massachusetts General Hospital and in-licensed to Petitioner. This technology is used in hundreds of laboratories and hospitals across the country for a variety of research applications, especially cancer research.

In October 2023, Patent Owner Tecan Genomics, Inc. (“Tecan”) sued IDT, alleging that IDT’s products induce infringement of the ’241 patent, among others. Based on its disclosures in district court as of the date of this petition, Tecan does not market a competing product or practice the ’241 patent.

The ’241 patent claims a method of “detecting duplicate sequencing reads” following NGS by using a random identifier sequence appended to the DNA fragments prior to sequencing.

By 2013, when the priority application was filed, the ’241 patent’s method was well known. Scientists in the DNA sequencing field had long recognized—and solved—the problem of determining whether an observed sequence was a duplicate created during NGS library preparation or a copy contained in the original sample.

The solution, as reflected in multiple prior art publications, was to label DNA fragments with random identifier sequences prior to NGS and use the labels to identify and remove duplicate sequences created during library preparation. The remaining elements recited in the claims were also well known at the time. Adaptors with the other features recited in the dependent claims were conventional by that point, as were methods of attaching them to DNA fragments via ligation or priming reactions. The remainder of the method recites conventional steps performed in any NGS reaction.

In fact, every element of the method claimed in the '241 patent was expressly disclosed in at least two references, including the Kivioja and Bielas references that are the basis for this Petition. Kivioja, a 2011 article in *Nature Methods*, EX1012, teaches the use of a random identifier sequence that “can improve accuracy of almost any next-generation sequencing method” and uses the random identifier sequence and nucleic acid fragment sequence to differentiate original fragment sequences from duplicates—precisely the same use as in the later '241 claims. EX1012.0001-03. Kivioja thus anticipates, or at a minimum, renders obvious, the claims of the '241 patent.

Bielas, WO 2013/123442, EX1013, teaches a method using ligated adaptors with exactly the features claimed in the '241 patent and the use of those adaptors in an NGS method that permits each sequenced fragment to be “connected back to the

original molecule.” EX1013.0009. Bielas discloses every element of the method of the '241 patent and anticipates, or at a minimum, renders obvious, the claims.

Accordingly, Petitioner respectfully requests that the Board institute *inter partes* review and cancel the Challenged Claims.

I. BACKGROUND

The '241 patent relates to methods for preparing libraries of nucleic acids for NGS and information processing. EX1079.¶30. NGS was introduced for commercial use around 2005 and revolutionized sequencing by dramatically cutting down time and cost. EX1079.¶¶80-83; EX1050.0001-02; EX1071.0002. While NGS harnessed new advances in computer technology and molecular fabrication to increase throughput and efficiency, the biology and chemistry underlying NGS was decades old. EX1079.¶¶62-66, ¶¶80-84; EX1070.0001-06; EX1009.0001, 0002-03; EX1014.0022; EX1050.0001-02; EX1060.0009; EX1062.0034-35; EX1068.0020; EX1008.0001. NGS was rapidly adopted across academia and industry, and there was an explosion of articles and patent applications for NGS-related processes based on applying those old molecular biology tools to NGS, often covering methods that were simultaneously being described elsewhere. EX1079.¶¶80-84; EX1070.0001-06; EX1009.0002-03; EX1014.0022; EX1050.0002; EX1060.0009; EX1062.0034-35.

A. Technical Background

The following primer is based on EX1079, the Declaration of Dr. Peter Sims, a professor at Columbia, who has been teaching and researching in the NGS field for many years.

1. Biological Roles of DNA and RNA

a. DNA

DNA encodes genetic information in the sequence of four repeated nucleotides: adenine (“A”), cytosine (“C”), guanine (“G”), and thymine (“T”). EX1079.¶¶31-35, ¶46, ¶50; EX1014.0011-12. Two strands of nucleotides in DNA are “annealed” to one another based on a specific interaction between the nucleotides in each strand. EX1079.¶36; EX1014.0012. Based on this pairing, two paired DNA strands are said to be “complementary” to one another. EX1079.¶¶36-37; EX1014.0012.

Each strand of DNA is directional with each nucleotide attached from the 3' end of one to the 5' end of the next, and thus each strand of DNA has a 5' and a 3' end. EX1079.¶35, ¶38; EX1014.0011-12. The two DNA strands run in the opposite direction to one another—the 5' end of one strand is complementary to the 3' end of the other. EX1079.¶38.

DNA is generally synthesized one strand at a time by a “polymerase” that starts from a short strand called a “primer” annealed to a template strand. EX1079.¶¶42-45; EX1014.0011-12; EX1065.0001-02.

DNA sequencing has long been an important goal of biological research to study the relationship between genotype and phenotype and the underlying process of genetic heritability. EX1079.¶¶57-59; EX1009.0001. DNA sequencing is thus useful in fields from archaeology to zoology. EX1079.¶57.

b. RNA

RNA is a polymer related to DNA that is generally single stranded and plays a critical translational function. EX1079.¶¶39-41; EX1066.0001. DNA in cells is transcribed to make RNA by a “transcriptase.” EX1079.¶47. RNA in turn can be translated into the proteins that make up the basic building blocks of a cell. EX1079.¶¶48-49; EX1067.0001.

RNA can also be “reverse transcribed” back to complementary DNA (“cDNA”) by a “reverse transcriptase.” EX1079.¶52, ¶61.

Because RNA also plays a critical biological role and reflects the intermediate step between genetic information and proteins, scientists have also been interested in RNA sequencing. EX1079.¶51, ¶60.

2. Pre-NGS DNA and RNA Research Tools

Beginning in the second half of the 20th century, scientists began to use the cell’s biological tools, such as polymerases and transcriptases, for research purposes, including to manipulate and sequence DNA and RNA. EX1079.¶¶67-79.

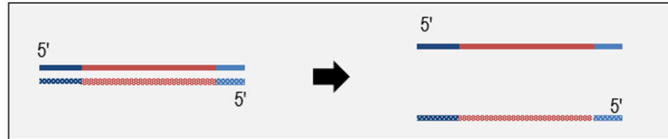
a. Polymerase Chain Reaction

Discovered in the 1980s, a polymerase chain reaction (“PCR”) is a method of exponentially copying DNA (including cDNA) and thus is a frequently-used tool to increase DNA concentration for various purposes, *e.g.*, prior to sequencing. EX1079.¶¶67, ¶¶71-72. In a PCR reaction, DNA fragments are denatured to single strands and combined with a polymerase, free nucleotides, and primers. EX1079.¶¶68. The primers anneal to the single-stranded DNA fragments and the DNA polymerase regenerates a complementary strand. EX1079.¶¶69-70. The double-stranded products are denatured again and the process repeats. EX1079.¶¶69-70.

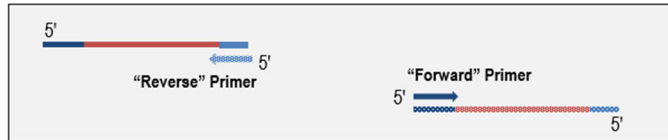
Because each strand can be a template in the next round of the reaction, PCR is said to “amplify” DNA because the process is exponential and each round of the reaction doubles the number of DNA molecules. EX1079.¶¶67-70. For a PCR reaction to proceed exponentially, one “forward” primer anneals on one side of the fragment on one strand and one “reverse” primer anneals to a sequence on the other side of the fragment on the complementary strand, as shown in the figure¹ below. EX1079.¶¶68-69.

¹ This figure uses a standard convention to depict DNA strands as colored bars that is discussed in further detail in Dr. Sims’s declaration. EX1079.¶¶69.

1. Denature template DNA fragment into single strands.



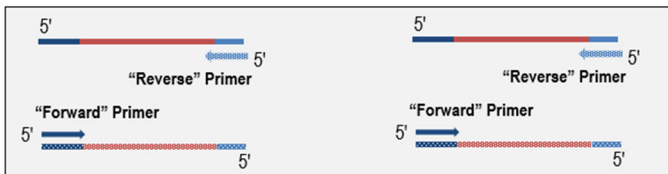
2. Anneal primers to each template strand.



3. Generate complementary DNA strands using a polymerase.



4. Repeat.



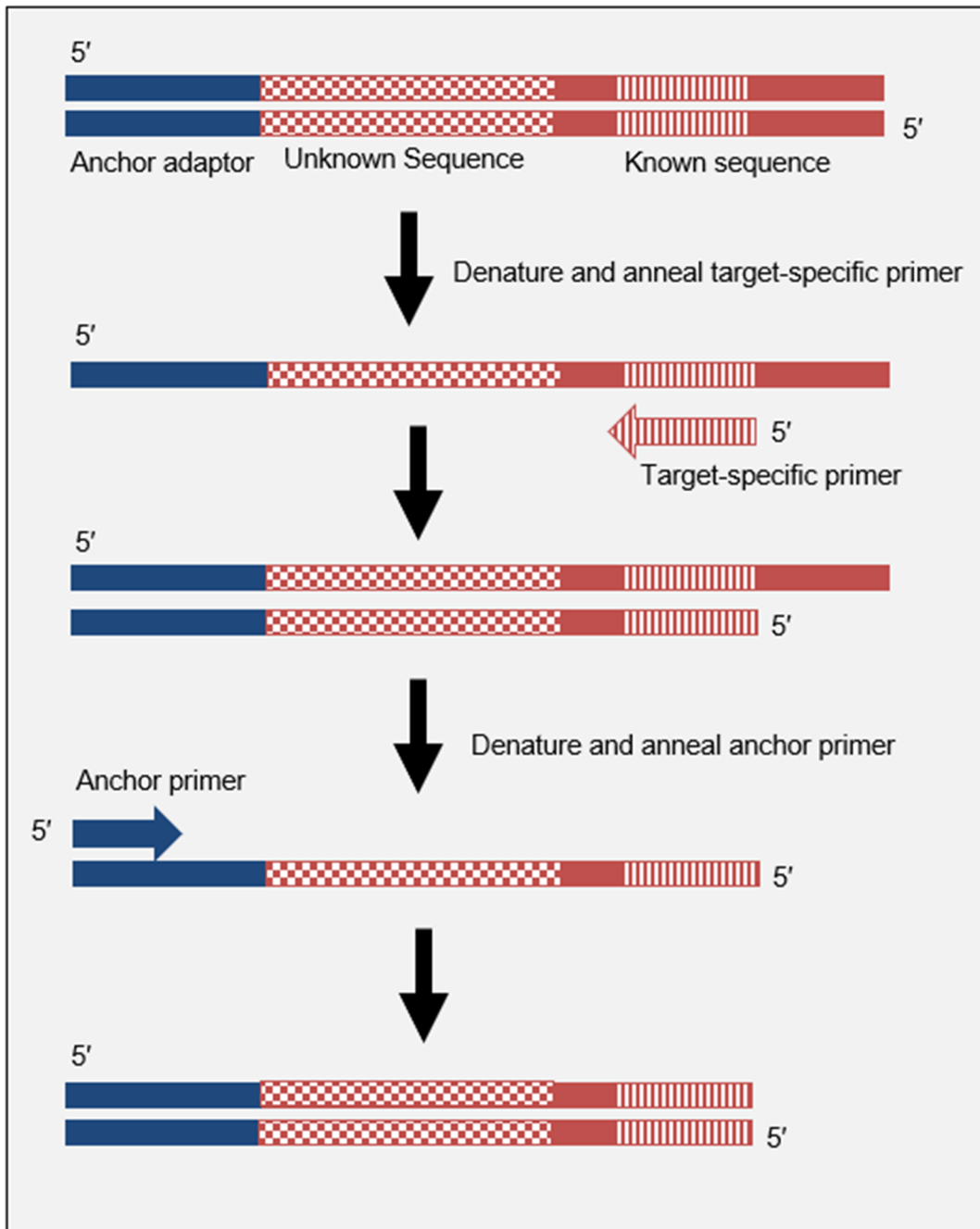
b. Anchored PCR

One challenge with traditional PCR is that amplification requires knowing at least a portion of the sequence to be amplified at the beginning and the end so that specific forward and reverse primers can be made (as opposed to using random primers to amplify all of the DNA). EX1079.¶¶73-79; EX1058.0001-02; EX1007.0001-02. But for some research applications, being able to amplify an unknown sequence is desirable, *e.g.*, for genetic mutations implicated in cancer. EX1079. ¶73.

Beginning in the 1990s, scientists developed a technique, called anchored PCR, to amplify such sequences. EX1079.¶73-79; EX1058.0001-02; EX1007.0001-02. In anchored PCR, a synthetic sequence, called an “adaptor,” is added to one or

both ends of the DNA prior to PCR. EX1079.¶56. One known method of adding an adaptor was to use an enzyme called a “ligase.” EX1079.¶53. Ligases directly join two pieces of DNA or RNA together via a process called “ligation.” EX1079.¶¶53-55; EX1069 at 0001-06. One common application was to join a synthetic sequence to a naturally occurring sequence, *e.g.*, as an adaptor sequence for anchored PCR. EX1079.¶56.

The anchored PCR technique is summarized in the figure below. EX1079.¶74. Once the anchor sequence is added, a PCR reaction is conducted with two primers: one complementary to the anchor sequence, and one complementary to a known sequence. EX1079.¶¶74-79; EX1058.0002. The result of this PCR reaction is to amplify fragments with the known sequence on one side and the anchor on the other, with potentially unknown sequence between the two. EX1079.¶74, ¶79; EX1007 at 0002.



c. Sequencing By Synthesis ("SBS")

In the 1970s, Frederick Sanger developed an eponymous method of sequencing DNA by using the natural action of DNA polymerase. . EX1079.¶62-66; EX1068.0020; EX1050.0001; EX1009.0001-02. Sanger sequencing, or

“sequencing by synthesis” (“SBS”) involved denaturing double-stranded DNA, annealing primers, and using a polymerase to synthesize a complementary strand. EX1079.¶¶63-64; EX1068.0020. The addition of each nucleotide to the growing strand in the DNA synthesis reaction was measured using radioactive or fluorescently-labeled nucleotides. EX1079.¶63.

3. Next Generation Sequencing (“NGS”)

Beginning in the mid-2000s, sequencing technology was revolutionized by the introduction of NGS. EX1079.¶80, ¶86; EX1050.0001-02; EX1008.0001; EX1014. While NGS used sequencing by synthesis chemistry, NGS devices incorporated modern fabrication and imaging techniques to permit exponentially higher throughput using many parallel reactions. EX1079.¶80; EX1050.0001-02; EX1008.0001. Multiple different NGS platforms were launched in the mid-2000s, but the ones that were most widely adopted incorporated a few common features. EX1079.¶84; EX1009.0003; EX1050.0001-02; EX1060.0009; EX1062.0034-35. Specifically, these NGS platforms used a solid support, such as a chip or a bead, to which fragments of DNA were attached. EX1079.¶82, ¶84; EX1050.0002. NGS platforms then performed millions of parallel SBS reactions, which were each measured individually using modern microscopy or other analytical techniques. EX1079.¶84-85. Because of the massively parallel nature of NGS, sequencing could be conducted using far less space and reagents than before, which led to much

greater throughput at a lower cost. EX1079.¶81, ¶85; EX1050.0001-02; EX1071.0002.

The first NGS platform, Roche/454 pyrosequencing (“454”), was introduced in 2004 and became the first commercially available NGS platform in 2005. EX1079.¶83, ¶¶87-91; EX1070.0001-06; EX1009.0002; EX1014.0022. By 2013, several other NGS platforms were available, including, among others, Life Technologies/Ion Torrent “semiconductor sequencing,” EX1079.¶¶98-99; EX1014.0027-28, and Solexa/Illumina “reversible terminator sequencing,” which by 2013 was the dominant NGS platform, EX1079.¶¶92-97; EX1050.0002; EX1014.0023-24; EX1060.0009; EX1062.0034-38; EX1063.0003-04; EX1054.0012-15.

4. Library Preparation And Adaptors for NGS

In 2013, 454, Illumina, and Ion Torrent all attached fragments to be sequenced to a solid support prior to sequencing. EX1079.¶82, ¶84, ¶¶100-103; EX1008 at 0001-02; EX1009.0003. All three platforms did this by annealing the fragments to short DNA strands of known sequence that were fixed to the support. EX1079.¶¶100-101; EX1008.0001-02; EX1009.0003. In addition, all three platforms required a primer binding site for the SBS reaction primers. EX1079.¶102. In order to add these synthetic sequence adaptors as “handles” to the

fragments for sequencing, NGS required a “library preparation” process before sequencing. EX1079.¶103.

During library preparation, DNA is fragmented and adaptors are attached that contain, at minimum, a sequence complementary to the strand on the solid support and a binding site for the SBS primer. EX1079.¶100. To ensure sufficient concentration of sample for NGS, following attachment of the adaptors, the fragments are often amplified in one or more PCR steps. EX1079.¶101-102.

By 2013, scientists recognized that adaptors could be modified to incorporate additional synthetic sequences serving other functions, such as sample indexes for multiplex sequencing and random identifier sequences to distinguish original fragment sequences from PCR copies generated during library preparation. EX1079.¶103.

5. Sample Indexes

For certain NGS applications, the number of fragments in a particular sample was much smaller than the available space on an NGS device. EX1079.¶104. With NGS’s capability of sequencing numerous fragments in a single run, scientists began to simultaneously sequence multiple samples, by “multiplexing” pooled samples. EX1079.¶¶104-112; EX1073.0001-09; EX1072; EX1055.0001-02; EX1064.0003.

With certain modifications to the adaptors added during library preparation, an NGS platform could simultaneously sequence DNA or cDNA from multiple

samples. EX1079.¶¶104-112. By 2013, matching a sequence to its sample was generally accomplished by using synthetic sequences called “sample indexes” or “sample barcodes” unique to a particular sample. EX1079.¶¶110-111; EX1064.0003; EX1055.0002. When pooled samples are sequenced, a particular sequence read can be “demultiplexed” to its original sample using the sample index. EX1079.¶¶104-107. Sample indexes for Illumina sequencers were in use at least as of 2008. EX1079.¶107; EX1072. By the priority date, some Illumina devices sequenced the sample index in an SBS reaction independently from the fragment sequence, while others sequenced them as part of the main read. EX1079.¶¶110-111.

NGS platforms output sequencing data in certain file formats. EX1079.¶84, ¶97; EX1060.0009; EX1062.0034-35; EX1061.0080-81. For example, one file format output by an Illumina device is called FASTQ. EX1079.¶84; EX1060.0009; EX1062.0034-35. The files contain data for each sequencing read performed and by 2013, it was customary for FASTQ files to be separated by sample, or “demultiplexed,” using the sample index prior to further analysis. EX1079.¶97; EX1060.0009; EX1062.0034-38; EX1061.0080-81; EX1063.0003-04; EX1054.0012-15.

6. Molecular Tags

Because PCR was a standard component of NGS library preparation, scientists realized they needed a way to ascertain whether a particular group of seemingly identical sequencing reads were copies of the same original fragment from the sample, or if there were actually fragments in the original sample with the same sequence. EX1079.¶113. The solution multiple research groups independently discovered was to add sequences, sometimes called “molecular tags,” “unique molecular identifiers,” “molecular barcodes,” or “cyphers” to NGS adaptors prior to amplification so that PCR copies that originated from the same DNA fragment could be identified as duplicates. EX1079.¶¶118-170; EX1059; EX1021; EX1022; EX1023; EX1024; EX1025; EX1026; EX1027; EX1028; EX1012; EX1029; EX1030; EX1031; EX1032.

By 2013, scientists understood that library preparation and subsequent sequencing could introduce errors due to, *inter alia*, polymerase’s natural error rate, or because of nonspecific priming. EX1079.¶109, ¶¶114-117, ¶135, ¶456; EX1025.0001; EX1013.0004. Molecular tags allowed scientists to determine whether a change observed in a sequence (compared to other fragments or a reference genome) was the result of a mutation in the original fragment or an error introduced during PCR or sequencing. EX1079.¶¶117-118.

Molecular tags also permitted researchers to count the number of original fragments in a sample, *e.g.*, to count the number of copies of a gene in a patient sample. EX1079.¶113. This was of particular interest for cancer research because cancer cells often have too many copies of particular oncogenic genes. EX1079.¶231; EX1074.0001-11.

For these reasons, even prior to widespread adoption of NGS, scientists recognized the benefit of being able to identify PCR copies that originated from the same DNA fragment. EX1079.¶103, ¶113. A 2003 paper proposed random molecular tags added via PCR or ligation “to count the number of individual molecules pertaining to the same molecular species.” EX1059.0001-09; EX1079.¶¶119-120. And as early as 2004, scientists began tagging “individual genomic fragments” prior to PCR amplification with random sequences called “molecular sequence barcodes” to identify “redundant and contaminant sequences.” EX1021.0001-03; EX1079.¶¶121-123. These random sequences allowed scientists “to evaluate cloned sequences for redundancy and contamination,” quantify original sample molecules, and correct “for skewing that can arise from PCR amplification or the cloning” of the sample. EX1021.0002-03; EX1079.¶¶121-123. This method served “as a highly accurate method for positive identification of desired sequences.” EX1021.0002; EX1079.¶¶121-123.

The prior art also taught how to modify the length of the random sequence to account for the number of fragments in the original sample. EX1079.¶128. Because there are four different nucleotides that can be incorporated into any one “spot” in a polynucleotide, there are 4^n possibilities for uniquely labeling original fragments where “n” is the number of nucleotides in the random sequence. EX1022.0004; EX1079.¶128. The prior art made clear that as long as there were a sufficient number of tags compared to the number of original fragments, each fragment could be made unique by combining the molecular tag and fragment sequence. EX1079.¶¶125-129, ¶¶143-145; EX1022.0004; EX1027.0002.

Once Illumina released its HiSeq 2000 instrument in 2010, greatly increasing the availability of NGS, the field exploded. EX1079.¶86, ¶89, ¶129. Scientists simultaneously, and frequently, began publishing papers on the use of random sequence tags to identify duplicate copies created by PCR and attribute observed sequences to original fragments:

- In 2010, Hiatt proposed randomized 20-nucleotide tags in adaptor-ligated fragments “to identify molecules that are clonally derived” from the same fragment following PCR. EX1023.0001-04; EX1079.¶¶130-132.
- In 2010, König incorporated random three-nucleotide tags in cDNA during reverse transcription to “discriminate between unique cDNA products and PCR duplicates.” EX1024.0007; EX1079.¶¶133-134.

- In 2011, Kinde added 12-14-nucleotide tags via PCR to identify PCR duplicates, enhance the accuracy of NGS, and detect rare mutations. EX1025.0001, 0004-06; EX1079.¶¶135-138.
- In 2011, Casbon ligated adaptors with partially random 12-nucleotide tags to fragments prior to PCR. EX1026.0001-06. The tags were “used to determine whether a putative variant is associated with a single template molecule or, alternatively, multiple template molecules and hence the probability that it derives from a polymerase error or true variant.” EX1026.0001; EX1079.¶¶139-142.
- In 2011, Fu ligated adaptors with 14-nucleotide tags to fragments prior to PCR to count the absolute number of original molecules in a sample. EX1027.0001-02, 0004-05; EX1079.¶¶143-145.
- In 2011, Jabara added 8-nucleotide tags to cDNA libraries of viral RNA to “directly remove error, correct for PCR resampling, and capture the fluctuation of minor variants in the viral population within a host.” EX1028.0002; EX1079.¶¶146-149.
- In 2011, Kivioja described a method of using random tags in combination with the sequences of the fragments to which they were attached as a “unique molecular identifier” associated with a particular original fragment to enable removal of copies from sequencing data and counting

of original fragments. EX1012.0001-03, 0005; EX1079.¶¶150-159.

- In 2011, Tollervey used König’s protocol of random molecular tags prior to PCR to identify “3.7 million reads representing unique cDNA molecules.” EX1029.0002; EX1079.¶¶160-161.
- In 2012, Shiroguchi ligated adaptors containing 20-nucleotide pre-defined tags to cDNA prior to PCR in order to count the number of fragments in the original sample. EX1030.0001-05; EX1079.¶¶162-163.
- In 2012, Schmitt reported a method of ligating “standard Illumina sequencing adapters” containing 12-nucleotide tags to fragments to detect and remove PCR errors and thereby discover rare genetic variants. EX1031.0001-06; EX1079.¶¶164-169.
- In 2013, Bielas filed a patent application disclosing a method of ligating adaptors with random molecular tags prior to PCR to differentiate “true mutations” in the original fragments from PCR artifacts. EX1013.0027, 0032; EX1079.¶¶455-486.

II. THE ’241 PATENT

The ’241 patent claims priority to a provisional application filed on November 13, 2013. EX1005.0001. The ’241 patent is the fourth issued patent in a chain of divisional and continuation applications claiming priority to the ’826 provisional.

Id.

The '241 patent's claims are directed to a "method for detecting duplicate sequencing reads." EX1005 at 25:32-33. Claim 1, the only independent claim, recites three steps:

A method for detecting duplicate sequencing reads, the method comprising:

obtaining amplicons each comprising an amplified fragment of a nucleic acid with an appended adaptor, wherein each adaptor comprises an identifier site comprising a plurality of nucleotides unique to the amplified fragment;

sequencing the amplicons to generate sequence reads that include identifier and target sequence; and

identifying sequence reads with identical identifier and target sequences as duplicates.

EX1005 at 25:32-43.

A. Prosecution History

The '241 patent issued from an application filed on July 21, 2021. EX1005.0001. The Examiner did not issue any rejections prior to issuing a Notice of Allowability on March 17, 2023. EX1020.0001-10427. In the Notice, the Examiner stated that the pending independent claim (what would become claim 1 in the issued '241 patent) could only claim priority to July 21, 2021, the filing date of

the non-provisional application being examined, and the Patent Owner did not submit any response. EX1020.10433. While the Examiner stated that the prior art of record “does not teach or suggest using a combination of target sequence and identifier sequence for duplicate removal,” EX1020.10434, the Patent Owner did not submit either the Kivioja or Bielas references to the Office at any point during prosecution.

B. The Person Of Ordinary Skill In The Art

Because the '241 patent is directed to library preparation and data interpretation methods for NGS, the POSA could have had academic training in any of a variety of fields that used NGS, including chemistry, molecular biology, biochemistry, or medicine. EX1079.¶184. The POSA would have a PhD or equivalent training in one of those fields and several years of work experience (or commensurately less education and more work experience). EX1079.¶184. The POSA further would have had specific experience with implementing and designing library preparation methods for Illumina, the leading NGS platform in 2013. EX1079.¶185. This would have included specific experience with, and understanding of, the sequences contained in adaptors for Illumina sequencing at the priority date, and the chemistry used to attach those adaptors to DNA fragments prior to sequencing. EX1079.¶184.

As a professor of systems biology and director of the Sequencing Core at Columbia University, Dr. Sims exemplifies the POSA in this case. EX1079.¶¶4-14. Dr. Sims has been using NGS platforms, including Illumina, and NGS library preparation techniques continuously since the late 2000s. EX1079.¶186. In addition to using NGS regularly in his research, Dr. Sims has published papers and received patents relating to his own innovative NGS techniques. EX1079.¶¶184-186.

C. Claim Construction

Where relevant claim terms are defined in the '241 patent, EX1005 at 4:33-6:21, Petitioner applies those definitions, as set forth in this section.

1. “appended”

The '241 patent does not place any limitation on how the adaptors are “appended” to the fragments to obtain amplicons. The plain and ordinary meaning of “appended” is simply “added” and does not imply any particular restrictions on the means of adding the “appended” adaptor. EX1079.¶403; *see 3M Innovative Properties Co. v. Avery Dennison Corp.*, 350 F.3d 1365, 1371-72 (Fed. Cir. 2003) (favoring structural claim interpretations). The '241 patent states that “[i]n some embodiments, appending the adaptor to the nucleic acid fragments generated by the methods described herein can be achieved using a ligation reaction or a priming reaction.” EX1005 at 9:34-38. Thus, “appended” must encompass attachment by at

least ligation and priming reactions, confirming the breadth of the term's ordinary meaning.

2. “identifier site”

The '241 patent defines “identifier site” as a molecular tag attached to fragments prior to amplification, specifically, “a nucleic acid sequence that comprises random bases and is used to identify duplicate sequencing reads.” EX1005 at 4:45. An “identifier site” “can be designed as a set of sequences, or it can be semi-random, or it can be completely random.” EX1005 at 4:51-53. An “identifier site” can also “be a fixed length, or it can be a variable length.” EX1005 at 4:53-54.

3. “indexing site”

The '241 patent defines “indexing site” as a sample index, specifically, “a nucleic acid sequence that acts as an index for multiple polynucleotide samples, thus allowing for the samples to be pooled together into a single sequencing run, which is known as multiplexing.” EX1005 at 4:37-40.

III. IDENTIFICATION OF THE CHALLENGE (37 C.F.R. §42.104(b)) AND STATEMENT OF THE PRECISE RELIEF REQUESTED (37 C.F.R. §42.22(a))

Petitioner respectfully requests institution of IPR and cancellation of claims 1-16, for the reasons in the table below and explained further below. In support of

the proposed grounds, this Petition includes the declaration of Dr. Peter A. Sims, EX1079, and is further supported by the references cited herein.

Ground	References	Basis	Claims Challenged
I	Kivioja (EX1012)	§102	1-16
II	Kivioja	§103	4, 6, 11, 16
III	Bielas (EX1013)	§102	1-3, 6-16
IV	Bielas	§103	4, 5, 11, 12, 15, 16

A. Priority Date

To the extent the Challenged Claims are entitled only to a 2021 priority date, and Tecan could prove that the Archer AMP products infringe, the Challenged Claims would all be invalid based on the prior sale and use of the Archer products. Accordingly, Petitioner assumes that Tecan will assert the earliest claimed priority date of November 13, 2013, contrary to the Examiner's findings, and for purposes of this Petition only, without prejudice to its right to challenge that priority date in any subsequent proceeding, has assumed that the November 13, 2013 priority date applies.

B. The References In the Grounds Are Prior Art

Kivioja published online on November 20, 2011 and included supplemental materials available on the journal's website. EX1012.0001, 0003. The online supplemental material was made available contemporaneously with the article and is cited extensively in the article such that the POSA would have considered them one reference. EX1079.¶¶206-207. To the extent the article and supplemental

material are not a single prior art reference, the supplemental material is incorporated by reference. *See Arbutus Biopharma Corp. v. ModernaTX, Inc.*, 65 F.4th 656, 662-63 (Fed. Cir. 2023); *Advanced Display Sys., Inc. v. Kent State Univ.*, 212 F.3d 1272, 1283-84 (Fed. Cir. 2000). Kivioja is §102(a)(1) prior art.

Bielas, published on August 22, 2013, EX1013.0001, is prior art under at least §102(a)(1).

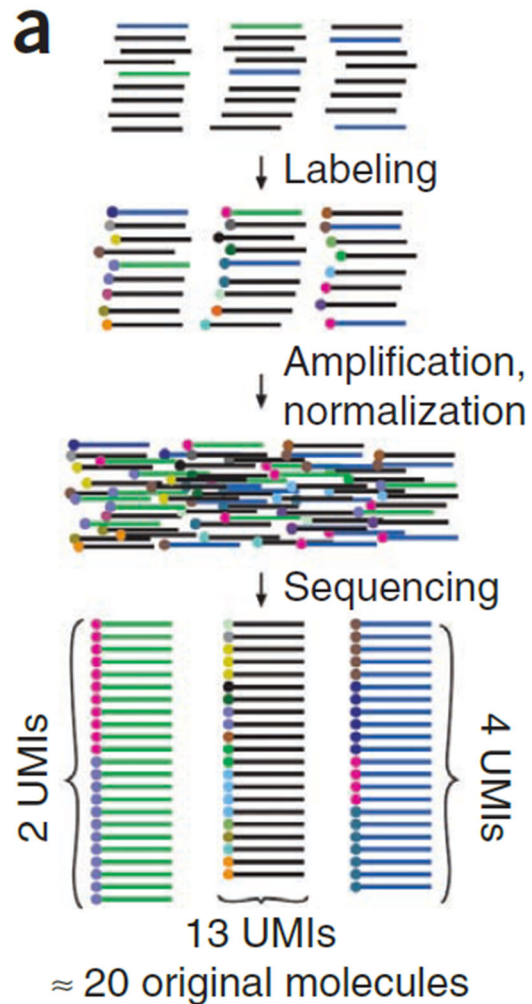
IV. GROUND I: ANTICIPATION OVER KIVIOJA

A. Kivioja Disclosed Methods With All Of The Elements Claimed In The '241 Patent.

Kivioja discloses every element of claims 1-16 of the '241 patent. Kivioja disclosed a method of library preparation to “improve the accuracy of almost any next-generation sequencing method.” EX1012.0001; EX1079.¶236. Kivioja explained that “errors introduced during amplification”—that is, PCR error—hinder interpretation of sequencing data. EX1012.0002; EX1079.¶236. Kivioja solved this problem by adding a random molecular tag sequence prior to amplification and sequencing, and using that tag sequence in combination with the fragment sequence to associate observed sequences with a particular original fragment. EX1012.0001, 0005; EX1079.¶¶236-240.

Kivioja showed its method diagrammatically in Figure 1(a), reproduced in this paragraph. EX1012.0001. Kivioja fragmented a sample and then labeled the fragments with random molecular tags. EX1012.0001. After amplification and

sequencing, Kivioja taught that duplicate reads with identical unique label-fragment combinations corresponded to a single “UMI” or unique original sequence. EX1012.0001. As shown in Figure 1(a), Kivioja collapsed the amplification products to 20 original molecules, i.e., they identified duplicate molecules that were the result of PCR and associated them to a particular original labeled fragment. EX1012.0001. Thus, in Figure 1(a), Kivioja disclosed generally the steps of ’241 claim 1:

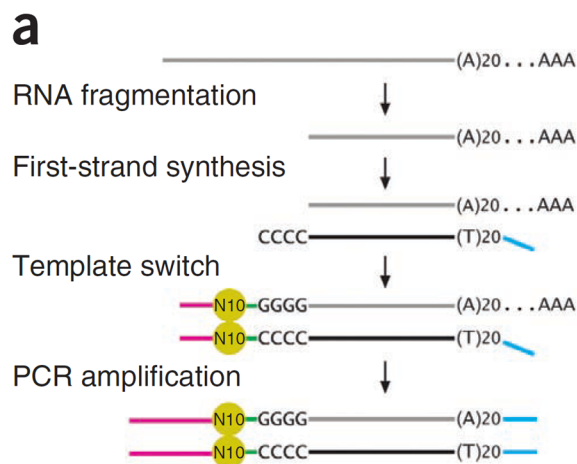


(a) obtaining amplicons comprising a fragment and an adaptor with an identifier site/molecular tag, (b) sequencing the amplicons, and (c) identifying sequence reads with identical identifier/molecular tag and fragment sequences as duplicates. EX1012.0001.

Kivioja also provided several proof of concept experiments, including one experiment using mRNA molecules from a *Drosophila* fly. EX1012.0002-05; EX1079.¶241. Kivioja showed this reaction in a diagram in Figure 3(a).

EX1012.0002. In that experiment, the adaptor contained a “10-base-pair random label,” i.e. a molecular tag, shown as the **gold** “N10” in Figure 3(a) below.

EX1012.0002; EX1079.¶401.



Thus, Kivioja taught obtaining amplicons of fragments with an appended adaptor comprising an identifier site as recited in claim 1, as shown in the final step in Figure 3(a) reproduced above. EX1079.¶¶401-409. Because Kivioja exemplified the UMI method in RNA, Kivioja used a reaction called an RNA template switch to attach the adaptor to the 5' end of the fragments. EX1012.0002-04; EX1079.¶¶401-407. After the RNA template switch reaction, Kivioja obtained a set of cDNA molecules corresponding to the original RNA molecules with the adaptor sequence added at the 5' end. EX1012.0004; EX1079.¶¶242-246, ¶¶405-407. Then, Kivioja amplified the cDNA in a PCR reaction. EX1012.0004; EX1079.¶¶247, 408. Via these steps, Kivioja obtained a set of amplicons comprising the amplified fragment

sequence with an appended adaptor containing an identifier site. EX1079.¶¶409-412.

Kivioja further taught the next step recited in the '241 claim—the amplicons were then sequenced to generate sequence reads that included the identifier and the target/fragment sequences. EX1012.0002, 0004; EX1079.¶¶247, 4¶¶13-415.

Finally, Kivioja taught the last step recited in the '241 patent of identifying sequence reads with identical identifier and target sequences as duplicates. Kivioja explained that after sequencing, “the mapped reads with the same gene, position, and label were collected to one UMI and the number of such reads was recorded as the copy number of that UMI.” EX1012.0005. In other words, Kivioja explained that the combination of the fragment sequences (mapped against known *Drosophila* genes) and the random label sequence were used to determine whether particular sequence reads were unique or PCR duplicates. EX1079.¶¶248, ¶¶416-419. Kivioja then collected sequence reads with identical random label and fragment sequence to a single UMI, i.e. unique fragment. EX1079.¶¶248, ¶¶416-419.

B. Kivioja Anticipates Claim 1

Kivioja discloses every element of claim 1 arranged as in the claim. EX1079.¶394.

1. “A method for detecting duplicate sequencing reads, the method comprising”

To the extent the preamble is limiting and entitled to patentable weight, Kivioja discloses this element in the method of collapsing labeled fragments to a single UMI based on the random molecular tag and fragment sequence. EX1012.0001, 0005.

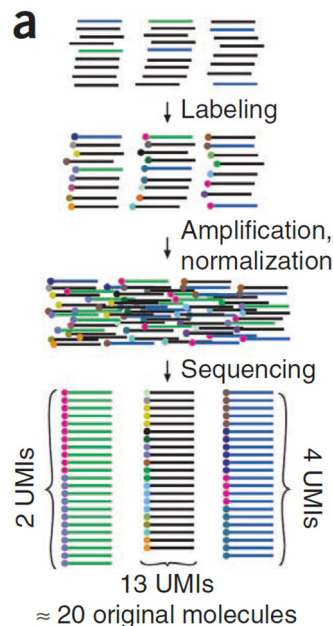
Kivioja expressly contemplated duplicate identification as part of their methods. EX1012.0005; EX1079.¶¶236-240, ¶¶395-398. Kivioja taught that one of the principal benefits of the method was for digital karyotyping. EX1012.0001-02. A karyotype is a common method of diagnosis wherein the number of chromosomes in a cell are counted. EX1079.¶257. Kivioja explains that the number of original fragments can be determined by associating all observed sequences with a particular original fragment using the UMI. EX1012.0001-02; EX1079.¶258, ¶¶397-398.

Kivioja then exemplifies this by using its mRNA sequencing method to demonstrate that “one can reproducibly detect the number of molecules after different numbers of PCR cycles without normalization.” EX1012.0002-03; EX1079.¶¶258-259, ¶¶397-398. This method necessarily involves the determination of whether particular observed sequences from the Illumina sequencing run were original fragments or PCR copies. EX1079.¶260, ¶¶397-398. Indeed, Kivioja explains that “the number of UMIs in the library acts as a molecular

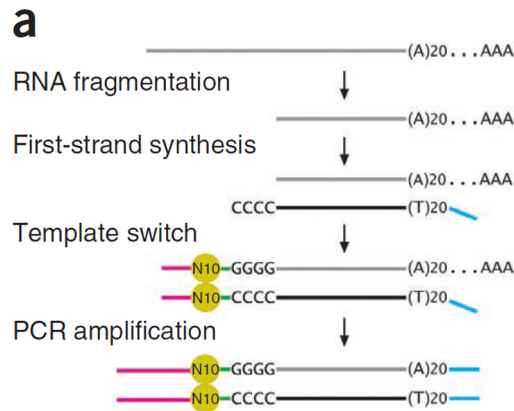
memory of the number of molecules in the starting sample.” EX1012.0001. Thus, while Kivioja does not use the word “duplicate,” the POSA would have understood that Kivioja teaches a method of detecting duplicate sequencing reads. EX1079.¶260, ¶¶397-398.

2. “obtaining amplicons each comprising an amplified fragment of a nucleic acid with an appended adaptor”

Kivioja discloses this limitation. In Figure 1(a), reproduced below, Kivioja teaches appending adaptors with random molecular tags (referred to as “labeling”) to nucleic acid fragments and then amplifying those labeled fragments prior to sequencing. EX1012.0001; EX1079.¶¶399-409; *supra* §IV.A. As shown in the penultimate step in Figure 1(a), Kivioja discloses a method of obtaining amplicons with molecular tags appended to them.



Kivioja exemplified this concept in its mRNA example. As described above, *supra* §IV, Kivioja added an adaptor to the 5' end of fragmented mRNA using a technique called a “template switch,” summarized in Figure 3(a) and reproduced below. EX1012.0002.



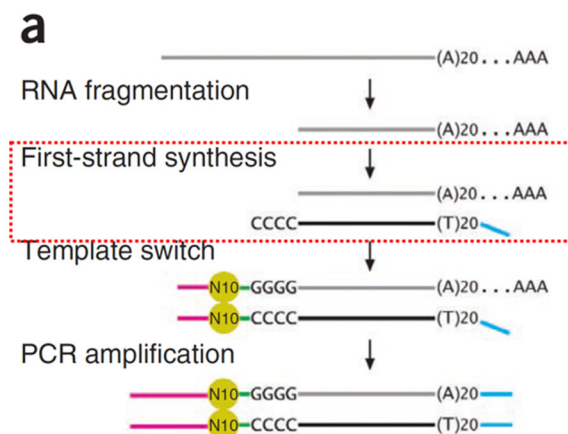
As the legend explains, the appended adaptor includes an Illumina linker sequence (in **magenta**), followed by a random 10 base sequence label shown (**gold N10**), and a sample index (**green**). EX1012.0002. Kivioja discloses the sequence of the 5' adaptor, as shown below and reproduced from EX1012.0014.

5'-adaptor	ACACTCTTCCCTACACGACGCTCTCCGATCT(NNN)dU(NNNN)dU(NNN)[GACTT]rGrGrGrG
------------	--

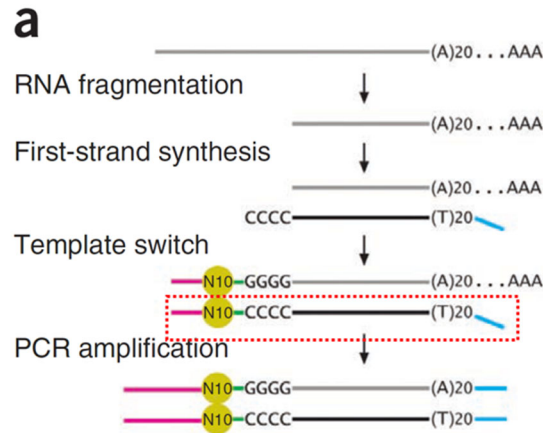
As Kivioja explains, the “N”s in the adaptor are “a random ten base DNA sequence label.” EX1012.0014; EX1079.¶402.

Template switching is a method used to reverse transcribe RNA to cDNA, as Kivioja did in the mRNA experiments. EX1012.0004; EX1079.¶¶403-407. Kivioja used a reverse transcriptase enzyme to generate a complementary strand of cDNA from the RNA template molecules (the “first strand synthesis” step annotated in

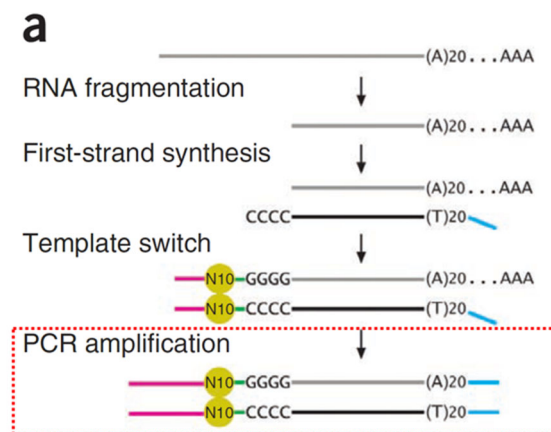
Figure 3(a) below), starting from a short primer that annealed to one end of the RNA molecules and had a noncomplementary adaptor tail (shown in blue). EX1012.0002, 0004; EX1079.¶¶405-407.



The 5' adaptors discussed above were in the reaction mixture along with the short primer with a noncomplementary adaptor tail. EX1012.0002, 0004, 0014; EX1079.¶406. The reverse transcriptase added the so-called “5' adaptor” to the end of the newly transcribed cDNA strand. EX1079.¶406. The final product was a strand of cDNA, complementary to the original RNA strand, with the 5' adaptor appended, as shown in the annotation to Figure 3(a) below from EX1012.0002. EX1079.¶406.



Kivioja then PCR amplified the fragments to obtain its amplicons—that is, a set of double stranded, amplified cDNA molecules comprised of an adaptor containing a random molecular tag appended to the cDNA fragments, as shown in the annotation to Figure 3(a) below, reproduced from EX1012.0002. EX1079.¶408.



While the term “appended,” which is not defined in the ’241 patent, does not limit how an adaptor is attached to a fragment, to the extent that Tecan contends that it does, the ’241 patent expressly contemplates “appending” to include a priming reaction. EX1005.0016 at 9:34-36; EX1079.¶404. Specifically, the ’241 patent states that “[i]n some embodiments, appending the adaptor to the nucleic acid

fragments . . . can be achieved using . . . a priming reaction.” EX1005.0016 at 9:34-

36. Because Kivioja initiated cDNA synthesis with a short primer (discussed above in relation to Figure 3(a)) and because the first strand cDNA itself serves as a primer for copying the 5' adaptor used in the template switch method, the POSA would have recognized that the mRNA template switch is a type of “priming reaction.” EX1079.¶404.

3. “wherein each adaptor comprises an identifier site comprising a plurality of nucleotides unique to the amplified fragment”

Kivioja discloses this limitation. As Kivioja explains, the purpose of the molecular tags was to “generate a library in which each molecule ha[d] a distinct identifying sequence.” EX1012.0001; EX1079.¶411.

As explained, Kivioja’s 5' adaptor used in the mRNA experiments comprised a “10-base-pair random label”—that is, an identifier site comprised of 10 nucleotides—that was used to uniquely label each cDNA fragment generated from the template switch reaction. EX1012.0002, 0014; EX1079.¶412. Kivioja explained that the mRNA experiment had enough random molecular tags to uniquely label each molecule. EX1079.¶412; EX1012.0005. Thus, each adaptor comprised an identifier site with a plurality of nucleotides unique to each amplified fragment. EX1079.¶¶410-412.

4. “sequencing the amplicons to generate sequence reads that include identifier and target sequence, and”

Kivioja discloses this limitation. Kivioja explains that its methods are designed for use with “any next generation sequencing method.” EX1012.0001. For the mRNA experiment, Kivioja sequenced the amplified adaptor-cDNA fragments on an Illumina device. EX1012.0004; EX1079.¶247. The POSA would have known this generated sequence reads that included the identifier site and target sequence. EX1079.¶¶247, ¶¶356-360, ¶¶413-415. The POSA also would have recognized that Kivioja’s “5’ adaptors” placed the identifier site downstream of the sequencing primer, so that both the identifier and target sequence were sequenced together. EX1079.¶¶242, ¶¶356-360, ¶¶413-415.

5. “identifying sequence reads with identical identifier and target sequences as duplicates.”

Kivioja discloses this limitation. Kivioja shows identifying duplicates based on the identifier and target sequences in Figure 1(a), where sequences are collapsed to unique fragments (UMIs) based on the colored label sequence and the colored fragment sequence together. EX1012.0001; EX1079. ¶¶256-260, ¶¶274-278.

Kivioja exemplified this in the mRNA experiment and explains that after sequencing, “the combination of label sequence and the 5’ mapped position of the RNA fragment forms the UMI.” EX1012.0002. Kivioja further explains that “the mapped reads with the same gene, position, and label were collected to one UMI.”

EX1012.0004. For each “gene” of interest, to identify a read with the same “position” Kivioja mapped the fragment portion of the read to known *Drosophila* genes, meaning Kivioja necessarily used the fragment sequence as part of the information used to determine a duplicate. EX1079.¶¶274-278, ¶¶361-363, ¶¶416-419; EX1012.0005. Kivioja combined that mapped position of the fragment with the label sequence. EX1012.0005; EX1079.¶¶274-278, ¶¶361-363, ¶¶416-419. Thus, Kivioja discloses identifying duplicates sequencing reads as reads with identical identifier sites (the “label”) and target sequences (the “gene” and “position”). EX1079.¶¶274-278, ¶¶361-363, ¶¶416-419; *supra* §IV.A.1. Kivioja’s performance of this method is confirmed in its Figure 1(a), where different sequences with the same magenta identifier are grouped separately, demonstrating that Kivioja’s “UMI” consists of both the identifier and the target sequence.

C. Claim 2

Claim 2 depends from claim 1 and adds the step of “removing a duplicate read from the sequence reads.” EX1005 at 25:44-45. Kivioja anticipates this claim. Kivioja shows this in Figure 1a, where the duplicates are combined to a single UMI. In addition, Kivioja explains that in the mRNA experiment duplicate reads “are collected to one UMI” and treated as a single original fragment. EX1012.0005; EX1079.¶¶284-286, ¶¶364-366, ¶¶420-421. The POSA, moreover, would have understood that Kivioja’s method of counting the original number of molecules in a

sample requires deduplication as a predicate step to counting the original fragments.

EX1079.¶¶284-286, ¶¶364-366, ¶¶420-421.

D. Claim 3

Claim 3 depends from claim 1 and adds that the identifier site is “sequenced with” the target sequence. EX1005 at 25:46-47. The ’241 patent does not define “sequenced with.” In this context, the plain and ordinary meaning of sequencing one sequence “with” another sequence is that both sequences are included in a single SBS “read” on the NGS device. EX1079.¶368. Kivioja anticipates this claim.

As shown in the annotated figure below, reproduced from EX1012.0014, the random molecular tag (in yellow) in Kivioja’s adaptor was 3’ to the sequencing primer binding site (green). EX1079.¶425. And as explained above, *supra* §IV.B.2, the nucleic acid fragment was appended to the 3’ side of the adaptor. EX1079.¶ 425.

Supplementary Table 1. Sequences for oligomers used in mRNA-seq. Sequences in square brackets and parentheses represent an index sequence that was used to enable multiplexed sequencing, and a random ten base DNA sequence label, respectively. dU and rG represent deoxyuridine and guanine ribonucleotide, respectively.

Oligomer	Sequence (5’-3’)
Oligo-dT adapter	CAAGCAGAAGACGGCATACGAGCTCTTCCGATCTTTTTTTTTTTTTTTTTTTTVN
5’-adapter	ACACTCTTTCCCTACACGACGCTCTTCCGATCT(NNN)dU(NNNN)dU(NNN)[GACTT]rGrGrGrG
Paired-end compatible oligo-dT adapter	CTCGGCATTCTGCTGAACCGCTCTTCCGATCTTTTTTTTTTTTTTTTTTTTVN

The POSA would have known that the placement of the sequencing primer binding site on the 5’ side of the random molecular tag and nucleic acid fragment

means the random molecular tag/identifier site would have been sequenced with the nucleic acid fragment/target sequence. EX1079.¶242, ¶¶367-370, ¶¶422-425.

Furthermore, Kivioja sequenced the adaptor-nucleic acid fragments from the mRNA experiments using Illumina single-read sequencing in one experiment and paired-end sequencing in the other. EX1012.0004; EX1079.¶¶171-175, ¶¶424-425. In at least one read in both experiments, the sequences were read from the green adaptor sequence through the gold identifier, and into the fragment sequence. EX1079.¶242, ¶¶367-370, ¶¶422-425. Thus, in the mRNA single read experiments, Kivioja disclosed sequencing the identifier and fragment sequence together. EX1079.¶¶422-425.

E. Claim 4

Claim 4 depends from claim 1 and adds that the identifier site is “sequenced separately” from the target sequence. EX1005 at 25:48-49. The '241 patent does not define “sequenced separately.” In this context, the plain and ordinary meaning of sequencing one sequence “separately” from another sequence is that the sequences are read in two separate reads on the NGS device, e.g. in two reads in paired-end sequencing. EX1079.¶377. Kivioja anticipates this claim too.

As just explained, *supra* §IV.D, Kivioja used Illumina paired-end sequencing for one of its mRNA experiments. EX1012.0004; EX1079.¶377. The use of paired end sequencing generated two reads: one from the green adaptor sequence through

the identifier and into the fragment, and one from the other end of the fragment that would read the fragment sequence but not the identifier. EX1079.¶¶171-175, ¶377. That is because Kivioja's random molecular tag was in the so-called "5' adaptor" that was only added to one side of the fragment and would not have been sequenced with the nucleic acid fragment in the sequencing read primed from the other end (at least for fragments longer than the maximum read length of 54 nucleotides). EX1079.¶377, ¶¶426-428; EX1012.0014, 0002.

F. Claim 5

Claim 5 depends from claim 1 and adds that "the amplicons include amplified fragments of cDNA." EX1005 at 25:50-51. Kivioja anticipates this claim. As Kivioja explains, for the mRNA experiment, the libraries for sequencing "were generated by fragmenting total RNA and reverse transcribing it to cDNA." EX1012.0002; EX1079.¶429; *supra* §IV.B.2.

G. Claim 6

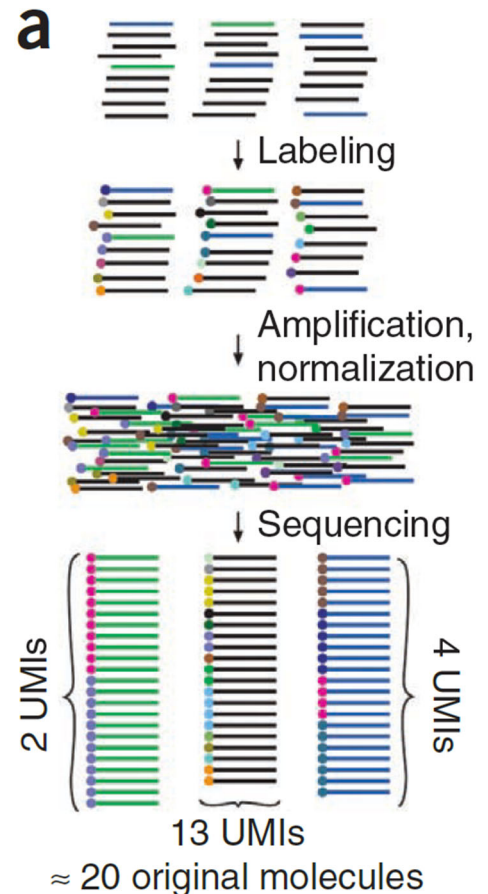
Claim 6 depends from claim 1 and adds that “the amplicons include amplified fragments of genomic DNA.” EX1005 at 25:52-53. Kivioja anticipates this claim. In Figure 1, reproduced below, Kivioja explains that “DNA species (green, blue, and black lines) are labeled with a collection of random labels (colored filled circles).” EX1012.0001. These DNA samples are then used in the subsequent steps of the method. EX1079.¶431.

Kivioja also explains that its methods are applicable to “genomic DNA” specifically. EX1012.0002; EX1079.¶431. While Kivioja’s genomic DNA proof-of-concept method did not use molecular tags/identifier sites, EX1012.0004, the POSA would have understood that the DNA fragments used in the method described and shown

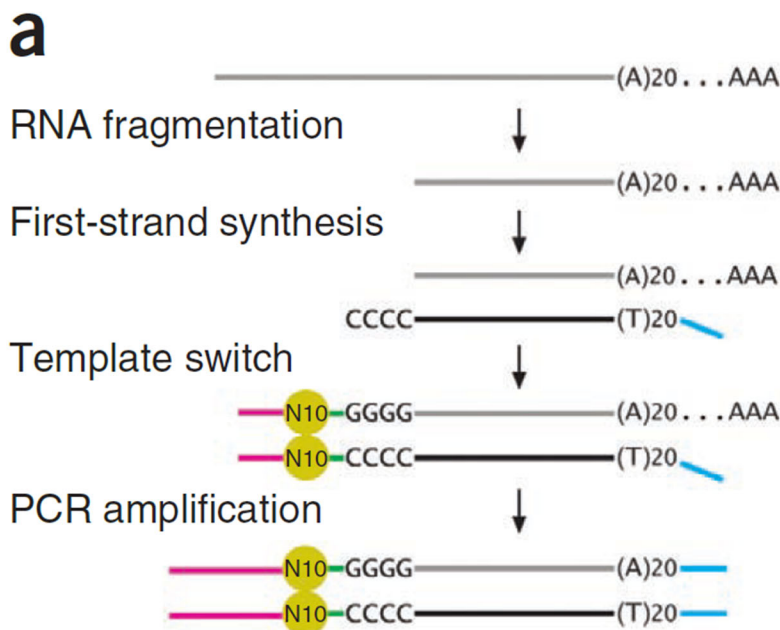
in Figure 1 could be genomic DNA or DNA from other sources, EX1079.¶¶430-431.

H. Claim 7

Claim 7 depends from claim 1 and adds the limitation that “each adapter includes a primer binding site.” EX1005 at 25:54-55. Kivioja anticipates this claim. In Figure 3(a), reproduced below, Kivioja depicts the preparation of a library of



nucleic acid fragments appended with adaptors, including the portion in magenta and gold. EX1012.0002; EX1079.¶¶433-434; *supra* §IV.A.



The magenta sequence was an “Illumina linker.” EX1012.0002. That linker sequence included binding sites for multiple primers. First, it included a primer binding site for the PCR amplification reaction to generate the library of amplicons. EX1012.0002, 0004; EX1079.¶434. Second, the magenta sequence included the primer binding site for the forward read primer in the SBS read on the Illumina device. EX1012.0014; EX1079.¶434; *supra* §IV.B.4.

I. Claim 8

Claim 8 depends from claim 7 and further adds that the primer binding site is “a universal target sequence primer binding site.” EX1005 at 25:56-57. The ’241 patent defines a universal primer as “a primer that can be used for amplifying a

number of different sequences.” EX1005 at 5:59-60. Kivioja anticipates this claim. Kivioja used a standard Illumina sequencing primer binding site that is the same in all of the adaptors and thus is a universal target sequence primer binding site. EX1079.¶¶336-337, ¶¶435-436; EX1012.0014, 0002-04; *supra* §IV.B.4, §IV.H.

J. Claim 9

Claim 9 depends from claim 1 and adds the limitation that “each unique identifier is at least 6 nucleotides in length.” EX1005 at 25:58-59. Kivioja anticipates this claim. Kivioja used a 10-nucleotide identifier site in its mRNA experiments. EX1079.¶¶437-438, EX1012.0003, 0014.

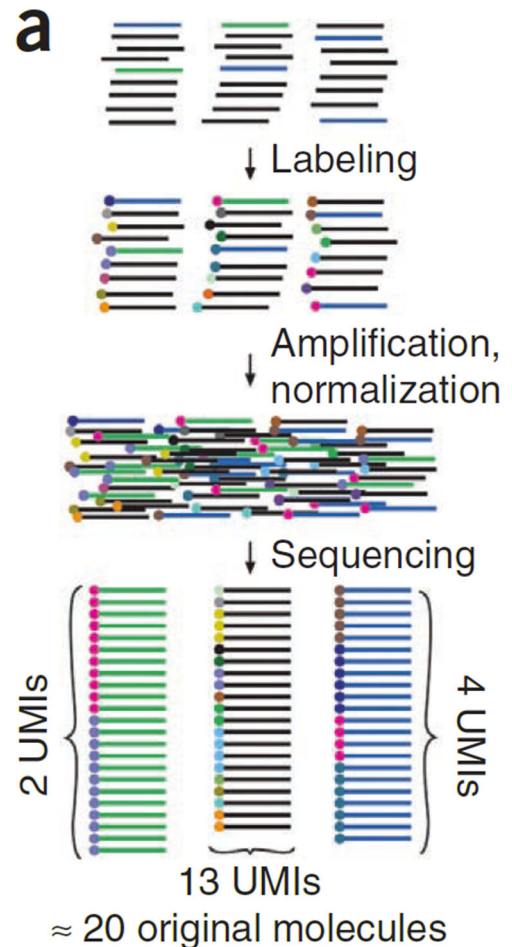
K. Claim 10

Claim 10 depends from claim 1 and requires that the adaptors be “appended to the fragments using a ligation reaction or a priming reaction.” EX1005 at 26:32-34. Kivioja anticipates this claim. As explained, *supra* §IV.A, Kivioja’s method appends adaptors to nucleic acid fragments using a priming reaction, EX1079.¶¶404-407, ¶¶439-440; EX1012.0002, 0004, 0014.

L. Claim 11

Claim 11 depends from claim 1 and adds that “each unique identifier is at a junction between the adapter and the nucleic acid fragment.” EX1005 at 26:35-37.

Kivioja anticipates this claim. While in Kivioja’s mRNA proof of concept experiment, Kivioja did not place the identifier at the junction between the adaptor and the fragment, Kivioja depicts exactly that arrangement in Figure 1. As shown in Figure 1(a), reproduced on the right of this page, EX1012.0001, the labels/identifier sites (colored circles) are at the junction between the adaptor (which includes the labels) and the nucleic acid fragment, EX1079.¶251, ¶¶441-443.



M. Claim 12

Claim 12 depends from claim 1 and adds that the method comprises “determining the genomic start position for each sequencing read.” EX1005 at 26:38-39. Kivioja anticipates this claim. Kivioja states that “the mapped genomic position of either end [of a nucleic acid fragment] can be used as a UMI.” EX1012.0002. In the mRNA experiment, Kivioja explains that after sequencing

“[t]he combination of the label sequence and the 5' mapped position of the RNA fragment forms the UMI,” EX1012.0002-03, and the POSA would have understood that the “5' mapped position” is the genomic start position, EX1079.¶¶446.

N. Claim 13

Claim 13 depends from claim 1 and adds the limitation that “each adaptor comprises an indexing site.” EX1005 at 26:40-41. Kivioja anticipates this claim. The adaptor Kivioja used in the mRNA experiments includes “an index sequence that was used to enable multiplexed sequencing,” as shown below in orange. EX1012.014; EX1079.¶¶447-448.

Supplementary Table 1. Sequences for oligomers used in mRNA-seq. Sequences in square brackets and parentheses represent an index sequence that was used to enable multiplexed sequencing, and a random ten base DNA sequence label, respectively. dU and rG represent deoxyuridine and guanine ribonucleotide, respectively.

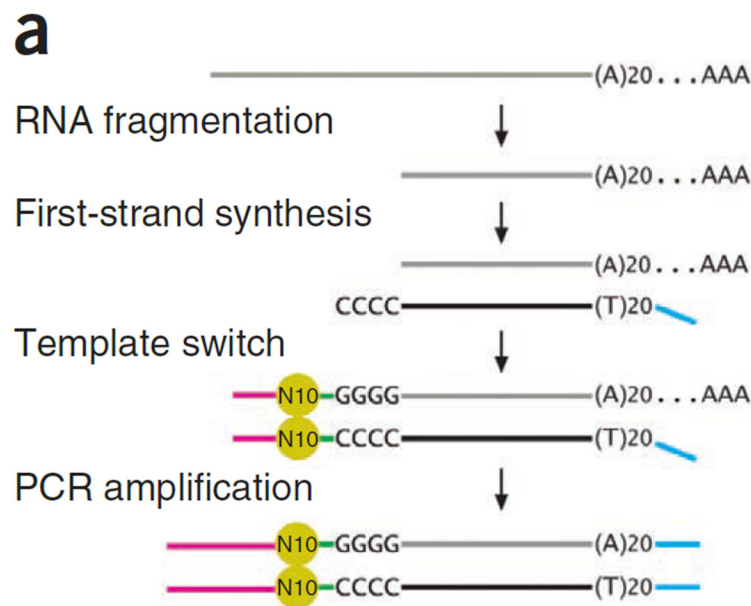
Oligomer	Sequence (5'-3')
Oligo-dT adapter	CAAGCAGAAGACGGCATACGAGCTCTTCCGATCTTTTTTTTTTTTTTTTTTTVN
5'-adapter	ACACTCTTTCCTACACGACGCTCTTCCGATCT(NNN)dU(NNNN)dU(NNN)[GACTT]rGrGrGrG
Paired-end compatible oligo-dT adapter	CTCGGCATTCTGCTGAACCGCTCTTCCGATCTTTTTTTTTTTTTTTTTTTVN

O. Claim 14

Claim 14 depends from claim 1 and adds that each identifier site is “at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length.” EX1005 at 26:43-44. Kivioja anticipates this claim. Kivioja used a 10-nucleotide identifier site in the mRNA experiments. EX1012.0003, 0014; EX1079.¶¶449-450.

P. Claim 15

Claim 15 depends from claim 1 and adds that the method comprises obtaining a sample comprising the nucleic acid, fragmenting the nucleic acid, appending adaptors to the fragments, and amplifying the fragments to obtain amplicons. EX1005 at 26:45-50. Kivioja anticipates this claim. For the mRNA experiments, Kivioja obtained an mRNA sample from *Drosophila*, fragmented the mRNA, appended an adaptor via the template switch priming reaction, and amplified those adaptor-nucleic acid fragments. EX1012.0004-05; EX1079.¶451. Kivioja's process was shown in Figure 3(a), which shows each of the claimed steps. EX1012.0002, EX1079.¶451.



Q. Claim 16

Claim 16 depends from claim 1 and adds that amplicons are obtained from two different samples, that each sample is appended to an adaptor containing a

sample index unique to the sample, and that after sequencing the reads are separated based on the sample index. EX1005 at 26:51-57. Kivioja anticipates this claim. Kivioja expressly discloses the use of unique sample indexes for multiplexed sequencing on Illumina devices. EX1012.0014, 0002-05; EX1079.¶452; *supra* §IV.N. The POSA would have known that the standard Illumina workflow for multiplexed sequencing included separating samples based on sample index. EX1079.¶452.

V. GROUND II: OBVIOUSNESS OVER KIVIOJA

To the extent claims 4, 6, 11, and 16 are not anticipated by Kivioja, they would have been obvious over Kivioja.

A. Claim 4

Claim 4 depends from claim 1 and adds that the identifier site is “sequenced separately” from the target sequence. EX1005 at 25:48-49. To the extent claim 4 is not anticipated by Kivioja, *supra* §IV.E, it is obvious over Kivioja. As discussed, *supra* §§IV.D-E, Kivioja disclosed the use of paired-end sequencing and adaptors with an identifier site, EX1012.0004; EX1079.¶377, ¶¶426-428. Because Kivioja’s identifier site was only in one of two adaptors that would have been appended to either end of the nucleic acid fragment, and because paired-end sequencing reads the adaptor-nucleic acid fragment from both ends, i.e., both strands get sequenced, one of the two reads would have started from the end without an identifier site.

EX1079.¶¶426-428; ¶¶171-175. Meanwhile, the sequencing read from the 5' end would have separately sequenced the identifier site. EX1079.¶¶426-428; ¶¶171-175.

Moreover, the POSA would have recognized that there were benefits to sequencing the fragment separately from the identifier, including maximizing the length of the read used for the fragment sequence. EX1079.¶¶377-378, ¶¶426-428. Based on Kivioja's teachings, the POSA would have been motivated to sequence the identifier site separately from the nucleic acid fragment and would have had a reasonable expectation of success in doing so.

B. Claim 6

Claim 6 depends from claim 1 and adds the limitation that "the amplicons include amplified fragments of genomic DNA." EX1005 at 25:52-53. To the extent Kivioja does not anticipate claim 6, *supra* §IV.G, the POSA would have been motivated to practice the method of claim 6 because Kivioja explains that its methods are applicable to genomic DNA and provides a proof-of-concept experiment that uses genomic DNA (though not with identifier sites), EX1012.0002, 0004; EX1079.¶¶245-246, ¶¶430-432. The POSA would have been motivated to practice the Kivioja method with genomic DNA because the same rationales for using molecular tags with mRNA apply to some contexts of genomic DNA, including for example, as Kivioja explains where there are "high- and low-abundance species in a single reaction." EX1012.0002. For the same reasons, the

POSA would have had a reasonable expectation of success in practicing the method of claim 6 based on Kivioja.

C. Claim 11

Claim 11 depends from claim 1 and adds the limitation that “each unique identifier is at a junction between the adapter and the nucleic acid fragment.” EX1005 at 26:35-37. To the extent Kivioja does not anticipate claim 11, *supra* §IV.L, it is rendered obvious by Kivioja. First, Kivioja shows that adaptors with identifier sites can be appended directly to nucleic acid fragments, as in Figure 1(a). EX1012.0001; EX1079.¶400. Second, the POSA would have understood that an adaptor could be designed with an identifier site in various positions within the adaptor, including at the junction between the adaptor and nucleic acid fragment, but that there were a limited number of positions available given the small number of features in the adaptor and the need to position the identifier vis-à-vis the read sequencing primer binding site. EX1079.¶441-445. The POSA would have understood that the choice between the small number of available positions was a routine design choice for a particular experiment. EX1079.¶445. *See Uber Tech., Inc. v. X One, Inc.*, 957 F.3d 1334, 1340 (Fed. Cir. 2020); *In re Brandt*, 886 F.3d 1171, 1174, 1175-78 (Fed. Cir. 2018); *Vicor Corp. v. SynQor, Inc.*, 869 F.3d 1309, 1326 (Fed. Cir. 2017). Nor does the '241 patent disclose any benefit or unexpected properties of placing the identifier site at the junction with the fragment (as opposed

to any other suitable location in the adaptor). EX1079.¶445. Based on Kivioja's disclosures, the POSA would have had a reasonable expectation of success in practicing Kivioja's methods with the identifier site at the junction with the fragment. EX1079.¶445.

D. Claim 16

Claim 16 depends from claim 1 and adds that amplicons are obtained from two different samples, that each sample is appended to an adaptor containing a sample index unique to the sample, and that after sequencing the reads are separated based on the sample index. EX1005 at 26:51-57. To the extent claim 16 is not anticipated by Kivioja, *supra* §IV.Q, it is rendered obvious over Kivioja. Kivioja included a sample index in the adaptor used in the mRNA experiment and discloses that the sample index "was used to enable multiplex sequencing." EX1012.0014; EX1079.¶452. Kivioja also used Illumina devices for sequencing. EX1012.0004. The POSA would have understood that sample indexes allowed fragments from different samples to be uniquely labeled, pooled together, and sequenced together. EX1079.¶453. The POSA also would have known that the standard Illumina workflow separated sequencing reads based on sample indexes. EX1079.¶453. The POSA would have been motivated to pool and multiplex samples to maximize the use of the space on the Illumina device and save costs and time, and thus these limitations would have been obvious to the POSA based on the standard Illumina

workflows at the time. EX1079.¶¶452-453. For these reasons, the POSA likewise would have had a reasonable expectation of success in pooling samples and practicing the method of claim 16. EX1079.¶453.

VI. GROUND III: ANTICIPATION OVER BIELAS

A. Bielas Disclosed Methods With All Of The Elements Claimed In The '241 Patent

Bielas is a published patent application that teaches the benefits of using in NGS library preparation random molecular tags, which Bielas calls “Cyphers.” EX1013. Bielas uses Cyphers to identify PCR copies of original fragments after Illumina sequencing. EX1013.0026-32. Examples 1 and 3 of Bielas disclose all of the elements of Claims 1-3 and 6-16, as arranged in those claims, and thus anticipate them. EX1013.0026-32.

Bielas teaches that Cyphers are random molecular tags that are appended to nucleic acid fragments prior to PCR amplification and sequencing. E.g., EX1013.0010, 0026, 0038. Cyphers “allow a person of ordinary skill in the art to more accurately distinguish true mutations (*i.e.*, naturally arising *in vivo* mutations) of a nucleic acid molecule from artifact ‘mutations’ (*i.e.*, *ex vivo* mutations or errors) of a nucleic acid molecule that may arise for various reasons, such as a downstream amplification error, a sequencing error, or physical or chemical damage.” EX1013.0007. Thus, Bielas teaches a method of using random molecular tags to differentiate between, and deduplicate, PCR copies to determine if a particular

mutation is present in the original fragment (a “true mutation”) or created by PCR (an “artifact mutation”). EX1079.¶¶456-486, ¶¶487-491.

Bielas appends Cyphers to DNA fragments by ligating an adaptor with all of the features recited in the '241 patent. Example 1 of Bielas discloses a method wherein DNA from a sample is fragmented and then ligated into a circular piece of DNA called a vector that contains the adaptors. EX1013.0026. Specifically, Bielas states “the end-repaired DNA fragments are ligated into . . . the library of dual cypher vectors shown in Figure 2” EX1013.0026.

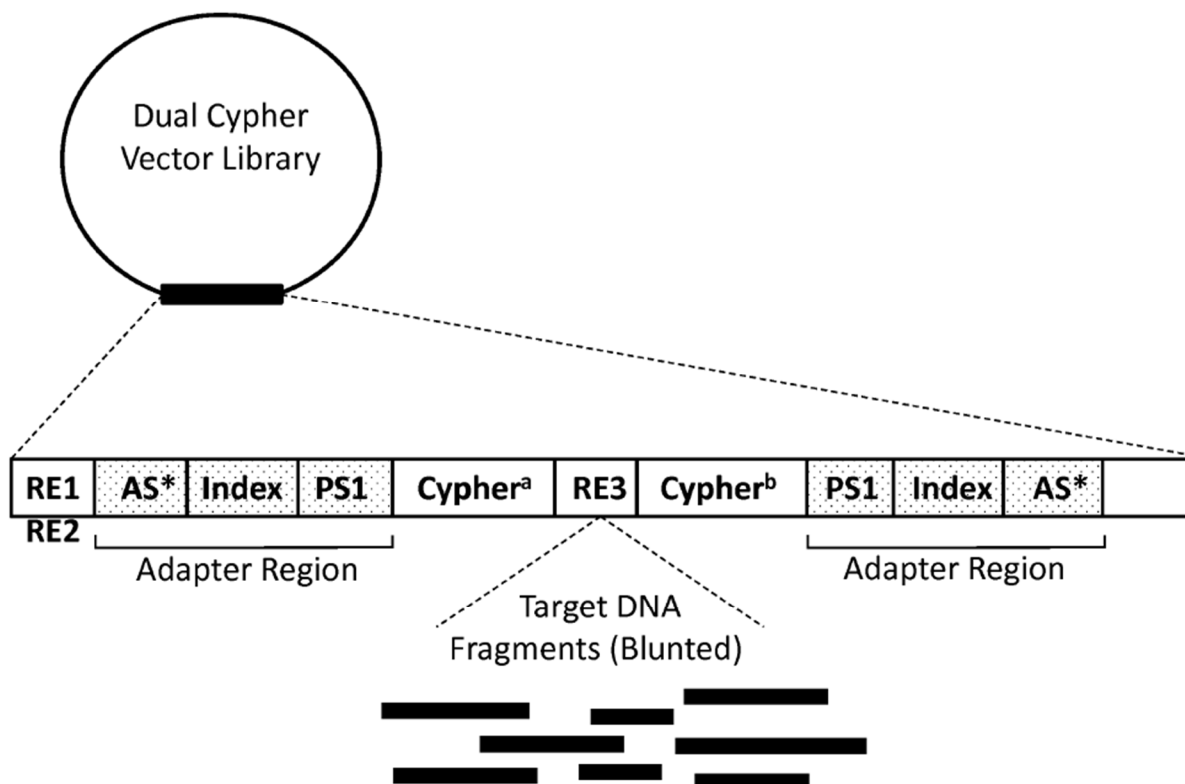
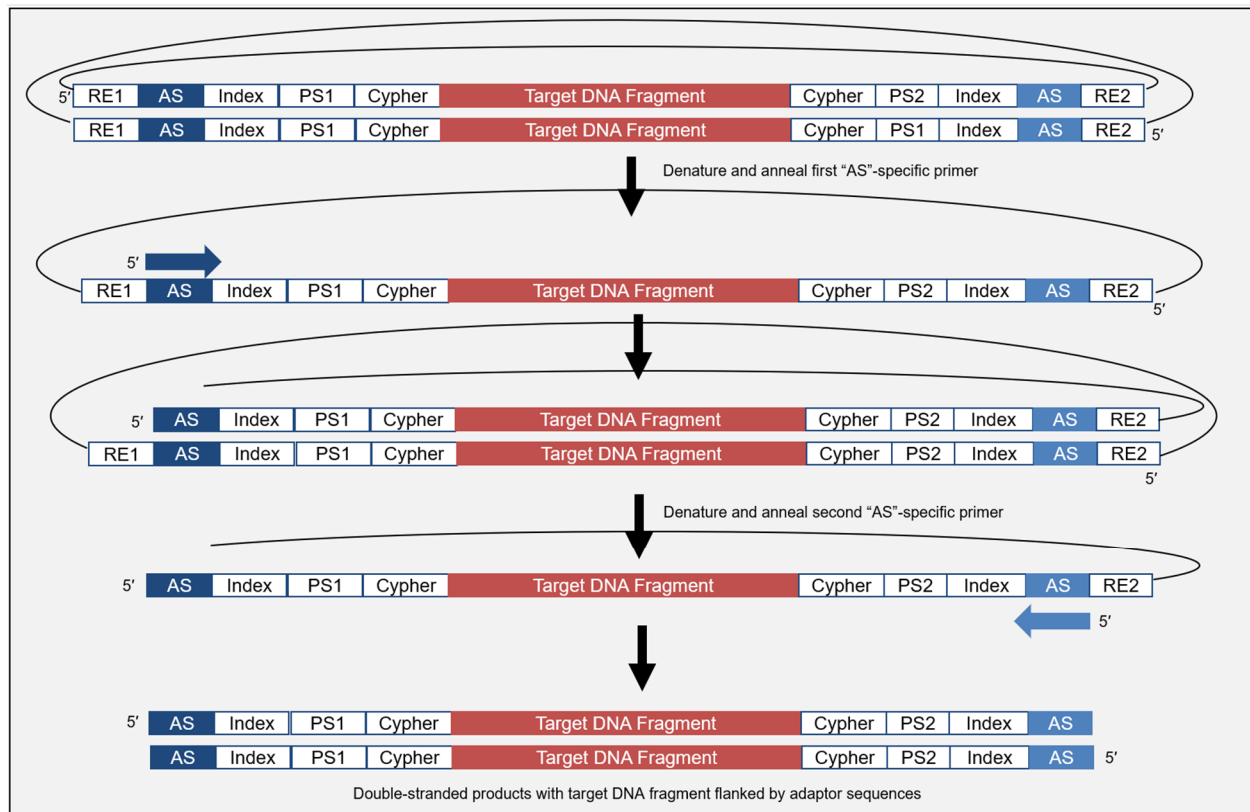


Fig. 2

EX1013.0041.

Bielas cut the vectors at RE3 and inserted the fragments at that site. EX1079.¶460-461; EX1013.0026-27. Then, Bielas used a PCR reaction, shown below, to amplify only the adaptor-fragment region out of the vector. EX1079.¶462; EX1013.0026-27.



The result of this PCR reaction was a library of double-stranded adaptor-ligated fragments with the structure AS-Index-PS1-Cypher-Fragment-Cypher-PS2-Index-AS. EX1079.¶463; EX1013.0026-27, 0041. For the features on the 5' side of the fragment, i.e., AS-Index-PS1-Cypher, Bielas explains that

1. “AS” is the Illumina common adaptor sequence used for annealing to the solid support and also used as the primer binding site for sequencing the sample index, EX1013.0023, 0026-27; EX1079.¶464;
2. “Index” is a sample index for multiplexing, EX1013.0027; EX1079.¶465;
3. “PS1” is a primer binding site for the Illumina SBS primer, EX1013.0012, 0022-23, 0027; EX1079.¶¶466-467; and
4. “Cypher” is the random molecular tag sequence, EX1013.0014, 0041; EX1079.¶468.

These adaptor-ligated fragments were then sequenced on an Illumina NGS device. EX1013.0027.

After sequencing, Bielas explains the combined Cypher-fragment sequences are used for de-duplication—“[t]he unique cypher tags are used to computationally deconvolute the sequencing data and map all sequence reads to single molecules (*i.e.*, distinguish PCR and sequencing errors from real mutations).” EX1013.0027; EX1079.¶470. As discussed further below, *infra* §VI.B.1.a, §VI.B.1.e, this process of deconvoluting sequences necessarily involved de-duplicating PCR copies and collapsing them to an original fragment sequence. EX1079.¶470, ¶¶489-491, ¶¶508-509.

Example 3 of Bielas uses a similar method. EX1013.0028-32. In that example, Bielas created a library of fragments containing pieces of the TP53 gene,

EX1013.0028-32, and ligated those fragments into vectors similar to those in Example 1. EX1013.0028-29; EX1079.¶¶471-486. The authors provided the DNA sequence of the circular vector, which the POSA would have recognized contained standard Illumina sequences. EX1013.0028-29; EX1079.¶¶471-480; EX1056; EX1057; EX1075; EX1076; EX1077; EX1078.

Bielas again used PCR to amplify the adaptor-fragments out of the vector. EX1013.0031; EX1079.¶481. The final adaptor-ligated fragments again had the structure AS-Index-PS1-Cypher-Fragment-Cypher-PS2-Index-AS. EX1079.¶481; EX1013.0028-31. The POSA would understand that the AS sequence was the standard Illumina flow cell sequence that also contains a primer binding site to read the sample index, Index is a sample index, PS1 is the Illumina primer binding site for sequencing the fragment, and Cypher is the random molecular tag. EX1079.¶¶474-480. The authors sequenced the adaptor-fragments on an Illumina MiSeq device. EX1013.0029.

Bielas explains that “all sequencing reads with identical cypher pairs and their reverse complements were grouped into families to create a consensus sequence,” i.e., the adaptor-ligated fragments were de-duplicated using the Cypher and the fragment sequence. EX1013.0031; EX1079.¶¶483-486. Using this process, “true mutations (i.e., those that arise naturally in a cell during replication) are readily

detectable using the cypher system of the instant disclosure.” EX1013.0032. As further explained *infra* §VI.B.1.e, Bielas illustrates this process in Figure 3B:

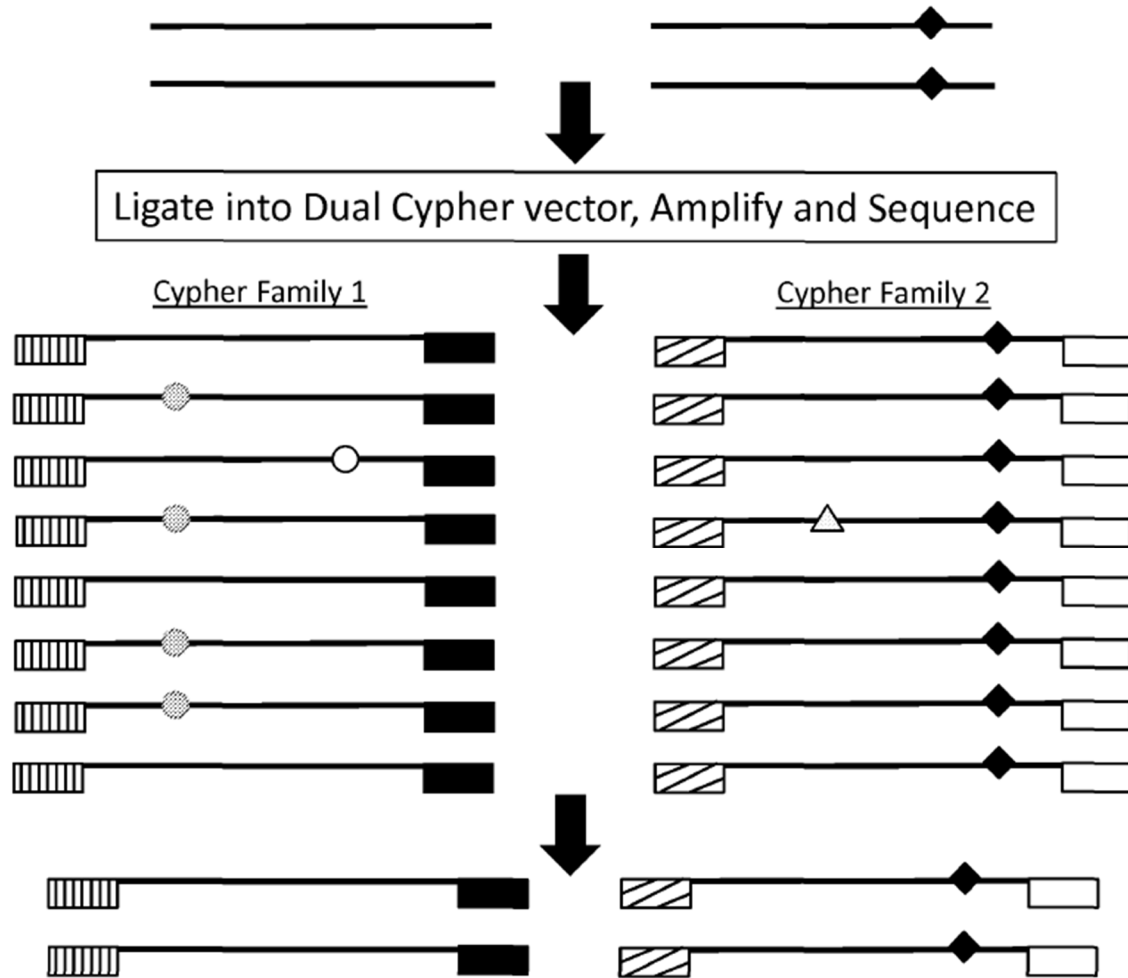


Fig. 3B

EX1013.0031-32, 0043.

In summary, Bielas disclosed not one, but two, examples of methods that practiced every step of claims 1-3 and 5-16.

B. Bielas Anticipates Claim 1.

Bielas discloses every element of claim 1 as arranged in the claim both in Example 1 and Example 3.

1. Claim 1

a. “A method for detecting duplicate sequencing reads, the method comprising:”

Bielas discloses this limitation. Despite not using the word “duplicate,” Bielas teaches differentiating between “true mutations” and “artifact ‘mutations’” using the combination of the Cypher/identifier site and the fragment so that each sequence “can be connected back to the original molecule.” EX1013.0007, 0009. In Example 1, Bielas explains that reads were “computationally deconvolute[d]” using the Cypher sequence and the mapped position of the fragment genes. EX1013.0026-27; EX1079.¶¶489-491, ¶¶612-613. Likewise, in Example 3, Bielas explains that a consensus sequence was derived using the Cypher and the fragment sequence, and then the “errors introduced during library preparation and during sequencing were computationally eliminated from the consensus sequence.” EX1013.0028-32. As discussed further below, *infra* §VI.B.1.e, the POSA would have understood that in order to “deconvolute” sequences to particular original fragments, the sequences were necessarily de-duplicated to remove PCR copies of the same molecule from the data set. EX1079.¶¶489-491, ¶¶612-613.

b. “obtaining amplicons each comprising an amplified fragment of a nucleic acid with an appended adaptor”

Bielas discloses this limitation. Example 1 and Example 3 of Bielas both involved ligation of a fragment of a nucleic acid into a circular vector, which appended an adaptor made up of AS-Index-PS1-Cypher to the 5' end of the fragment, as shown below in the annotated version of Bielas Figure 2. EX1013.0026, 0029-31; EX1079.¶¶492, ¶¶614-617.

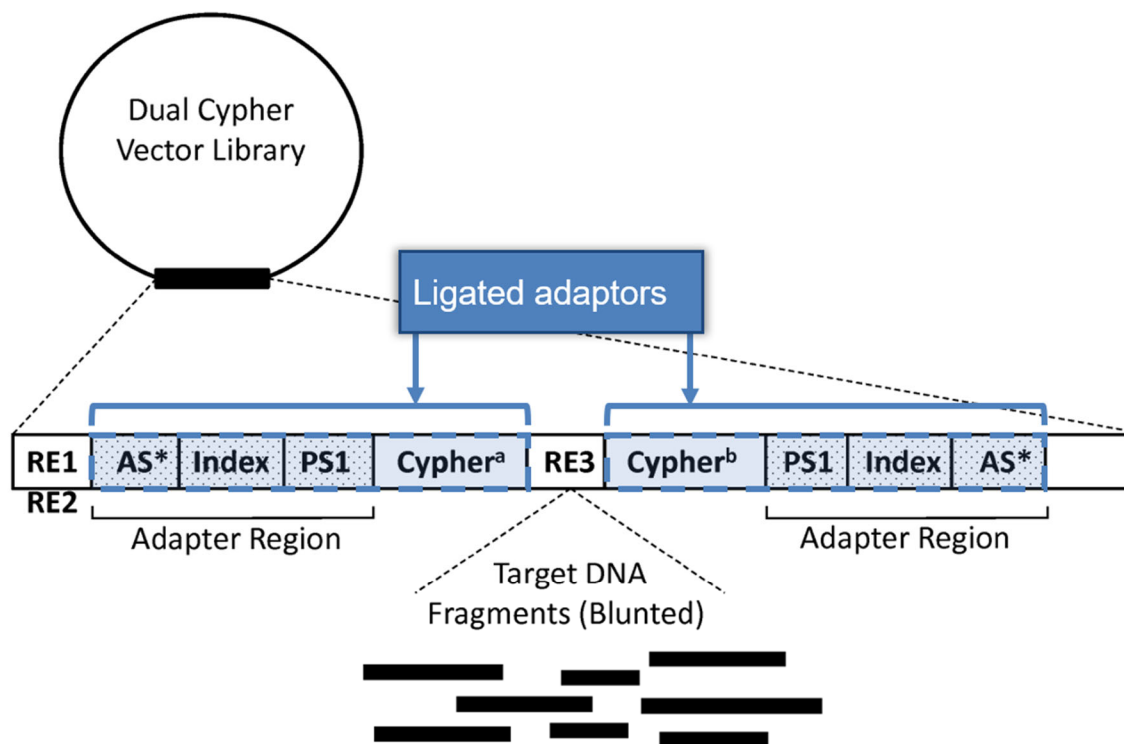


Fig. 2

To append the adaptor, Bielas used a ligation reaction. EX1013.0026; EX1079.¶¶492, ¶¶614-617. As explained above, *supra* §II.C.1, “append” does not put any limitation on the method by which an adaptor is attached to a fragment,

EX1079.¶403, but to the extent Tecan argues otherwise, ligation is one of the two methods of appending expressly disclosed in the '241 patent. EX1005 at 9:34-36.

c. “wherein each adaptor comprises an identifier site comprising a plurality of nucleotides unique to the amplified fragment”

Bielas discloses this limitation. The '241 patent defines an identifier site as “a nucleic acid sequence that comprises random bases and is used to identify duplicate sequencing reads.” EX1005 at 4:43-45. In both Example 1 and Example 3, Bielas discloses an adaptor with a random Cypher/identifier site to identify duplicate sequencing reads. EX1013.0026, 0028-29; EX1079.¶¶618-620; *infra* §VI.B.1.e.

In Example 1, Bielas used the adaptor in Figure 2. EX1013.0026; EX1079.¶619. The adaptor includes a Cypher sequence in the 5' region. EX1013.0041; EX1079.¶¶460-468, ¶619. Bielas teaches that the Cypher sequences are “unique” and “used to computationally deconvolute the sequencing data.” EX1013.0027. The POSA would have understood that “unique” Cyphers in each adaptor meant that the adaptors comprised an identifier site with “a plurality of nucleotides unique to the amplified fragment.” EX1079.¶¶618-620.

In Example 3, Bielas discloses a 7-nucleotide random Cypher in the 5' region of the adaptor, designated as NNNNNNN—a standard convention for random nucleotides. EX1013.0028, 0016; EX1079¶504. The POSA would have understood

that Cyphers comprised of 7 random nucleotides meant that the adaptors comprised an identifier site with “a plurality of nucleotides unique to the amplified fragment.” EX1079.¶¶618-620.

d. “sequencing the amplicons to generate sequence reads that include identifier and target sequence, and”

Bielas discloses this limitation. In both Example 1 and Example 3, Bielas sequenced the adaptor-ligated fragments on Illumina devices to generate a population of sequencing reads that included the identifier and target sequence. EX1013.0027, 0031; EX1079.¶469, ¶482, ¶507, ¶¶621-623.

e. “identifying sequence reads with identical identifier and target sequences as duplicates.”

Bielas discloses this limitation. EX1079.¶¶624-626. Bielas teaches that sequenced fragments can be “computationally deconvoluted” using Cypher-fragment sequences to distinguish “true mutations” found in original fragments from “artifact mutations” generated during library preparation. EX1013.0024-25, 0027, 0037. This process required Bielas to first identify duplicate sequencing reads as those comprising an identical Cypher/identifier site and fragment sequence as other reads. EX1079.¶¶508-509, ¶¶624-626.

In Example 1, Bielas explains that “[t]he unique cypher tags are used to computationally deconvolute the sequencing data and map all sequence reads to single molecules (*i.e.*, distinguish PCR and sequencing errors from real mutations)”

and that “sequence alignment” was performed using a conventional Illumina software package (called Eland). EX1013.0027. “Sequence alignment” entailed mapping the fragment sequences to their positions in the then-known human genome so as to determine any mutations from the germline. EX1013.0027; EX1079.¶¶470, 490, 645. In other words, to de-duplicate all of the sequence reads to “single molecules,” i.e. original fragment sequences, Bielas relied on the Cypher sequence *and* the mapped fragment sequence.

Likewise, in Example 3, Bielas explains that “all sequencing reads with identical cypher pairs and their reverse complements were grouped into families to create a consensus sequence” and then “errors introduced during library preparation and during sequencing were computationally eliminated from the consensus sequence and only mutations present in all reads of a cypher family were counted as true mutations.” EX1013.0031-32. To perform this comparison, Bielas looked at both the Cypher sequence, to group reads, and the fragment sequence, to ascertain whether a particular sequence contained a mutation. EX1013.0031-32, 0043; EX1079.¶¶483-486. The authors then de-duplicated reads with identical cypher and fragment sequences to a single consensus read in order to compare those reads to the reference genome and identify “true mutations.” EX1013.0031-32, 0043; EX1079.¶¶483-486.

Bielas diagrammed this process in Figure 3B (reproduced below with annotations). EX1013.0043. Original double stranded fragments are shown at the top of the figure. EX1079.¶486; EX1013.0043. These fragments are then ligated to the adaptors containing the Cyphers/identifier sequences shown as boxes on either end of the fragment. EX1079.¶486; EX1013.0043. These fragments are then amplified, creating multiple different copies of the same original fragment, annotated as copies 1, 2, and 3 below. EX1079.¶486; EX1013.0043. These copies are then de-duplicated and collapsed to a single fragment in the final step. EX1079.¶486; EX1013.0043.

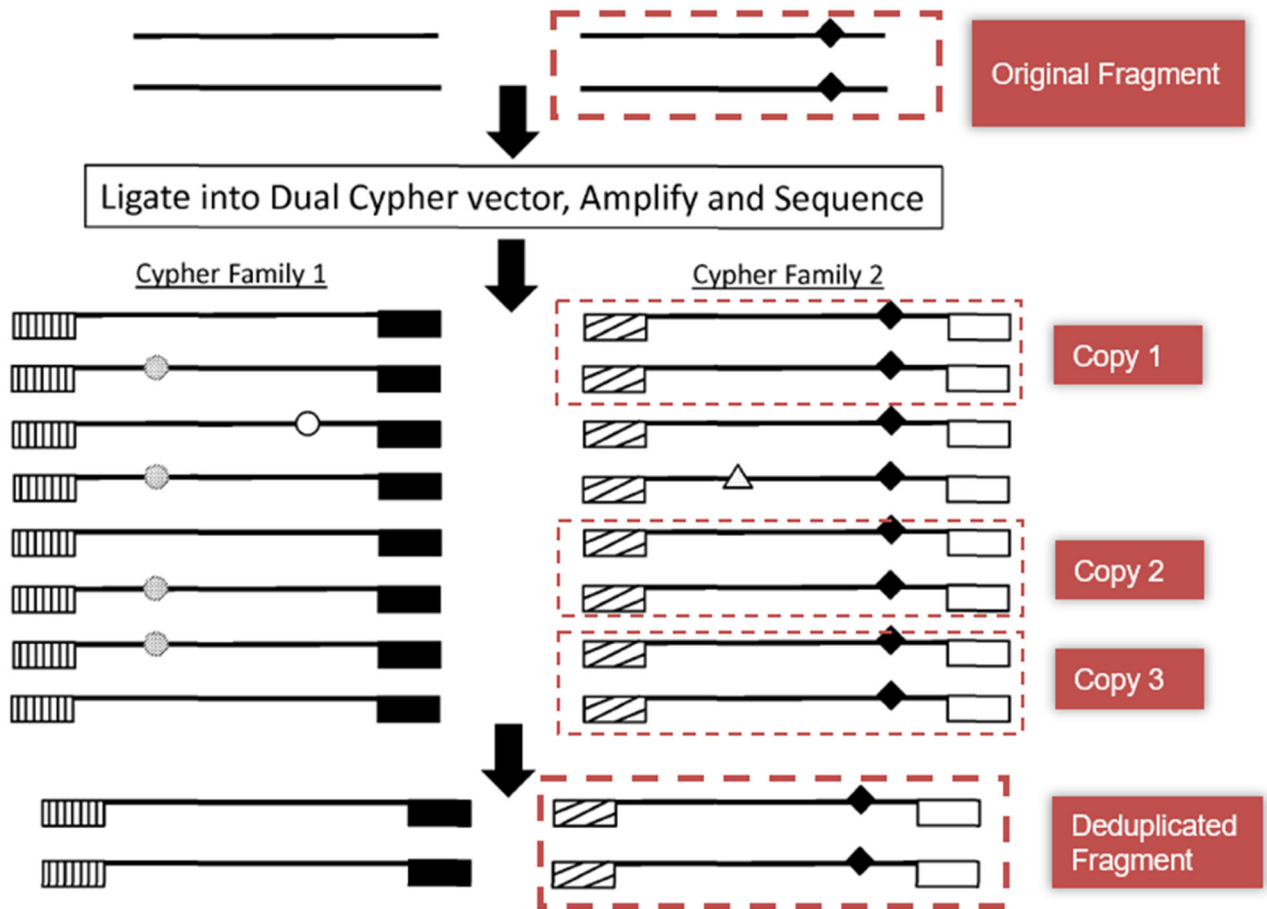


Fig. 3B

Thus, in Example 1 and Example 3, Bielas identified duplicate sequencing reads by using the Cypher/identifier site and fragment sequence.

2. Claim 2

Claim 2 depends from claim 1 and adds a limitation of “removing a duplicate read from the sequence reads.” EX1005 at 25:44-45. Bielas anticipates this claim. In Example 1 and Example 3, reads with duplicate Cypher-fragment sequences were “deconvoluted” to “original molecules,” i.e., they were de-duplicated, as shown in Figure 3B. *Supra* §VI.A, §VI.B.1.e; EX1079.¶¶520-523, ¶¶627-628; EX1013.0043.

3. Claim 3

Claim 3 depends from claim 1 and adds the limitation that the identifier site is “sequenced with” the target sequence. EX1005 at 25:46-47. As discussed above, *supra* §IV.D, the plain and ordinary meaning of this claim is sequencing the identifier and the target sequence in one SBS read. EX1079.¶368. Bielas anticipates this claim. In both Example 1 and Example 3, Bielas disclosed the use of adaptors in which the Cypher/identifier site and the nucleic acid fragment/target sequence are on the 3' side of the sequencing primer binding site. EX1013.0041, 0028-29; EX1079.¶585. The POSA would have understood that Bielas therefore sequenced the identifier site with the nucleic acid fragment in at least one read (from PS1 through the Cypher and fragment sequence). EX1079.¶¶584-588, ¶¶629-630.

4. Claim 6

Claim 6 depends from claim 1 and adds the limitation that “the amplicons include amplified fragments of genomic DNA.” EX1005 at 25:52-53. Bielas anticipates this claim. Bielas used genomic DNA fragments in Example 1 and Example 3. EX1013.0028, 0030-32; EX1079.¶635.

5. Claim 7

Claim 7 depends from claim 1 and adds the limitation that “each adapter includes a primer binding site.” EX1005 at 25:54-55. Bielas anticipates this claim. The adaptors used in Bielas contained multiple primer binding sites. First, the adaptors included the primer binding sites for use in the PCR reaction to copy the

adaptor-ligated fragments out of the vector constructs. EX1079.¶636; EX1013.0027-30. In addition, the adaptors used in Example 1 and Example 3 include two standard Illumina primer binding sites: a primer binding site to read the sample index and a primer binding site to read the nucleic acid fragment and identifier site. EX1079.¶¶460-486, ¶636; EX1013.0027-30, 0041; *supra* §VI.A, §VI.B.3.

6. Claim 8

Claim 8 depends from claim 7 and further adds the limitation that the primer binding site is “a universal target sequence primer binding site.” EX1005 at 25:56-57. The '241 patent defines a universal primer as “a primer that can be used for amplifying a number of different sequences.” EX1005 at 5:59-60. Bielas anticipates this claim. In Examples 1 and 3, Bielas’s adaptors included a standard Illumina sequencing/target sequence primer binding site that is the same in all of the adaptors, and thus a “universal target sequencing primer binding site.” EX1079.¶¶558-559, ¶¶637-638; EX1013.0027-30, 0041; *supra* §VI.A, §VI.B.3, §V.B.5.

7. Claim 9

Claim 9 depends from claim 1 and adds the limitation that “each unique identifier is at least 6 nucleotides in length.” EX1005 at 25:58-59. Bielas anticipates this claim. Bielas teaches several values for the cypher length, including ranges of 6-10. EX1013.0010, and 7-9, EX1013.0021, and specific values of six, seven, or

eight nucleotides, EX1013.0015; EX1079.¶639. In Example 3, Bielas used a 7-nucleotide identifier. EX1013.0028-29; EX1079.¶640.

8. Claim 10

Claim 10 depends from claim 1 and requires that the adaptors be “appended to the fragments using a ligation reaction or a priming reaction.” EX1005 at 26:32-34. Bielas anticipates this claim. In both Examples 1 and 3, Bielas ligated the adaptors to the fragments. EX1013.0026, 0028-29; EX1079.¶641.

9. Claim 11

Claim 11 depends from claim 1 and adds that “each unique identifier is at a junction between the adapter and the nucleic acid fragment.” EX1005 at 26:35-37. Bielas anticipates this claim. In both Examples 1 and 3, the identifier site is located where the adaptor joins the nucleic acid fragment. EX1013.0028-29, 0041; EX1079.¶¶642-643, ¶¶606-608. Bielas used in Example 1 the adaptor shown in Figure 2. EX1013.0027, 0041; EX1079.¶¶460-470, ¶¶604-609, ¶¶642-643; *supra* §VI.A. Bielas cut at “RE3” and inserted the nucleic acid fragments at that point. EX1013.0023; EX1079.¶¶460-461. Furthermore, Bielas disclosed the sequence of the adaptor used in Example 3, which shows that the Cypher/identifier site was at the junction between the adaptor and nucleic acid fragment. EX1013.0028-29; EX1079.¶¶471-480.

10. Claim 12

Claim 12 depends from claim 1 and adds that the method comprises “determining the genomic start position for each sequencing read.” EX1005 at 26:38-39. Bielas anticipates this claim. In Example 1, Bielas explains that sequencing reads were “computationally deconvolute[d]” using the Cypher and the position of the fragment mapped against the reference genome. EX1013.0026-27; EX1079.¶645. Likewise, in Example 3, Bielas explains that “[s]equences were then compared to wild-type TP53 sequence.” EX1013.0006; EX1079.¶646. When comparing the fragment sequence to known human genes in Example 1 and Example 3, Bielas determined the genomic start position. EX1079.¶¶644-646.

11. Claim 13

Claim 13 depends from claim 1 and adds that “each adaptor comprises an indexing site.” EX1005 at 26:40-41. In both Example 1 and Example 3, Bielas disclosed the use of an adaptor that includes a sample index. EX1079.¶¶495-497, 648; EX1013.0028-31, 0041. In Example 1, Bielas states that the adaptor contains “an index sequence if multiplex sequencing is desired.” EX1013.0027. Additionally, Bielas shows the structure of the adaptor in Figure 2, which includes an “Index” site, which the POSA would have known to be a sample index. EX1013.0027 (citing Figure 2); EX1013.0041 (Figure 2); EX1079.¶¶495-497. In

Example 3, Bielas provides the adaptor sequence which includes in the 5' region a standard Illumina sample index. EX1013.0028-29; EX1079.¶¶495-497.

12. Claim 14

Claim 14 depends from claim 1 and adds that each identifier site is “at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides in length.” EX1005 at 26:43-44. Bielas anticipates this claim. Among other values and ranges that fall within the range of at least 2-10, including 6-10. EX1013.0010, and 7-9, EX1013.0021, Bielas teaches that the Cypher can be specifically six, seven, or eight nucleotides, EX1013.0015; EX1079.¶649. In Example 3, Bielas used a 7-nucleotide identifier. EX1013.0028-29; EX1079.¶649; *supra* §VI.B.7.

13. Claim 15

Claim 15 depends from claim 1 and adds that the method comprises obtaining a sample comprising the nucleic acid, fragmenting the nucleic acid, appending adaptors to the fragments, and amplifying the fragments to obtain amplicons. EX1005 at 26:45-50. Bielas anticipates this claim. In Example 1, Bielas obtained samples of genomic DNA, fragmented the DNA, ligated the fragments to adaptor-containing vectors, and then amplified the adaptor-ligated fragments. EX1013.0026-27; EX1079.¶¶460-470, ¶¶487-511, ¶650.

14. Claim 16

Claim 16 depends from claim 1 and further adds that amplicons are obtained from two different samples, that each sample is appended to an adaptor containing a

sample index unique to the sample, and that after sequencing the reads are separated based on the sample index. EX1005 at 26:51-57. Bielas anticipates this claim. As described, Bielas discloses the use of a sample index in an adaptor in Example 1 and Example 3, including a standard Illumina sample index sequence in Example 3. EX1013.0028-29, 0041; EX1079.¶¶465, ¶¶478-479, ¶652; *supra* §V.A, §V.B.9. The purposes of these sample indexes was to pool multiple samples for multiplexed sequencing. EX1079.¶652. Bielas, moreover, discloses that the adaptor-nucleic acid fragments were sequenced on Illumina devices. EX1013.0027, 0031; EX1079.¶¶469, ¶482; *supra* §VI.A. The POSA would have known that the standard Illumina workflow for multiplexed sequencing included separating samples based on sample indexes that were unique to a particular sample. EX1079.¶653.

VII. GROUND IV: OBVIOUSNESS OVER BIELAS

Claims 4 and 5 would have been obvious over Bielas. To the extent claims 11, 12, 15 and 16 are not anticipated by Bielas, they are at a minimum obvious over Bielas.

A. Claim 4

Claim 4 depends from claim 1 and adds that the identifier site is “sequenced separately” from the target sequence. EX1005 at 25:48-49. As discussed above, *supra* §IV.E, the plain and ordinary meaning of this claim is to sequence the identifier and target sequence in two different SBS reaction reads on the Illumina

device. EX1079.¶¶631-633, ¶377. This claim would have been obvious based on Bielas. EX1079.¶¶631-633, ¶¶589-592.

Bielas disclosed the use of Illumina devices to sequence the adaptor-nucleic acid fragments in Example 1 and Example 3. EX1013.0027-31. Bielas further disclosed the use of adaptors appended to either end of the nucleic-acid fragment, EX.1013.0028-29, 0041; EX1079.¶¶456-486, which the POSA would have understood to be the standard for use on Illumina devices, EX1079.¶¶471-480; EX1075; EX1076; EX1077; EX1078; EX1056.0003; EX1057.0005, 0039-44; EX1053.0004; EX1054; EX1055; EX1008.0005.

The POSA would have known that the use of adaptors on either end of the fragment allowed for paired-end sequencing on the Illumina devices. EX1079.¶¶631-633; ¶¶589-592; ¶¶171-175, ¶251. In paired end sequencing, the Illumina device would first read one adaptor through the identifier and into the fragment sequence. EX1079.¶¶631-633, ¶590, ¶¶171-175; EX.1013.0028-29, 0041. Then, in the second read, the device would read the fragment from the other direction through the second adaptor sequence on the other end of the fragment, but not the first identifier site that was sequenced in the first read. EX1079.¶¶631-633, ¶590, ¶¶171-175; EX.1013.0028-29, 0041. Accordingly, in paired end reads, the POSA would recognize that some identifier sites were read separately from the fragment read on the paired-end strand. EX1079.¶¶631-633, ¶¶171-175, ¶¶590-592;

EX.1013.0028-29, 0041. Given the known benefits of paired-end sequencing, including maximizing the length of the fragment read, the POSA would have been motivated to use paired-end sequencing in the methods disclosed by Bielas, and would have had a reasonable expectation of success in doing so. EX1079.¶¶171-175, ¶251.

Furthermore, the choice of sequencing the identifier with the fragment sequence or separately via paired-end sequencing or otherwise was the type of routine design choice that the POSA routinely encountered in NGS experimental design. EX1079.¶¶631-633, ¶592, ¶¶586-588. *See Uber Tech.*, 957 F.3d at 1340. The POSA would have recognized that there were only two choices—sequence the identifiers “with” the fragment sequence or “separately.” EX1079.¶¶171-175, ¶¶590-592. The ’241 patent does not disclose any unexpected properties of sequencing the identifiers “separately” from the fragment sequences. EX1079.¶¶631-633, ¶588.

B. Claim 5

Claim 5 depends from claim 1 and adds that “the amplicons include amplified fragments of cDNA.” EX1005 at 25:50-51. While Bielas uses genomic DNA in Example 1 and Example 3, EX1013.0027-31, the POSA would have been motivated to use the Bielas method with cDNA and would have had a reasonable expectation of success in doing so, EX1079.¶634, ¶¶456-486. Indeed, Bielas expressly teaches

that the method is suitable for use with cDNA. EX1013.0007, 0014; EX1079.¶634. The POSA also would have known that there was no significant difference between genomic DNA and cDNA that would hinder the application of the Bielas method to cDNA. EX1079.¶634.

C. Claim 11

Claim 11 depends from claim 1 and adds the limitation that “each unique identifier is at a junction between the adapter and the nucleic acid fragment.” EX1005 at 26:35-37. To the extent claim 11 is not anticipated by Bielas, *supra* §V.B.9, it is obvious over Bielas. EX1079.¶¶642-643, ¶¶604-609. Bielas discloses the structure of the adaptors used in Example 1 and Example 3, and discloses the sequence of the adaptor used in Example 3. EX1013.0028-29, 0041; EX1079.¶¶460-468, ¶¶471-481; EX1076; EX1077; EX1078; EX1079. For the 5' adaptor used in both examples, the Cypher/identifier site is at the 3' end of the adaptor where it is appended to the nucleic acid fragment. EX1013.0028-29, 0041; EX1079.¶471, ¶460, ¶468; EX1075. For the 3' adaptor used in both examples, the Cypher/identifier site is at the 5' end of the adaptor where it is appended to the nucleic acid fragment. EX1013.0028-29, 0041; EX1079.¶471, ¶460, ¶468; EX1075. Moreover, the POSA would have understood that it could be beneficial in some experimental designs to use an adaptor with the identifier site oriented like those in Bielas because the identifier site could be read with the nucleic acid fragment,

primed from the read sequencing primer. EX1079.¶¶642-643, ¶609, ¶¶584-588. Based on Bielas's teachings, the POSA would have been motivated to practice the method of claim 11 and would have had a reasonable expectation of success in doing so. EX1079.¶¶642-643, ¶609.

D. Claim 12

Claim 12 depends from claim 1 and adds that the method comprises “determining the genomic start position for each sequencing read.” EX1005 at 26:38-39. To the extent claim 12 is not anticipated by Bielas, *supra* §V.B.10, it is obvious over Bielas. EX1079.¶644-647. As explained in §V.B.10, Bielas taught that sequencing reads generated in Example 1 and Example 3 were aligned with a reference genome. EX1013.0006, 0027; EX1079.¶¶645-647. The POSA would have known that the genomic start position could be determined by aligning a sequencing to a reference genome. EX1079.¶¶645-647. The POSA would have recognized that sequence alignment was a standard part of NGS data processing with obvious benefits, including to detect mutations from the reference genome. EX1079.¶¶645-647. Thus, based on Bielas, the POSA would have been motivated to practice the method of claim 12 and would have had a reasonable expectation of success in doing so. EX1079.¶647.

E. Claim 15

Claim 15 depends from claim 1 and adds that the method comprises obtaining a sample comprising the nucleic acid, fragmenting the nucleic acid, appending adaptors to the fragments, and amplifying the fragments to obtain amplicons. EX1005 at 26:45-50. To the extent claim 15 is not anticipated by Bielas, *supra* §VI.B.13, it is obvious over Bielas. Bielas taught each of the limitations of this claim in Example 1. EX1013.0026-32; EX1079.¶650. Bielas further taught library preparation methods that include each of these limitations, and specifically explained that libraries of nucleic acids for use in the method could be generated by fragmentation using several techniques. EX1013.0010-11; EX1079.¶650. Thus, based on Bielas's teachings, the POSA would have been motivated to practice the method of claim 15 and would have had a reasonable expectation of success in doing so. EX1079.¶651.

F. Claim 16

Claim 16 depends from claim 1 and further adds that amplicons are obtained from two different samples, that each sample is appended to an adaptor containing a sample index unique to the sample, and that after sequencing the reads are separated based on the sample index. EX1005 at 26:51-57. To the extent claim 16 is not anticipated by Bielas, *supra* §VI.B.14, it is obvious over Bielas. In both Example 1 and Example 3, Bielas discloses the use of a sample index standard for use on

Illumina devices. EX1013.0027-31, 0041; EX1079.¶¶465, ¶¶478-479, ¶652. Bielas further discloses that the method is suitable for sample multiplexing, which Bielas explains involves using a unique sample index for each sample and then separating reads based on the unique index. EX1013.0015-16, 0022; EX1079.¶¶465, ¶¶478-479, ¶653. The POSA further would have understood that pooling samples with unique sample indexes prior to sequencing on an Illumina device was a routine technique used in NGS to save costs and time, and would have resulted in sequencing data separated by sample index, i.e. separated by sample. EX1079.¶¶652-653. Thus, based on Bielas's teachings, the POSA would have been motivated to practice the method of claim 16 and would have had a reasonable expectation of success in doing so. EX1079.¶653.

VIII. DISCRETIONARY DENIAL IS NOT APPROPRIATE

The Board should not deny institution under 35 U.S.C. §325(d), or 35 U.S.C. §314(a) and *Apple Inc. v. Fintiv, Inc.*, IPR2020-00019, Paper 11 (PTAB Mar. 20, 2020).

A. Discretionary Denial Under §325(d) Is Not Appropriate

The Petition is based on prior art and arguments that were not before the Examiner or substantially similar to those before the Examiner. First, Kivioja and Bielas were not cited during prosecution, nor were any related or substantially-similar references. EX1020; EX1079.¶¶654-656. Nor did the Examiner have the

benefit of the additional evidence presented by Petitioner here, including Dr. Sims's declaration. Second, to the extent the Examiner allowed the claims on the grounds that the prior art did not teach de-duplication using both the identifier and fragment sequence, *supra* §II.A, the Examiner materially erred because the prior art, including Kivioja and Bielas, taught using the fragment and the identifier sequence to de-duplicate, *supra* §§IV-VII; EX1079.¶¶654-656. *See Advanced Bionics, LLC v. MED-EL Elektromedizinische Geräte G.m.b.H.*, IPR2019-01469, Paper 6, at 8-9 (PTAB Feb. 13, 2020).

B. Discretionary Denial Under § 314(d) Is Not Appropriate.

The parties' litigation in *Tecan Genomics, Inc. v. Invitae Corp., et al.*, C.A. No. 23-cv-1114-GBW (D. Del.) does not warrant discretionary denial under the relevant factors. *See Fintiv*, IPR2020-00019, Paper 11, at 6.

First factor. The first factor is neutral because no party has requested a stay in district court, though Petitioner will promptly seek a stay after filing this Petition.

Second factor. Trial is scheduled for June 22, 2026—after the Board's projected decision date. This factor weighs in favor of institution.

Third factor. The case is still early in fact discovery, favoring institution.

Fourth and fifth factors. Petitioner and Patent Owner are both parties to the district court action. Petitioner has not presented any grounds, arguments, or evidence in district court, though Petitioner expects to serve initial invalidity

contentions soon after the filing of this Petition. If this trial is instituted, however, Petitioner stipulates it will not pursue any arguments in district court that could have been raised herein under 35 U.S.C. §311(b). Thus, there is no prospect for conflicting decisions and duplicative efforts, and these factors cannot weigh against institution. Katherine K. Vidal Memorandum 7-8 (June 21, 2022).

Sixth factor. The Petition raises “a compelling unpatentability challenge” and that “alone demonstrates that the PTAB should not discretionarily deny institution under *Fintiv*.” *CommScope Techs. LLC v. Dali Wireless, Inc.*, IPR2022-01242, Paper 23, at 3 (PTAB Feb. 27, 2023). Any doubt should be resolved in favor of institution because it is “highly likely” that Petitioner will “prevail with respect to at least one challenged claim.” *Id.* at 3-4.

IX. STANDING (37 C.F.R. § 42.104(a)) AND PROCEDURAL STATEMENTS

Petitioner certifies the '241 patent is available for IPR and that Petitioner is not barred or estopped from requesting IPR on the grounds herein. Petitioner is submitting a Power of Attorney and an Exhibit List pursuant to §42.10(b) and §42.63(e), respectively, as well as all fees required under 37 C.F.R. §42.15(a), concurrently with this Petition. If any additional fees are due during this proceeding, the Office is authorized to charge such fees to Deposit Acct. No. DA506403.

X. MANDATORY NOTICES UNDER 37 C.F.R. §42.8

A. Real Party-in Interest (37 C.F.R. §42.8(b)(1))

The real parties-in-interest are Integrated DNA Technologies, Inc., and its corporate parent, Danaher Corporation.

B. Related Matters (37 C.F.R. §42.8(b)(2))

To the best of Petitioner’s knowledge, the ’241 patent is, or has been, involved in only two matters. First, on October 6, 2023, the Patent Owner filed a complaint against Petitioner for infringement of the ’241 patent in *Tecan Genomics, Inc. v. Invitae Corp.*, C.A. No. 23-cv-1114-GBW (D. Del.) (the “District Court Action”). Second, the Patent Owner asserted the ’241 patent in a related district court action: *Tecan Genomics, Inc. v. QIAGEN Sciences, LLC*, C.A. No. 23-cv-1115-GBW (D. Del.).

U.S. Patents 9,546,399, EX1003, and 11,098,357, EX1004, which like the ’241 patent also claim priority to U.S. provisional application No. 61/903,826,² are asserted in the District Court Action and in a related district court action: *Tecan Genomics, Inc. v. QIAGEN Sciences, LLC*, C.A. NO. 23-cv-1115-GBW (D. Del.).

² U.S. Patent 10,570,448 and U.S. Patent Application 2024/0141426 also claim priority to the same provisional application. Neither are the subject of any matters of which Petitioner is aware.

Petitioner will concurrently file petitions for IPR against U.S. Patents 9,546,399 and 11,098,357.

C. Lead and Backup Counsel and Service Information (37 C.F.R. § 42.8(b)(3) and (b)(4))

Lead Counsel	Backup Counsel
Thomas S. Fletcher (Reg. No. 72,383) Williams & Connolly LLP 680 Maine Avenue SW Washington, DC 20024 Phone: 202-434-5497 Fax: 202-434-5029 tfletcher@wc.com	Jessamyn S. Berniker (Reg. No. 72,328) Williams & Connolly LLP 680 Maine Avenue SW Washington, DC 20024 Phone: 202-434-5474 Fax: 202-434-5029 jberniker@wc.com
	Nicholas G. Vincent (Reg. No. 79,788) Williams & Connolly LLP 680 Maine Avenue SW Washington, DC 20024 Phone: 202-434-5470 Fax: 202-434-5029 nvincent@wc.com

IDT consents to service by e-mail at tfletcher@wc.com, jberniker@wc.com, nvincent@wc.com, and IDT-Tecan-IPRs@wc.com.

XI. CONCLUSION

IDT respectfully requests institution and cancellation of claims 1-16.

Dated: September 30, 2024

Respectfully submitted,

/Thomas S. Fletcher/
Thomas S. Fletcher (Reg. No. 72,383)
WILLIAMS & CONNOLLY LLP
680 Maine Avenue SW
Washington, DC 20024
Phone: 202-434-5497

IPR2024-01506
Patent No. 11,725,241

Fax: 202-434-5029
tfletcher@wc.com

*Lead Counsel for Petitioner
Integrated DNA Technologies, Inc.*

CERTIFICATION UNDER 37 C.F.R. § 42.24(d)

I hereby certify that the foregoing complies with the type-volume limitations of 37 C.F.R. § 42.24(d) and contains 13,094 words, excluding those portions exempted under 37 C.F.R. § 42.24(a)(1).

/Thomas S. Fletcher/
Thomas S. Fletcher
Reg. No. 72,383

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. § 42.6(e) and 37 C.F.R. § 42.105, I hereby certify that on September 30, 2024, I caused the foregoing Petition for *Inter Partes* Review, Power of Attorney, Exhibit List, and the accompanying exhibits to be served on Patent Owner by depositing them for shipment with Federal Express, Standard Overnight delivery, to the following correspondence address of record listed on USPTO Patent Center:

BROWN RUDNICK LLP
One Financial Center
Boston, MA 02111

A courtesy electronic copy has also been sent to Timothy J. Beavers (tbeavers@goodwinlaw.com), Linnea P. Cipriano (lcipriano@goodwinlaw.com), John P. Padro (jpadro@goodwinlaw.com), and Scott T. Weingaertner (sweingaertner@goodwinlaw.com), counsel for Plaintiffs in *Tecan Genomics, Inc. v. Invitae Corp. et al.*, C.A. No. 23-cv-1114-GBW (D. Del.).

Dated: September 30, 2024

/Thomas S. Fletcher/
Thomas S. Fletcher
Reg. No. 72,383