

Foresight Diagnostics, Inc

v.

Personalis, Inc.

IPR2023-00224 and IPR2023-00317

U.S. Patent Nos. 11,384,394 and 11,408,033

Patent Owner Personalis's Demonstratives For Oral Hearing

Judge Ulrike W. Jenks

Judge Robert A. Pollock

Judge Timothy G. Majors

March 19, 2024

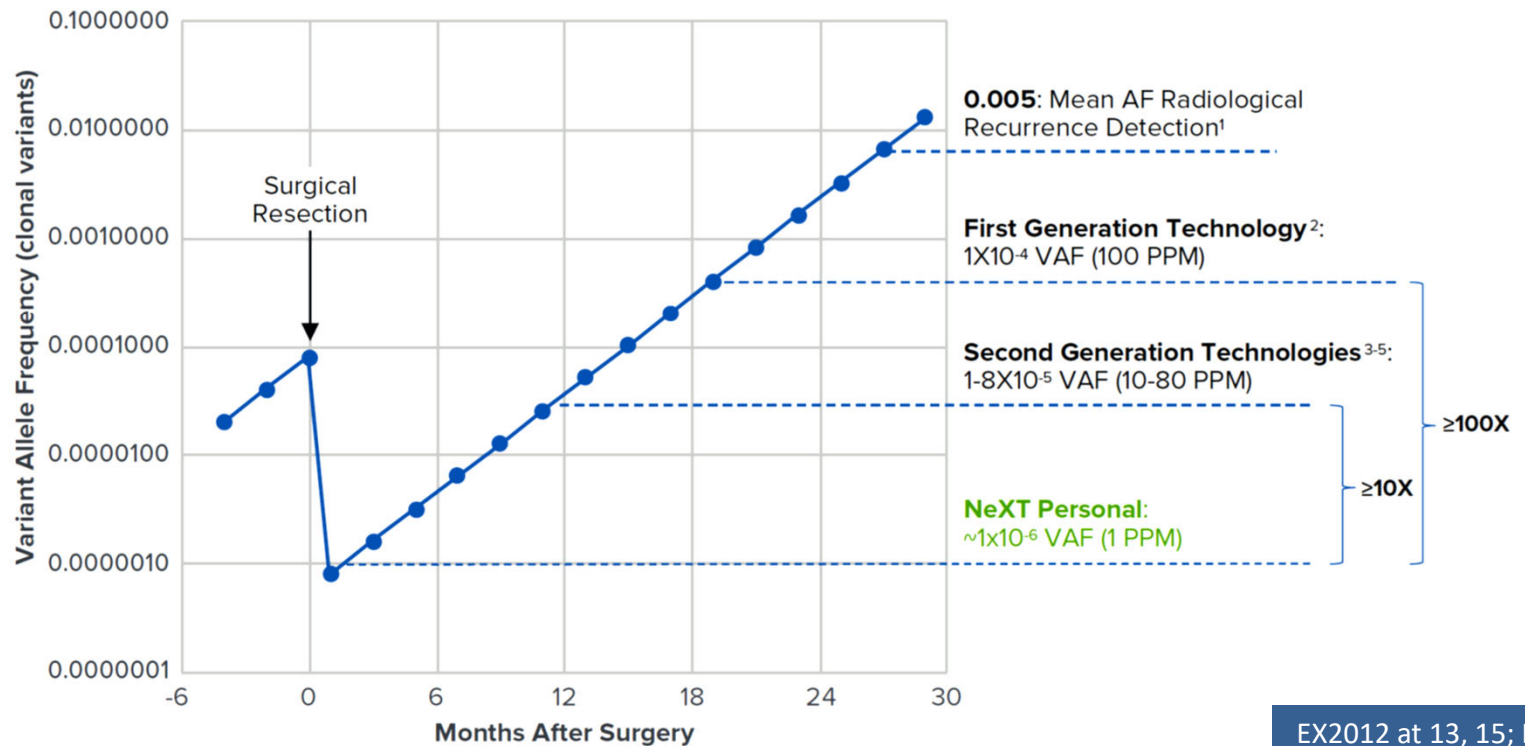
DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Personalis EX2201



NeXT Personal™

The Next Generation in Molecular Residual Disease Testing and Variant Monitoring for Solid Tumors



DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

EX2012 at 13, 15; EX2031 ¶178
IPR2023-00224 POR at 1, 81-83

Foresight's Challenges

IPR2023-00224

Challenge	Reference(s)
§ 103	Leary
§ 103	Leary + Ley
§ 103	Leary + Chan + Liao

U.S. 11,384,394

IPR2023-00317

Challenge	Reference(s)
§ 102	Forschew
§ 103	Forschew + Wagle
§ 103	Wagle + Chan

U.S. 11,408,033

ForsheW

Monitoring levels of ctDNA

Various methods have been suggested to monitor changes in mutation load in plasma. These can have enhanced sensitivity compared to TAm-Seq for tracking individual mutations, but require design of personalized assays (3, 18, 19). None of these methods have been widely adopted. We therefore applied TAm-Seq as a generic tool to measure changes in the frequency of ctDNA over time.

Citation 18 is Leary (EX1002)

The intermediate scale of sequencing is most likely to have immediate impact on clinical genomics. Targeted sequencing has been applied for tumor DNA (34, 35) and cyst fluid (33) to detect mutations down to 5% AF, but has not been applied for analysis of circulating tumor nucleic acids.

Citation 35 is Wagle (EX1033)

Previously proposed methods for personalized monitoring of tumor dynamics relied on expensive custom-designed probes (3) or identification of rearrangements using whole-genome sequencing (18, 19). These have better analytical sensitivity than currently achieved by TAm-Seq, but are difficult to implement on a routine basis. TAm-Seq strikes a balance between sensitivity and ease of use and could facilitate study and application of circulating DNA.

EX1030 (ForsheW) at 8, 9, 10
IPR2023-00224 POR at 33
IPR2023-00317 POR at 71

Claim 1

U.S. 11,384,394

1. A method for analyzing nucleic acid samples isolated from an individual, comprising:

- (a) generating a first subset of nucleic acid molecules from a first nucleic acid sample obtained from said individual;
- (b) conducting a first sequencing assay on said first subset of nucleic acid molecules to yield a first result comprising a first nucleic acid sequence wherein said first sequencing assay comprises whole genome sequencing;
- (c) generating a second subset of nucleic acid molecules from a second nucleic acid sample of said individual by contacting said second nucleic acid sample with a plurality of **capture probes**, wherein said plurality of **capture probes** hybridize to one or more polymorphisms which are based on or extracted from one or more databases of polymorphisms, observed in a population of one or more samples, or a combination thereof, wherein said first sample or said second sample is obtained from said subject at a first time point;
- (d) conducting a second sequencing assay on said second subset of nucleic acid molecules to yield a second result comprising a second nucleic acid sequence;
- (e) using said plurality of **capture probes** to generate an additional subset of nucleic acid molecules from an additional nucleic acid sample isolated from an additional sample obtained from said subject at an additional time point different from said first time point;
- (f) conducting a third sequencing assay on said additional subset of nucleic acid molecules from said additional nucleic acid sample to yield a third result comprising a third nucleic acid sequence; and
- (g) generating a biomedical report that includes biomedical information of said subject, which biomedical information identifies the presence or absence of said one or more polymorphisms identified by said second result or said third result.

capture probes

U.S. 11,408,033

1. A method for analyzing a nucleic acid sample obtained from an individual, comprising:

- (a) producing, with the aid of a computer processor, a plurality of **capture probes**, wherein said plurality of **capture probes** hybridize to a plurality of polymorphisms, wherein said plurality of polymorphisms are in sequences encoding genes with known biomedically interpretable variants, and wherein said plurality of polymorphisms are based on or extracted from one or more databases of polymorphisms and are observed in a population of one or more samples;
- (b) contacting said nucleic acid sample with said plurality of **capture probes** produced in (a);
- (c) conducting a sequencing assay on a subset of nucleic acid molecules generated from said nucleic acid sample in step (b) to yield a result comprising a nucleic acid sequence, thereby analyzing said nucleic acid sample; and
- (d) repeating steps (b)-(c) on a subsequently obtained biological sample from said individual.

Construction of Capture Probes

Patent Owner

“a nucleic acid sequence that hybridizes to target DNA, either in solution or on a solid support, so that one can physically capture and isolate the target DNA”

Petitioner

“encompass[es] at least PCR primer capture probes and solution-based hybridization capture probes”

Luminara and Phillips

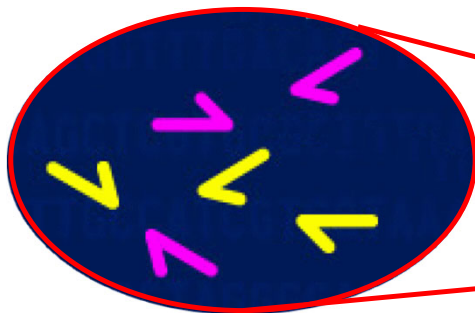
Absent lexicography or disavowal, we do not depart from the plain meaning of the claims.

Luminara Worldwide v. Liown Elecs., 814 F.3d 1343, 1353 (Fed. Cir. 2016)
IPR2023-00224 POR at 16-17

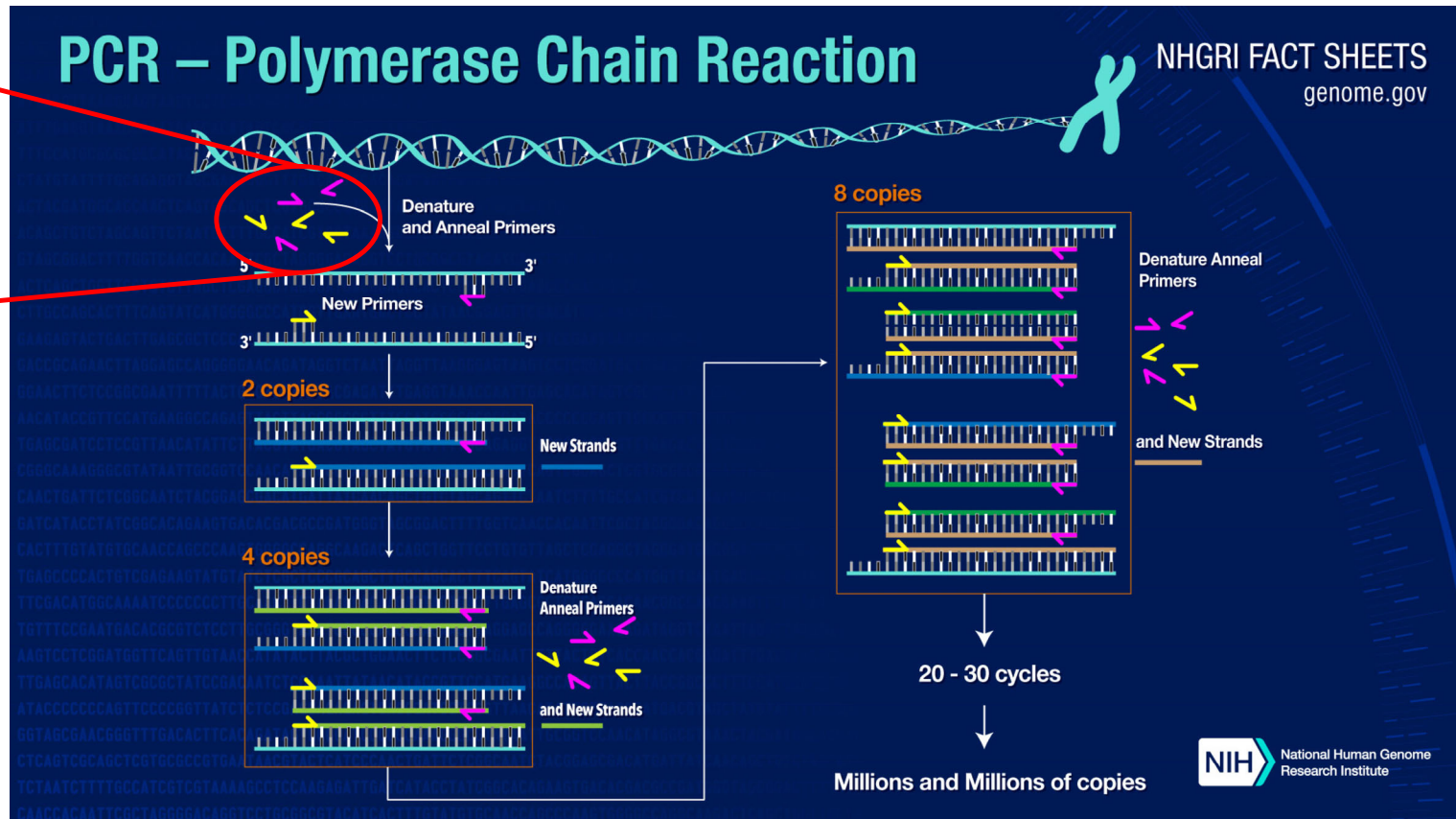
We have frequently stated that the words of a claim are generally given their ordinary and customary meaning. We have made clear, moreover, that the ordinary and customary meaning of a claim term is the meaning that the term would have to a person of ordinary skill in the art in question at the time of the invention, i.e., as of the effective filing date of the patent application.

Phillips v. AWH Corp., 415 F.3d 1303, 1312-13 (Fed. Cir. 2005) (en banc)
IPR2023-00224 POR at 17

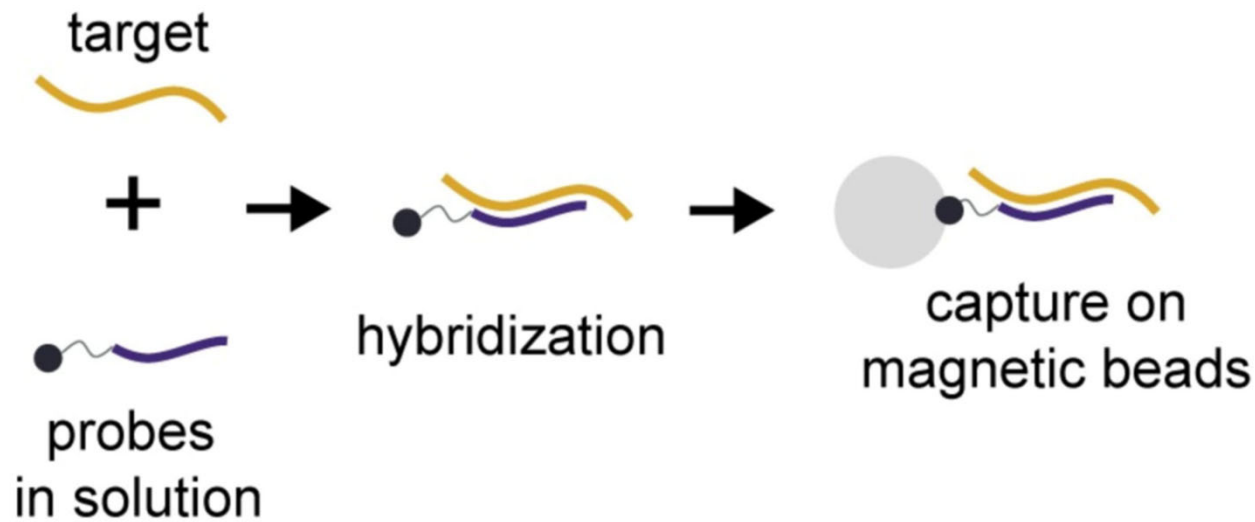
Polymerase Chain Reaction (PCR)



PCR primers
copy
DNA



Capture Probes



IPR2023-00224 Petition at 14; POR at 21-22; Sur-Reply at 3

Wagle's [capture probe] approach enriches by isolating the targets and removing them from non-targeted nucleic acids in the sample. *Forsheew's* [PCR primer] approach copies the targets and thereby enriches them over non-targeted nucleic acids. Quackenbush ¶183.

Dr. Quackenbush

Q. Okay. Is the term “PCR primer capture probe” something that you see commonly used in the literature?

A. So I don't believe the term “PCR capture probe” occurs in the literature. ...

EX2018 (Quackenbush Trans.) at 166:10-167:17

Q. And I just want to know, have you ever seen the term “PCR primer capture probe” used in the literature?

A. So for clarity in my presentation, I refer to them as “PCR primer capture probes” in addition to “capture probes” and also “PCR capture probes,” I believe, although I don't recall seeing that in the literature, although it may appear. I'm not sure. We could easily do a PubMed search and see.

EX2018 (Quackenbush Trans.) at 168:4-169:1
IPR2023-00224 POR at 18-19

Mamanova

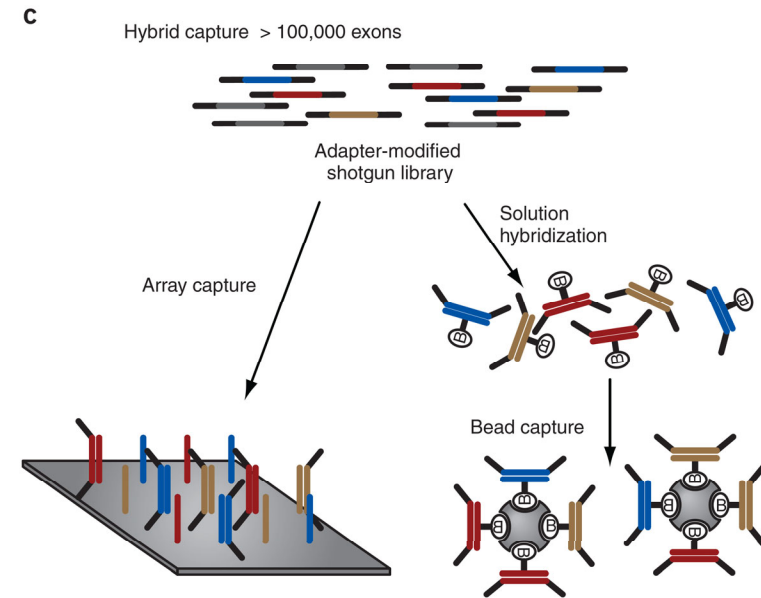
HYBRID CAPTURE

On-array capture

The principle of **direct selection** is well-established: a shotgun fragment library is hybridized to an immobilized probe, nonspecific hybrids are removed by washing and **target DNA** is eluted.

In-solution capture

The principle difference between the Agilent and NimbleGen solution capture products is the nature of the **capture probes**: the NimbleGen product uses 60-90-mer DNA **capture probes**, whereas the Agilent one uses 150-mer RNA **capture probes**.



Mertes

ENRICHMENT TECHNIQUES

Current techniques for targeted enrichment can be categorized according to the nature of their core reaction principle (Figure 1):

- (i) 'Hybrid capture': wherein nucleic acid strands derived from the input sample are hybridized specifically to preprepared DNA fragments complementary to the targeted regions of interest, either in solution or on a solid support, so that one can physically capture and isolate the sequences of interest;
- (ii) 'Selective circularization': also called molecular inversion probes (MIPs), gap-fill padlock probes and selector probes, wherein single-stranded DNA circles that include target region sequences are formed (by gap-filling and ligation chemistries) in a highly specific manner, creating structures with common DNA elements that are then used for selective amplification of the targeted regions of interest;

- (iii) PCR amplification: wherein polymerase chain reaction (PCR) is directed toward the targeted regions of interest by conducting multiple long-range PCRs in parallel, a limited number of standard multiplex PCRs or highly multiplexed PCR methods that amplify very large numbers of short fragments.

Given the operational characteristics of these different targeted enrichment methods, they naturally vary in their suitability for different fields of application. For example, where many megabases need to be analyzed (e.g. the exome), hybrid capture approaches are attractive as they can handle large target regions, even though they achieve suboptimal enrichment over the complete region of interest. In contrast, when small target regions need to be examined, especially in many samples, PCR-based approaches may be preferred as they enable a deep and even coverage over the region of interest, suitable for genetic variance analysis.

Mertes

Table I: Currently employed targeted enrichment techniques

Enrichment technique	Vendor	Features	Pros	Cons	Number of loci (target size)	Library prep for NGS
Hybrid capture Solid support	Agilent SureSelect, Roche NimbleGen SeqCap EZ	Medium to large target regions, custom and preconfigured target regions (i.e. whole exome), Multiplexing possible, ready to use kits	Ease of production, large target sets	Large amount of input DNA, high-tech equipment (3–10 µg)	10 ⁴ –10 ⁶ (1–50 Mb)	Before enrichment
In solution						
Circularization Molecular inversion probes Selector probes	HaloGenomics	Custom kits and clinically relevant panel kits	No dedicated instruments, high specificity, input DNA (<1 µg)	exome kit not available yet	10 ² –10 ⁴ (0.1–5 Mb) 10–200 (0.1–1.5 Mb)	During enrichment (incorporated into hybridization probes)
PCR Long range	Invitrogen SequalPrep, Qiagen SeqTarget system	Smaller target regions, coverage by tiling	Relatively easy to set up and automatable, even coverage	PCR conditions largely influence effectiveness, >10 µg DNA for Large sets	10 ² –10 ⁴ (0.1–5 Mb)	After enrichment
Multiplex	Multiplicom, Fluidigm	Smaller target regions, coverage by tiling, multiplex PCR of 150–200 amplicons (150–450 bp)	Easy to perform, reasonably economical in terms of, even coverage			
Micro droplet	RainDance	Smaller target regions, coverage by tiling, micro droplet PCR of up to 20 000 amplicons (150–1500 bp)	Even coverage, low input DNA	Relatively expensive, specialist equipment	10 ³ –10 ⁴ (up to 10 Mb)	

Dr. Quackenbush

Q. Is it fair that you view Mertes as a reliable source for understanding what the skilled artisan understood about hybrid capture and PCR in the January 2013 time frame?

A. Mertes is one piece of the puzzle.

Part of the reason I cite to Mertes and Mamanova is they provide complementary sources of information.

The biomedical literature is vast, tens of thousands of papers published every year. I pulled out two as representatives.

But I wouldn't want to reply on any particular limitation laid out. I'm not using these as primary references to argue or to present the case for obviousness in the case of the '394 patent but rather really to provide background on capture technology.

This is in the "Background" section of my report.

Mertes's Seven Metrics

Descriptive metrics for targeted DNA enrichment experiments

To allow meaningful comparison of enrichment methods and experiments that employ them, and to rationally decide which technologies are most suitable when designing a research project, it is important that an objective set of descriptive metrics are defined and then widely used when reporting enrichment datasets. A series of metrics need to be considered, and the importance of each can be weighted according to specific needs and objectives of any experiment. A proposal for such a set of metrics is soon to be published, and it contains the following (Nilsson *et al.*, manuscript in preparation):

- (i) Region of interest (size): ROI;
- (ii) Average read depth (in ROI): D;
- (iii) Fraction of ROI sufficiently covered (at a specified D): F;
- (iv) Specificity (fraction of reads in ROI): S;
- (v) Enrichment Factor (D for ROI versus D for rest of genome): EF;
- (vi) Evenness (lack of bias): E and
- (vii) Weight (input DNA requirement): W.

Mamanova's Seven Parameters

There are several enrichment approaches, each with unique advantages and disadvantages. Here we describe our experiences with leading target-enrichment technologies, the optimizations that we have performed and typical results that can be obtained using each. We also provide detailed protocols for each technology so that end users can find the best compromise between sensitivity, specificity and uniformity for their particular project.

Several approaches to target enrichment have been developed (Fig. 1), and there are several parameters by which the performance of each can be measured, which vary from one approach to another: (i) sensitivity, or the percentage of the target bases that are represented by one or more sequence reads; (ii) specificity, or the percentage of sequences that map to the intended targets; (iii) uniformity, or the variability in sequence coverage across target regions; (iv) reproducibility, or how closely results obtained from replicate experiments correlate; (v) cost; (vi) ease of use; and (vii) amount of DNA required per experiment, or per megabase of target.

EX1006 (Mertes) at 380; EX1007 (Mamanova) at 111
IPR2023-00224 POR at 25-26

Foresight's Founder

A challenge with hybrid capture approaches is that a larger genomic space provides more opportunities for artifactual mutations to arise. One strategy for reducing this problem is to first assess the pattern of stereotypic artifacts in a group of normal samples and informatically account for that background when assessing patient samples for the presence of oncogenic mutations (29, 39, 40). An additional limitation is that hybrid capture-based workflows typically have longer turnaround times, since the target enrichment process often takes days (instead of hours with PCR), and are more expensive, since they involve sequencing a larger genomic space. Finally, despite the advantages of overcoming stochastic sampling error, the larger genomic space provided by hybrid capture strategies may provide diminishing additional value in some contexts (41).

Dr. Quackenbush

77. When hybrid capture baits are contacted with the nucleic acid sample (the “pool”), the baits hybridize to the sequences of interest. This hybridization results in a “catch” of nucleic acids bearing the targeted sequences, which are “caught” through the hybridization of strand-to-strand base-pairing. The catch is then pulled down or fished out using beads that bind the biotin that was incorporated into the baits, and the catch can then be amplified using PCR before sequencing, for example using NGS workflows. Ex. 1005 (Gnirke) at 183; Fig. 1 (reproduced below). *See also* Ex. 1006 (Mertes 2011) at 374 (“By selective recover[y] and subsequent sequencing of genomic loci of interest, costs and efforts can be reduced significantly compared with whole-genome sequencing.”)

Examiner Zhang

EAST Search History (Prior Art)

L11	38	L10 and ((enrich\$5 captur\$5 pull-down pulldown) with probe)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2022/04/05 15:01
L12	34	L11 and ((enrich\$5 captur\$5 pull-down pulldown) with (polymorphism inDel SNV mutation insertion deletion))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2022/04/05 15:01
L13	37	L10 and ((enrich\$5 captur\$5 pull-down pulldown) with probe with (polymorphism SNP inDel SNV mutation insertion deletion variant variation))	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	OR	ON	2022/04/05 15:02

Dr. Quackenbush

Q. In PCR, the capture doesn't take place simply by virtue of the primer hybridizing to the target DNA. Is that correct?

A. So I'm not sure where in the patent it's explicitly stated that capture has to occur at the point of hybridization. In fact, it would be hard for me to think of any technology where capture would simply occur the moment hybridization occurred.

...

If one -- in the same way that if one used hybridization capture probes, the simple act of hybridizing wouldn't provide you a purified sample. Those have to be extracted from solution in some way.

Claim 1

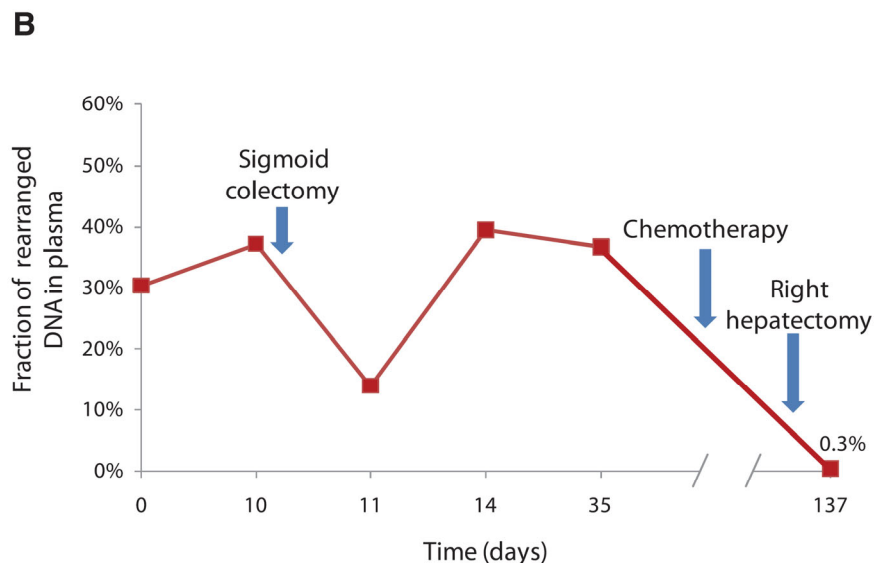
U.S. 11,384,394

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- (d) conducting a second sequencing assay on said second subset of nucleic acid molecules to yield a second result comprising a second nucleic acid sequence;
- (e) using said plurality of capture probes to generate an additional subset of nucleic acid molecules from an additional nucleic acid sample isolated from an additional sample obtained from said subject at an additional time point different from said first time point;
- (f) conducting a third sequencing assay on said additional subset of nucleic acid molecules from said additional nucleic acid sample to yield a third result comprising a third nucleic acid sequence; and
- (g) generating a biomedical report that includes biomedical information of said subject, which biomedical information identifies the presence or absence of said one or more polymorphisms identified by said second result or said third result.

(c) generating a second subset of nucleic acid molecules from a second nucleic acid sample of said individual by contacting said second nucleic acid sample with a plurality of capture probes, wherein said plurality of capture probes hybridize to one or more polymorphisms which are based on or extracted from one or more databases of polymorphisms, observed in a population of one or more samples, or a combination thereof, wherein said first sample or said second sample is obtained from said subject at a first time point;

Leary's detection method is PCR

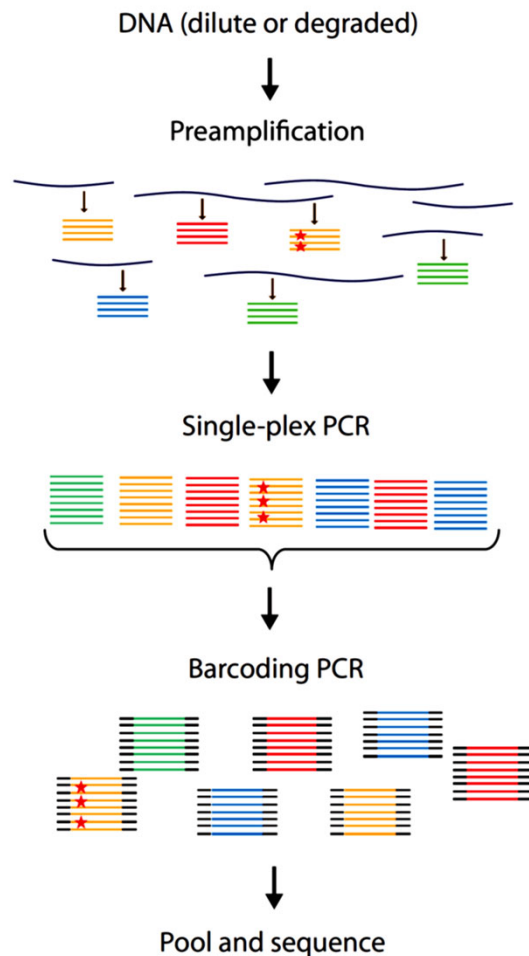


Development of PARE biomarkers from rearranged sequences

Each of the rearranged sequences identified through PARE was unique, as no identical rearrangement was found in any of the other five tumor samples. To determine the utility of these rearranged sequences to serve as potential biomarkers, we designed PCR assays to detect them in the presence of increasing amounts of normal DNA.

Fig. 4. (B) Plasma samples from patient Hx402 were analyzed at different time points using digital PCR to determine the fraction of genomic equivalents of plasma DNA containing the chromosome 4:8 rearrangement. The fraction of rearranged DNA at day 137 was 0.3%, consistent with residual metastatic lesions present in the remaining lobe of the liver.

B



Three rounds of PCR

Fig. 1. (B) Workflow overview. Multiple regions were amplified in parallel. An initial preamplification step was performed for 15 cycles using a pool of the target-specific primer pairs to preserve representation of all alleles in the template material. The schematic diagram shows DNA molecules that carry mutations (red stars) being amplified alongside wild-type molecules. Regions of interest in the preamplified material were then selectively amplified in individual (single-plex) PCR, thus excluding nonspecific products. Finally, sequencing adaptors and sample-specific barcodes were attached to the harvested amplicons in a further PCR.

ForsheW

Table 2. Mutations identified by TAm-Seq in a set of 62 plasma samples from ovarian cancer patients. Forty mutations were identified by TAm-Seq using stringent parameters for mutation calling. Plasma samples described in this table are distinct from those in Table 1, but patients included overlap. Additional data on patients and mutations are provided in table S6.

Sample number	Plasma volume per amplification reaction (μl)	DNA amount per amplification reaction (ng)	Gene	Protein change	Mean depth (sequencing reads)	Mean AF using TAm-Seq	Mean AF using digital PCR
1	70	0.9	TP53	p.R273C	640	0.260	0.167
2	160	4.2	TP53	p.R248Q	340	0.244	0.150
3	160	5.7	TP53	p.R248Q	640	0.507	0.410
4	120	9.9	TP53	p.R213X	810	0.059	0.035
5	120	1.4	TP53	p.C141Y	680	0.021	0.013
6	120	2.1	TP53	p.C141Y	720	0.044	0.038
7	190	17.9	TP53	p.I195N	800	0.091	0.081
8	160	14.8	TP53	p.R175H	510	0.608	0.627
9	160	10.7	TP53	p.R175H	550	0.526	0.604
10	160	6.1	TP53	p.R175H	530	0.651	0.682
11	160	4.9	TP53	p.R175H	490	0.526	0.581
13	160	2.8	TP53	p.C135R	480	0.039	0.045
14	160	2.5	TP53	p.C135R	610	0.046	0.120
15	160	3.0	TP53	p.C135R	470	0.091	0.068
16 [†]	130	3.7	TP53	p.R196P	1070	0.088	0.135

Wagle

Pooling and Hybrid Capture

DNA libraries were pooled by mixing 300 ng of each library in a single 1.5-mL polypropylene sample tube, lyophilizing by the use of a speedvac evaporator, and resuspending in 4 μ L of nuclease-free water. This entire amount (3,600 ng DNA in 4 μ L) was used for hybrid selection. Solution-phase hybrid capture was performed as previously described (51) with 3 modifications to the hybrid selection step (Basic Protocol 3).

Third, during PCR enrichment of the captured DNA (“the catch”), PCR was performed with primers P5 (5'-AAT GAT ACG GCG ACC ACC GA-3') and P7 (5'-CAA GCA GAA GAC GGC ATA CGA-3'), both at 100 μ M, instead of PCR primers PE1.0 and PE2.0.

DeRienzo And Quackenbush

Validation of SNV by Sanger sequencing

Selected candidate SNVs were further characterized using PCR and conventional Sanger sequencing to confirm tumor-specific single-nucleotide mutations. Gene-specific primer pairs were used to PCR amplify genomic DNA from tumor and normal specimens (Supplementary Table S2) as previously described (16).

Target capture, sequencing, and data analysis

Library preparation, custom exome capture, and next-generation sequencing were performed at the Center for Cancer Computational Biology. Library construction and custom exome capture were performed using the SureSelect XT2 for Illumina protocol (Agilent) according to the manufacturer's instructions. Libraries were combined to 3 library pools, diluted to a 2 nmol/L working stock and sequenced at a final concentration of 12 pmol/L on a paired end flowcell with 50 cycles in each direction.

Dr. Quackenbush

Q. So if you had chosen to use solution-phase hybridization capture probes for the process described under the validation of SNV by Sanger sequencing, how would you go about doing that?

A. Again, there are a variety of different ways one could hypothetically do that. Ultimately, whatever one did one would have to do targeted PCR on whatever was captured to allow the Sanger sequencing reactions all to start at the same place because all would have to have primer sequences introduced at the same location.

So it would be an additional step, after which one would have to do targeted PCR with specific primers, but all the methods would rely on nucleic acid hybridization through complementary base-pairing.

Mr. West

Q. Do you remember being asked whether you ever tried to use PCR primers to capture DNA for sequencing?

A. That's not what I remember thinking the question was at the time. I've seen a preliminary draft transcript of the session and now understand that the question did include that term, "capture."

And so I guess I would clarify that PCR is not a capture process. It makes copies of DNA, and that in PCR, it wouldn't be usual that the original molecule that created all the copies doesn't even end up in the result at all. All you end up with is the copies.

And so I don't consider PCR to be a capture process, and I wouldn't use that term.

Antedation



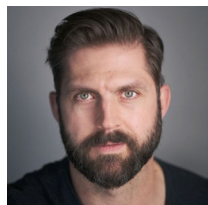
John West

Founding Chief Executive Officer of Personalis (now retired)
Co-Inventor of '394 and '033 patents



Jonathan MacQuitty

Chairman of Personalis's Board of Directors (2011-2022)
Board Member of 15 prominent life science companies



Doug Zeman

Associate Director of Scientific Computing and
Information Technology at Personalis

80+

Over 80 supporting and corroborating exhibits

Exemplary Conception Corroboration

Overview of Personalis Accuracy Program

Characterize errors Analysis Rates by variant type & Q-score Underlying mechanisms Medical interpretation impact Regulatory impact Methods Comparison with literature Studies on limited regions Whole genome analysis: Single genome metrics Same person twice Family inheritance (MIE) Platform comparison Arrays ILMN / CGI / Ion T... Sanger capillary Prioritize solutions Bio-informatic approaches Laboratory approaches Content approaches	Informatic Solutions Advances in 3rd party alg's Aligner for > 200bp reads New versions, current alg's Other alg's (e.g. IobSTR) Advances in our alg's: SV's : Zygosity, accuracy Combine SV's & SNP's Broader use of Breakseq New Personalis alg's Alignment degeneracy Genome-aware alignment VNTR-specific Advances in reference Ethnic major-allele ref's Iterative Admixed InDel's / SV's Flag or Filter Out For problems not yet solved	Lab, Content Solutions Combine technologies WGS + exome Longer reads and/or inserts Multi-vendor Arrays (genotypes & SV's) Electrophoretic sizing Sanger validation Content-based semi-custom Array (genotypes & SV's) Pullout Custom sample preps Problematic motifs Degenerate regions Phasing Content Gold standard genomes Known healthy genomes
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Personalis Confidential May 8, 2012

5

Market Developments

- Illumina / Roche
 - Financial pressure on Illumina may drive more aggressive approach to clinical potential
 - Illumina will be asked whether it is developing that opportunity as fast as Roche might
 - MiSeq moving to 510k; **Cancer-gene pullout panel released**
 - 2 x 250 reads now "summer" and only MiSeq
- Complete Genomics
 - Delivery times being quoted as 12-16 weeks
- Oxford Nanopore
 - No data seen yet, but tentative meeting in UK in July
- Life Ion Proton
 - Expect HapMap genome for analysis in the next month

Personalis Confidential May 8, 2012

4

Publications & Upcoming Presentations

- **Seminal paper by Mike Snyder & team published in Cell :**
 - Ongoing clinical use of genome-scale sequencing
 - **Not just a genome once in your life**
- Multiple publications by Rong Chen
- JW invited to speak at CHI "The Clinical Genome" meeting
 - June 13, San Francisco
- JW invited to speak at Institute of Medicine meeting
 - Washington, DC, July

Personalis Confidential May 8, 2012

3

Laboratory assays – **Whole genome Plus**

- Illumina or CGI **whole genome sequence with short-insert paired-end reads** (may be provided by customer). Coverage & read length recommendations TBD
- **Targeted re-sequencing using array or oligo-based pullout:**
 - Additional coverage to fill in systematic gaps due to bias of biochemistry / molecular biology used for whole genome
 - Long-fragment pullout to resolve repeats, particularly in approx 3,000 genes with alignment problems due to gene-family homologies
- Whole genome and/or exome-focused genotyping to provide:
 - Technologically orthogonal validation of numerous specific medically interpretable alleles
 - Calibration of the sequencing error model
 - Independent assessment of Copy Number Variation
- Electrophoretic assay of certain VNTR's which are difficult to assess with NGS
- FISH assay of chromosomal translocations, difficult to assess with NGS
- RNA-seq from same sample, for expression, allelic bias, genetic variant-driven splice variation, partial phasing of individual genomes

Personalis Confidential Jan 13, 2012

2

Laboratory assays – **"Exome plus"**

- **Exon re-sequencing by pullout from customer sample** (may be provided by customer)
- **Additional re-sequencing by pullout, to capture regions outside the exons which are in VarMed, Regulome, lincRNA catalog & possibly others**
- **Other elements from the list of assays which supplement the Whole Genome product.** Selection likely to be based on customer's specific goals and pricing

Personalis Confidential Jan 13, 2012

3

Categories of experimental data to assess error types, mechanisms, frequencies

- **Repeatability :**
 - **Same person twice (or more ?)**, trying to keep everything else constant
 - Alternatives: Genomes of identical twins, or children where identical
- Controlled process experiments :
 - **Same person sequenced multiple times**, each time varying just one parameter of the process (many of these can be done computationally)
- Concordance of other technology platforms on the same sample
- Compare process QC metrics from many sources of raw genome data, even low coverage (e.g. 1,000 Genomes)
- Mendelian inheritance errors (MIE's) determined from family genome sets
- Absolute accuracy :
 - Genomes of individuals known to have clear Mendelian diseases
 - Sequencing of complex synthesized oligo sets (synthetic genome)
 - Targeted modifications of DNA from a naturally occurring genome

IPR2023-00317 POR at 14-23

EX2112 Slides 3, 4, 5; EX2109 Slides 2, 3; EX2046 Slide 14
EX2032 (West Dec.) ¶¶54, 55, 57, 92, 93, 97, 125, 127

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

***Medtronic* 68 F.4th 1298**

In an IPR, the petitioner bears the ultimate burden of persuasion on invalidity, which never shifts to the patent owner. However, when a patent owner attempts to antedate an asserted prior art reference, the patent owner takes on a temporary burden of production. Once that burden is met, the burden shifts back to the petitioner.

Medtronic, Inc. v. Teleflex Innovations SARL, 68 F.4th 1298, 1303 (Fed. Cir. 2023)
IPR2023-00224 Sur-Reply at 20

***Medtronic* 86 F.4th 902**

An asserted prior art reference can be antedated based on a constructive reduction to practice by a showing of
(1) conception prior to the filing date of the asserted reference and
(2) reasonably continuous diligence from just before the date the asserted reference was filed until the date that the patent owner filed its priority application.

Medtronic, Inc. v. Teleflex Innovations SARL, 86 F.4th 902, 906 (Fed. Cir. 2023)
IPR2023-00224 Sur-Reply at 24

Burroughs

The Burroughs Wellcome inventors claim conception of these inventions prior to the NIH experiments, based on the draft British patent application. That document is not itself a conception, for conception occurs in the inventors' minds, not on paper. The draft simply corroborates the claim that they had formulated a definite and permanent idea of the inventions by the time it was prepared.

Burroughs Welcome v. Novopharm, Inc., 40 F.3d 1223, 1230 (Fed. Cir. 1994)
IPR2023-00224 Sur-Reply at 23

Burroughs

For conception, we look not to whether one skilled in the art could have thought of the invention, but whether the alleged inventors actually had in their minds the required definite and permanent idea.

Burroughs Welcome v. Novopharm, Inc., 40 F.3d 1223, 1232 (Fed. Cir. 1994)
IPR2023-00224 Sur-Reply at 23

Fleming

Importantly, the law does not impose an impossible standard of independence on corroborative evidence by requiring that every point of a reduction to practice be corroborated by evidence having a source totally independent of the inventor; indeed, such a standard is the antithesis of the rule of reason.

Fleming v. Escort Inc., 774 F.3d 1371, 1377 (Fed. Cir. 2014)
IPR2023-00224 Sur-Reply at 24

Conception

(12) **United States Patent**
Bartha et al.

(10) **Patent No.:** **US 11,384,394 B2**

(45) **Date of Patent:** **Jul. 12, 2022**

(54) **METHODS AND SYSTEMS FOR GENETIC ANALYSIS**

(71) Applicant: **Personalis, Inc.**, Menlo Park, CA (US)

(72) Inventors: **Gabor T. Bartha**, Los Altos, CA (US); **Gemma Chandratillake**, Cambridge (GB); **Richard Chen**, Burlingame, CA (US); **Sarah Garcia**, Palo Alto, CA (US); **Hugo Yu Kor Lam**, Sunnyvale, CA (US); **Shujun Luo**, Castro Valley, CA (US); **Mark R. Pratt**, Roseburg, OR (US); **John West**, Cupertino, CA (US)

(73) Assignee: **Personalis, Inc.**, Menlo Park, CA (US)

All inventors, even those who contribute to only one claim or one aspect of one claim of a patent, must be listed on that patent.

Vapor Point LLC v. Moorhead, 832 F.3d 1343, 1348-49 (Fed. Cir. 2016)

EX1001 at title page
IPR2023-00224 Sur-Reply at 21

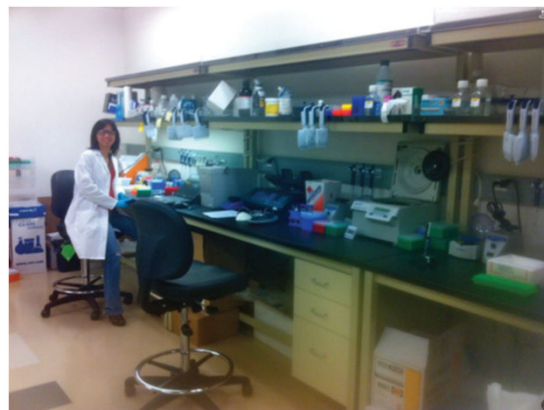
DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Exemplary Diligence

May 2012



November 2012



DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

EX2032 (West Dec.) ¶¶327, 399
IPR2023-00224 POR at 61-76

Exemplary Diligence

Bartha hired May 1
Conception by May 8
Lease space for lab

Luo hired June 18
Lab construction

In-house sequencing

Lab construction completed
HiSeq-2000 installed

Custom capture probe
libraries ordered

Capture probe libraries received

Continued work on
Accuracy Program

Communications with
Patent Counsel
Patent Application Filed

Communications with
Patent Counsel
Continued work

May '12

June

July

Aug.

Sep.

Oct.

Nov.

Dec.

Jan. '13

EX2043	EX2057	EX2066	EX2075	EX2083	EX2090	EX2099	EX2104	EX2108
EX2047	EX2058	EX2067	EX2076	EX2084	EX2091	EX2100	EX2105	
EX2048	EX2059	EX2068	EX2077	EX2085	EX2092	EX2101	EX2106	
EX2049	EX2060	EX2069	EX2078	EX2086	EX2093	EX2102		
EX2050	EX2061	EX2070	EX2079	EX2087	EX2094	EX2103		
EX2051	EX2062	EX2071	EX2080	EX2088	EX2095	EX2111		
EX2052	EX2063	EX2072	EX2081	EX2089	EX2096			
EX2053	EX2064	EX2074	EX2082		EX2097			
EX2054-2056	EX2065				EX2098			

EX2032 (West Dec.) ¶¶34-35, 325-409
IPR2023-00224 POR at 61-76

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

Claim 1 Of The '394 Patent

U.S. 11,384,394

1. A method for analyzing nucleic acid samples isolated from an individual, comprising:

- (a) generating a first subset of nucleic acid molecules from a first nucleic acid sample obtained from said individual;
- (b) conducting a first sequencing assay on said first subset of nucleic acid molecules to yield a first result comprising a first nucleic acid sequence wherein said first sequencing assay comprises whole genome sequencing;
- (c) generating a second subset of nucleic acid molecules from a second nucleic acid sample of said individual by contacting said second nucleic acid sample with a plurality of capture probes, wherein said plurality of capture probes hybridize to one or more polymorphisms which are based on or extracted from one or more databases of polymorphisms, observed in a population of one or more samples, or a combination thereof, wherein said first sample or said second sample is obtained from said subject at a first time point;
- (d) conducting a second sequencing assay on said second subset of nucleic acid molecules to yield a second result comprising a second nucleic acid sequence;
- (e) using said plurality of capture probes to generate an additional subset of nucleic acid molecules from an additional nucleic acid sample isolated from an additional sample obtained from said subject at an additional time point different from said first time point;
- (f) conducting a third sequencing assay on said additional subset of nucleic acid molecules from said additional nucleic acid sample to yield a third result comprising a third nucleic acid sequence; and
- (g) generating a biomedical report that includes biomedical information of said subject, which biomedical information identifies the presence or absence of said one or more polymorphisms identified by said second result or said third result.

**whole genome sequencing
(WGS)**

TI Group Automotive

Our validity analysis is a two-step procedure: The first step involves the proper interpretation of the claims. The second step involves determining whether the limitations of the claims as properly interpreted are met by the prior art.

TI Group Automotive Sys. v. VDO N.Am., 375 F.3d 1126, 1139 (Fed. Cir. 2004)
IPR2023-00224 Sur-Reply at 10

Construction of Whole Genome Sequencing

Board & Patent Owner

“a non-targeted approach to sequencing all or almost all of a genome”

Petitioner

“a non-targeted approach to generating sequencing reads and mapping those reads back to a reference genome (rather than a genome subset)”

Dr. Quackenbush

Q. Now, we've talked a lot about whole-genome sequencing. When did you first become familiar with whole-genome sequencing?

A. So it's hard to answer that question because the term has taken on many different flavors of meaning.

Leary 2010

Description of the approach

The PARE (personalized analysis of rearranged ends) approach, shown schematically in Fig. 1, requires the identification of patient-specific rearrangements in tumor samples. To determine the feasibility of identifying such alterations using next-generation sequencing approaches, we initially analyzed four tumor samples (two colon and two breast tumors) and their matched normal tissue samples using the Applied Biosystems SOLiD System (Table 1). Genomic DNA from each sample was purified, sheared, and used to generate libraries with mate-paired tags ~1.4 kb apart.

SOLiD library preparation and sequencing

Mate-pair libraries were generated for the SOLiD platform as described (15). In brief, genomic DNA was sheared into ~1.4-kb fragments and used as template in emulsion PCR. Fragments were coupled to beads via an adapter sequence and clonally amplified. A 3' modification of the DNA fragments allowed for covalent attachment to a slide. Sequencing primers hybridized to the adapter sequence and four fluorescently labeled di-base probes were used in ligation-based sequencing. Each nucleotide is sequenced twice in two different ligation reactions, resulting in two-base encoding, which has been shown to reduce sequencing artifacts.

EX1002 (Leary 2010) at 1, 5

Leary 2012

Detection of Chromosomal Alterations in the Circulation of Cancer Patients with Whole-Genome Sequencing

Sequencing and analyses of Illumina fragment libraries

All samples were sequenced on Illumina HiSeq and Genome Analyzer II instruments. Sequence reads were analyzed and aligned to human genome hg18 with the ELAND algorithm using CASAVA 1.7 software (Illumina). Reads were mapped with the default seed-and-extend algorithm, allowing a maximum of two mismatched bases in the first 32 bp of sequence. Paired reads with duplicate start positions or reads with mapping scores of <50 were removed from further analysis.

EX2183 (Leary 2012) at Title, 8
IPR2023-00224 Sur-Reply at 9

Dr. Quackenbush

Q. Are you familiar with the SOLiD sequencing platform?

A. Yes. The SOLiD sequencer was developed by Applied Biosystems. As it used -- rather than sequencing by synthesis, it was technology based on sequencing by ligation in which a series of probes -- I think they were four bases in length -- were hybridized to and ligated -- hybridized to DNA and ligated together to produce a signal.

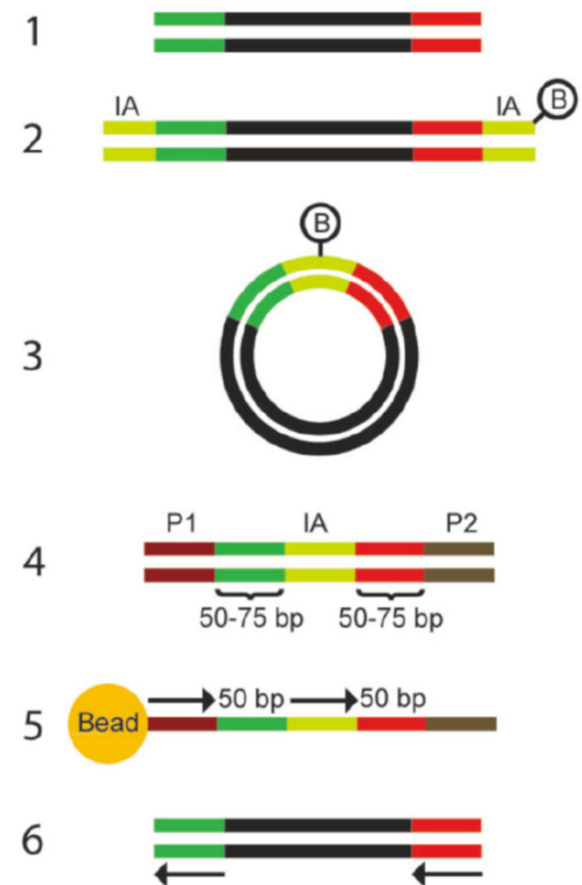
And this was done sequentially with primers that differed in length to create different offsets for the former hybridizations.

That's my best recollection of the technology today. There could be details that I've gotten wrong, since that technology has been off the market for quite some time.

Leary 2010

Description of the approach

The PARE (personalized analysis of rearranged ends) approach, shown schematically in Fig. 1, requires the identification of patient-specific rearrangements in tumor samples. To determine the feasibility of identifying such alterations using next-generation sequencing approaches, we initially analyzed four tumor samples (two colon and two breast tumors) and their matched normal tissue samples using the Applied Biosystems SOLiD System (Table 1). Genomic DNA from each sample was purified, sheared, and used to generate libraries with mate-paired tags ~1.4 kb apart. Libraries were digitally amplified by emulsion polymerase chain reaction (PCR) on magnetic beads (21), and 25-bp mate-paired tags were sequenced using the sequencing-by-ligation approach (15, 22). An average of 198.1 million 25-bp reads was obtained for each sample, where each read aligned perfectly and was uniquely localized in the reference human genome (hg18), resulting in 4.95 Gb of mappable sequence per sample. An average of 40 million mate-paired reads, where both tags were perfectly mapped to the reference human genome, was obtained for each sample. The total amount of genome base pairs covered by the mate-paired analysis (that is, distance between mate-paired tags $\times$ number of mate-paired tags) was 53.6 Gb per sample, or an 18-fold physical coverage of the human genome.



Dr. Quackenbush

Q. How does having the 1.4-kilobase separation help the Leary protocol versus if they had just sheared the DNA strands down to strands around 50-kilobases long?

A. If they're only 50 bases long?

Q. Yeah, 50 bases long. Thank you.

A. So, you know, think about it, right. You've got a place in the genome where the two chromosomes are broken, right. They would have to do a lot more sequencing of smaller fragments to get the same physical coverage of the genome.

So they would have had to do 28 times more sequencing. ...

Dr. Quackenbush

Q. Do you agree with Dr. Furneaux that Leary's mate pairs likely overlapped?

A. So it says, "the data presented in Leary confirms that the mate-paired tags in Leary are very likely overlapping to a very significant degree."

So I would concur that they overlap. I don't know what Dr. Furneaux is referring to when he says "a very significant degree."

Dr. Quackenbush

Q. What degree do you think that Leary's mate pairs overlapped by?

A. I haven't done that calculation.

So -- I haven't done that calculation. So I don't know.

Dr. Furneaux

100. The following calculation illustrates this in the case of Co84 normal sample:

- There are 5,457,003,650 total bases sequenced. These sequenced bases come from 25 bp tags, and the tags are placed into 3 kb bins. *See* Leary (EX1002) at Table 1; *id.* at page 3.
- Leary also explains on page 6 that there are 849,434 bins. *Id.* at 6. These 849,434 bins cover only $849,434 \text{ bins} \times (3000 \text{ bases of the genome per bin}) = 2,548,302,000$ bases.
- Therefore, of the 5,457,003,650 bases sequenced, only about $5,457,003,650 \times 2,548,302,000 / 3,200,000,000 = 4,345,654,161$ are actually placed into a bin.

- Therefore, there are $(4,345,654,161 \text{ sequenced bases}) / (849,434 \text{ bins}) = 5,115$ sequenced bases/bin.
- There are 3000 bases of the genome per bin.
- Therefore, there are $(5,115 \text{ sequenced bases/bin}) / (3000 \text{ nucleotides/bin}) = 1.70$ sequenced bases/nucleotide.

In other words, based on the data in Leary, it appears that each base that is sequenced is, on average, actually sequenced about 1.7 times. Therefore, in reality, the mate-paired analysis for Co84 normal covers, in a best-case scenario, only about 1.71 Gb = $2.9 / 1.70$ of the genome, not 2.9 Gb.

At best, Leary sequenced only 53-60% of the genome.

Dr. Quackenbush

66. Even accepting for the sake of argument Dr. Furneaux's math that Leary sequenced 1.71 Gb of the genome, Ex2031, ¶98, such sequence coverage (1.71 Gbp/2.86 Gbp = 60%)⁷ was still sufficient to constitute "almost all of the genome" as it would have been understood by a POSA. *See* Section II.A.1.b).

⁷ If the incorrect 3.2 Gbp denominator is used, then it would be about 53%.

Leary / Chan / Liao

Leary's "PARE"

Chan's "cancer genome scanning"

} not whole genome sequencing

Liao

BACKGROUND: Massively parallel sequencing has recently been used in noninvasive prenatal diagnosis. The current costs of this technology are still relatively expensive, however, and sample throughput is still relatively low when it is used as a molecular diagnostic tool. Rather than nonselectively sequencing the genome, target enrichment provides a logical approach for more efficient and cost-effective massively parallel sequencing because it increases the proportion of informative data from the targeted region(s). Existing applications of targeted sequencing have mainly been qualitative analyses of genomic DNA. In this study, we investigated its applicability in enriching selected genomic regions from plasma DNA and the quantitative performance of this approach.

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

EX1009 (Liao) at 92
IPR2023-00224 POR at 77-78

Ley

Although we performed whole-genome sequencing on this cancer sample, we restricted our initial validation studies to the 1–2% of the genome that encodes genes. This raises the issue of whether sequencing the complementary DNA transcriptome of this tumour would have been a faster, cheaper and more efficient way of finding the mutations. Although this approach will undoubtedly be an important adjunct to whole-genome sequencing, there are several advantages to the approach we used: (1) coverage models for whole-genome libraries are at present better understood than for cDNA libraries, where transcript abundance can vary over many orders of magnitude; (2) even if the transcriptome had been sequenced, extensive characterization of the normal genome would have been required to distinguish inherited variants from somatic mutations; and (3) relevant non-synonymous mutations could be missed by cDNA sequencing, including mutations that result in RNA instability (splice variants, nonsense mutations), and/or mutations in genes expressed at low levels, or in only a small subset of tumour cells.

Ley

We performed 98 full runs on the Illumina Genome Analyser to achieve the targeted level of 90% diploid coverage as determined by coverage of the high-quality SNP set.

EX1012 (Ley) at 67
IPR2023-00224 Sur-Reply at 11

Our results strongly support the notion that hypothesis-driven (for example, candidate gene-based) examination of tumour genomes by PCR-directed or capture-based methods is inherently limited, and will miss key mutations.

EX1012 (Ley) at 70
IPR2023-00224 Petition at 15, 57

Mertes

CHALLENGES AND FUTURE PERSPECTIVES

The main reason that targeted enrichment has been developed as an adjunct for NGS in recent years is that it was needed to make extensive sequencing affordable for subregions of complex genomes. The alternative of fully sequencing many complete genomes to high average coverage ($\sim 30\times$ or higher) to enable things like genetic variation analysis, was simply not affordable. Another reason for assaying, e.g. exome rather than whole-genome sequencing is the simpler data interpretation of the former. This is a crucial consideration as it is generally much more challenging to find the functional impact of variants in noncoding regions.

Blighe

Ultimately, the method for detecting genomic variation that offers the highest resolution is next generation sequencing (Van de Wiel *et al.*, 2011 and Manolio *et al.*, 2009). However, sequencing technologies are still in their infancy and are not yet ready for use in diagnostics (Meldrum *et al.*, 2011). Such methods also generate tremendous amounts of data - much more than microarrays - that equally require intensive interpretation. For example, solely from pilot data, the 1,000 Genomes Project (The 1,000 Genomes Project Consortium, <http://www.1000genomes.org/>) has already discovered around 15 million SNPs and 1 million insertions and deletions, but to what end? Whole genome sequencing may merely provide another more expensive way of determining potential targets that then have to be examined, as usual, in the laboratory. However, targeted sequencing could be a way forward if it can be developed to cover key somatic mutations and disease-causing SNPs/CNVs.

Sathirapongsasuti And Quackenbush

1 INTRODUCTION

The development of next-generation sequencing has enabled routine large-scale resequencing projects, permitting us to perform increasingly more comprehensive DNA variant analysis. However, the cost and analytical complexity of sequencing still limit the number of whole genomes that can be sequenced in any single project (Teer and Mullikin, 2010). In fact, the analysis of complete human genome sequence often interprets DNA alterations in protein coding regions primarily. This is in practice a reasonable strategy since ~85% of the disease-causing mutations are found in the coding regions or canonical splice sites (Choi *et al.*, 2009). Thus, whole-exome sequencing presents an effective alternative to whole-genome sequencing and provides an unbiased, cost-effective and time-efficient tool for the study of the genetic basis for disease.

Sims (2014)

Genomics is extending its reach into diverse fields of biomedical research from agriculture to clinical diagnostics. Despite sharp falls in recent years¹, sequencing costs remain substantial and vary for different types of experiment. Consequently, in all of these fields investigators are seeking experimental designs that generate robust scientific findings for the lowest sequencing cost. Higher coverage of sequencing (BOX 1) inevitably requires higher costs.

Foresight's Founders

Whole-Genome Sequencing

In theory, application of whole-genome sequencing (WGS) to posttreatment plasma could allow detection of an even greater number of mutations than the aforementioned NGS approaches and not require custom panel design. However, due to assay background error rates, there are diminishing returns of increasing the number of tracked mutations past a certain point (see below). Therefore, recent work suggests that when combined with customized bioinformatics, direct WGS of plasma can potentially achieve similar but not superior limits of MRD detection as the other NGS approaches (48). Additionally, the amount of sequencing required for the much larger targeted genomic space makes the per sample costs of WGS significantly higher.

Belden and Polaris

[O]bviousness concerns whether a skilled artisan not only ***could have made*** but ***would have been motivated to make*** the combinations or modifications of prior art to arrive at the claimed invention.

Belden Inc. v. Berk-Tek LLC, 805 F.3d 1064, 1073 (Fed. Cir. 2015)
IPR2023-00224 Sur-Reply at 14-15

[M]erely stating that a particular placement of an element is a design choice does not make it obvious.

Polaris Indus. v. Artic Cat, Inc., 882 F.3d 1056, 1069 n.4 (Fed. Cir. 2018)
IPR2023-00224 Sur-Reply at 16

'394 Patent Claims 17 & 18

17. The method of claim 1, wherein said whole genome sequencing produces at least 750,000,000 sequencing reads.

18. The method of claim 1, wherein said whole genome sequencing produces at least 1,000,000,000 sequencing reads.

Dr. Quackenbush

And [Leary] is a very cost-effective strategy of identifying translocations. Because rather than having to sequence every base, she relies on this additional information about the length of the fragments.

Really smart because it lets you find these things very, very efficiently. Because they don't end at the same place, she can narrow down where they are, which is what allows her to design her PCR capture probes and perform her subsequent analysis.

So she optimized for physical coverage because she's looking for translocations. If she wanted to optimize for other types of mutation or polymorphism detection, she could have chosen a different strategy.

'394 Patent Claims 17 & 18

17. The method of claim 1, wherein said whole genome sequencing produces at least 750,000,000 sequencing reads.

18. The method of claim 1, wherein said whole genome sequencing produces at least 1,000,000,000 sequencing reads.

Dr. Quackenbush

Q. For what people would call sequencing a genome in 2013, how many times would you do it?

A. It depends on your application.

Q. For sequencing an entire genome, a whole-genome sequencing. Would someone say 10 X would be sufficient for whole-genome sequencing?

A. So it would depend on your application. ...

So the depth of coverage is going to depend on your application. ...

And again, it's going to depend on your particular application.

'394 Patent Limitation 1(f) And Claim 19

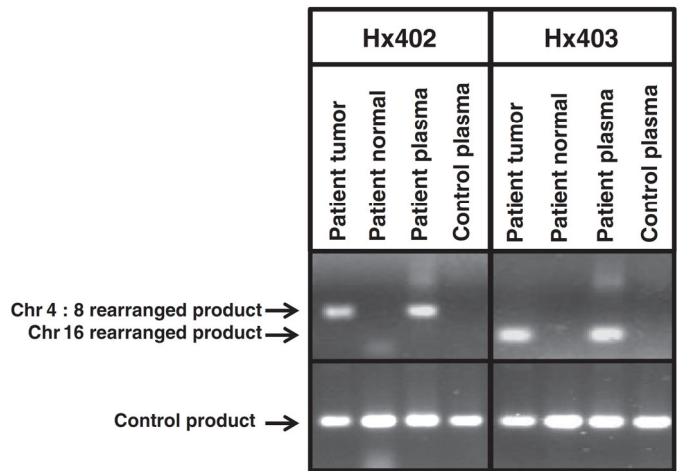
(1)(f) conducting a third sequencing assay on said additional subset of nucleic acid molecules from said additional nucleic acid sample to yield a third result comprising a third nucleic acid sequence; and

19. The method of claim 1, further comprising:

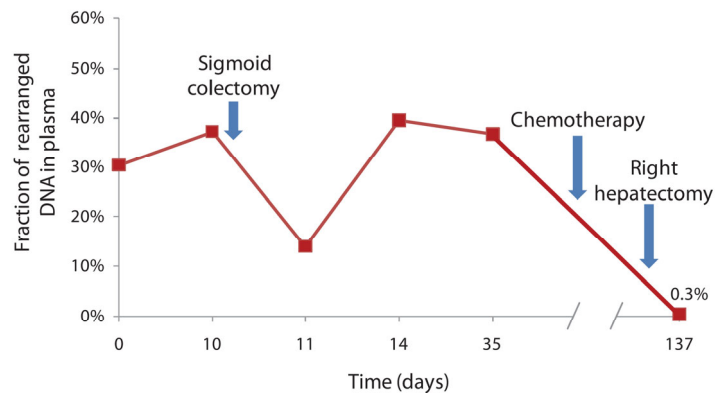
- (i) repeating said third sequencing assay one or more times on one or more subsequent samples to yield an additional result comprising a fourth nucleic acid sequence; and
- (ii) generating an additional biomedical report that includes biomedical information of said subject, which biomedical information identifies the presence or absence of said one or more polymorphisms identified by said additional result.

Leary Does Not Teach Or Suggest '394 Patent Limitation 1(f) Or Claim 19

A



B



A chromosome 4:8 translocation associated with an amplification was used in tumor Hx402, and an intrachromosomal rearrangement associated with a homozygous deletion of chromosome 16 was used in tumor Hx403. PCR amplification of plasma DNA using primers spanning the break-points produced products of the expected sizes only in the plasma samples from patients with disease and not in plasma from healthy controls (Fig. 4A). Sequencing of the PCR products from plasma DNA identified the identical breakpoints observed in the tumor DNA samples.

We next determined whether circulating tumor-derived rearranged DNA could be quantitatively measured in patient plasma. Digital PCR analyses of plasma samples from patient Hx402 indicated that rearranged tumor DNA was detectable at all six time points analyzed throughout the course of treatment (Fig. 4B).

DEMONSTRATIVE EXHIBIT – NOT EVIDENCE

EX1002 (Leary) at 4, Fig. 4
IPR2023-00224 POR at 33-34, 35-36
IPR2023-00224 Petition at 41-44, 53-54

'033 Patent Limitation 1(d)

U.S. 11,408,033

1. A method for analyzing a nucleic acid sample obtained from an individual, comprising:

- (a) producing, with the aid of a computer processor, a plurality of capture probes, wherein said plurality of capture probes hybridize to a plurality of polymorphisms, wherein said plurality of polymorphisms are in sequences encoding genes with known biomedically interpretable variants, and wherein said plurality of polymorphisms are based on or extracted from one or more databases of polymorphisms and are observed in a population of one or more samples;
- (b) contacting said nucleic acid sample with said plurality of capture probes produced in (a);
- (c) conducting a sequencing assay on a subset of nucleic acid molecules generated from said nucleic acid sample in step (b) to yield a result comprising a nucleic acid sequence, thereby analyzing said nucleic acid sample; and
- (d) repeating steps (b)-(c) on a subsequently obtained biological sample from said individual.

(d) repeating steps (b)-(c) on a subsequently obtained biological sample from said individual.

No Motivation Based On Wagle And Chan

In other words, Wagle did not repeat steps (b)-(c) because of the sample type and also because it was an interrogation of tumor tissue to personalize cancer treatment (as opposed to tumor monitoring over time) and did not require repeating.

IPR2023-00317 Petition at 67

No motivation to modify Wagle to repeat steps (b)-(c) on:
(1) Subsequently obtained tumor samples; or
(2) Plasma samples that included cell-free tumor DNA.

No Motivation For (1) Subsequently Obtained Tumor Samples

Forsheew:

“Biopsies are invasive and costly and only provide a snapshot of mutations present at a given time and location.”

EX1030 (Forsheew) at 1
IPR2023-00317 POR at 78

Foresight's Founders:

“Analysis of ctDNA has the potential to revolutionize detection and monitoring of tumors. Noninvasive access to malignant DNA is particularly attractive for solid tumors, which cannot be repeatedly sampled without invasive procedures.”

No Motivation For (1) Subsequently Obtained Tumor Samples

Forsheew

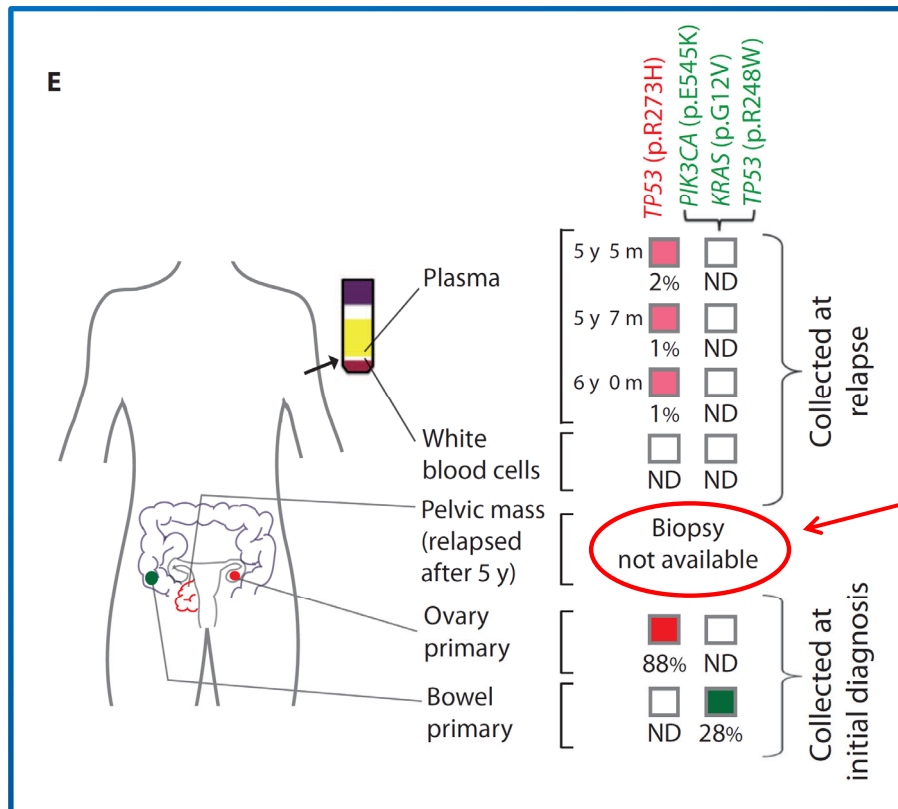


Fig. 4. (E) Retrospective analysis of samples from synchronous primary tumors (bowel and ovarian) collected at the time of initial surgery and three plasma samples collected at relapse. In primary tumors from this patient (not included in the other sets of samples analyzed), a *TP53* mutation was identified in the ovarian cancer (red box), and mutations in *PIK3CA*, *KRAS*, and *TP53* were identified in the bowel cancer (green box). At relapse, a biopsy was not performed on the pelvic mass.

No Motivation For (2) Cell-Free Tumor DNA

Forsheew:

“Circulating DNA is fragmented to an average length of 140 to 170 base pairs (bp) and is present in only a few thousand amplifiable copies per milliliter of blood, of which only a fraction may be diagnostically relevant.”

EX1030 (Forsheew) at 1; IPR2023-00317 POR

Foresight’s Founders in 2015:

“Tumors continually shed DNA into the blood where it can be detected as circulating tumor DNA (ctDNA). Although this phenomenon has been recognized for decades, techniques that are sensitive and specific enough to robustly detect ctDNA have only become available recently.”

The Scientist



2022 Top 10 Innovations

This year's crop of winning products features many with a clinical focus and others that represent significant advances in sequencing, single-cell analysis, and more.

We are happy to announce the new products that our panel of independent judges has chosen as this year's Top 10 Innovations.



THE SCIENTIST STAFF

NeXT Personal™ Personalis

TRACERx



NEW YORK – Cancer Research UK, University College London, and the Francis Crick Institute said Wednesday that they have adopted Personalis' NeXT Personal, a personalized residual disease detection platform, in their TRACERx study.

"Thanks to the TRACERx study, we've already seen the importance of ctDNA in predicting cancer recurrence in an initial cohort of patients," the study's principal investigator Charles Swanton, who is affiliated with all three of the institutions, said in a statement. "Personalis' highly sensitive [test] will allow us to achieve a rich understanding of the complex tumor ecosystem, including by tracking clinically relevant ctDNA variants at recurrence, and ultimately allowing us to make more informed decisions about patient care," he added.

AstraZeneca

DEVELOPMENTAL THERAPEUTICS—MOLECULARLY TARGETED AGENTS AND TUMOR BIOLOGY

MATRIX plasma-in-plasma contrived samples assessment and detection of MRD using the Personalis NeXT Personal assay.

Paul Labrousse; AstraZeneca, Waltham, MA

Conclusions: The MATRIX *plasma-in-plasma approach* is a robust option for assessing the sensitivity of MRD assays to low levels of ctDNA. This approach addresses all parts of an MRD assay for both the plasma and tumor/normal tissues, and is the closest thing to real clinical samples while allowing direct interrogation of sensitivity. Additionally, it allows assessment of any genomic or epigenomic biological feature. The Personalis NeXT Personal assay shows ultra-high sensitivity with reproducible data down to the 1-3 PPM range, and expansion of the personalized panel fixed clinical content allows tracking of more driver and resistance mutations with no impact to the MRD sensitivity. Research Sponsor: AstraZeneca.