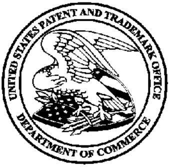

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NOTICE OF ALLOWANCE AND FEE(S) DUE

115823 7590 07/16/2020

Wilson Sonsini Goodrich & Rosati / Guardant Health
650 Page Mill Road
Palo Alto, CA 94304

EXAMINER	
HORLICK, KENNETH R	
ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 07/16/2020

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/593,633	10/04/2019	AmirAli TALASAZ	42534-704.314	1021

TITLE OF INVENTION: SYSTEMS AND METHODS TO DETECT RARE MUTATIONS AND COPY NUMBER VARIATION

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1000	$0.00	$1000.00	$0	10/16/2020

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

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III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

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Guardant - EXHIBIT 2010
Tempus AI, Inc. v. Guardant Health, Inc.
IPR2025-01435

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Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

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INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

115823 7590 07/16/2020

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I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)

(Signature)

(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/593,633	10/04/2019	AmirAli TALASAZ	42534-704.314	1021

TITLE OF INVENTION: SYSTEMS AND METHODS TO DETECT RARE MUTATIONS AND COPY NUMBER VARIATION

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	$1000	$0.00	$1000.00	$0	10/16/2020

EXAMINER	ART UNIT	CLASS-SUBCLASS
HORLICK, KENNETH R	1637	506-004000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1______________________

2______________________

3______________________

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ❑ Individual ❑ Corporation or other private group entity ❑ Government

4a. Fees submitted: ❑Issue Fee ❑Publication Fee (if required) ❑Advance Order - # of Copies ______________

4b. Method of Payment: *(Please first reapply any previously paid fee shown above)*

❑ Electronic Payment via EFS-Web ❑ Enclosed check ❑ Non-electronic payment by credit card (Attach form PTO-2038)

❑ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. __________

5. **Change in Entity Status** (from status indicated above)

❑ Applicant certifying micro entity status. See 37 CFR 1.29

❑ Applicant asserting small entity status. See 37 CFR 1.27

❑ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.
NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.
NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature ______________________ Date ______________________

Typed or printed name ______________________ Registration No. ______________________

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16/593,633	10/04/2019	AmirAli TALASAZ	42534-704.314	1021

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EXAMINER	
HORLICK, KENNETH R	
ART UNIT	PAPER NUMBER
1637	

DATE MAILED: 07/16/2020

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)

(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Notice of Allowability	**Application No.** 16/593,633	**Applicant(s)** TALASAZ, AmirAli	
	Examiner KENNETH R HORLICK	**Art Unit** 1637	**AIA (FITF) Status** Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1.☑ This communication is responsive to the resonse filed 06/15/20.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ______.

2.☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ______; the restriction requirement and election have been incorporated into this action.

3.☑ The allowed claim(s) is/are 31-60 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see **http://www.uspto.gov/patents/init_events/pph/index.jsp** or send an inquiry to **PPHfeedback@uspto.gov.**

4.☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a) ☐All b) ☐ Some *c) ☐ None of the:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. ______ .
3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: ______ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5.☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date ______ .

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6.☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1.☐ Notice of References Cited (PTO-892)
2.☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date ______.
3.☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material ______.
4.☐ Interview Summary (PTO-413), Paper No./Mail Date. ______.
5. ☑ Examiner's Amendment/Comment
6. ☐ Examiner's Statement of Reasons for Allowance
7. ☐ Other ______.

/KENNETH R HORLICK/
Primary Examiner, Art Unit 1637

EXAMINER'S COMMENTS

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

2. The declaration under 37 CFR 1.132 filed 06/15/20 is sufficient to overcome the rejection of claims 31-60 based upon Salk et al. (U.S. 10,604,804) applied under 35 U.S.C. 102 or 103.

3. Any inquiry concerning this communication or earlier communications from the examiner should be directed to KENNETH R HORLICK whose telephone number is (571)272-0784. The examiner can normally be reached on Mon. - Thurs. 8:30 - 6:30.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see https://ppair-my.uspto.gov/pair/PrivatePair. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

07/09/20

/KENNETH R HORLICK/
Primary Examiner, Art Unit 1637

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings in the above-referenced patent application.

Listing of Claims:

1.-30. (Cancelled).

31. (Currently amended) A method for detecting a presence or an absence of one or more somatic genetic variants, the method comprising:

(a) non-uniquely tagging a plurality of cell-free deoxyribonucleic acid (cfDNA) molecules obtained from a human subject with adapters comprising molecular barcodes ~~from a plurality of cfDNA molecules obtained from a human subject~~ to produce non-uniquely tagged parent polynucleotides, wherein the adapters are ligated to both ends of molecules of cfDNA from the plurality of cfDNA molecules;

(b) amplifying a plurality of the non-uniquely tagged parent polynucleotides to produce progeny polynucleotides with associated molecular barcodes;

(c) sequencing a plurality of the progeny polynucleotides to produce sequencing reads of the progeny polynucleotides with associated molecular barcodes; and

(d) detecting, from among a plurality of the sequencing reads, **[[a]]** the presence or the absence of the one or more somatic genetic variants, based at least in part on the associated molecular barcodes of the sequencing reads.

32. (Previously presented): The method of claim 31, wherein the human subject has a cancer or is suspected of having a cancer.

33. (Previously presented): The method of claim 31, wherein the non-uniquely tagging comprises blunt-end ligation or sticky-end ligation.

34. (Previously presented): The method of claim 31, wherein the molecular barcodes are from a set of molecular barcodes having 5 to 1,000 different nucleotide sequences that are 5 to 20 nucleotides in length.

35. (Previously presented) The method of claim 31, further comprising selectively

enriching the progeny polynucleotides for target regions associated with cancer.

36. (Previously presented) The method of claim 31, further comprising filtering out one or more of the sequencing reads that fail to meet a quality control threshold.

37. (Currently amended) The method of claim 31, further comprising mapping~~aligning~~ a plurality of the sequencing reads to a human reference sequence to produce aligned sequencing reads.

38. (Currently amended) The method of claim 37, further comprising grouping a plurality of the ~~aligned~~ mapped sequencing reads into families based on sequence information of the molecular barcodes, a start base position of a given ~~aligned~~ mapped sequencing read from among the ~~aligned~~mapped sequencing reads at which the given ~~aligned~~ mapped sequencing read is determined to start ~~aligning~~ mapping to the human reference sequence, and a stop base position of the given ~~aligned~~ mapped sequencing read at which the given ~~aligned~~ mapped sequencing read is determined to stop ~~aligning~~ mapping to the human reference sequence.

39. (Previously presented) The method of claim 38, further comprising generating a set of consensus sequences from among the sequencing reads in the families.

40. (Previously presented) The method of claim 39, further comprising determining the presence or absence of the one or more somatic genetic variants in the set of consensus sequences as compared to the human reference sequence.

41. (Previously presented) The method of claim 40, further comprising quantifying a number of consensus sequences from the set of consensus sequences that comprise one or more somatic genetic variants as compared to the human reference sequence.

42. (Previously presented) The method of claim 38, wherein the detecting comprises generating a base call at a genetic locus of the human reference sequence for a family from among the families.

43. (Previously presented) The method of claim 38, further comprising quantifying a number of the families.

44. (Previously presented) The method of claim 31, wherein the one or more somatic genetic variants comprise a single nucleotide variant (SNV), a copy number variation (CNV), an

insertion or deletion (indel), a gene fusion, or any combination thereof.

45. (Previously presented) The method of claim 31, wherein the detecting further comprises detecting cancer in the human subject when the presence of the one or more somatic genetic variants is detected.

46. (Previously presented) The method of claim 31, further comprising generating a tumor mutation profile of the human subject based on the detected presence or absence of the one or more somatic genetic variants.

47. (Previously presented) The method of claim 46, wherein the tumor mutation profile is generated based on the presence or absence of one or more somatic genetic variants detected from different samples obtained from the human subject at different time points.

48. (Previously presented) The method of claim 31, further comprising performing (a)- (d) in combination with immune repertoire profiling.

49. (Previously presented) The method of claim 31, wherein the one or more somatic genetic variants are located in a microsatellite region.

50. (Previously presented) The method of claim 49, wherein the one or more somatic genetic variants comprise an indel.

51. (Currently amended) A method for detecting a presence or an absence of one or more somatic genetic variants, the method comprising:

   (a) non-uniquely tagging a plurality of cell-free deoxyribonucleic acid ~~molecules~~ (cfDNA) molecules from a human subject with molecular barcodes ~~from a plurality of cfDNA molecules obtained from a human subject~~ to produce non-uniquely tagged parent polynucleotides, wherein the molecular barcodes are ligated to both ends of molecules of cfDNA from the plurality of cfDNA molecules;

   (b) amplifying a plurality of the non-uniquely tagged parent polynucleotides to produce progeny polynucleotides with associated molecular barcodes;

   (c) selectively enriching the progeny polynucleotides for target regions associated with

cancer, whereby enriched progeny polynucleotides are generated;

(d) sequencing a plurality of the enriched progeny polynucleotides to produce sequencing reads of the enriched progeny polynucleotides with associated molecular barcodes;

(e) ~~aligning~~ mapping a plurality of the sequencing reads to a human reference sequence to produce ~~aligned~~ mapped sequencing reads;

(f) grouping a plurality of the aligned sequencing reads into families based on sequence information of the molecular barcodes, a start base position of a given mapped~~aligned~~ sequencing read from among the ~~aligned~~ mapped sequencing reads at which the given ~~aligned~~ mapped sequencing read is determined to start ~~aligning~~ mapping to the human reference sequence, and a stop base position of the given ~~aligned~~ mapped sequencing read at which the given ~~aligned~~ mapped sequencing read is determined to stop ~~aligning~~ mapping to the human reference sequence; and

(g) detecting, from among a plurality of the families, the presence or the absence of the one or more somatic genetic variants comprising a single nucleotide variant (SNV), a copy number variation (CNV), an insertion or deletion (indel), a gene fusion, or any combination thereof.

52. (Previously presented) The method of claim 51, wherein the plurality of cfDNA molecules comprises between 1 nanogram (ng) and 100 ng of cfDNA molecules.

53. (Previously presented) The method of claim 51, wherein the molecular barcodes are from a set of molecular barcodes having 5 to 1,000 different nucleotide sequences that are 5 to 20 nucleotides in length.

54. (Previously presented) The method of claim 51, wherein the molecular barcodes are part of adapter sequences.

55. (Currently amended) The method of claim 51, further comprising filtering out one or more of the sequencing reads or the ~~aligned~~ mapped sequencing reads that fail to meet a set accuracy, quality score, or mapping score threshold.

56. (Currently amended) The method of claim 51, further comprising generating a set of

consensus sequences from among the ~~aligned~~ mapped sequencing reads in the families.

57. (Previously presented) The method of claim 56, further comprising calculating a number of consensus sequences from the set of consensus sequences that comprise one or more somatic genetic variants as compared to the human reference sequence.

58. (Previously presented) The method of claim 51, further comprising quantifying a number of the families.

59. (Previously presented) The method of claim 51, wherein 0.1% to 1.0% of the cfDNA molecules in the plurality of cfDNA molecules is from one or more cancer genomes.

60. (Previously presented) The method of claim 51, wherein the detecting further comprises detecting cancer in the human subject when the presence of the one or more somatic genetic variants is detected.

SUMMARY OF THE INTERVIEW

Applicant is appreciative of Examiner Horlick for extending the courtesy of a telephonic interview to Applicant's representative(s) Timothy Hott, on May 13, 2020, and to Timothy Hott and Jacqueline Stroncek on June 11, 2020. During the interview, amendments and arguments consistent with those detailed herein were discussed.

REMARKS

Claims 31-60 were pending prior to the entry of these amendments. Claims 31, 37, 38, 51, 55, and 56 have been amended. No new matter is added by these amendments. Accordingly, upon entry of these amendments, claims 31-60 will be pending.

Reconsideration and allowance are respectfully requested in light of the abovementioned amendments and the following remarks.

Claim amendments

Claims 31, 37, 38, 51, 55, and 56 have been amended for clarity and antecedent basis purposes. These amendments are fully supported by the application as originally filed (U.S. Application No. 16/593,633, now U.S. Patent Publication No. 20200087736A1, referred to herein as "Application") at, for example, paragraphs [0221]-[0223], and throughout the specification.

35 U.S.C. § 102

Claims 31-33, 35-51, 54-58, and 60 stand rejected under 35 U.S.C. § 102(a)(2) as being anticipated by Salk et al. (US 10,604,804, hereinafter referred to as "Salk"). Applicant respectfully submits that claims 31 and 51 are novel over Salk because Salk does not meet all of the elements of the instant claims. For example, Salk does not disclose a tagging method for cfDNA molecules, much less "non-uniquely tagging a plurality of cell-free deoxyribonucleic acid molecules (cfDNA) molecules from a human subject with molecular barcodes to produce non-uniquely tagged parent polynucleotides," as recited in claims 31 and 51. Nor does Salk disclose "(e) mapping a plurality of the sequencing reads to a human reference sequence to produce mapped sequencing reads; (f) grouping a plurality of the mapped sequencing reads into families" as recited in claim 51.

1. Salk does not disclose tagging cell-free DNA molecules as recited in claims 31 and 51.

As explained in the Declaration of Jay Shendure, M.D., PhD., submitted concurrently herewith (the "Declaration," attached hereto as Appendix), a person of ordinary skill in the art would not view Salk as teaching, or even contemplating, application of its methods to cfDNA. (Declaration, ¶ 12) The "Duplex Consensus Sequencing" or "DCS" method disclosed by Salk is described exclusively as applicable to cellular DNA samples. (Declaration, ¶ 13) Cellular DNA samples are discussed throughout Salk and provide the only examples of application of DCS. *Id.* Salk does not even mention applying DCS to cfDNA, let alone suggest that DCS might be applicable to such samples. *Id.*

To the extent that other types of nucleic acids are even mentioned in Salk, a person of ordinary skill in the art would **not** understand these portions of Salk to be contemplating application of DCS to the detection of rare variants in cfDNA. (Declaration, ¶ 15) Observing that others have applied fundamentally different methods to fetal aneuploidy (Salk, 1:45-46) is not disclosure of application of the DCS method to cfDNA. *Id.* Further, single molecule counting of circulating microRNAs (i.e., Salk's only disclosed circulating nucleic acid) is not disclosure of applying the DCS method to cfDNA at least because (1) circulating microRNA is not cfDNA, and (2) single molecule counting is not the DCS method. *Id.*

2. Salk does not disclose "non-uniquely tagging" as recited in claims 31 and 51.

As explained in the Declaration, Salk would not be viewed by a person of ordinary skill in the art as disclosing or teaching "non-uniquely tagging" as that term is used in the Application. (Declaration, ¶ 29) Paragraph 252 of the instant specification provides a definition of "non-uniquely tagged."

> A set of polynucleotides in the composition that map to a mappable base position in a genome can be non-uniquely tagged, that is, the number of different identifiers can be at least [] 2 and fewer than the number of polynucleotides that map to the mappable base position.

(Declaration, ¶ 30)

The instant claims are directed to a tagging method that uses at least 2 and fewer than the number of polynucleotides that map to the given mappable base position—far fewer tags than the

total number of polynucleotides in the sample and is in marked contrast to the tagging methods described by Salk. (Declaration, ¶ 34) Salk does not use the term "non-uniquely tagged" in the specification, nor does Salk contemplate non-unique tagging as claimed. *Id.* In contrast to the claimed method, the DCS method of Salk critically relies on **flooding** a sample **with an excess number of tags**, such that every DNA fragment is labeled with distinct or "unique" tags. (Declaration, ¶ 35 (referencing Salk)) Furthermore, Salk exclusively describes methods that use ***more*** tags than polynucleotide fragments in the sample. Salk refers to its disclosed methods as providing ***"unique"*** tagging. (Declaration, ¶ 36 (referencing Salk))

Salk **never** instructs that the number of tags — which Salk refers to as "Single Molecule Identifiers" or "SMIs" —should be ***less*** than the number of polynucleotides in a sample, let alone fewer than the number of polynucleotides in a set of the composition that map to a given mappable base position. (Declaration, ¶ 37 (referencing Salk)) Where Salk does relate the number of barcodes to polynucleotides in a sample, it instructs that the barcodes should be in ***excess of*** the sample polynucleotides. *Id.*; *see also* Salk 3:55-61 (describing Salk's DCS method generally); 6:67-7:4. With respect to the "hybrid method" of Salk, this is expressly characterized as providing "unique molecular identifiers" – rather than non-unique barcodes. (Salk, 9:31-35). Absent information about the particular sample size, how many nucleic acid fragments are tagged, and express disclosure of using far fewer tags (disclosure that is absent from Salk) one could not reasonably conclude Salk is contemplating anything other than what is expressly stated throughout the Salk reference—i.e., flooding a sample with an excess number of tags or "unique" tagging. *See e.g.*, Salk 3:55-61; 6:67-7:4. Finally, Salk's "hybrid method" uses sheared ends of polynucleotide molecules. Salk, 9:32. As explained in the Shendure declaration and below, shearing is not compatible with cfDNA. (Declaration, ¶¶ 17-18)

3. Salk does not disclose "mapping" and "grouping" as recited in claim 51.

Additionally, Salk does not disclose "mapping a plurality of the sequencing reads to a human reference sequence to produce aligned sequencing reads" and "grouping a plurality of the mapped sequencing reads" as recited in claim 51.

Accordingly, Applicant respectfully requests that the § 102 rejection of independent claims 31 and 51 be withdrawn.

Claims 32, 33, 35-50, 54-58, and 60 depend from an include all elements of claims 31 and 51 and recite additional elements of particular advantage and utility. Salk does not meet all the elements of claims 31 and 51, much less the unique combination of claims 32, 33, 35-50, 54-58, and 60. Accordingly, Applicant respectfully requests that the § 102 rejection of claims 32, 33, 35-50, 54-58, and 60 also be withdrawn.

Finally, Salk was filed as a continuation application (U.S. Application No. 16/411,045) on May 13, 2019, claiming priority to USSN 15/660,785 (the '758 application). The '785 application claims priority to USSN 14/386,800, which is a U.S. national stage application of International Application No. PCT/US2013/032665, filed Mar. 15, 2013 claiming priority to U.S. Provisional Patent Application No. 61/613,413, filed Mar. 20, 2012; U.S. Provisional Patent Application No. 61/625,623, filed Apr. 17, 2012; and U.S. Provisional Patent Application No. 61/625,319, filed Apr. 17, 2012.

In articulating its rejection, the Office refers to certain claims of Salk. ("Salk et al. discloses methods comprising these steps as well as the steps in the dependent claims; see claims 1-2, 7-11, and 16-28. The claimed methods cannot be distinguished from the patent methods of Salk et al." Office action, p. 3) To the extent that the claims of Salk are referenced by the Office with respect to the instant claims, Applicant submits that at least "(b) attaching tags comprising barcodes . . . to generate non-uniquely tagged parent polynucleotides, wherein each non-uniquely tagged parent polynucleotide is substantially unique with respect to other non-uniquely tagged parent polynucleotides in the bodily sample," amongst other elements of Salk's claims, do not find support in the disclosure of its priority documents and therefore are not prior art under § 102. (*See* MPEP 2136.06 "For prior art purposes, a U.S. patent or patent application publication that claims the benefit of an earlier filing date under 35 U.S.C. 120 of a prior nonprovisional application (i.e., a continuation, divisional, or continuation-in-part application) would be accorded the earlier filing date as its prior art date under pre-AIA 35 U.S.C. 102(e), *provided the earlier-filed application properly supports the subject matter relied upon in any rejection in*

compliance with 35 U.S.C. 112(a) or pre-AIA 35 U.S.C. 112, first paragraph." MPEP 211 "The disclosure of a continuation application must be the same as the disclosure of the prior-filed application; i.e., the continuation must not include anything which would constitute new matter if inserted in the original application.")

35 U.S.C. § 103

Claims 34, 52, 53, and 59 stand rejected under 35 U.S.C. § 103 as being unpatentable over Salk et al.

Initially, Applicant submits that claims 31 and 51 are not obvious over Salk because Salk does not teach, suggest, or disclose all elements of claims 31 and 51 (as stated above). Claims 34, 52, 53, and 59 depend from and include all elements of claims 31 and 51 and recite additional elements of particular advantage and utility.

Second, the teachings and disclosure of Salk provide no reasonable expectation of success for at least "**non-uniquely tagging** a plurality of **cell-free deoxyribonucleic acid (cfDNA) molecules**" to "detect[], from among a plurality of the sequencing reads, the presence or the absence of the one or more somatic genetic variants" as recited in claims 31 and 51, let alone dependents thereof.

As provided in Dr. Shendure's declaration, a person of ordinary skill in the art would not view Salk as teaching, or even contemplating, application of its methods to cfDNA. (Declaration, ¶ 12) In fact, there are numerous aspects of Salk's method that would be recognized as incompatible with cfDNA analysis. (Declaration, ¶ 12 and ¶ 16) This includes Salk's random fragmentation process, sample processing that instructs discarding fragments of the size expected in cfDNA, and Salk's requirement of excessive amounts of DNA not available in cfDNA samples. (Declaration, ¶ 12)

(a) Salk requires excessive amounts of DNA for cfDNA sample analysis.

The "Duplex Consensus Sequencing" or "DCS" method disclosed by Salk is described exclusively as applicable to **cellular** DNA samples. (Declaration, ¶ 13) Cellular DNA samples are discussed throughout Salk and provide the only examples of application of DCS. *Id.* Salk describes a method designed for cellular DNA samples which includes numerous aspects that are

fundamentally incompatible with cfDNA samples. (Declaration, ¶ 14) Salk describes methods which require large amounts of cellular DNA. (Declaration, ¶ 21) For example, Salk discloses using 3 µg of human DNA. (*Id.,* referencing Salk, 20:50-51). While cellular DNA might be available in such large quantities, cfDNA is not. (Declaration, ¶ 22) For instance, 1 mL of human serum typically contains only about 10 ng of cfDNA. *Id.* That is, the amount of cfDNA in 1 mL is over 2-orders of magnitude less than what Salk discloses is used for DCS. *Id.* It is simply not feasible to routinely obtain cfDNA in microgram quantities from patients. *Id.*

(b) The DCS method disclosed in Salk cannot be adapted for cfDNA without fundamental modification.

As provided in the Declaration of Dr. Shendure, adapting Salk to detect somatic genetic variants in cfDNA is not just a matter applying the technique to a more dilute DNA sample but would require fundamental modification and redesign of the DCS method. (Declaration, ¶ 23)

By way of example, the enzymatic steps involved in preparation of samples for DCS analysis (e.g., end repair, T-tailing, ligation, Salk, 20:50-21:8)) work less efficiently with dilute samples. *Id.*

Other aspects of Salk's method are not compatible with cfDNA analysis. For example, Salk's method also relies on acoustic shearing of cellular DNA. (Declaration, ¶ 17) Acoustic shearing is not compatible with cfDNA. (Declaration, ¶ 18) Cell-free DNA is already fragmented and has a peak size of around 160 nucleotides. *Id.* Further reduction in size, such as by shearing, would result in significant destruction of sample DNA that is already present in only minute quantities. *Id.* Moreover, sheared cfDNA would be even more difficult to isolate and sequence due the reduction in fragment length. *Id.*

(c) Salk characterizes polynucleotide fragments that are of the length of cfDNA as sub-optimal.

As provided in the Declaration of Dr. Shendure, Salk characterizes polynucleotide fragments of the size that would be found in cfDNA samples as being sub-optimal for its method

and instructs that such fragments are discarded during sample preparation. (Declaration, ¶ 19) For example, Salk instructs selection of fragments in the optimal range of 200-500 base pairs. *Id.* The vast majority of cfDNA fragments, however, are about 140 to 170 base pairs—which is outside of Salk's optimal size range. (Declaration, ¶ 20) Salk, in fact, instructs that fragments of less than 200 base pairs (e.g., fragments about 140 to 170 base pairs) should be discarded in the sample preparation steps of the DCS method. *Id.* Discarding fragments outside the optimal range of about 200-500 base pairs is not compatible with cfDNA analysis as it would result in discarding the vast majority of informative cfDNA fragments. *Id.*

(d) Salk discloses the use of SMIs, resulting in data loss, which is incompatible with methods for analyzing cfDNA samples.

As provided in the Declaration of Dr. Shendure, the Salk DCS method also loses significant amounts of data through its reliance on random (i.e., degenerate) SMI tags. (Declaration, ¶ 24) Data loss, for all practical purposes, is no different than loss of physical sample and is incompatible with cfDNA samples. *Id.* According to Salk, approximately 1% of bases are incorrectly identified by NGS. (Declaration, ¶ 25) These errors are incorporated throughout the polynucleotide fragment, including in the SMI tag. *Id.* Taking Salk's 12-base SMI tags as an example, where every fragment includes two tags (one at each end), even a relatively low error rate of 1% will result in an error in about every fourth sequence read. *Id.* Base misincorporation errors in the SMI tag cannot be easily corrected. (Declaration, ¶ 26) Rather, an error in the SMI tags would substantially limit the grouping of the polynucleotide into the appropriate family as required by Salk, particularly so when there are large numbers of families. *Id.* As a result, polynucleotides with sequence errors in the SMI tag are unlikely to be usable for error correction and must be discarded. *Id.*

Unlike the disclosure of Salk, which requires (1) excessive amounts of DNA, (2) a protocol that cannot be adapted to cfDNA without fundamental modification (including which molecules to keep for analysis), and (3) the use of tags that potentially introduce data loss, an embodiment of the claims, "Digital Sequencing Technology workflow enables the vast majority of starting molecules to be converted and sequenced." (Declaration, ¶ 27, Application [0377])

"This is critically important for detection of rare variants as there may only be a handful of somatically mutated molecules in an entire 10mL tube of blood." *Id.* This advantage is not contemplated by the disclosure of Salk. As such, there would be no reasonable expectation of using the disclosure of Salk to arrive at the instant claims.

(e) *The inapplicability of Salk to cfDNA was recognized in the art.*

The inapplicability of the Salk method to cfDNA samples has been recognized and documented in the literature. For example, in 2017 (nearly five years after the earliest claimed filing date of Salk), Perakis et al. noted that the method had yet to be applied to cfDNA samples. Perakis further stated that "[p]rospects of success are limited since the method is relatively inefficient when limited amounts of input DNA—as it is most likely the case for cfDNA—are used." Perakis et al., "Advances in Circulating Tumor DNA Analysis," Adv. Clin Chem (2017) p. 30 (IDS submitted October 22, 2019).

Applicant respectfully submits that at least for the foregoing reasons, claims 31 and 51 are not obvious over the cited references, and neither are the claims depending therefrom. Withdrawal of the rejections is respectfully requested.

***

It shall be understood herein that any instance in which Applicant has addressed certain comments set forth by the Office shall not be construed as a concession to other comments or arguments advanced by the Office. Any circumstance in which Applicant has amended or canceled a claim also does not mean that Applicant concedes to the arguments or positions advanced by the Office with respect to that claim or other claims pending herein.

CONCLUSION

In view of the foregoing, Applicant believes that all claims now pending in this Application are in condition for allowance. Should the Examiner have any questions, the Examiner is encouraged to contact the undersigned at 650-849-3293.

The Commissioner is hereby authorized to charge any additional fees or credit any overpayment in connection with this paper to Deposit Account No. 23-2415, Attorney Docket No. 42534-704.314.

Respectfully submitted,

Dated: June 15, 2020

By: /Jacqueline Stroncek/
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Confirmation No.: 1021
Inventor: AmirAli TALASAZ	Examiner: Kenneth R HORLICK
Application No.: 16/593,633	Group Art Unit: 1637
Filed: 10/04/2019	Customer No. 115823
Title: SYSTEMS AND METHODS TO DETECT RARE MUTATIONS AND COPY NUMBER VARIATION	

DECLARATION OF JAY SHENDURE, M.D., PH.D. UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

I, Jay Shendure, hereby declare as follows:

I. Qualifications

1. I am currently the Director of the Brotman-Baty Institute for Precision Medicine, as well as the Director of the Allen Discovery Center for Cell Lineage. I am an Investigator for the Howard Hughes Medical Institute, a tenured Professor at the University of Washington (Department of Genome Sciences), and an Affiliate Professor at the Fred Hutchinson Cancer Research Center (Division of Human Biology).

2. I graduated *summa cum laude* with a Bachelor of Arts in Molecular Biology from Princeton University in 1996. In 2005, I earned a Ph.D. in Genetics from Harvard University. Then, in 2007, I earned my M.D. from the Harvard Medical School.

3. I am the founder of Bellwether Bio, Inc. ("Bellwether"). Bellwether researched and developed next generation cancer diagnostics techniques using cell-free

DNA. Bellwether was acquired by Guardant Health on April 8, 2019. I continue to serve as a Scientific Consultant to Guardant Health pursuant to that acquisition.

4. I also serve on the Scientific Advisory Boards or as a consultant for numerous other corporations, including Nanostring (Scientific Advisory Board); Maze Therapeutics (Scientific Advisory Board); Camp4 Therapeutics (Scientific Advisory Board); Phase Genomics (Founder; Scientific Advisory Board); GenePeeks (Scientific Advisory Board); Adaptive Biotechnologies (Scientific Advisory Board); and Stratos Genomics (Scientific Advisory Board).

5. I am also a member of the Editorial Boards of numerous publications in the field, including Science, Genome Medicine, Molecular Case Studies, Genetics, Human Molecular Genetics, Human Genetics, Genome Biology, and Genome Research.

6. I have authored or co-authored numerous articles and publications, including: Diversity of Human Copy Number Variation and Multicopy Genes, 300 (6004) SCIENCE 641 (2010); Copy Number Variation and False-positives in Prenatal Aneuploidy Screening, 372 (17) NEW ENGLAND J. MED. 1639 (2015); Cell-free DNA Comprises an In Vivo Nucleosome Footprint that Informs Its Tissues-Of-Origin, 164 CELL 57 (2016); Fragment Length of Circulating Tumor DNA, 12(7) PLOS GENETICS (2016); Noninvasive Whole-Genome Sequencing of a Human Fetus SCIENCE TRANSLATIONAL MEDICINE Jun 6:4(137) (2012).

7. I am a named inventor on numerous patents and patent publications, including: U.S. Patent No. 7,425,431, "*Polony Fluorescent in situ Sequencing Beads*"; U.S. Patent No. 8,865,410, "*Error Detection in Sequence Tag Directed Subassemblies of Short Sequencing Reads*"; U.S. Patent No. 9,809,904, "*Methods for Retrieval of Sequence-Verified DNA Constructs*"; PCT/US2015/042310, "*Methods of Determining Tissues and/or Cell Types Giving Rise to Cell-Free DNA, and Methods of Identifying a Disease or Disorder Using Same*"; U.S. Publication No. 2016/0357903, "*A Framework for Determining the Relative Effects of Genetic Mutations*"; U.S. Publication No. 2015/0105267, "*Whole Genome Sequencing of a Human Fetus*"; U.S. Publication No. 2008/0269068, "*Multiplex Decoding of Sequence Tags in Barcodes.*"

8. I have been involved in numerous research projects, both completed and ongoing, with numerous organizations. Examples of these research projects include:

Massively Parallel Genome Sequencing of Antibiotic-Resistant Emerging Pathogens (NIH/NIAID, 2009-012); *Ultrasensitive Identification and Precise Quantitation of Low Frequency Somatic Mutations by Molecular Counting* (NCI/NIH, 2011-2014).

9. For my work in the field, I have been honored with various awards. These awards include: Richard and Carol Hertzberg Prize for Technology Innovation from the University of California, San Diego (2018); Cell "40 under 40" (2014); NIH Director's Pioneer Award (2013); Curt Stern Award from the American Society of Human Genetics (2012); James Tolbert Shipley Prize from the Harvard Medical School (2007).

10. My professional *curriculum vitae* is attached.

II. Scope of Work

11. I have been asked to review Salk (U.S. Pat. No. 10,604,804) for disclosure relating to, and the applicability of, the methods described therein to cfDNA samples and for disclosure relating to non-uniquely tagging polynucleotides. My billing rate is $500 per hour. My compensation is not contingent on the outcome of this matter or the specifics of my testimony.

III. Salk does not Disclose Cell-Free DNA

12. Having reviewed Salk in detail, it is my opinion that a person of ordinary skill in the art would not view Salk as teaching, or even contemplating, application of its methods to cfDNA. In fact, there are numerous aspects of Salk's method that would be recognized as incompatible with cfDNA analysis. This includes Salk's random fragmentation process, sample processing that instructs discarding fragments of the size expected in cfDNA, and Salk's requirement of excessive amounts of DNA not available in cfDNA samples. Further explanation of my analysis is set forth in detail below.

13. The "Duplex Consensus Sequencing" or "DCS" method disclosed by Salk is described exclusively as applicable to cellular DNA samples. Cellular DNA samples are discussed throughout Salk and provide the only examples of application of DCS. Example 1 applies the method to cellular DNA isolated from "normal human colonic mucosa." Salk, 20:13-14. Example 2 applies the method to "human mitochondrial DNA" isolated from cells. *Id.*, 25:23-24. Example 3 applies the method to DNA isolated from yeast cells. *Id.*, 26:56-57. Example 4 applies the method to plasmid DNA isolated from bacterial cells. *Id.*, 28:10-15.

Salk does not even mention applying DCS to cfDNA, let alone suggest that DCS might be applicable to such samples.

14. The background section of Salk (1:41-48) observes that other methods employing "deep sequencing" may be useful for analysis of certain biological specimens. Salk also mentions that "single-molecule counting" can be applied to "quantitation of circulating microRNAs." (18:63-19:8) However, I have reviewed the Salk reference in detail and am unable to find disclosure regarding application of Salk's DCS method to cfDNA.

15. A person of ordinary skill in the art would not understand these portions of Salk to be contemplating application of DCS to the detection of rare variants in cfDNA. Observing that others have applied fundamentally different methods to fetal aneuploidy (1:45-46) is not disclosure of application of the DCS method to cfDNA. Further, single molecule counting of circulating microRNAs (*i.e.*, Salk's only disclosed circulating nucleic acid) is not disclosure of applying the DCS method to cfDNA at least because (1) circulating microRNA is not cfDNA, and (2) single molecule counting is not the DCS method.

16. Besides containing no disclosure regarding application of the DCS method to cfDNA, the method of Salk includes numerous aspects that are fundamentally incompatible with cfDNA samples.

17. For example, Salk's method relies on acoustic shearing of cellular DNA. Salk, 3:52-55 ("FIG. 1 illustrates an overview of Duplex Consensus Sequencing. Sheared double-stranded DNA that has been end-repaired and T-tailed is combined with A-tailed SMI adaptors and ligated according to one embodiment."), 4:33-35 ("...randomly sheared fragments containing a particular genomic site are identified..."), 20:51-53 ("DNA was ... sheared on the Covaris AFA system").

18. Such acoustic shearing, however, is not compatible with cfDNA. Cell-free DNA is already fragmented and has a peak size of around 160 nucleotides. Further reduction in size, such as by shearing, would result in significant destruction of sample DNA that is already present in only minute quantities. Moreover, sheared cfDNA would be even more difficult to isolate and sequence due the reduction in fragment length.

19. Additionally, Salk characterizes polynucleotide fragments of the size that would be found in cfDNA samples as being sub-optimal for its method and instructs that

such fragments are discarded during sample preparation. For example, Salk instructs selection of fragments in the optimal range of 200-500 base pairs. Salk, 19:65-66 (identifying "the optimal range of ~200-500 bp" for its DCS method), 22:43-46.

20. The vast majority of cfDNA fragments, however, are about 140 to 170 base pairs—which is outside of Salk's optimal size range. Salk, in fact, instructs that fragments of less than 200 base pairs (*e.g.*, fragments about 140 to 170 base pairs) should be discarded in the sample preparation steps of the DCS method. Salk, 20:58-64 ("DNA fragments larger than the optimal range of ~200-500 bp were removed…."), ("this step allows fragments of approximately 200 bp or greater to bind to the beads."), 23:36-40 (describing "size-selection for fragments in the range of ~200-500 bp by size-selective binding to Ampure XP beads."). Discarding fragments outside the optimal range of about 200-500 base pairs is not compatible with cfDNA analysis as it would result in discarding the vast majority of informative cfDNA fragments.

21. Salk describes methods with high levels of sample and data loss and, as such, require large amounts of cellular DNA that are simply not available when working with cfDNA. For example, Salk discloses using 3 μg of human DNA. Salk, 20:50-51.

22. While cellular DNA might be available in such large quantities, cfDNA is not. For instance, 1 mL of human serum typically contains only about 10 ng of cfDNA. That is, the amount of cfDNA in 1 mL is over 2-orders of magnitude less than what Salk discloses is used for DCS. It is simply not feasible to routinely obtain cfDNA in microgram quantities from patients.

23. Adapting Salk to detect somatic genetic variants in cfDNA is not just a matter applying the technique to a more dilute DNA sample but would require fundamental modification and redesign of the DCS method. By way of example, the enzymatic steps involved in preparation of samples for DCS analysis (*e.g.*, end repair, T-tailing, ligation, Salk, 20:50-21:8)) work less efficiently with dilute samples.

24. The Salk DCS method also loses significant amounts of data through its reliance on random (*i.e.*, degenerate) SMI tags. Data loss, for all practical purposes, is no different than loss of physical sample and is incompatible with cfDNA samples.

25. According to Salk, approximately 1% of bases are incorrectly identified by NGS. Salk, 1:67-2:9 ("Polymerase mistakes during pre-amplification generate point mutations resulting from base mis-incorporations and rearrangements due to template switching [26, 27], cluster amplification, cycle sequencing and image analysis, approximately 1% of bases are incorrectly identified, depending on the specific platform and sequence context [2, 28]."). These errors are incorporated throughout the polynucleotide fragment, including in the SMI tag. Taking Salk's 12-base SMI tags as an example, where every fragment includes two tags (one at each end), even a relatively low error rate of 1% will result in an error in about every fourth sequence read.

26. Base misincorporation errors in the SMI tag cannot be easily corrected. Rather, an error in the SMI tags would substantially limit the grouping of the polynucleotide into the appropriate family as required by Salk, particularly so when there are large numbers of families. *E.g.*, Salk, Fig. 3, 4:12-37. As a result, polynucleotides with sequence errors in the SMI tag are unlikely to be usable for error correction and must be discarded.

27. As the instant application explains in paragraph 377, loss of sample is not trivial when the purpose of the method is the identification of rare somatic variants in cfDNA.

> Unlike conventional sequencing library preparation protocols, whereby the majority of extracted circulating DNA fragments are lost due to inefficient library conversion, our Digital Sequencing Technology workflow enables the vast majority of starting molecules to be converted and sequenced. This is critically important for detection of rare variants as there may only be a handful of somatically mutated molecules in an entire 10mL tube of blood. The efficient molecular biology conversion process developed enables the highest possible sensitivity for detection of rare variants.

28. Accordingly, a person of ordinary skill in the art would not understand Salk to teach a method for detecting somatic genetic variants in cell free DNA ("cfDNA").

IV. Salk does not Disclose Non-Uniquely Tagging Parent Polynucleotides

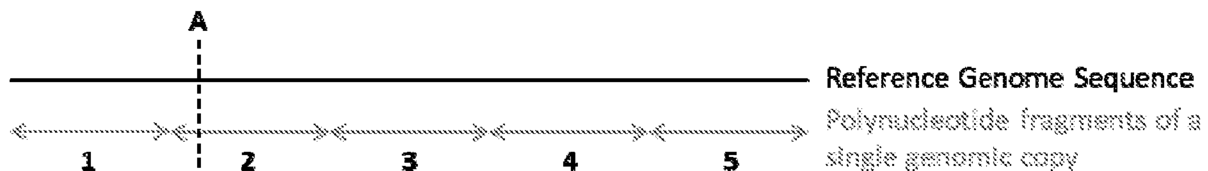
29. It is my opinion that a person of ordinary skill in the art would not view Salk as disclosing or teaching "non-uniquely tagging" as that term is used in the instant application. Further explanation of my analysis is set forth in detail below.

30. Paragraph 252 of the instant specification provides a definition of "non-uniquely tagged."

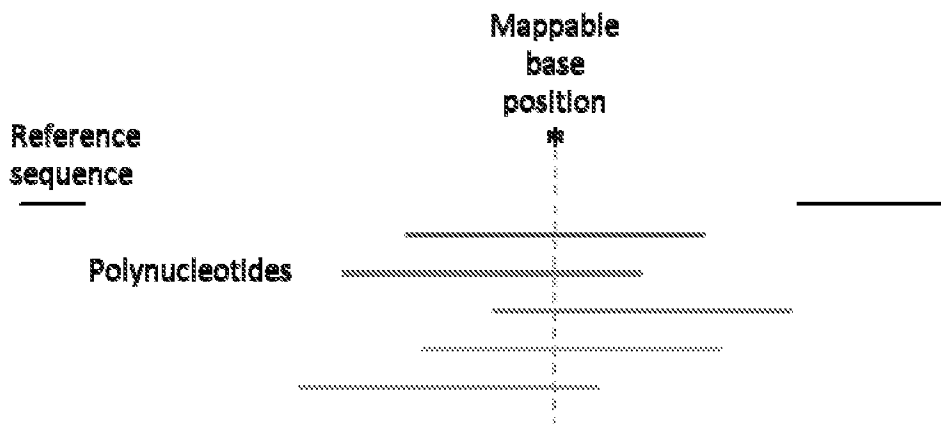
> A set of polynucleotides in the composition that map to a mappable base position in a genome can be non-uniquely tagged, that is, the number of different identifiers can be at least [] 2 and fewer than the number of polynucleotides that map to the mappable base position.

31. The number of polynucleotides that map to a given mappable base position reflects the number of genomic copies in a sample. Such a set of polynucleotides in a sample is less than the total number of fragments in a sample.

32. Consider as an example, a single copy of a genome that is fragmented into five polynucleotides, all of which are mappable to the reference sequence. Such a sample includes five polynucleotides. Yet, at any given mappable base position of the reference sequence (*e.g.*, locus "A," below), there is only one polynucleotide fragment (fragment "2" in this example).



33. As another example, consider a sample comprised of five haploid copies of the human nuclear genome that is quantitatively fragmented. The entire sample might comprise millions of polynucleotides, but no more than 5 polynucleotides that map to a given mappable base position.



34. Accordingly, the instant claims are directed to a tagging method that uses far fewer tags than the total number of polynucleotides in the sample and is in marked contrast to the tagging methods described by Salk. Salk does not use the term "non-uniquely tagged" in the specification, nor does Salk contemplate non-unique tagging as claimed.

35. In contrast to the claimed method, the DCS method of Salk critically relies on flooding a sample with an excess number of tags, such that every DNA fragment is labeled with distinct or "unique" tags. *E.g.*, Salk, 3:55-61 ("Because every adaptor contains a unique, double-stranded, complimentary n-mer random tag on each end (n-mer =12 bp according to one embodiment), every DNA fragment becomes labeled with two distinct SMI sequences…).

36. Furthermore, Salk exclusively describes methods that use more tags than polynucleotide fragments in the sample. Salk refers to its disclosed methods as providing "unique" tagging. *E.g.*, Salk, 16:15-19 ("The double-stranded target nucleic acid complex is then amplified by a method known in the art (e.g., a PCR or non-PCR method known in the art), resulting in a set of uniquely labeled, amplified SMI-target nucleic acid products."), 19:25-31 ("Because the use of SMI tags (or 'double-stranded SMI sequences') allows every molecule to be uniquely labeled prior to PCR duplication, true PCR duplicates may be unambiguously identified by virtue of having a common (i.e., the same or identical) SMI sequence.").

37. Salk never instructs that the number of tags — which Salk refers to as "Single Molecule Identifiers" or "SMIs" —should be less than the number of polynucleotides in a sample. Where Salk does relate the number of barcodes to polynucleotides in a sample, it

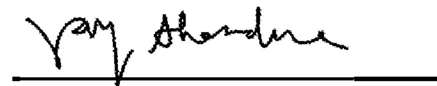
instructs that the barcodes should be in excess of the sample polynucleotides. *E.g.*, Salk, 3:55-61 ("Because every adaptor contains a unique, double-stranded, complimentary n-mer random tag on each end (n-mer =12 bp according to one embodiment), every DNA fragment becomes labeled with two distinct SMI sequences…), 6:67-7:4 ("The first and/or second degenerate or semi-degenerate nucleotide n-mer sequences may be any suitable length to produce a sufficiently large number of unique tags to label a set of sheared DNA fragments from a segment of DNA.").

38. A person of ordinary skill in the art would not understand Salk to teach a tagging method based on the number of polynucleotides that map to a given mappable base position.

V. Concluding Statements

39. I declare that all statements made herein are true to the best of my knowledge, or if made upon information and belief, are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent under examination thereon.

Respectfully submitted,



June 9, 2020

Date

Jay Shendure, M.D., PhD.

UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/593,633	10/04/2019	AmirAli TALASAZ	42534-704.314	1021

115823 7590 05/20/2020
Wilson Sonsini Goodrich & Rosati / Guardant Health
650 Page Mill Road
Palo Alto, CA 94304

EXAMINER	
HORLICK, KENNETH R	

ART UNIT	PAPER NUMBER
1637	

NOTIFICATION DATE	DELIVERY MODE
05/20/2020	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

Patents@guardanthealth.com
patentdocket@wsgr.com

Office Action Summary	Application No. 16/593,633	Applicant(s) TALASAZ, AmirAli	
	Examiner KENNETH R HORLICK	Art Unit 1637	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b)☑ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on _____; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 31-60 is/are pending in the application.
5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 31-60 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
Certified copies:
a)☐ All b)☐ Some** c)☐ None of the:
1.☐ Certified copies of the priority documents have been received.
2.☐ Certified copies of the priority documents have been received in Application No. _____.
3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)
Paper No(s)/Mail Date _____.

3) ☑ Interview Summary (PTO-413)
Paper No(s)/Mail Date 5/13/20.

4) ☐ Other: _____.

Notice of Pre-AIA or AIA Status

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA.

NEW GROUNDS OF REJECTION

2. In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

3. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –(a)(2) the claimed invention was described in a patent issued under section 151, or in an application for patent published or deemed published under section 122(b), in which the patent or application, as the case may be, names another inventor and was effectively filed before the effective filing date of the claimed invention.

Claims 31-33, 35-51, 54-58, and 60 are rejected under 35 U.S.C. 102(a)(2) as being anticipated by Salk et al. (US 10,604,804; effective filing date 03/20/12).

Claims 31-33 and 35-50 are drawn to methods comprising non-uniquely tagging a plurality of cell-free DNA molecules with adapters comprising barcodes, wherein adapters are ligated to both ends of the molecules; amplifying said molecules; sequencing a portion of said amplified molecules to produce sequencing reads; and detecting among the sequence reads one or more somatic genetic variants. Claims 51, 54-58, and 60 are drawn to similar methods comprising the additional steps of aligning sequencing reads; and grouping such reads into families.

Salk et al. discloses methods comprising these steps as well as the steps in the dependent claims; see claims 1-2, 7-11, and 16-28. The claimed methods cannot be distinguished from the patented methods of Salk et al.

4. The following is a quotation of 35 U.S.C. 103 which forms the basis for all obviousness rejections set forth in this Office action:

> A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 34, 52, 53, and 59 are rejected under 35 U.S.C. 103 as being unpatentable over Salk et al.

These claims are drawn to the methods as described and rejected above, with further limitations regarding the set of barcodes, quantity of cell-free DNA molecules; and percentage of said molecules that are from one or more cancer genomes.

While Salk et al. does not disclose these further limitations, it is submitted that they are within the category of routine optimization of known-important reaction parameters, which as established in U.S. patent practice does not support unobviousness (see M.P.E.P. 2144.05). Thus, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the application was filed to optimize these known-important parameters in the method disclosed and claimed in Salk et al.

CONCLUSION

5. No claims are free of the prior art.

6. Any inquiry concerning this communication or earlier communications from the examiner should be directed to KENNETH R HORLICK whose telephone number is (571)272-0784. The examiner can normally be reached on Mon. - Thurs. 8:30 - 6:30.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at http://www.uspto.gov/interviewpractice.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see https://ppair-my.uspto.gov/pair/PrivatePair. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

05/13/20

/KENNETH R HORLICK/
Primary Examiner, Art Unit 1637

Applicant-Initiated Interview Summary	**Application No.** 16/593,633	**Applicant(s)** TALASAZ, AmirAli	
	Examiner KENNETH R HORLICK	**Art Unit** 1637	**AIA (FITF) Status** Yes

All participants (applicant, applicants representative, PTO personnel):

(1) KENNETH R. HORLICK. (3) _____.

(2) TIMOTHY HOTT. (4) _____.

Date of Interview: 13 May 2020.

Type: ☑ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☑ No.
If Yes, brief description: _____.

Issues Discussed ☐101 ☑112 ☑102 ☑103 ☐Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: N/A.

Identification of prior art discussed: Salk et al. (US 10,604,804).

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

There was a discussion regarding support and enablement for the claims of the '804 patent. No agreement was reached..

Applicant recordation instructions: The formal written reply to the last Office action must include the substance of the interview. (See MPEP section 713.04). If a reply to the last Office action has already been filed, applicant is given a non-extendable period of the longer of one month or thirty days from this interview date, or the mailing date of this interview summary form, whichever is later, to file a statement of the substance of the interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/KENNETH R HORLICK/
Primary Examiner, Art Unit 1637

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record
A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) 1.133 Interviews
Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.
All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiners responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicants correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:
- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:
1) A brief description of the nature of any exhibit shown or any demonstration conducted,-
2) an identification of the claims discussed,
3) an identification of the specific prior art discussed,
4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
5) a brief identification of the general thrust of the principal arguments presented to the examiner,
   (The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
6) a general indication of any other pertinent matters discussed, and
7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicants record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiners version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, Interview Record OK on the paper recording the substance of the interview along with the date and the examiners initials.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings in the above-referenced patent application. The foregoing amendments are without prejudice and do not constitute an admission regarding the patentability of the amended subject matter and should not so be construed. Applicant reserves the right to pursue the subject matter of the canceled claims in this or any other appropriate patent application.

Listing of Claims:

1.-30. (Cancelled).

31. (Currently amended): A method for detecting one or more somatic genetic variants, the method comprising:
    (a) non-uniquely tagging a plurality of cell-free deoxyribonucleic acid (cfDNA) molecules with adapters comprising molecular barcodes from a plurality of cfDNA molecules obtained from a human subject to produce non-uniquely tagged parent polynucleotides, wherein the adapters are ligated to both ends of molecules of cfDNA from the plurality of cfDNA molecules;
    (b) amplifying a plurality of the non-uniquely tagged parent polynucleotides to produce progeny polynucleotides with associated molecular barcodes;
    (c) sequencing a plurality ~~portion~~ of the progeny polynucleotides to produce sequencing reads of the progeny polynucleotides with associated molecular barcodes; and
    (d) detecting, from among a plurality of the sequencing reads, a presence or absence of the one or more somatic genetic variants, based at least in part on the associated molecular barcodes of the sequencing reads.

32. (Previously presented): The method of claim 31, wherein the human subject has a cancer or is suspected of having a cancer.

33. (Previously presented): The method of claim 31, wherein the non-uniquely tagging comprises blunt-end ligation or sticky-end ligation.

34. (Previously presented): The method of claim 31, wherein the molecular barcodes are from a set of molecular barcodes having 5 to 1,000 different nucleotide sequences that are 5 to 20 nucleotides in length.
35. (Previously presented): The method of claim 31, further comprising selectively enriching the progeny polynucleotides for target regions associated with cancer.
36. (Previously presented): The method of claim 31, further comprising filtering out one or more of the sequencing reads that fail to meet a quality control threshold.
37. (Currently amended): The method of claim 31, further comprising aligning a plurality of the sequencing reads to a human reference sequence to produce aligned sequencing reads.
38. (Currently amended): The method of claim 37, further comprising grouping a plurality of the aligned sequencing reads into families based on sequence information of the molecular barcodes, [[the]] a start base position of a given aligned sequencing read from among the aligned sequencing reads at which the given aligned sequencing read is determined to start aligning to the human reference sequence, and a stop base position of the given aligned sequencing read at which the given aligned sequencing read is determined to stop aligning to the human reference sequence.
39. (Previously presented): The method of claim 38, further comprising generating a set of consensus sequences from among the sequencing reads in the families.
40. (Previously presented): The method of claim 39, further comprising determining the presence or absence of the one or more somatic genetic variants in the set of consensus sequences as compared to the human reference sequence.
41. (Previously presented): The method of claim 40, further comprising quantifying a number of consensus sequences from the set of consensus sequences that comprise one or more somatic genetic variants as compared to the human reference sequence.
42. (Previously presented): The method of claim 38, wherein the detecting comprises generating a base call at a genetic locus of the human reference sequence for a family from among the families.
43. (Previously presented): The method of claim 38, further comprising quantifying a number of the families.

44. (Previously presented): The method of claim 31, wherein the one or more somatic genetic variants comprise a single nucleotide variant (SNV), a copy number variation (CNV), an insertion or deletion (indel), a gene fusion, or any combination thereof.
45. (Previously presented): The method of claim 31, wherein the detecting further comprises detecting cancer in the human subject when the presence of the one or more somatic genetic variants is detected.
46. (Previously presented): The method of claim 31, further comprising generating a tumor mutation profile of the human subject based on the detected presence or absence of the one or more somatic genetic variants.
47. (Previously presented): The method of claim 46, wherein the tumor mutation profile is generated based on the presence or absence of one or more somatic genetic variants detected from different samples obtained from the human subject at different time points.
48. (Previously presented): The method of claim 31, further comprising performing (a)-(d) in combination with immune repertoire profiling.
49. (Previously presented): The method of claim 31, wherein the one or more somatic genetic variants are located in a microsatellite region.
50. (Previously presented): The method of claim 49, wherein the one or more somatic genetic variants comprise an indel.
51. (Currently amended): A method for detecting a presence or absence of one or more somatic genetic variants, the method comprising:
    (a) non-uniquely tagging a plurality of cell-free deoxyribonucleic acid molecules (cfDNA) molecules with molecular barcodes from a plurality of cfDNA molecules obtained from a human subject to produce non-uniquely tagged parent polynucleotides, wherein the molecular barcodes are ligated to both ends of molecules of cfDNA from the plurality of cfDNA molecules;
    (b) amplifying a plurality of the non-uniquely tagged parent polynucleotides to produce progeny polynucleotides with associated molecular barcodes;
    (c) selectively enriching the progeny polynucleotides for target regions associated with cancer, whereby enriched progeny polynucleotides are generated;

(d) sequencing a plurality ~~portion~~ of the enriched progeny polynucleotides to produce sequencing reads of the enriched progeny polynucleotides with associated molecular barcodes;

(e) aligning a plurality of the sequencing reads to a human reference sequence to produce aligned sequencing reads;

(f) grouping a plurality of the aligned sequencing reads into families based on sequence information of the molecular barcodes, [[the]] a start base position of a given aligned sequencing read from among the aligned sequencing reads at which the given aligned sequencing read is determined to start aligning to the human reference sequence, and a stop base position of the given aligned sequencing read at which the given aligned sequencing read is determined to stop aligning to the human reference sequence; and

(g) detecting, from among a plurality of the families, the presence or absence of the one or more somatic genetic variants comprising a single nucleotide variant (SNV), a copy number variation (CNV), an insertion or deletion (indel), a gene fusion, or any combination thereof.

52. (Previously presented): The method of claim 51, wherein the plurality of cfDNA molecules comprises between 1 nanogram (ng) and 100 ng of cfDNA molecules.
53. (Previously presented): The method of claim 51, wherein the molecular barcodes are from a set of molecular barcodes having 5 to 1,000 different nucleotide sequences that are 5 to 20 nucleotides in length.
54. (Previously presented): The method of claim 51, wherein the molecular barcodes are part of adapter sequences.
55. (Previously presented): The method of claim 51, further comprising filtering out one or more of the sequencing reads or the aligned sequencing reads that fail to meet a set accuracy, quality score, or mapping score threshold.
56. (Previously presented): The method of claim 51, further comprising generating a set of consensus sequences from among the aligned sequencing reads in the families.

57. (Previously presented): The method of claim 56, further comprising calculating a number of consensus sequences from the set of consensus sequences that comprise one or more somatic genetic variants as compared to the human reference sequence.
58. (Previously presented): The method of claim 51, further comprising quantifying a number of the families.
59. (Previously presented): The method of claim 51, wherein 0.1% to 1.0% of the cfDNA molecules in the plurality of cfDNA molecules is from one or more cancer genomes.
60. (Previously presented): The method of claim 51, wherein the detecting further comprises detecting cancer in the human subject when the presence of the one or more somatic genetic variants is detected.