

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): ELTOUKHY et al.	Confirmation No.: 1052
Serial Number: 16/601,168	Customer No.: 115823
Filing Date: October 14, 2019	Group Art Unit: 1637
Title: METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	Examiner: Kenneth R. HORLICK

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Commissioner for Patents
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AMENDMENT AND RESPONSE TO NON-FINAL OFFICE ACTION

Sir:

This paper is in response to the Office Action mailed on February 18, 2020. The shortened statutory period for reply expires May 18, 2020; therefore, this response is timely filed. Applicant respectfully requests reconsideration of the above-referenced application in view of the following remarks:

Amendments to the Claims begin on page 2 of this paper.

Remarks begin on page 9 of this paper.

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 classifying consensus sequences generated from sequencing reads derived from ~~detecting a presence or absence of somatic genetic variants in~~ double-stranded cell-free deoxyribonucleic acid (cfDNA) molecules from a sample of a human subject, the method comprising:

(a) ~~non-uniquely tagging ligating adapters comprising molecular barcodes to~~ a population of double-stranded cfDNA molecules obtained from the sample with at least a 10X molar excess, relative to the double-stranded cfDNA molecules in the population, of adapters comprising molecular barcodes ~~relative to the double-stranded cfDNA molecules in the population~~ to generate non-uniquely tagged parent polynucleotides,

wherein the double-stranded cfDNA molecules that map to a mappable base position of a reference sequence are tagged with a number of different molecular barcodes ranging from at least 2 and fewer than a number of double-stranded cfDNA molecules that map to the mappable base position, and

wherein at least 20% of the double-stranded cfDNA molecules are tagged attached with the adapters comprising molecular barcodes at both ends of a molecule of the double-stranded cfDNA molecules;

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

- (c) ~~selectively~~ enriching a plurality of the progeny polynucleotides for target regions of interest associated with cancer, whereby enriched progeny polynucleotides are generated;
 - (d) sequencing at least a subset of the enriched progeny polynucleotides to produce a set of sequencing reads;
 - (e) mapping a plurality of the sequencing reads to the one or more reference sequence[[s]];
 - (f) grouping a plurality of the sequencing reads mapped in (e) into families of sequencing reads based at least on (i) sequence information from the molecular barcodes and (ii) the beginning base position and ending base position of the sequencing reads that map to the ~~one or more~~ reference sequence[[s]]; ~~and~~
 - (g) generating a consensus sequence of each family from among a plurality of families ~~detecting, from among a plurality of families of sequencing reads, the presence or absence of one or more somatic genetic variants; and~~
 - (h) classifying a plurality of consensus sequences from the set of consensus sequences as
 - (1) paired consensus sequences generated from sequencing reads representing a Watson strand and a Crick strand of a non-uniquely tagged parent polynucleotide or
 - (2) unpaired consensus sequences generated from sequencing reads representing either a Watson strand or a Crick strand of a non-uniquely tagged parent polynucleotide.
32. (Cancelled).
33. (Previously presented): The method of claim 31, wherein the population of double-stranded cfDNA molecules comprises 1 nanogram (ng) to 100 ng of double-stranded cfDNA molecules.
34. (Previously presented): The method of claim 31, wherein the sample is blood, plasma, or serum.
35. (Currently amended): The method of claim 31, wherein ligating the adapters comprising molecular barcodes are attached to the double-stranded cfDNA molecules by ~~comprises~~ blunt-end ligation or sticky-end ligation.

36. (Currently amended): The method of claim 31, wherein at least 40% of the double-stranded cfDNA molecules are non-uniquely tagged with adapters comprising molecular barcodes at both ends of the cfDNA molecules.
37. (Currently amended): The method of claim 31, wherein more than a 90X molar excess of adapters relative to the double-stranded cfDNA molecules in the population is used to generate the non-uniquely tagged parent polynucleotides.
38. (Currently amended): The method of claim 31, wherein the molecular barcodes are from a set of molecular barcodes ~~comprising molecular barcodes~~ having 2 to 1,000 different molecular barcode sequences.
39. (Currently amended): The method of claim 31, wherein the molecular barcodes are from a set of molecular barcodes ~~comprising molecular barcodes~~ having 5 to 100 different molecular barcode sequences that have a length of 5 to 20 nucleotides.
40. (Currently amended): The method of claim 31, wherein the target regions of interest ~~associated with cancer~~ comprise genetic sequences of a plurality of genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.
41. (Previously presented): The method of claim 31, further comprising amplifying a plurality of the enriched progeny polynucleotides prior to sequencing.
42. (Cancelled).
43. (Cancelled).
44. (Cancelled).
45. (Cancelled).
46. (Cancelled).
47. (Currently amended): The method of claim 31 ~~[[46]]~~, further comprising:

- (i) calculating a first quantitative measure of paired consensus sequences that map to a locus of ~~the one or more~~ reference sequence[[s]], and
- (ii) calculating a second quantitative measure of unpaired consensus sequences that map to a locus of the ~~one or more~~ reference sequence[[s]].

48. (Currently amended): The method of claim 47, further comprising:

- (iii) calculating a third quantitative measure of double-stranded cfDNA molecules that map to a locus of the ~~one or more~~ reference sequence[[s]] for which neither complementary strand of the double-stranded cfDNA molecules is detected in said set of consensus sequences, wherein the third quantitative measure is calculated based at least in part on the first and second quantitative measures.

49. (Previously presented): The method of claim 48, further comprising:

- (iv) calculating a fourth quantitative measure of a total number of double-stranded cfDNA molecules in the population of double-stranded cfDNA molecules in the sample, wherein the total number of double-stranded cfDNA molecules comprises unseen double-stranded cfDNA molecules in the sample, and wherein the total number of double-stranded cfDNA molecules is determined based at least in part on the first, second, and third quantitative measures.

50. (Cancelled).

51. (Currently amended): The method of claim 49 [[50]], comprising determining the first, second, third, and fourth[[],] ~~and fifth~~ quantitative measures with a programmed computer processor.

52. (Cancelled).

53. (Cancelled).

54. (Cancelled).

55. (Cancelled).

56. (Cancelled).

57. (Currently amended): A method for classifying unique sequence reads generated from sequencing reads derived from double-stranded ~~detecting a presence or absence of somatic genetic variants in~~ cell-free deoxyribonucleic acid (cfDNA) molecules from a bodily fluid sample of a human subject, the method comprising:

(a) ~~ligating adapters comprising molecular barcodes to tagging~~ a population of double-stranded cfDNA molecules obtained from the bodily fluid sample with at least a 10X molar excess of adapters comprising molecular barcodes, relative to the double-stranded cfDNA molecules in the population, to generate tagged parent polynucleotides,

wherein at least 20% of the cfDNA molecules are ~~tagged~~ ligated with the adapters comprising the molecular barcodes at both ends of a molecule of the double-stranded cfDNA molecules;

(b) amplifying a plurality of the tagged parent polynucleotides to produce progeny polynucleotides;

(c) sequencing at least a plurality of the progeny polynucleotides to produce a set of sequencing reads; ~~and~~

(d) mapping a plurality of sequencing reads from the set of sequencing reads to a reference sequence ~~detecting, from among a plurality of sequencing reads in the set of sequencing reads that map to one or more reference sequences, the presence or absence of one or more somatic genetic variants using sequence information from the molecular barcodes in combination with mapping positions of the sequencing reads that map to the one or more reference sequences;~~

(e) determining unique sequence reads from the set of sequencing reads based at least on the molecular barcode sequences, wherein a unique sequence read is representative of a tagged parent polynucleotide from among the tagged parent polynucleotides; and

(f) classifying a plurality of the unique sequence reads as either (1) paired sequences generated from sequencing reads representing a Watson strand and a Crick strand of a tagged parent polynucleotide or (2) unpaired sequences generated from sequencing reads representing either a Watson strand or a Crick strand of a tagged parent polynucleotide.

58. (Currently amended): The method of claim 57, further comprising selectively enriching a plurality of the progeny polynucleotides for target regions of interest ~~associated with cancer.~~

59. (Currently amended): The method of claim 57, wherein determining unique sequence reads ~~the detecting~~ comprises grouping the plurality of sequencing reads in the set of sequencing

reads that map to ~~the one or more~~ reference sequence[[s]] into families, wherein a family comprises sequencing reads of progeny polynucleotides amplified from the same tagged parent polynucleotide.

60. (Cancelled).

61. (New): The method of claim 57, further comprising:

- (i) calculating a first quantitative measure of paired sequences that map to a locus of the reference sequence, and
- (ii) calculating a second quantitative measure of unpaired sequences that map to a locus of the reference sequence.

62. (New): The method of claim 61, further comprising:

- (iii) calculating a third quantitative measure of double-stranded cfDNA molecules that map to a locus of the reference sequence for which neither complementary strand of the double-stranded cfDNA molecules is detected in said set of unique sequence reads, wherein the third quantitative measure is calculated based at least in part on the first and second quantitative measures.

63. (New): The method of claim 62, further comprising:

- (iv) calculating a fourth quantitative measure of a total number of double-stranded cfDNA molecules in the population of double-stranded cfDNA molecules in the sample, wherein the total number of double-stranded cfDNA molecules comprises unseen double-stranded cfDNA molecules in the sample, and wherein the total number of double-stranded cfDNA molecules is determined based at least in part on the first, second, and third quantitative measures.

64. (New): The method of claim 63, comprising determining the first, second, third, and fourth quantitative measures with a programmed computer processor.

65. (New): The method of claim 57, wherein the molecular barcodes are from a set of molecular barcodes having 2 to 10,000 different molecular barcode sequences.

66. (New): The method of claim 57, wherein the molecular barcode sequences are predetermined sequences.

67. (New): The method of claim 57, wherein at least 40% of the double-stranded cfDNA molecules are tagged with adapters comprising molecular barcodes at both ends of the cfDNA molecules.
68. (New): The method of claim 57, wherein more than a 90X molar excess of adapters relative to the double-stranded cfDNA molecules in the population is used to generate tagged parent polynucleotides.
69. (New): The method of claim 57, further comprising quantifying a number of unique sequence reads identified from the set of sequencing reads.
70. (New): The method of claim 57, wherein determining unique sequence reads is further based on (1) a start base position of a given sequencing read from among the set of sequencing reads at which the given sequencing read starts aligning to the reference sequence, and (2) a stop base position of the given sequencing read at which the given sequencing read stops aligning to the reference sequences.
71. (New): The method of claim 31, further comprising determining a quantity of consensus sequences generated from among a plurality of families.

REMARKS

Claims 31-60 were pending prior to entry of the above-referenced claim amendments. Claims 32, 42-46, 50, 52-56 and 60 are hereby cancelled without disclaimer or prejudice. Claims 31, 35-40, 47-48, 51, 57-59 are amended. Claims 61-71 are newly added. Support for these amendments may be found throughout the application as filed, for example, at least at paragraphs [0021], [00115], [00119], and [00179]-[00180] as filed. No new matter is believed to be added by these amendments. Therefore, claims 31, 33-41, 47-49, 51, 57-59 and 61-71 are pending and set for examination.

Nonstatutory Double Patenting Rejections

Claims 31-60 were rejected on the ground of non-statutory double patenting as allegedly being unpatentable over claims 1-33 of U.S. Patent No. 9,902,992. Claims 31-60 were rejected on the ground of non-statutory double patenting as allegedly being unpatentable over claim 10 of U.S. Patent No. 9,920,366. Claims 57-60 were provisionally rejected on the ground of non-statutory double patenting as allegedly being unpatentable over claim 42 of copending Application No. 15/892,178.

Without conceding in the basis of the rejections, and solely to expediate prosecution of this application, Applicant submits herewith terminal disclaimers over the '366 patent and the '178 application. Accordingly, Applicant respectfully requests that the respective nonstatutory double patenting rejections be withdrawn.

Applicant respectfully requests the Examiner to reconsider the non-statutory double patenting rejection over the '992 patent in view of the aforementioned claim amendments. If only a non-statutory double patenting rejection remains following entry of this response, Applicant respectfully requests the Examiner to telephone the undersigned attorney of record such that a Terminal Disclaimer may be considered and timely filed.

Applicant submits that the nonstatutory double patenting rejections to claims 32, 42-46, 50, 52-56 and 60 are moot in view of the cancellation of these claims.

Rejection under 35 U.S.C. §103

Claims 31-60 were rejected under 35 U.S.C. §103 as allegedly being unpatentable over Schmitt et al. (U.S. 9,752,188) in view of Sacko et al. (U.S. 2010/0264331).

Without conceding in the basis of the rejection, and to expediate prosecution, independent claims 31 and 57 have been amended to clarify the claimed subject matter. Applicant submits that the asserted combination of Schmitt et al. and Sacko et al. does not meet all of the elements of independent claims 31 and 57 as amended, much less the elements of dependent claims 33-41, 47-49, 51, and 58-59. Moreover, a person of ordinary skill in the art would recognize that there was no reasonable expectation of success in applying the method of Schmitt et al. to cfDNA. Indeed, as disclosed in Perakis et al., “Advances in Circulating Tumor DNA Analysis,” *Adv. Clin Chem* (2017) pp 1-81 (IDS submitted 1/14/20), “[p]rospects of success are limited since the [Schmitt et al.] method is relatively inefficient when limited amounts of input DNA—as it is most likely the case for cfDNA—are used” (page 30, internal citation omitted).

Therefore, independent claims 31 and 57 and dependent claims 33-41, 47-49, 51, and 58-59 are not obvious over Schmitt et al. in view of Sacko et al. For purposes solely to expedite the prosecution of this application, claims 32, 42-46, 50, 52-56 and 60 have been cancelled, thereby rendering the rejection moot as to these claims.

Accordingly, Applicant respectfully requests that the §103 rejection of the above-mentioned claims over Schmitt et al. in view of Sacko et al. be withdrawn.

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 cancelled 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.

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CONCLUSION

This paper fully addresses the rejections raised in the Office Action mailed February 18, 2020. Applicant believes that the present application is now in condition for allowance and respectfully requests that the Examiner expedite the prosecution of this application to allowance. The Commissioner is authorized to charge any underpayment, or credit any overpayment, to Deposit Account No. 60-2231 (Attorney Docket No. GH0004US-CON2).

Respectfully submitted,

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