

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Inventor(s): ELTOUKHY et al.	Confirmation No.: 3448
Serial Number: 16/672,267	Customer No.: 115823
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Title: METHODS AND SYSTEMS FOR DETECTING GENETIC VARIANTS	Examiner: Kenneth R. HORLICK

Mail Stop Amendment  
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 March 3, 2020. The shortened statutory period for reply expires June 3, 2020, therefore, this response is timely filed. Applicants respectfully request 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 7 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. – 60. (Cancelled).
61. (New): A method, comprising:
  - (a) providing a population of cell-free deoxyribonucleic acid (cfDNA) molecules having first and second complementary strands;
  - (b) tagging a plurality of the cfDNA molecules in the population with a set of duplex tags comprising molecular barcodes from a set of molecular barcodes to produce tagged parent polynucleotides, wherein the duplex tags are attached at both ends of a molecule of the cfDNA molecules;
  - (c) amplifying a plurality of the tagged parent polynucleotides to produce amplified progeny polynucleotides;
  - (d) sequencing at least a subset of the amplified progeny polynucleotides to produce a set of sequence reads; and
  - (e) reducing and/or tracking redundancy in the set of sequence reads to generate a plurality of consensus sequences representative of original cfDNA molecules from among the tagged parent polynucleotides, wherein the plurality of consensus sequences are generated from (i) paired reads corresponding to sequence reads generated from a first tagged strand and a second tagged complementary strand derived from a cfDNA molecule from among the tagged parent polynucleotides or (ii) unpaired reads corresponding to sequence reads generated from a first tagged strand having no second tagged complementary strand derived from a cfDNA molecule from among the tagged parent polynucleotides.

62. (New): The method of claim 61, wherein the sample is obtained from a subject having cancer.

63. (New): The method of claim 61, wherein the plurality of cfDNA molecules comprises 1 nanogram (ng) to 100 ng of cfDNA molecules.

64. (New): The method of claim 61, wherein the molecular barcodes are ligated to the cfDNA molecules using more than a 10X molar excess of duplex tags as compared to the cfDNA molecules.

65. (New): The method of claim 64, wherein at least 20% of the cfDNA molecules from the sample are tagged with the duplex tags.

66. (New): The method of claim 61, wherein tagging comprises non-uniquely tagging the plurality of the cfDNA molecules with the set of duplex tags comprising molecular barcodes from the set of molecular barcodes, wherein the 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 cfDNA molecules that map to the mappable base position

67. (New): The method of claim 61, wherein the molecular barcodes in the set of molecular barcodes have predetermined sequences.

68. (New): The method of claim 61, wherein the molecular barcodes in the set of molecular barcodes have 5 to 10,000 different molecular barcode sequences and are 5 to 20 base pairs in length.

69. (New): The method of claim 61, further comprising enriching the amplified progeny polynucleotides for target regions of interest prior to sequencing.

70. (New): The method of claim 69, wherein the target regions of interest 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.

71. (New): The method of claim 61, further comprising amplifying a plurality of the enriched progeny polynucleotides prior to sequencing.

72. (New): The method of claim 61, wherein the molecular barcodes are part of sequencing adapters.

73. (New): The method of claim 72, wherein the adapter is a Y-shaped adapter.

74. (New): The method of claim 61, wherein reducing and/or tracking redundancy in the set of sequence reads comprises mapping a plurality of the sequence reads to a reference sequence.

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

(f) determining quantitative measures of at least two of (i) paired reads, (ii) unpaired reads, (iii) read depth of the paired reads and (iv) read depth of the unpaired reads.

76. (New): The method of claim 75, further comprising:

(g) estimating with a programmed computer processor a quantitative measure of total cfDNA molecules based on said quantitative measures of at least two of (i) paired reads, (ii) unpaired reads, (iii) read depth of the paired reads and (iv) read depth of the unpaired reads.

77. (New): The method of claim 76, wherein (f) comprises determining quantitative measures of paired reads and unpaired reads, and wherein in (g), the quantitative measure of total cfDNA molecules is determined based on the quantitative measures of paired reads and unpaired reads.

78. (New): A method, comprising:

(a) providing a population of double-stranded cell-free deoxyribonucleic acid (cfDNA) molecules having first and second complementary strands;

(b) non-uniquely tagging a plurality of the double-stranded cfDNA molecules in the population with a set of duplex tags comprising molecular barcodes from a set of molecular barcodes to produce 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;

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

(d) sequencing at least a subset of the amplified progeny polynucleotides to produce a set of sequence reads;

(e) reducing and/or tracking redundancy in the set of sequence reads;

(f) sorting sequence reads into paired reads and unpaired reads, wherein (i) a paired read corresponds to sequence reads generated from a first tagged strand and a second tagged complementary strand derived from a double-stranded cfDNA molecule from among the non-uniquely tagged parent polynucleotides, and (ii) an unpaired read corresponds to sequence reads generated from a first tagged strand having no second tagged complementary strand derived from a double-stranded cfDNA molecule from among the non-uniquely tagged parent polynucleotides; and

(g) determining quantitative measures of at least two of (i) paired reads, (ii) unpaired reads, (iii) read depth of the paired reads and (iv) read depth of the unpaired reads; and

79. (New): The method of claim 78, wherein the sample is blood, plasma, or serum.

80. (New): The method of claim 78, wherein the plurality of double-stranded cfDNA molecules comprises 1 nanogram (ng) to 100 ng of double-stranded cfDNA molecules.

81. (New): The method of claim 78, wherein the tagging comprises ligating the molecular barcodes to double-stranded cfDNA molecules.

82. (New): The method of claim 78, wherein the molecular barcodes in the set have 2 to 10,000 different molecular barcode sequences.

83. (New): The method of claim 78, wherein the molecular barcodes in the set have 5 to 10,000 different molecular barcode sequences and are 5 to 20 base pairs in length.

84. (New): The method of claim 78, further comprising enriching the amplified progeny polynucleotides for target regions of interest prior to sequencing.

85. (New): The method of claim 84, wherein the target regions of interest 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.

86. (New): The method of claim 78, further comprising amplifying a plurality of the enriched progeny polynucleotides prior to sequencing.

87. (New): The method of claim 78, wherein reducing and/or tracking redundancy in the set of sequence reads comprises collapsing a plurality of the sequence reads to generate consensus sequences representative of original double-stranded cfDNA molecules from among the non-uniquely tagged parent polynucleotides.

88. (New): The method of claim 87, further comprising mapping a plurality of the sequence reads and/or consensus sequences to a reference sequence.

89. (New): The method of claim 78, further comprising:

(h) estimating with a programmed computer processor a quantitative measure of total double-stranded polynucleotide molecules based on said quantitative measures of at least two of (i) paired reads, (ii) unpaired reads, (iii) read depth of the paired reads and (iv) read depth of the unpaired reads.

90. (New): The method of claim 89, wherein (g) comprises determining quantitative measures of paired reads and unpaired reads, and wherein in (h), the quantitative measure of total double-stranded cfDNA molecules is determined based on the quantitative measures of paired reads and unpaired reads.

### **REMARKS**

Claims 31-60 were pending prior to entry of the above-referenced claim amendments, but are hereby cancelled without disclaimer or prejudice. Claims 61-90 have been newly added, support for which may be found throughout the application as published, for example, at least at paragraphs [0061], [0113], [0117], [0133], [0171], [0199], [0208], [0236], [0261]. No new matter is added by these amendments. Accordingly, claims 61-90 are now pending and set for examination.

#### **I. Nonstatutory Double Patenting Rejection**

Claims 31-60 were rejected on the ground of nonstatutory double patenting as allegedly unpatentable over claims 1-33 of U.S. Patent No. 9,902,992. Claims 31-60 were rejected on the ground of nonstatutory double patenting as allegedly unpatentable over claims 1-21 of U.S. Patent No. 9,920,366. Claims 31-60 were provisionally rejected on the ground of nonstatutory double patenting as allegedly unpatentable over claims 31-60 of copending Application No. 16/601,168. Claims 31-60 were provisionally rejected on the ground of nonstatutory double patenting as allegedly unpatentable over claims 31-60 of copending Application No. 16/714,579.

Without conceding in the basis of the rejections, Applicant has cancelled claims 31-60. Accordingly, the nonstatutory double patenting rejections to claims 31-60 are moot in view of the cancellation of these claims.

#### **II. Prior Art Rejection— 35 U.S.C. §103**

Claims 31-60 were rejected under 35 U.S.C. §103 as allegedly unpatentable over Schmitt et al. (U.S. 9,752,188) in view of Deciu et al. (U.S. 2013/0288244) and further in view of Sacko et al. (U.S. 2010/0264331).

Without conceding in the basis of the rejection, Applicant has cancelled claims 31-60. Accordingly, the 35 U.S.C. §103 rejection to claims 31-60 is moot in view of the cancellation of these claims.

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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.

**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. 708.304/GH0004US-CON3).

Respectfully submitted,  
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