



(51) International Patent Classification:

C12Q 1/6809 (2018.01) C12Q 1/6886 (2018.01)

(21) International Application Number:

PCT/US2021/037668

(22) International Filing Date:

16 June 2021 (16.06.2021)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/039,886 16 June 2020 (16.06.2020) US

(71) Applicant: **GRAIL, LLC**. [US/US]; 1525 O'Brien Drive, Menlo Park, CA 94025 (US).

(72) Inventors: **MAUNTZ, Ruth, E.**; C/o Grail, Inc., 1525 O'Brien Drive, Menlo Park, CA 94025 (US). **LARSON, Matthew**; C/o Grail, Inc., 1525 O'Brien Drive, Menlo Park, CA 94025 (US). **SHENOY, Archana**; C/o Grail, Inc., 1525 O'Brien Drive, Menlo Park, CA 94025 (US). **JAMSHIDI, Arash**; C/o Grail, Inc., 1525 O'Brien Drive, Menlo Park, CA 94025 (US). **BURKHARDT, David**; C/o Grail, Inc., 1525 O'Brien Drive, Menlo Park, CA 94025 (US).

(74) Agent: **GIERING, Jeffery, C.** et al.; Greenberg Traurig, LLP, 18565 Jamboree Road, Suite 500, Irvine, California 92612 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: METHODS FOR ANALYSIS OF CELL-FREE RNA

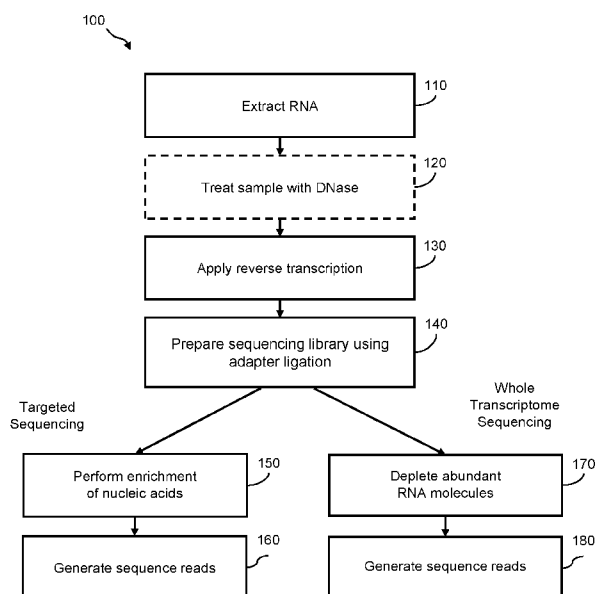


FIG. 1

(57) Abstract: Methods for measuring subpopulations of cell-free ribonucleic acid (RNA) molecules are provided. In some embodiments, methods of generating a sequencing library from a plurality of RNA molecules in a test sample obtained from a subject are provided, as well as methods for analyzing the sequencing library to detect, e.g., the presence or absence of a disease.

Published:

— *with international search report (Art. 21(3))*

(88) Date of publication of the international search report:

03 March 2022 (03.03.2022)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/037668

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/6809; C12Q 1/6886 (2021.01)

CPC - C12Q 1/6886; C12Q 2600/112; C12Q 2600/158; G01N 2800/7028 (2021.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2020/0081008 A1 (PRIME GENOMICS INC.) 12 March 2020 (12.03.2020) entire document	1, 14-34
Y	WO 2019/246625 A1 (BLUESTAR GENOMICS INC.) 26 December 2019 (26.12.2019) entire document	1, 14-34
Y	WO 2019/200410 A1 (FREENOME HOLDINGS INC.) 17 October 2019 (17.10.2019) entire document	25, 28
A	US 2020/0105375 A1 (GRAIL INC.) 02 April 2020 (02.04.2020) entire document	1, 14-34
A	US 2019/0316184 A1 (NATERA INC.) 17 October 2019 (17.10.2019) entire document	1, 14-34
P, X	US 2020/0199671 A1 (GRAIL INC.) 25 June 2020 (25.06.2020) entire document	1, 14-34

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

04 November 2021

Date of mailing of the international search report

DEC 07 2021

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Harry Kim

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/037668

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 39, 40
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 14-34

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-38 are drawn to methods of detecting cancer.

The first invention of Group I+ is restricted to a plurality of target cell-free RNAs, selected to be a first target cfRNA AARD and a second target cfRNA ABCA12, and methods comprising the same. It is believed that claims 1 and 14-34 read on this first named invention and thus these claims will be searched without fee to the extent that they read on AARD and ABCA12.

Applicant is invited to elect additional target cfRNAs to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be a plurality of target cell-free RNAs, selected to be a first target cfRNA ABCC11 and a second target cfRNA ABCC8, and methods comprising the same. Additional target cfRNAs will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for detecting cancer requiring the selection of alternative target cfRNAs where "A method of detecting cancer in a subject, the method comprising: (a) measuring a plurality of target cell-free RNA (cfRNA) molecules in a sample of the subject, wherein the plurality of target cfRNA molecules are selected from one or more transcripts of Table 11; and (b) detecting the cancer, wherein detecting the cancer comprises detecting one or more of the target cfRNA molecules above a threshold level."

Additionally, even if Groups I+ were considered to share the technical features of a method of detecting cancer in a subject, the method comprising: (a) measuring a plurality of target cell-free RNA (cfRNA) molecules in a sample of the subject; and (b) detecting the cancer, wherein detecting the cancer comprises detecting one or more of the target cfRNA molecules above a threshold level; a method of measuring a plurality of target cell-free RNA (cfRNA) molecules in a sample, the method comprising: (a) enriching for the plurality of target cfRNA molecules, or cDNA molecules thereof, to produce an enriched sample of polynucleotides; and (b) sequencing the polynucleotides of the enriched sample, or amplification products thereof. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2019/0316184 A1 to Natera, Inc. discloses a method of detecting cancer in a subject (a method for monitoring and detection of early relapse or metastasis of cancer ... a sample of blood or urine or a fraction thereof from a patient, Para. [0003]), the method comprising: (a) measuring a plurality of target cell-free RNA (cfRNA) molecules in a sample of the subject (levels of total or particular ... cfRNA, Para. [0311]; wherein a higher relative allele frequency compared to the other single nucleotide variants of the plurality of single nucleotide variant loci is indicative of a clonal single nucleotide variant in the breast cancer, Para. [0018]; nucleic acids isolated from a sample of blood or urine or a fraction thereof from a patient, Para. [0003]); and (b) detecting the cancer (detected and the presence of the at least 2 or at least 5 SNVs is indicative of early relapse or metastasis of breast cancer, Para. [0007]), wherein detecting the cancer comprises detecting one or more of the target cfRNA molecules above a threshold level (detecting early relapse or metastasis of MIBC when two or more patient-specific single nucleotide variants are detected above a predetermined confidence threshold, Para. [0078]; levels of total or particular ... cfRNA, Para. [0311]); a method of measuring a plurality of target cell-free RNA (cfRNA) molecules in a sample (levels of total or particular ... cfRNA, Para. [0311]; wherein a higher relative allele frequency compared to the other single nucleotide variants of the plurality of single nucleotide variant loci is indicative of a clonal single nucleotide variant in the breast cancer, Para. [0018]; nucleic acids isolated from a sample of blood or urine or a fraction thereof from a patient, Para. [0003]), the method comprising: (a) enriching for the plurality of target cfRNA molecules, or cDNA molecules thereof, to produce an enriched sample of polynucleotides (the sample is enriched for cancer cells or for DNA or RNA from cancer cells, Para. [0533]; levels of total or particular ... cfRNA, Para. [0311]; wherein a higher relative allele frequency compared to the other single nucleotide variants of the plurality of single nucleotide variant loci is indicative of a clonal single nucleotide variant in the breast cancer, Para. [0018]; nucleic acids isolated from a sample of blood or urine or a fraction thereof from a patient, Para. [0003]); and (b) sequencing the polynucleotides of the enriched sample, or amplification products thereof (a SNV amplification/sequencing reaction is performed on one or more tumor samples from the individual, Para. [0207]; the sample is enriched for cancer cells or for DNA or RNA from cancer cells, Para. [0533]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.