PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

LOVEJOY Brett, A.
Morgan, Lewis & Bockius LLP
One Market, Spear Street Tower
San Francisco, CA 94105
United States of America

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	16 Mar 2020
Applicant's or agent's file reference 1184965006WO	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2019/063551	27 Nov 2019	29 Nov 2018

International Patent Classification (IPC) or both national classification and IPC
IPC (20200101) C12Q 1/6876 C12Q 1/6874 G16B 30/20
CPC (20180501) C12Q 1/6876 C12Q 1/6874 G16B 30/20

Applicant
XGENOMES CORP.

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized officer
Israel Patent Office Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel Email address: pctoffice@justice.gov.il	16 Mar 2020	MAZEL Alexander Telephone No. 972-73-3927174

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/063551

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   [X] the international application in the language in which it was filed.

   [ ] a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. [X] This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(b))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. [ ] forming part of the international application as filed:

      [ ] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2019/063551

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	2,3,19-21,25,26,28-41,43,44,46,49-54	YES
	Claims	1,4-18,22-24,27,42,45,47,48,55,56	NO
Inventive step (IS)	Claims		YES
	Claims	1-56	NO
Industrial applicability (IA)	Claims	1-56	YES
	Claims		NO

2. Citations and explanations:

2.1 Reference is made to the following documents:

D1: US6221592 (WISCONSIN ALUMI RESEARCH FOUNDATION), 24 April 2001 (2001/04/24).

D2: US2018327829 (Mir Kalim U), 15 November 2018 (2018/11/15).

D3: WO2004070005 (454 CORPORATION, ; CHEN, YI-JU, ; LEAMON, JOHN, H, ; LOHMAN, KENTON, ; RONAN, MICHAEL, T, ; SRINIVASAN, MAITHREYAN, ; ROTHBERG, JONATHAN, ; WEINER, MICHAEL), 19 August 2004 (2004/08/19).

D4: Levy-Sakin, M., & Ebenstein, Y. (2013). Beyond sequencing: optical mapping of DNA in the age of nanotechnology and nanoscopy. *Current opinion in biotechnology*, *24*(4), 690-698.

2.2 Claim 1 relates to a method of sequencing a single elongated target polynucleotide molecule comprising:
a) seeding a plurality of separately resolvable origins of polynucleotide synthesis along the single, elongated target polynucleotide molecule;
b) contacting the target polynucleotide molecule with a polymerase and labeled nucleotides;
c) incorporating a labeled nucleotide, using the polymerase, into a plurality of sequence fragments complimentary to the target polynucleotide molecule in a template-directed reaction originating from the origins of polynucleotide synthesis;
d) detecting and storing in computer memory respective identity and positions of the labeled nucleotide incorporated into each of the plurality of sequence fragments; and
e) repeating steps (c) and (d) until a threshold fraction of adjacent sequence fragments merge and result in continuous sequence reads spanning two or more adjacent sequence fragments.

Independent claim 42 relate to a method of obtaining a long-contiguous sequencing read comprising
obtaining a first short read;
obtaining a second short read adjacent to the first read;
obtaining further short reads adjacent to the first and / or second short read; and
stitching at least two short reads together to obtain a contiguous long read.

Independent claims 55 and 56 relate to methods of sequencing target polynucleotides, wherein said methods are variations of the method set forth in said claim 1.

Dependent claims 40-41 relate to methods of haplotype resolved sequencing, said methods based on the method of present claim 1. Dependent claims 2-39 and 43-54 detail inter alia about target polynucleotides, preparation & disposing thereof; labeled nucleotides; origins of polynucleotide synthesis and obtaining & seeding thereof; and optical & computational means to detect identity & positions of incorporated labeled nucleotides to merge them in continuous sequence read.

2.3 Novelty

2.3.1 D1 (whole document, for example abstract; column 20, lines 18-20) discloses a method for optical sequencing of an elongated single target nucleic acid molecule, wherein numerous loci are simultaneously investigated on the same molecule. Claims 48 and 49 of D1 disclose determining the nucleotide sequence of an individual nucleic acid, said determining inter alia includes: (1) elongating and fixing the nucleic acid molecule onto a surface; (2) annealing at least one primer to said nucleic acid molecule; (3) exposing the nucleic acid molecule annealed with primer(s) to polymerase that produces a primer(s) extension products(s) by addition of labeled nucleotides; (4) imaging said nucleic acid molecule to produce an image and to detect presence the labeled nucleotide in the primer(s) extension product(s). Claims 19 of D1 discloses a method for determining a nucleotide sequence of an individual double stranded (ds) nucleic acid molecule, said method inter alia comprises (1) nicking the double stranded nucleic acid molecule elongated and fixed onto a surface; (2) extending the nicked strand by adding nucleotide(s) comprising a label to 3' terminus of the nicked site; and (3) imaging the added label. According to D1 (column 15, lines 35-50), the steps of said methods are repeated a desired number of times to determine the nucleotide sequence of the nucleotide acid molecule. According D1 (column 9, lines 51-67; column 34, lines 19-48), computer analysis of images of the labeled nucleotides is performed in order to locate addition of said labeled nucleotides along the backbone of the nucleic acid molecule that is stretched and immobilized on a surface and to obtain the nucleotide sequence of said nucleic acid molecule. The framework of the analysis is centered on the accumulation of fluorescence intensities at addition sites, or "spot" histories, as a function of position, (x, y), and addition cycle, I(s). This scheme is outlined in figure 5. Positional data of fluorescence intensities accumulated after each cycle are used to link labeled nucleotide additions for a given nick, or gap site. Each target nucleotide molecule contains 10-20 nick sites, varying in the size of the target molecule and the frequency of nick sites. D1 (see for example column 32, lines 50-67; column 77 (line 40) - column 78 (line 56)) reveals optical systems that are used for imaging as well as algorithms and approaches for computer analysis of the obtained images. Said computer analysis implies storing in computer memory of identity and position of the added labeled nucleotides. Moreover, D1 (column 58, lines 10-23) explicitly discloses that a computer equipment used for analysis of the images comprises a RAM memory. According D1, the assembling the addition sites of the labeled nucleotides along nucleic acid molecule backbone and verification and eventual assembly of the finished sequence is an integral part of the image analysis and processing (column 34, line 30 - column 35, line 7). According to some embodiments of D1 (column 66, lines 42-57), the sequence of target nucleic acid obtained by said methods is compared to known (i.e. reference) sequence. Thus, D1 discloses or implies all traits of the present claims 1, 7, 12, 14, 15, 23, 24, 27, 42, 55 and 56.

In addition, D1 discloses the following features of the present dependent claims. According

claim 23 of D1, the method of claim 19 further comprises opening the nicked sites on the double stranded nucleic acid molecule. According to D1 (column 84, lines 8-20), the origins of polynucleotide synthesis are separated by 3-6 kb. According to claim 50 of D1, the added nucleotides comprise four bases and four labels. Moreover, according to description of D1 (column 16, lines 33-50), the different bases are labeled by different fluorochromes with different spectral characteristics so that types of nucleotide bases are identified simultaneously according to the different fluorescence image. D1 perform experiments on intact DNA molecules and chromosomes extracted from cells (see column 28, lines 15-25; column 67 lines 50-55). D1 (column 75, lines 38-41) teaches to perform the invention with single-stranded templates and random hexamer primers. According D1 (column 22, lines 45-57) nucleic acid molecules from 300 bp to mammalian chromosome size (greater than 1000 kb) are used.

Thus, D1 also discloses or implies each and every trait of the present claims 4, 6, 8-11, 13, 16, 18 and 22.

2.3.2 D2 (claims 1, 7, paragraph [601]) relates to a method for template-directed sequencing-by-synthesis of an array of target polynucleotide extracted from cells, the method comprising:

(a) providing an array of target polynucleotides in a fluidic vessel;

(b) contacting the array of polynucleotides with a solution comprising (i) polymerization complex and (ii) reversibly terminating and differently labeled A,C,G, and T/U nucleotides;

(c) incorporating one of the differently labeled nucleotides, using the polymerization complex, into a chain complementary to at least one of the array of polynucleotides;

(d) binding imaging tags to the differently labeled nucleotides of step (c);

(e) imaging and storing the identity and position of the imaging tags of step (d);

(f) reversing termination (b)-(e);

(g) repeating steps (b)-(e) and assembling a sequence for each of the array of target polynucleotides from the stored identity and position of the imaging tags.

According claims 6-8 of D2, the method further comprises seeding the incorporation at multiple locations on the stretched and elongated target polynucleotide disposed on a surface. According paragraph [0161] of D2, said surface has 3D gel architecture. Super-resolution imaging methods such as STORM or DNA PAINT are used for localization of the labeled nucleotides (see D2, claims 16, 20). According to D2 (description, paragraph [[0382]), the method is applied to a single long polynucleotide such as 100 kbs or longer length of genomic DNA. According paragraphs [0289] and [0495] of D2, the method is carried out wherein the target polynucleotide is placed into nano- or micro-channels.

Thus, D2 discloses or implies each and every feature of the present claims 1, 4-10, 13-17, 45, 47 and 48.

2.3.3 Therefore, the subject-matter of claims 1, 4-18, 22-24, 27, 42, 45, 47, 48, 55 and 56 is not novel and therefore does not comply with PCT Article 33(2).

2.3.4 The subject-matter of claims 2, 3, 19-21, 25, 26, 28-41, 43, 44, 46, 49-54 is novel and therefore complies with PCT Article 33(2).

2.4 Inventive step

2.4.1 As the present claims 1, 4-18, 22-24, 27, 42, 45, 47, 48, 55 and 56 are lack of novelty, no inventive step can be acknowledged for them.

2.4.2 Additional features of the dependent claims 19-21, 40, 41 and 49-51 are obvious to the skilled person in light of combination of D3 with D1.

2.4.2.1 D3 (claim 1) relates to a method of sequencing a nucleic acid molecule comprising the steps of : (a) hybridizing two or more sequencing primers to a single strands of the nucleic acid molecule, wherein all the primers except for one are reversibly blocked primers; (b) incorporating at least one base onto the nucleic acid molecule by polymerase elongation from an unblocked primer; (c) preventing further elongation of said unblocked primer (d) deblocking one of the reversibly blocked primers into an unblocked primer; (e) repeating steps (b) to (d) until at least one of the reversibly blocked primers are deblocked and used for determining a sequence. According embodiments of D3 (see for example page 8, line 22 - page 9, line 2) more than ten sequencing primers are simultaneously hybridized to the sequenced nucleic acid molecule. All said primers are reversibly blocked except one. An elongation is performed from the unblocked primer in presence of a polymerase and dNTPs. After the elongation from the unblocked primer is completed or terminated, one of the blocked primers is unblocked and the sequencing from the second primer proceeds. The process is repeated until all blocked primers are unblocked and used in the sequencing. The sequence is determined by conventional sequencing methods. Thus, the process of nucleic acid sequencing of D3 shares many traits with the DNA sequencing methods set forth in the present claim 1 and D1.

2.4.2.2 The process of sequencing of D3 is described and depicted in further details at page 9 (line 24) - page 10 (line 11). Said details imply that the process of sequencing results in adjacent sequenced fragments of the target nucleic acid molecule, said adjacent fragments mutually abutting or overlapping. Moreover, D3 (page 8, lines 12-21) explicitly teaches about overlaps of 50 bases between the fragments. Thus, D3 discloses or implies additional traits of the present dependent claims 19-21 in a context of the sequencing method that is similar to the methods set forth in the present claim 1 and D1. Therefore, the present claims 19-21 are trivial and obvious to the skilled person in light of combination of D1 and D3.

2.4.2.3 D3 (page 18, lines 3-11) teaches to use the method for sequencing nucleic acid molecule for determining a molecular haplotype of a DNA sample at multiple loci. In light of similarity the method for nucleic acid sequencing of D3 to the method set forth in D1, the possibility to use the method of sequencing set forth in D1 for haplotype resolving as set forth in the present claims 40 and 41 is trivial and obvious to the skilled person. Therefore, the present claims 40 and 41 are not inventive in light of combination D1 and D3.

2.4.2.4 According to D3 (page 13 (line 23) - page 14 (line 25); figure 1 (B-E)), prior to DNA sequencing the strands of the target nucleotide acid molecule are separated, attached via oligonucleotide linkers to a matrix (beads) and amplified using primers associated with said beads. Thus, D3 discloses or implies additional traits of the present dependent claims 49-51 in context of the sequencing method that is similar to the methods set forth in the present claim 1 and D1. Therefore, in light of combination of D1 and D3, the present claims 49-51 are trivial and obvious to the skilled person. Moreover, the skilled person has motivation to use said teachings of D3 in context of the method set forth in D1, since advantages of application of said teachings to the method of D1 are obvious to the skilled person. Thus, combination of D1 and D3 takes away inventiveness of the present claims 49-51.

2.4.3 D4 (whole document, especially abstract; page 694, left column (first full paragraph - left

column, first paragraph); figures 2 and 3) teaches that optical mapping is useful complement to next generation sequencing in order to perform assembly of genomic DNA and haplotype resolution. According D4, optical mapping includes inter alia labeling epigenetic marks such DNA methylation. D4 implies that labeling of epi-mark is orthogonal to the labeling used for DNA sequencing. D4 teaches that that epi-marks are resolved using super-resolution, e.g. STORM.

In light of D4 it is obvious and trivial for the skilled person to complement the method for DNA sequencing disclosed in D1 with analysis of epi-marks by super-resolution methods. Therefore, the combination of D1 and D4 takes away inventiveness of the present claims 53 and 54.

2.4.4 The additional features of the dependent claims 2, 3, 25, 26, 28-39, 43, 44, 46 and 52 do not confer an inventive step to said claims because said features are trivial and obvious to the skilled person.

2.4.4 Thus, the subject-matter of claims 1-56 does not involve an inventive step and therefore does not comply with PCT Article 33(3).

2.5. Industrial applicability

The subject-matter of the present claims 1-56 is considered to be industrially applicable under Article 33(4) PCT.