# PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:

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# PCT

NOTIFICATION OF DECISION CONCERNING REQUEST FOR RECTIFICATION

(PCT Rule 91.3(a) and (d))

| | |
|---|---|
| Date of mailing *(day/month/year)* | 02 Feb 2020 |
| Applicant's or agent's file reference<br>1184965006WO | **REPLY DUE** **NONE**<br>However, see last paragraph below |
| International application No.<br>PCT/US2019/063551 | International filing date *(day/month/year)* 27 Nov 2019 |
| Applicant<br>XGENOMES CORP. | |

The applicant is hereby notified that this International Searching Authority has considered the request for rectification of obvious mistakes in the international application/in other documents submitted by the applicant to this Authority, and has decided:

1. a. ☐ to authorize the rectification:

   ☐ as requested by the applicant.

   ☐ to the extent set forth below*:

   b. ☐ The rectification **will be or has been taken into account** for the purposes of the international search (Rule 43.6*bis*(a)).

   ☐ The rectification **has not been taken into account** because it was authorized by this Authority after this Authority has begun to draw up the international search report (Rule 43.6*bis*(b)).

2. ☒ to refuse to authorize the rectification or part of it for the following reasons*:

   The numbering of the claims 1a-b, 2a, 3a-b, 19b, 19d is not numbered in consecutive Arabic numerals. Need to fixed.

A copy of this notification, together with a copy of the applicant's request for rectification, has been sent to the receiving Office and to the International Bureau.

* **If the authorization of the rectification has been refused in whole or in part**, the applicant may request the International Bureau, within two months from the date of the refusal and subject to the payment of a special fee, to publish the request for rectification and the reasons for refusal by this Authority and any further brief comments that may be submitted by the applicant together with the international application. See Rule 91.3(d) and, for the amount of the fee, see the *PCT Applicant's Guide*, Volume I, Annex B2(IB).

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|---|---|
| Name and mailing address of the ISA/<br>Israel Patent Office<br>Technology Park, Bldg.5, Malcha, Jerusalem, 9695101, Israel<br>Email address: pctoffice@justice.gov.il | Authorized officer<br>TSODIKOV David<br>DavidTs@justice.gov.il<br>Telephone No. 972-73-3927317 |

# PATENT COOPERATION TREATY

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| In re Application of: | XGENOMES CORP. |
| International Application No.: | PCT/US2019/063551 |
| International Filing Date: | 27 NOVEMBER 2019 |
| Title: | SEQUENCING BY COALESCENCE |

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## REQUEST FOR RECTIFICATION OF OBVIOUS ERRORS IN THE INTERNATIONAL APPLICATION UNDER RULE 91.1

Staff:

The US/RO has found a discrepancy in the numbering of the claims as filed in the subject International Application. A Rectification of Obvious Errors under Rule 91.1 is therefore respectfully requested.

The claims as filed were incorrectly numbered 1-47 and 50-51 (numerals 48 and 49 were inadvertently skipped). Applicant hereby submits a new set of claims correctly numbered 1-49. No claims have been deleted and new matter has been added by way of this correction.

Dated **January 7, 2020**

Respectfully submitted,

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Attorney Docket: 1184965006WO
PCT/US2019/063551
CORRECTED CLAIMS

**What is claimed:**

1. 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 complementary to the target polynucleotide molecule in a template-directed reaction originating from the origins of polynucleotide synthesis;

(d) detecting and storing in computer memory the 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.

1a: A method according to 1 where (the threshold fraction is low and) gaps remain, such gaps are filled by other polynucleotides that have been sequenced, where the same gaps are not present.

1b: A method according to 1 where (the threshold fraction is high and) negligible number of gaps remain, a substantially complete genome sequence is obtained without sequencing of other polynucleotides.

2. The method of claim 1 where step (b) comprises simultaneously contacting the target polynucleotide molecule with a polymerase and four types of differently labeled nucleotides comprising A, C, G, and T/U.

2a. The method of claim 1 and 2 where the nucleotides are reversible terminators and identifying the identity and positions of the labeled nucleotide is via detecting a signal from the labeled nucleotide and repeating of step b or c is preceded by reversing the termination.

3. The method of claim 1 where step (b) comprises contacting the target polynucleotide molecule with a polymerase and a single type of labeled nucleotide selected from the group consisting of A, C, G, and T/U.

3a. The method of claim 3 where the incorporation of the nucleotide is detected by detecting a spatially resolvable signal.

3b. The method of claim 3a where the spatially resolvable signal is due to one or more labels on the polymerase or nucleotide.

4. The method of claim 1, wherein the single target polynucleotide is a chromosome.

5. The method of claim 1, wherein the single target polynucleotide is about $10^2$, $10^3$, $10^4$, $10^5$, $10^6$, $10^7$, $10^8$ , $10^9$ bases in length.

6. The method of claim 1, wherein the single target polynucleotide is single stranded.

7. The method of claim 1, wherein the single target polynucleotide is double stranded.

8. The method of claim 1, further comprising extracting the single target polynucleotide molecule from a cell, organelle, chromosome, virus, exosome or body fluid or substance with minimal degradation.

9. The method of claim 1, wherein the target polynucleotide molecule is stretched.

10. The method of claim 1, wherein the target polynucleotide molecule is immobilized on a surface.

11. The method of claim 1, wherein the target polynucleotide molecule is disposed in a gel.

12. The method of claim 1, wherein the target polynucleotide molecule is disposed in a micro- or nano- fluidic channel.

13. The method of claim 1, wherein the target polynucleotide molecule is substantially intact.

14. The method of claim 1, wherein the merging of the adjacent sequence fragments comprises an overlap of at least 5 bases between the adjacent sequence fragments.

15. The method of claim 1, wherein the merging of the adjacent sequence fragments is determined by the relative positions of the adjacent sequence fragments abutting and/or overlapping.

16. The method of claim 1, wherein the merging of the adjacent sequence fragments is determined by the sequences of the adjacent sequence fragments overlapping.

17. The method of claim 1, wherein the adjacent separately resolvable origins of polynucleotide are separated by about 10, 50, 100, 250, 500, 750, 1,000, 5,000, or 10,000 bases.

18. The method of claim 1, wherein the adjacent separately resolvable origins of polynucleotide comprise natural sequences of the target polynucleotide.

19. The method of claim 1, wherein the adjacent separately resolvable origins of polynucleotide comprise (the 3' of) synthetic (origin-related) sequences annealed to the target polynucleotide.

19b. The method of claim 1, wherein the adjacent separately resolvable origins of polynucleotide synthesis comprise synthetic (origin-related) sequences incorporated/inserted into the target polynucleotide (e.g. via transposase).

19d: The method of claim 19c where the inserted sequence includes an indexing sequence adjacent to the origin-related sequence.

20. The method of claim 1, further comprising:

(f) ascertaining and storing the positions of the first and second locations in a computer memory;

(g) storing the position and identity of the differently labeled nucleotides incorporated into the first sequence fragment and the second sequence fragment in step (e); and

(h) ascertaining when the first and second sequence fragments coalesce and assembling the stored identity of the differently labeled nucleotides, thereby sequencing the single target polynucleotide.

21. The method of claim 19, further comprising computationally trimming an overlapping segment of adjacent sequence fragments.

22. The method of claim 1, further comprising:

(f) seeding a second plurality of separately resolvable origins of polynucleotide synthesis along the single, elongated target polynucleotide molecule;

(g) contacting the target polynucleotide molecule with the polymerase and labelled nucleotides;

(h) incorporating the labelled nucleotides, using the polymerase, into a second plurality of sequence fragments complementary to the target polynucleotide molecule, in a template-directed reaction and originating from the second plurality of separately resolvable origins of polynucleotide synthesis;

(i) identifying and storing the identity and positions of the labelled nucleotides incorporated into each of the second plurality of sequence fragments, thereby determining the sequences and relative positions of the second plurality of sequence fragments;

(j) repeating steps (g), (h) and (i) until a second threshold fraction of adjacent sequence fragments merge and result in continuous sequence reads spanning two or more adjacent sequence fragments; and

(k) combining the sequence reads from steps (e) and (j), thereby sequencing the target polynucleotide molecule.

23. The method of claim 1, wherein the sequence is determined without using another copy of the target polynucleotide molecule or reference sequence for the target polynucleotide molecule.

24. The method of claim 1, further comprising computationally trimming an overlapping segment of adjacent sequence fragments.

25. The method of claim 1, further comprising: (f) repeating steps (c) and (d) until a threshold fraction of adjacent sequence fragments overlap and result in redundant sequence reads spanning two or more adjacent sequence fragments.

26. The method of claim 24, further comprising: (g) identifying any inconsistencies in the redundant sequence reads as potential sequencing errors or ambiguities.

27. The method of claim 1, further comprising:
(f) degrading at least a fraction of the plurality of sequence fragments; and
(g) repeating steps (c) and (d), thereby resequencing the plurality of sequence fragments.

28. The method of claim 27, wherein a 3' to 5' exonuclease is used to degrade the fraction of the plurality of sequence fragments and optionally the degradation stops at the origin.

29. The method of claim 27, wherein the differently labeled nucleotides are degradable nucleotides

30. The method of claim 29, wherein the degradable nucleotides are 5' amide modified nucleotides and are cleaved by acid.

31. The method of claim 29, wherein the degradable nucleotides are RNA and are cleaved by an RNAse and/or alkali.

32. The method of claim 29, wherein the degradable nucleotides are RNA and further comprising the steps of:
(f) degrading at least one of the degradable nucleotides to leave an abasic site or nick; and
(g) repeating step (c) using the abasic site or nick as an origin of polynucleotide synthesis.

33. A method of haplotype resolved sequencing comprising:
sequencing a first target polynucleotide spanning a haplotype of a diploid genome using the method of claim 1;
sequencing a second target polynucleotide spanning a haplotype of the diploid genome using the method of claim 1,
wherein the first and second target polynucleotides are from different
homologous chromosomes (chromosome homologues); and
thereby determining the haplotypes on the first and second target polynucleotides.

34. A method of haplotype resolved sequencing of a polyploid genome comprising:
sequencing a first target polynucleotide spanning a first haplotype of a polyploid genome using the method of claim 1;
sequencing a second target polynucleotide spanning a second haplotype of the polyploid genome using the method of claim 1;
sequencing further target polynucleotide spanning further haplotypes of the polyploid genome using the method of claim 1
wherein the first and second and further target polynucleotides are from different homologous chromosomes (chromosome homologs); and thereby determining the first, second, and further haplotypes of the polyploid genome.

35. 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.

36. A method according to 35 where some of the reads are obtained from different polynucleotide molecules

37. A method according to 36 where some of the reads from different polynucleotides overlap sufficiently for the sequence of the different molecules to be aligned.

38. A method according to any of the previous claims where the reads are generated by identifying and storing the identity and positions of the labeled nucleotide incorporated into each of the plurality of sequence fragments by using super-resolution/single molecule localization.

39. A method according to claim 38 where the super-resolution/localization is virtual, and comprises using a reference sequence to assign unresolved signals from multiple origins to the correct origins.

40. A method according to claim 38 40 wherein the super-resolution single molecule localization is done via Stochastic Optical Reconstruction Microscopy (STORM), Super-resolution optical fluctuation imaging (SOFI), Microscopy or Points Accumulation for Imaging in Nanoscale Topography (PAINT) or other high resolution or nanometric localization method.

41. A method according to claim 40 where the PAINT comprises DNA PAINT

42. A method according to any one of claims 1-39 where the segments of the elongated polynucleotide that are sequenced are amplified in situ before sequencing.

43. A method according to claim 42 where the amplification occurs using the origin-related sequences inserted in 19b as primer binding sites or promoters.

44. A method according to previous claims where the target polynucleotides are contacted with a gel or matrix layer.

45. A method according to claim 1 where the origins are seeded, in close to random manner by incubating double stranded DNA with Nt.CViPII or derivatives.

46. A method according to claim 1 where sequencing is combined with analysis of epi-marks (e.g. methylation) by the of labeling of epi-marks orthogonally to sequencing.

47. A method according to claim 46 wherein the epi-marks are labeled such that they can be super-resolved or subjected to single molecule localization (e.g. by DNA PAINT).

48. A method of sequencing a target polynucleotide molecule comprising:

(a) seeding a plurality of separately resolvable origins of polynucleotide synthesis along each of a plurality of copies of the target polynucleotide molecule;

(b) contacting the plurality of copies with a polymerase and four types of differently labelled nucleotides simultaneously;

(c) incorporating the differently labelled nucleotides, using the polymerase, into a plurality of sequence fragments complementary to the target polynucleotide molecule and originating from the origins of polynucleotide synthesis;

(d) identifying and storing the identity and positions of the differently labelled nucleotides incorporated into each of the plurality of sequence fragments, thereby determining the sequences and relative positions of the plurality of sequence fragments;

(e) repeating steps (c) and (d) until a threshold number of nucleotides are sequenced; and

(f) assembling the plurality of sequence fragments, thereby determining the sequence of the elongated, target polynucleotide molecule.

49. A method of sequencing a single, elongated target polynucleotide molecule comprising:

(a) seeding a plurality of separately resolvable origins of polynucleotide synthesis along the target polynucleotide molecule;

(b) contacting the target polynucleotide molecule with a polymerase and four types of differently labelled nucleotides simultaneously;

(c) incorporating the differently labelled nucleotides, using the polymerase, into a plurality of sequence fragments complementary to the target polynucleotide molecule and originating from the origins of polynucleotide synthesis;

(d) identifying and storing the identity and positions of the differently labelled nucleotides incorporated into each of the plurality of sequence fragments, thereby determining the sequences and relative positions of the plurality of sequence fragments; and

(e) repeating steps (c) and (d) until a threshold number of nucleotides are sequenced; and

(f) comparing the sequences and relative positions of the plurality of sequence fragments to a reference sequence for the target polynucleotide molecule, thereby ascertaining any differences in sequence and/or structure between the target polynucleotide and the reference sequence.