

sequencing of one molecule. Bioinformatic procedures can be used to detect the different DNA fragments that are part of a same concatemer. Examples can also combine the two techniques.

[0005] Examples may include a method of determining a nucleic acid sequence. The method may include receiving a plurality of DNA fragments. The method may also include

5 concatemerizing a first set of the DNA fragments to obtain a first concatemer. The method may include performing single-molecule sequencing of the first concatemer to obtain a first sequence of the first concatemer. In some Examples, single-molecule sequencing may be performed using a nanopore, and the method may include passing the first concatemer through a first nanopore. A first electrical signal may then be detected as the first concatemer passes through the first
10 nanopore. The first electrical signal may correspond to a first sequence of the first concatemer.

[0005a] In one aspect, the present invention provides a method comprising: receiving a biological sample obtained from a subject, the biological sample including a bodily fluid comprising cell-free DNA fragments; extracting a plurality of the cell-free DNA fragments from the biological sample; concatemerizing a first set of the plurality of the cell-free DNA fragments
15 to obtain a first concatemer, wherein the cell-free DNA fragments of the first set have different sequences and are from different locations in a genome of the subject; and performing single-molecule sequencing of the first concatemer to obtain a first sequence of the first concatemers, the first sequence including the different sequences of the first set of the plurality of the cell-free DNA fragments as subsequences within the first sequence.

20 [0006] Other examples may include a method of determining a nucleic acid sequence. The method may include receiving a plurality of DNA fragments. A first set of DNA fragments may be concatemerized to obtain a first concatemer. A fluorescent-labeled nucleotide may be hybridized to the concatemer. A first fluorescent signal may be detected, with the first fluorescent signal corresponding to a specific nucleotide. The fluorescent label may then be
25 cleaved away and another fluorescent-labeled nucleotide may be added, and the process may be repeated.

[0007] Examples may include a method performed by a computer system. The method may include receiving a first sequence of a first concatemer generated by concatemerizing a first set of DNA fragments. The method may also include aligning subsequences of the first sequence to

identify fragment sequences corresponding to each DNA fragment of the first set of the plurality of the cell-free DNA fragments.

[0010b] In yet another aspect, the present invention provides a computer product comprising a computer readable medium storing a plurality of instructions for controlling the computer system to perform an operation disclosed herein.

[0011] Other examples are directed to systems, portable consumer devices, and computer readable media associated with methods described herein.

[0011a] In yet another aspect, the present invention provides a system comprising: the computer product disclosed herein; and one or more processors for executing instructions stored on the computer readable medium.

[0011b] In yet another aspect, the present invention provides a system comprising means for performing the method as disclosed herein.

[0011c] In yet another aspect, the present invention provides a system configured to perform the method as disclosed herein.

[0011d] In yet another aspect, the present invention provides a system comprising modules that respectively perform the steps as disclosed herein.

[0012] A better understanding of the nature and advantages of examples of the present invention may be gained with reference to the following detailed description and the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] **FIG. 1A** shows a simplified diagram of a nanopore device and a nucleic acid according to embodiments of the present invention.

[0014] **FIG. 1B** illustrates the process of concatemerizing DNA fragments according to embodiments of the present invention.

WHAT IS CLAIMED IS:

1. A method comprising:
receiving a biological sample obtained from a subject, the biological sample including a bodily fluid comprising cell-free DNA fragments;
5 extracting a plurality of the cell-free DNA fragments from the biological sample;
concatemerizing a first set of the plurality of the cell-free DNA fragments to obtain a first concatemer, wherein the cell-free DNA fragments of the first set have different sequences and are from different locations in a genome of the subject; and
performing single-molecule sequencing of the first concatemer to obtain a first
10 sequence of the first concatemer, the first sequence including the different sequences of the first set of the plurality of the cell-free DNA fragments as subsequences within the first sequence.
2. The method of claim 1, further comprising:
providing the first concatemer to a sequencing device as part of performing the single-molecule sequencing; and
detecting, using the sequencing device, a plurality of signals corresponding to the first concatemer, the plurality of signals corresponding to the first sequence of the first concatemer.
3. The method of claim 2, wherein the sequencing device includes a first nanopore, the method further comprising:
passing the first concatemer through the first nanopore; and
detecting first electrical signals as the first concatemer passes through the first nanopore, the first electrical signals corresponding to the first sequence of the first concatemer, wherein the plurality of signals includes the first electrical signals.
4. The method of claim 3, further comprising performing, by a computer system:
analyzing the first electrical signals to determine the first sequence; and
aligning subsequences of the first sequence to a reference genome to identify
fragment sequences corresponding to each of the first set of the plurality of the cell-free DNA fragments.
5. The method of claim 3, wherein passing the first concatemer through the first nanopore includes:

passing a first strand of the first concatemer through the first nanopore; and subsequently, passing a second strand of the first concatemer through the first nanopore.

6. The method of claim 5, further comprising analyzing, by a computer system, the first electrical signals for the first strand and the second strand to determine the first sequence.

7. The method of claim 3, wherein the first nanopore is one of a plurality of nanopores on a substrate.

8. The method of claim 7, further comprising:
concatemerizing a second set of the plurality of the cell-free DNA fragments to obtain a second concatemer;
passing the second concatemer through a second nanopore of the plurality of nanopores; and
detecting a plurality of second electrical signals as the second concatemer passes through the second nanopore, the second electrical signals corresponding to a second sequence of the second concatemer.

9. The method of claim 2, further comprising:
hybridizing a fluorescent-labeled nucleotide to the first concatemer, and
detecting a fluorescent signal, the fluorescent signal corresponding to a nucleotide.

10. The method of claim 1, further comprising performing, by a computer system:
aligning subsequences of the first sequence to a reference genome to identify fragment sequences corresponding to each of the first set of the plurality of the cell-free DNA fragments.

11. The method of claim 10, further comprising:
determining a size of each of the first set of the plurality of the cell-free DNA fragments based on the aligning of the subsequences.

12. The method of claim 10, wherein:

concatemerizing the first set of the plurality of the cell-free DNA fragments comprises concatemerizing the first set of the plurality of the cell-free DNA fragments and a second set of the plurality of the cell-free DNA fragments having known sequences, wherein the second set of the plurality of the cell-free DNA fragments are interspersed among the first set of the plurality of the cell-free DNA fragments, and

aligning subsequences of the first sequence comprises aligning subsequences to the known sequences to identify locations of the second set of the plurality of the cell-free DNA fragments in the first concatemer.

13. The method of claim 12, wherein the concatemerizing places one DNA fragment of the second set of the plurality of the cell-free DNA fragments between two DNA fragments of the first set of the plurality of the cell-free DNA fragments.

14. The method of claim 13, further comprising performing, by the computer system:

identifying, by the computer system, a starting base of a first subsequence corresponding to a first DNA fragment of the first set of the plurality of the cell-free DNA fragments based on identifying one of the known sequences of the second set of the plurality of the cell-free DNA fragments being before the first subsequence; and

identifying, by the computer system, an ending base of the first subsequence corresponding to the first DNA fragment of the first set of the plurality of the cell-free DNA fragments based on identifying one of the known sequences of the second set of the plurality of the cell-free DNA fragments being after the first subsequence.

15. The method of claim 12, wherein each DNA fragment of the second set of the plurality of the cell-free DNA fragments comprises a known sequence of less than or equal to 7 nucleotides.

16. The method of claim 12, wherein each of the second set of the plurality of the cell-free DNA fragments have a same known sequence.

17. The method of claim 10, wherein the aligning of the subsequences includes:

aligning sliding windows of the first sequence to the reference genome, each sliding window corresponding to a subsequence that is being aligned to the reference genome; and

identifying when two sliding windows align to different regions of the reference genome to distinguish between sequences of two DNA fragments of the first set of the plurality of the cell-free DNA fragments.

18. The method of claim 17, further comprising performing, by the computer system:

determine an ending or a beginning of a first subsequence of a first DNA fragment of the first set of the plurality of the cell-free DNA fragments based on the two sliding windows aligning to the different regions of the reference genome.

19. The method of claim 17, wherein the different regions of the reference genome comprise regions on different chromosomes.

20. The method of claim 17, wherein one or more windows between the two sliding windows are identified as not aligning to the reference genome.

21. The method of claim 20, further comprising determining a size of a first DNA fragment of the first set of the plurality of the cell-free DNA fragments, wherein determining the size of the first DNA fragment comprises:

determining a length of a longest subsequence that aligns to a single region of the reference genome.

22. The method of claim 1, wherein the biological sample is plasma or serum.

23. The method of claim 1, wherein the first set of the plurality of the cell-free DNA fragments are randomly distributed from a plurality of chromosomes.

24. A method comprising performing by a computer system:
receiving a first sequence of a first concatemer generated by concatemerizing a first set of DNA fragments, wherein
the first concatemer is obtained from a first set of a plurality of cell-free DNA fragments,
the plurality of the cell-free DNA fragments are extracted from a biological sample including bodily fluid, and
the biological sample is obtained from a subject; and
aligning subsequences of the first sequence to identify fragment sequences corresponding to each DNA fragment of the first set of the plurality of the cell-free DNA fragments.

25. The method of claim 24, wherein:
the first concatemer was generated by concatemerizing the first set of the plurality of the cell-free DNA fragments and a second set of the plurality of the cell-free DNA fragments, and
aligning subsequences of the first sequence comprises aligning subsequences to the second set of the plurality of the cell-free DNA fragments.

26. The method of claim 25, wherein each DNA fragment of the second set of the plurality of the cell-free DNA fragments comprise a known sequence of less than or equal to 7 nucleotides.

27. The method of claim 24, wherein aligning subsequences of the first sequence comprises aligning subsequences of the first sequence to a reference genome.

28. The method of claim 27, wherein the aligning of the subsequences includes:
aligning windows of the first sequence to the reference genome; and
identifying when two windows align to different regions of the reference genome.

29. The method of claim 28, wherein one or more windows between the two windows are identified as not aligning to the reference genome.

30. A computer product comprising a computer readable medium storing a plurality of instructions for controlling the computer system to perform an operation of any one of claims 24-29.

31. A system comprising:
the computer product of claim 30; and
one or more processors for executing instructions stored on the computer readable medium.

32. A system comprising means for performing of any one of claims 24-29.

33. A system configured to perform of any one of claims 24-29.

34. A system comprising modules that respectively perform the steps of any one of claims 24-29.