SINGLE-MOLECULE SEQUENCING OF PLASMA DNA

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 62/204,396, filed August 12, 2015, the contents of which are incorporated herein by reference for all purposes.

BACKGROUND

[0002] Noninvasive prenatal testing (NIPT) by maternal plasma DNA sequencing is now clinically available for screening fetal chromosomal aneuploidies (1). Unlike amniocentesis, maternal plasma DNA sequencing does not pose any miscarriage risk. These tests have close to 99% sensitivity and 99% specificity (1). Consequently, the clinical demand for NIPT has increased substantially since it first became commercially available in 2011.

[0003] Massively parallel sequencing is a core component of most of the currently used laboratory protocols for NIPT of chromosomal aneuploidies (2). Due to the high instrumentation cost, those tests are currently performed at reference laboratories. Oxford Nanopore Technologies has developed a nanopore-based DNA sequencing platform (3). Nanopore sequencers have a comparatively low equipment cost and have a small footprint. Each flow cell costs US$500-900 and can be used for multiple times for up to 48 hours. The sequencing speed is also relatively fast that can read 30 bases per second from each nanopore. Such features would be advantageous for use in clinical laboratories. But, current nanopore techniques are inefficient for samples (e.g., plasma) typically used for NIPT.

BRIEF SUMMARY

[0004] Examples improve the efficiency of single-molecule sequencing techniques when analyzing samples with relatively small DNA fragments. For example, a concentration of DNA fragments in a sample can be increased significantly, thereby allowing more DNA fragments to interact with a sequencing device (e.g., a nanopore). As another example, DNA fragments are combined into concatemers to make longer molecules that can be read, thereby allowing multiple DNA fragments (i.e., DNA fragments originally in the sample) to be efficiently read via the

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 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 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 plurality of DNA fragments; concatemerizing a first set of the plurality of DNA fragments to obtain a first concatemer; and performing single-molecule sequencing of the first concatemer to obtain a first sequence of the first concatemers.

[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 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 DNA fragments.

[0008] Some examples may include a method of sequencing cell-free DNA fragments. Cell-free DNA fragments may include plasma DNA fragments. The method may include receiving a biological sample including a plurality of DNA fragments. The biological sample may have a first concentration of DNA fragments. The method may also include concentrating the biological sample to have a second concentration of DNA fragments. The second concentration of DNA fragments may be 5 or more times higher than the first concentration of

DNA fragments. The method may further include passing the plurality of DNA fragments through nanopores on a substrate. For each of the plurality of DNA fragments, electrical signals may be detected as the DNA fragment passes through a nanopore. The electrical signals may correspond to the sequence of the DNA fragment.

[0009] Examples may include a method of sequencing cell-free DNA fragments. The method may include concentrating a biological sample to have a second concentration of DNA fragments 5 or more times higher than an initial concentration of DNA fragments. The method may further include a single-molecule sequencing technique. The DNA fragment may be hybridized with a fluorescent-labeled nucleotide. The method may also include detecting a signal from the fluorescent-labeled nucleotide, with the signal corresponding to the nucleotide. The fluorescent-labeled nucleotide may be cleaved off, and the process may be repeated to identify additional nucleotides and a sequence of the DNA fragment.

[0009a] In another aspect, the present invention provides a method comprising: receiving a biological sample including a plurality of DNA fragments, the biological sample having a first concentration of DNA fragments; concentrating the biological sample to have a second concentration of DNA fragments, the second concentration of DNA fragments being 5 or more times higher than the first concentration of DNA fragments; passing the plurality of DNA fragments through nanopores on a substrate; and for each of the plurality of DNA fragments: detecting electrical signals as the DNA fragment passes through a nanopore, the electrical signals corresponding to a sequence of the DNA fragment.

[0010] Examples may also include a computer product that includes a computer readable medium that stores a plurality of instructions to perform an operation of any of the methods of sequencing DNA described herein. Some examples may include a computer product and one or more processors for executing instructions stored on the computer readable medium. Additional examples include systems to perform any of the methods.

[0010a] In yet another aspect, the present invention provides 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; and aligning subsequences of the first sequence to

identify fragment sequences corresponding to each DNA fragment of a first set of the 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.