concentrations. Due to Poisson distribution, most of the beads and sample do not come together in an emulsion with a single bead and a single sample, and are thus lost. The cost of the beads is a substantial portion of the cost of the sequencing, and most of that cost is thrown away without ever generating any useful data. The current system enables utilization of virtually all of the sample, and the reuse of the beads, thus reducing the cost to the user.

[1004] Current DNA sequencing systems typically need whole genome amplification in order to have sufficient sample, as the sample is very inefficiently utilized. Such whole genome amplification methods typically introduce significant amounts of bias in amplification in different portions of the genome, and require higher levels of coverage to overcome said bias. Methods for localizing samples, and reagents into a volume wherein a desired reaction or binding may occur is another aspect which is envisioned for the system, which may eliminate or reduce the need for whole genome amplification, and thus reduce the coverage needed.

[1005] Thus, a need exists for improved systems and methods for extracting, amplifying and sequencing polynucleotides

Summary

[1006] The embodiments described herein relate to systems and methods for extracting, amplifying and sequencing polynucleotides. In some embodiments, the systems and methods can include a fully-automated, integrated platform, thereby reducing the cost, improving throughput and/or simplifying the methods of use.

[1006a] In a first aspect of the present disclosure, there is provided a method for sequencing a nucleic acid sample, the method comprising:

a. providing a plurality of particles, wherein each particle of said plurality is coupled to on average at most one nucleic acid molecule generated from said nucleic acid sample;

b. performing simultaneous nucleic acid amplification reactions on said nucleic acid molecules coupled to said particles, wherein said nucleic acid amplification reactions are performed while subjecting the particles to an electric field, thereby producing isolated, clonally-amplified nucleic acid molecules coupled to at least a subset of said plurality of particles;

c. performing a nucleic acid extension reaction using primers that hybridize to said clonally-amplified nucleic acid molecules;

d. measuring a local impedance change of each of said particles using a sensor array comprising a plurality of sensors to determine a sequence of each of said clonally-amplified nucleic acid molecules, when an individual sensor among said plurality of sensors comprises a pair of electrodes that are within the Debye length of a surface of the particle; and

e. based on the measured local impedance change, sequencing said nucleic acid sample.

[1007] ~~In one aspect, the~~ ~~invention~~present disclosure provides a method for isolating biological material, reactants, and/or reaction byproducts for a reaction, such as a nucleic acid amplification or sequencing reaction. The method comprises magnetically holding a bead carrying biological material (e.g., nucleic acid, which may be in the form of DNA fragments or amplified DNA) in a specific location of a substrate, and applying an electric field local to the bead to isolate the biological material or products or byproducts of reactions of the biological material. For example, the bead is isolated from other beads having associated biological material. The electric field in various embodiments concentrates reagents for an amplification or sequencing reaction, and/or concentrates and isolates detectable reaction by-products. For example, by isolating nucleic acids around individual beads, the electric field can allow for

clonal amplification, as an alternative to emulsion PCR. In other embodiments, the electric field isolates a nanosensor proximate to the bead, to facilitate detection of at least one of local pH change, local conductivity change, local charge concentration change and local heat. The beads may be trapped in the form of an array of localized magnetic field regions.

[1008] ~~In another aspect, the~~ inventionpresent disclosure also provides a method for conducting nucleic acid amplification and/or sequencing. The method comprises applying an electric field for confinement of a biological material to an environment, and conducting nucleic acid amplification and/or nucleic acid sequencing on the biological material. The confinement of the environment from an external environment via the electric field has the effect of isolating the biological material into a plurality of regions. The confinement creates a virtual well faciliating amplification and/or detection, and preventing contamination between virtual wells. In various embodiments, the biological material is associated with a plurality of beads, and the beads are held in place by a localized magnetic field in each of the plurality of regions. In certain embodiments, amplification within the virtual wells generates a clonal population of DNA associated with each of the beads, or on the surface of a sensor.

[1009] ~~In another aspect, the~~ inventionpresent disclosure further provides an automated method for separating a population of beads carrying amplified nucleic acids, from a population of beads not carrying amplified nucleic acids. The method comprises separating the populations of beads based on a charge associated with the beads. The separation may be implemented with electrophoresis. The bead separation may be based on a flow-through mechanism, and the beads may be reused in a subsequent amplification reaction, for example, by treating the beads so as to remove any amplified product and/or primer.

[1010] ~~In still other aspects, the~~ inventionpresent disclosure also provides a method for purifying DNA fragments from a biological material. The method comprises applying an electric field in a fluidic environment, said fluidic environment at least partially containing a filter medium. In this aspect, the electric field is adapted to separate a DNA fragment from a biological material as the biological material is conveyed through the filter medium. In various embodiments, the filter medium is a porous membrane or a medium providing a different mobility of the DNA fragments compared to a remainder of the biological material. Once purified, the DNA fragments can be used for DNA library construction, DNA amplification, DNA enrichment, and/or DNA sequencing, for example, using the methods and systems described herein.

[1011] ~~In yet another aspect, the invention~~present disclosure further provides a method for shearing DNA isolated from a biological material. The method comprises disposing a plurality of particles in a fluidic environment containing a population of DNA molecules, and causing flow of the particles in the fluidic environment to produce a shearing force on the DNA molecules in order to produce DNA fragments. In such embodiments, the shearing force produces blunt ends to aid in subsequent library construction.

[1012] ~~In another aspect, the invention~~present disclosure also provides a system for nucleic acid amplification and/or sequencing. The system comprises a substantially planar substrate coupled to a moiety for binding a nucleic acid to the substrate, and a means for separating the nucleic acid from the substrate such that the system is reusable for at least one of nucleic acid amplification and nucleic acid sequencing. During amplification, the system generates nucleic acid clones on the surface of the substrate. Amplification may involve either heating cycles or by isothermal amplification. In various embodiments, the system further comprises an instrument for detecting incorporation of a nucleotide in a sequencing reaction. The detection may be based on at least one of local pH change, local heat detection, local capacitance change and a local charge concentration and local conductivity change.

[1013] ~~In some aspects, the invention~~present disclosure further provides a system for detecting biological material or a biological reaction product or byproduct. The system comprises a substantially planar sensor array, the sensor array comprising a means for capturing a bead adjacent to each nanosensor in the array. The nanosensor is capable of detecting biological material or a biological reaction product or byproduct. The system further comprises a means for releasing a bead to facilitate reuse of the array, such as by magnetic, chemical, enzymatic means.

[1014] In some embodiments of the methods and systems described herein, an apparatus includes a substrate, a porous member and an electrode. The substrate defines a microfluidic channel configured to receive a sample. The porous member is disposed at least partially within the microfluidic channel. The electrode is configured to produce an electric field, and is coupled to the microfluidic channel such that at least a portion of the porous member may be disposed within the electric field. The porous member and the electric field may be cooperatively configured such that a nucleic acid may be separated from the sample when the sample is conveyed through the porous member.

[1015] In some embodiments, an apparatus includes a substrate, a plurality of particles and a flow mechanism. The substrate may define a microfluidic channel configured to receive a sample containing a plurality of DNA molecules. In other embodiments, the apparatus may be used as a probe and inserted into a well or other fluidic environment. The plurality of particles may be configured to be disposed within the microfluidic channel. The mechanism for producing the flow may be configured to produce a flow of the sample and the plurality of particles within the microfluidic channel such that the plurality of particles produces a shear force on the plurality of DNA molecules to produce a plurality of DNA fragments. In some embodiments, an on-chip peristaltic pump, made of multiple fluidic gates with orthogonal control and flow channels (Valve Technology), or an external pressure may generate the required flow in the channel.

[1016] The present ~~invention~~disclosure provides magnetic arrays and methods of using the magnetic arrays for polynucleotide amplification and sequence analysis, thereby providing fast, convenient, and/or low-cost DNA sequencing.

Brief Description of the Drawings

[1017] Figure 1A shows beads and a magnetic array with sensors which may be used to capture the beads.

[1018] Figure 1B shows the beads captured on the magnetic array in a one to one correspondence with the sensors.

[1019] Figure 1C shows the sensor array after the beads have been washed off ready for use for the next sample.

[1020] Figure 1D shows a schematic of a sensor array used with a set of nonspherical magnetic particles.

[1021] Figures 2A-2B Show a photomicrographs of a magnetic arrays according to an embodiment loaded with beads

[1022] Figures 3 and 4 are schematic illustrations of the reaction involved in incorporating nucleotides into a growing DNA strand, showing the release of pyrophosphate and concomitant increase in pH and heat release.

Claims:

1. A method for sequencing a nucleic acid sample, the method comprising:
    a. providing a plurality of particles, wherein each particle of said plurality is coupled to on average at most one nucleic acid molecule generated from said nucleic acid sample;
    b. performing simultaneous nucleic acid amplification reactions on said nucleic acid molecules coupled to said particles, wherein said nucleic acid amplification reactions are performed while subjecting the particles to an electric field, thereby producing isolated, clonally-amplified nucleic acid molecules coupled to at least a subset of said plurality of particles;
    c. performing a nucleic acid extension reaction using primers that hybridize to said clonally-amplified nucleic acid molecules; ~~and~~
    d. measuring ~~detecting~~ a local impedance change of each of said particles using a sensor array comprising a plurality of sensors to determine a sequence of each of said clonally-amplified nucleic acid molecules, when an individual sensor among said plurality of sensors comprises a pair of electrodes that are within the Debye length of a surface of the particle~~thereby sequencing said nucleic acid sample~~; and
    ~~d.~~e. based on the measured local impedance change, sequencing said nucleic acid sample.
2. The method of Claim 1, wherein said nucleic acid amplification reactions are emulsion free.
3. The method of Claim 1, further comprising, subsequent to (b), separating particles carrying clonally-amplified nucleic acid molecules from particles not carrying clonally-amplified nucleic acid molecules.
4. The method of Claim 1, wherein (b) is performed with the plurality of particles held adjacent to the sensor array.
5. The method of Claim 1, wherein (c) and (d) are performed simultaneously.
6. The method of Claim 1, wherein (c) and (d) are performed in different buffers.
7. The method of Claim 1, wherein the particles are beads.
8. The method of Claim 1, wherein the particles are magnetic.
9. The method of Claim 1, wherein, in (c), the particles are magnetically immobilized.
10. The method of Claim 1, wherein, in (d), an individual particle among said plurality of particles is adjacent to at least two sensors among said plurality of sensors.

11. The method of Claim 1, wherein the clonally-amplified nucleic acid molecules are sequenced with at least two sensors for each particle carrying clonally-amplified nucleic acid molecules.

12. The method of Claim 1, wherein the nucleic acid extension reactions comprise incorporation of a nucleotide into a nucleic acid strand primed for sequencing, said nucleic acid strand associated with said particle.

~~13. The method of Claim 1, wherein an individual sensor among said plurality of sensors measures said local impedance change of a particle among said plurality of particles within the Debye length of the surface of the individual sensor, particle, or nucleic acid molecules carried on the particle.~~

~~14.~~13. The method of Claim 1, wherein the electric field has DC and AC components.

~~15.~~14. The method of Claim 1, wherein the electric field is adapted to isolate or concentrate one or more of template DNA fragments, primers, polymerase, and dNTPs around each of the particles.

~~16.~~15. The method of Claim 1, wherein the electric field is applied to isolate or concentrate products of nucleic acid amplification reactions.

~~17.~~16. The method of Claim 1, wherein said plurality of sensors are part of a substantially planar sensor array.

~~18. A method for amplifying a nucleic acid sample, the method comprising:~~

~~a. providing a plurality of particles, wherein each particle of said plurality is coupled to on average at most one nucleic acid molecule generated from said nucleic acid sample;~~

~~b. immobilizing said plurality of particles adjacent to a substantially planar magnetic array using a magnetic field generated by said substantially planar magnetic array; and~~

~~c. performing simultaneous nucleic acid amplification reactions on said nucleic acid molecules coupled to said particles, wherein said nucleic acid amplification reactions are performed while subjecting the particles to an electric field, thereby producing isolated, clonally amplified nucleic acid molecules coupled to at least a subset of said plurality of particles.~~

~~19. The method of Claim 18, wherein said electric field is provided by at least one electrode adjacent to said substantially planar magnetic array.~~

20. The method of Claim 18, wherein the particles are beads.

21. The method of Claim 18, wherein the particles are magnetic.

22. A system, comprising:

a. at least one electrode that applies an electric field , wherein said electrode concentrates or isolates molecules local to each of a plurality of particles during nucleic acid amplification of nucleic acid molecules coupled to said plurality of particles; and

b. a substantially planar sensor array adjacent to or downstream of said at least one electrode, wherein said substantially planar sensor array comprises a plurality of sensors that sense nucleic acid incorporation events during sequencing of said nucleic acid molecules coupled to said plurality of particles, and wherein during sensing each sensor of said plurality detects charge changes within the Debye length of (i) a surface of an individual sensor among said plurality of sensors, (ii) an individual particle among said plurality of particles, or (iii) a nucleic acid molecule carried on said individual particle.

23. The system of Claim 22, wherein said at substantially planar sensor array is downstream of said at least one electrode.

24. The system of Claim 22, further comprising a magnetic array adjacent to said planar sensor array, wherein said magnetic array provides a magnetic field to position an individual particle among said plurality of particles at a given position on the magnetic array.

25. The system of Claim 24, wherein said individual sensor is adjacent to an individual position of the magnetic array

26. The system of Claim 24, wherein the electrode is part of the magnetic array or the magnetic array is part of the sensor array.

27. The system of Claim 24, wherein said magnetic array comprises a permanent magnetic material or a material that is configured to be magnetized by an electric field.

28. The system of Claim 22, wherein the electric field concentrates or isolates one or more of template DNA fragments, primers, polymerase, and dNTPs around each of the particles.

29. The system of Claim 22, wherein the particles are beads.

30. The system of Claim 22, wherein the particles are magnetic.

31. The system of Claim 22, wherein the substantially planar sensor array does not contain wells.

32. The system of Claim 22, wherein an individual position of the substantially planar sensor array comprises at least two sensors.

33. The system of Claim 22, wherein an individual sensor of said substantially planar sensor array is configured to measure at least one of local impedance change or local conductivity change.

34. The system of Claim 22, wherein an individual sensor of said substantially planar sensor array comprises electrodes that are configured to measure the impedance of a particle of said plurality of particles during sequencing of said nucleic acid molecules.

35. The system of Claim 22, further comprising a separation module, wherein the separation module separates particles among the plurality of particles that have nucleic acid molecules bound to or associated with the particles from particles that do not have nucleic acid molecules bound to or associated with the particles.

36. The system of Claim 35, wherein the particles are separated using electrophoresis.

37. The system of Claim 22, further comprising a fragmentation module, wherein the fragmentation module fragments nucleic acid molecules to generate nucleic acid fragments, and binding or associating the nucleic acid fragments with the particles.

38. The system of Claim 36, wherein the fragmentation module is fragments the nucleic acid molecules using shear force.

39. The system of Claim 22, wherein at least part of the system is reusable.

40. A system, comprising:

a. at least one electrode that applies an electric field, wherein said electrode is configured such that when said electrode is in use said electric field concentrates or isolates molecules local to each of a plurality of particles during nucleic acid amplification of nucleic acid molecules coupled to said plurality of particles; and

b. a substantially planar magnetic array adjacent to said at least one electrode, wherein said substantially planar magnetic array provides a magnetic field, and wherein said substantially planar magnetic array is configured such that when said substantially planar magnetic array is in use said magnetic field positions an individual particle

among said plurality of particles at a given position on the substantially planar magnetic array.

41. The system of Claim 40, wherein the particles are beads.

42. The system of Claim 40, wherein the particles are magnetic.

43. The system of Claim 40, wherein the electric field concentrates or isolates one or more of template DNA fragments, primers, polymerase, and dNTPs around each of the particles.

44. The system of Claim 40, wherein the electrode is part of the substantially planar magnetic array.