NUCLEIC ACID AMPLIFICATION

BACKGROUND

[0001] Nucleic acid amplification is very useful in molecular biology and has wide applicability in practically every aspect of biology, therapeutics, diagnostics, forensics and research. Generally, amplicons are generated from a starting template using one or more primers, where the amplicons are homologous or complementary to the template from which they were generated. Multiplexed amplification can also streamline processes and reduce overheads. This application relates to methods and reagents for nucleic acid amplification and/or analysis.

SUMMARY

[0002] Methods, reagents and products of nucleic acid amplification and/or analysis are provided herein. Amplification can make use of immobilized and/or soluble primers. A single set of primers can be mixed with different templates, or a single template can be contacted with multiple different primers, or multiple different templates can be contacted with multiple different primers. Amplicons generated from methods provided herein are suitable substrates for further analysis, e.g., sequence determination.

[0003] In some embodiments, the present teachings provide compositions, systems, methods, apparatuses and kits for nucleic acid amplification.

[0003a] In one ~~aspect of the disclosure~~embodiment, there is provided a method for nucleic acid amplification, comprising: (a) distributing at least two polynucleotides into an array of reaction chambers by introducing a single one of said polynucleotides into at least two of said reaction chambers; and (b) forming at least two substantially monoclonal nucleic acid populations by amplifying the polynucleotides within said at least two reaction chambers, wherein the said at least two reaction chambers are in fluid communication with each other during the amplifying.

[0003b] In one aspect,there is provided a method for nucleic acid amplification, comprising:

(a) distributing at least two polynucleotides into an array of reaction chambers or onto discrete supports by introducing a single one of said polynucleotides into at least two of said reaction chambers, or by introducing the at least two polynucleotides into a reaction chamber comprising discrete supports, wherein the at least two polynucleotides have different sequences; and

(b) forming at least two substantially monoclonal nucleic acid populations by amplifying the polynucleotides within said array of reaction chambers or within the reaction chamber comprising discrete supports, in the presence of a first immobilized primer and a second primer in solution, wherein the array of reaction chambers or the discrete supports are in fluid communication with each other during the amplifying, wherein the amplifying includes: contacting the at least two polynucleotides with a single reaction mixture containing reagents for nucleic acid synthesis, a recombinase and one or more accessory proteins in the reaction chambers. and wherein the reaction mixture further includes a sieving agent.

DETAILED DESCRIPTION OF THE DRAWINGS

[0004] **Figure 1** provides a schematic showing an embodiment of template walking. In an alternative embodiment, the immobilized primer comprises an adenosine-rich sequence designated as $(A)_n$, e.g., $(A)_{30}$, and the primer binding site for the immobilized primer on the template comprises a complementary T-rich sequence, e.g., $(T)_{30}$.

[0005] **Figure 2** depicts an overview of amplification on beads by template walking and deposition of beads onto a planar array for sequencing.

[0006] **Figure 3** depicts some alternative embodiments using semiconductor-based detection of sequencing by synthesis. Template walking can be used to generate a population of clonal amplicons on a bead or on the base or bottom of a reaction chamber. In an alternative embodiment, the immobilized primer comprises an adenosine-rich sequence designated as $(A)_n$,

CLAIMS

1. A method for nucleic acid amplification, comprising:

(a) distributing at least two polynucleotides into an array of reaction chambers or onto discrete supports by introducing a single one of said polynucleotides into at least two of said reaction chambers, or by introducing the at least two polynucleotides into a reaction chamber comprising discrete supports, wherein the at least two polynucleotides have different sequences; and

(b) forming at least two substantially monoclonal nucleic acid populations by amplifying the polynucleotides within said ~~at least two~~array of reaction chambers or within the reaction chamber comprising discrete supports, in the presence of a first immobilized primer and a second primer in solution, wherein the ~~said at least two~~ array of reaction chambers or the discrete supports are in fluid communication with each other during the amplifying, wherein the amplifying includes: contacting the at least two polynucleotides with a single reaction mixture containing reagents for nucleic acid synthesis, a recombinase and one or more accessory proteins in the reaction chambers, and wherein the reaction mixture further includes a sieving agent.

2. The method of claim 1, wherein the at least two polynucleotides have different target sequences and include a first primer binding site and a second primer binding site, and wherein the first immobilized primer can hybridize to the first primer binding site and the second primer in the solution can hybridize to the second primer binding site, optionally, wherein the array of reaction chambers or the reaction chamber comprising discrete supports, do not significantly comprise an immobilized primer that is homologous to a second non-overlapping portion of the same at least two polynucleotides or that can hybridize to the complement of the same at least two polynucleotides.

3. The method of claim 1, wherein the amplifying is performed without completely denaturing the at least two polynucleotides during the amplifying.

~~4. The method of claim 1, wherein the amplifying includes: contacting the at least two polynucleotides with a single reaction mixture containing reagents for nucleic acid synthesis.~~

~~5. The method of claim 1, wherein the amplifying includes: contacting the at least two polynucleotides with a recombinase in the reaction chambers.~~

~~6.~~4. The method of claim 1, wherein the amplifying is performed in an array of ~~at least two~~ reaction chambers that are operatively coupled to a sensor~~.~~,

~~7. The method of claim 6,~~ wherein the sensor can detect the presence of a nucleotide incorporation byproduct within the reaction chamber.

~~8.~~5. The method of claim ~~6~~4, wherein the reaction chamber includes a hydrophilic polymer matrix conformally disposed within the reaction chamber.

~~9.~~6. The method of claim ~~8~~5, wherein the hydrophilic polymer matrix includes a hydrogel polymer matrix~~.~~

~~10. The method of claim 8,~~ or wherein the hydrophilic polymer matrix is a cured-in-place polymer matrix.

~~11.~~7. The method of claim ~~8~~5, wherein the hydrophilic polymer matrix includes polyacrylamide, copolymers thereof, derivatives thereof, or combinations thereof.

~~12~~8. The method of claim ~~11~~7, wherein polyacrylamide is conjugated with an oligonucleotide primer.

~~13~~9. The method of claim ~~8~~5, wherein the reaction chamber has a ~~characteristic~~ diameter in a range of 0.1 micrometers to 2 micrometers.

~~14~~10. The method of claim ~~6~~4, wherein the sensor includes a field effect transistor (FET). ~~The FET can include an ion sensitive FET (ISFET).~~

~~15~~11. The method of claim 10, further including detecting the presence of one or more nucleotide incorporation byproducts at a site of the array using the FET~~.~~, optionally

~~16. The method of claim 14,~~ further including detecting a pH change occurring within the at least one reaction chamber using the FET.

~~17~~12. The method of claim ~~14~~10, further including introducing a nucleotide into the site; and detecting an output signal from the sensor resulting from incorporation of the nucleotide into ~~the~~a sequencing primer.

~~18. The method of claim 18, optionally~~ wherein the output signal is a current-based or a threshold voltage based ~~on a threshold voltage~~ output signal of the FET.

~~19~~13. The method of claim ~~14~~10, wherein the FET includes a floating gate conductor coupled to the reaction chamber.

~~20~~14. The method of claim ~~14~~10, wherein the FET includes a floating gate structure comprising a plurality of conductors electrically coupled to one another and separated by dielectric layers, and the floating gate conductor is an uppermost conductor in the plurality of conductors.

15. The method of claim 1, wherein the one or more accessory proteins include a recombinase loading factor or a single stranded binding protein, optionally wherein the recombinase loading factor comprises uvsY from T4 phage and the single stranded binding protein comprises gp32 protein from T4 phage or a modified gp32 protein from T4 phage.

16. The method of any one of the preceding claims, wherein the reaction mixture includes the sieving agent at a concentration of about 0.1 to about 20% weight per volume.

17. The method of any one of the preceding claims, wherein the sieving agent exhibits an average viscosity range of about 5 centipoise to about 15,000 centipoise when dissolved in water at 2 weight percent measured at about 25°C, or about 10 centipoise to about 10,000 centipoise as a 2% aqueous solutions measured at about 25°C, or about 15 centipoise to about 5,000 centipoise as a 2% aqueous solution measured at about 25°C.

18. The method of any one of the preceding claims, wherein the sieving agent comprises a polypeptide, or a polysaccharide polymer, or a polymer of glucose or galactose, or a glucopyranose polymer, or wherein the sieving agent comprises one or more polymers selected from the group consisting of: cellulose, dextran, starch, glycogen, agar, chitin, pectin or agarose.

19. The method of any one of the preceding claims, wherein the sieving agent comprises a cellulose derivative.

20. The method of claim 19, wherein the cellulose derivative is sodium carboxymethyl cellulose, sodium carboxymethyl 2-hydroxyethyl cellulose, methyl cellulose, hydroxyl ethyl cellulose, 2-hydroxypropyl cellulose, carboxy methyl cellulose, hydroxyl propyl cellulose, hydroxyethyl methyl cellulose, hydroxybutyl methyl cellulose, (hydroxypropyl)methyl cellulose or hydroxyethyl ethyl cellulose, or a mixture comprising any one or more of such polymers.

21. The method of claim 10, wherein the FET comprises an ion sensitive FET (ISFET).

22. The method of claim 1, wherein the amplifying is performed in a reaction chamber comprising different discrete beads or particles without the need for compartmentalization prior to amplification.

23. The method of claim 1 or claim 22, wherein the substantially monoclonal nucleic acid populations are formed on different beads or particles.