

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 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 non-immobilized 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 array of reaction chambers ~~or within the reaction chamber comprising discrete supports~~, in the presence of a first immobilized primer and a second non-immobilized 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.

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 non-immobilized 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.

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.

24. The method of claim 1, wherein the amplifying is performed without the need for compartmentalization prior to amplification.