METHODS AND COMPOSITIONS FOR IMPROVING EFFICIENCY OF NUCLEIC ACIDS AMPLIFICATION REACTIONS

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] The present application claims benefit of priority to US Provisional Patent Application No. 61/143,350, filed January 8, 2009, which is incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates to nucleic acid amplification reactions. The present invention encompasses hybrid polymerases with improved processivity as well as methods and compositions for reducing non-specific amplification during reactions such as PCR.

BACKGROUND OF THE INVENTION

[0003] Nucleic acid amplification reactions, such as polymerase chain reaction (PCR), are generally template-dependent reactions in which a desired nucleic acid sequence is amplified by treating separate complementary strands of a target nucleic acid with an excess of two oligonucleotide primers. The primers are extended to form complementary primer extension products which act as templates for synthesizing the desired nucleic acid sequence. In such processes, the nucleic acid sequence between the primers on the respective DNA strands is selectively amplified.

BRIEF SUMMARY ~~OF THE INVENTION~~

[0004] Accordingly, the present ~~invention~~disclosure provides methods and compositions for improving the efficiency and the specificity of nucleic acid synthesis and amplification reactions.

[0004a] In one aspect, there is provided a method of amplifying a target nucleic acid in a sample, the method comprising, incubating said target nucleic acid in a reaction mixture comprising: at least one primer, an exonuclease deficient hybrid polymerase comprising a polymerase domain and a heterologous DNA binding domain, wherein said incubating is under conditions that permit amplification of said target nucleic acid by said hybrid polymerase.

[0005] In one ~~aspect~~embodiment, the present ~~invention~~disclosure provides a method of amplifying a target nucleic acid. This method includes incubating the target nucleic acid with a reaction mixture that includes at least one primer, a hybrid polymerase, and an osmolyte. In a further ~~aspect~~embodiment, the incubating step is conducted under conditions that permit amplification of the target

nucleic acid by the hybrid polymerase In a still further ~~aspect~~embodiment, the hybrid polymerase comprises a polymerase domain and a DNA binding domain.

[0006] In a further ~~aspect~~embodiment, the ~~invention~~disclosure provides a kit for quantitative PCR. In an exemplary ~~aspect~~embodiment, the kit includes: a hybrid polymerase with reduced exonuclease activity, dNTPs, a buffer that includes sarcosine, and directions for using the hybrid polymerase in a nucleic acid amplification reaction.

[0007] In some embodiments, the ~~invention~~disclosure provides for a method of amplifying a target nucleic acid in a sample. In some embodiments, the method comprises

incubating said target nucleic acid in a reaction mixture comprising:

at least one primer,

a hybrid polymerase comprising a polymerase domain and a heterologous DNA binding domain, and

an additive selected from the group consisting of sarcosine and heparin, in a sufficient amount to improve efficiency of the amplification reaction by at least 10% compared to a reaction mixture lacking the additive,

wherein said incubating is under conditions that permit amplification of said target nucleic acid by said hybrid polymerase.

[0008] In some embodiments, the additive is sarcosine, which is at a concentration of less than 600 mM.

[0009] In some embodiments, the hybrid polymerase comprises a mutation in an exonuclease domain such that the polymerase substantially lacks a 3'-5' exonuclease activity. In some embodiments, the hybrid polymerase comprises a Family B-like polymerase that substantially lacks (e.g., has a mutation resulting in a substantial lack of) a 3'-5' exonuclease activity. In some embodiments, the hybrid polymerase comprises a polypeptide sequence that is substantially identical to SEQ ID NO:2, i.e., is at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity across the polymerase domain (i.e., all but SEQ ID NO:3) or the entire sequence of SEQ ID NO:2. In some embodiments, the hybrid polymerase comprises SEQ ID NO:2.

[0010] In some embodiments, the sample comprises an amplification inhibitor and wherein an aliquot of the sample is added to the reaction mixture in an amount such that the inhibitor is at a concentration capable of inhibiting wildtype Taq polymerase and wherein the hybrid

polymerase amplifies the target nucleic acid. In some embodiments, the sample is selected from blood or intact cells or food samples.

[0011] In some embodiments, the hybrid polymerase is complexed with one or more antibodies prior to a heating step during the incubating step. In some embodiments, the antibody comprises a heavy chain variable region and a light chain variable region, wherein the variable regions comprise complementary determining regions (CDRs), wherein:

(a) the heavy chain variable region CDRs comprise SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, and the light chain variable region CDRs comprise SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27; or

(b) the heavy chain variable region CDRs comprise SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, and the light chain variable region CDRs comprise SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33; or

(c) the heavy chain variable region CDRs comprise SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36, and the light chain variable region CDRs comprise SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39.

[0012] In some embodiments, signal from the amplification is detected in real-time.

[0013] In some embodiments, the heterologous DNA binding domain is substantially identical to (i.e., at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater) to SEQ ID NO:3.

[0014] In some embodiments, the amplification reaction is performed with extension times of less than 1, 2, 3, or 4 seconds.

[0015] In some embodiments, the polymerase and exonuclease domains are from a Family B DNA polymerase.

[0016] In some embodiments, the reaction mixture further comprises a double-stranded DNA binding dye.

[0017] The present ~~invention~~disclosure also provides for reaction mixtures. In some embodiments, the reaction mixture comprises:

a hybrid polymerase, wherein said polymerase comprises a polymerase domain and a heterologous DNA binding domain; and

(c) the heavy chain variable region CDRs comprise SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36, and the light chain variable region CDRs comprise SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39.

[0024] In some embodiments, the heterologous DNA binding domain is substantially identical to (i.e., at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater) to SEQ ID NO:3.

[0025] In some embodiments, the polymerase and exonuclease domains are from a Family B DNA polymerase.

[0026] In some embodiments, the reaction mixture comprises one or more different deoxyribonucleotide triphosphates.

[0027] In some embodiments, the reaction mixture further comprises a double-stranded DNA binding dye.

[0028] The present ~~invention~~disclosure also provides kits. In some embodiments, the kit comprises:

a hybrid polymerase comprising a polymerase domain and a heterologous DNA binding domain; and

an additive selected from the group consisting of sarcosine and heparin, in a sufficient amount to improve efficiency of an amplification reaction by at least 10% compared to a reaction mixture lacking the additive.

[0029] In some embodiments, the hybrid polymerase comprises a mutation in an exonuclease domain such that the polymerase substantially lacks a 3'-5' exonuclease activity. In some embodiments, the hybrid polymerase comprises a Family B-like polymerase that substantially lacks (e.g., has a mutation resulting in a substantial lack of) a 3'-5' exonuclease activity. In some embodiments, the hybrid polymerase comprises a polypeptide sequence that is substantially identical to SEQ ID NO:2, i.e., is at least 65%, 70%, 75%, 80%, 85%, 25 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity across the polymerase domain (i.e., all but SEQ ID NO:3) or the entire sequence of SEQ ID NO:2. In some embodiments, the hybrid polymerase comprise SEQ ID NO:2.

[0030] In some embodiments, the kit further comprises an antibody that is specific for the hybrid polymerase.

[0031] In some embodiments, the antibody comprises a heavy chain variable region and a light chain variable region, wherein the variable regions comprise complementary determining regions (CDRs), wherein:

(a) the heavy chain variable region CDRs comprise SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, and the light chain variable region CDRs comprise SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27; or

(b) the heavy chain variable region CDRs comprise SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, and the light chain variable region CDRs comprise SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33; or

(c) the heavy chain variable region CDRs comprise SEQ ID NO:34, SEQ ID NO:35_s and SEQ ID NO:36, and the light chain variable region CDRs comprise SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39.

[0032] In some embodiments, the heterologous DNA binding domain is substantially identical to (i.e., at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 15 97%, 98% or 99% or greater) to SEQ ID NO:3.

[0033] In some embodiments, the polymerase and exonuclease domains are from a Family B DNA polymerase.

[0034] In some embodiments, the kit further comprises one or more of:

one or more different deoxyribonucleotide triphosphates;

a double-stranded DNA binding dye; and

an oligonucleotide primer.

[0035] The present ~~invention~~disclosure also provides for an isolated antibody having binding specificity for a protein consisting of SEQ ID NO:2, wherein the antibody comprises a heavy chain and a light chain variable region, wherein the variable regions comprise complementary determining regions (CDRs), wherein:

(a) the heavy chain variable region CDRs comprise SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, and the light chain variable region CDRs comprise SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27; or

(b) the heavy chain variable region CDRs comprise SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, and the light chain variable region CDRs comprise SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33; or

(c) the heavy chain variable region CDRs comprise SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36, and the light chain variable region CDRs comprise SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39.

[0036] In some embodiments, the heavy chain and light chain variable regions comprise:

(a) SEQ ID NO:14 and SEQ ID NO:18, respectively;

(b) SEQ ID NO:15 and SEQ ID NO:19, respectively;

(c) SEQ ID NO:16 and SEQ ID NO:20, respectively; or

(d) SEQ ID NO:17 and SEQ ID NO:21, respectively;

[0037] The present ~~invention~~disclosure also provides for an isolated polynucleotide encoding a polypeptide comprising a heavy chain or light chain variable region as discussed above. .

[0038] The present ~~invention~~disclosure also provides for an antibody complexed with a polymerase, wherein the antibody comprises a heavy chain and/or light chain variable region as discussed above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] FIG. 1 shows the effect of sarcosine on efficiency of a qPCR reaction

[0040] FIG. 2 shows amplification curves for reactions containing different concentrations of heparin to those with no heparin added.

[0041] FIG. 3 shows amplification curves for different hybrid polymerases with change in annealing/extension temperature. Amplification curves for 25 pg, 250 pg, and 2500 pg input DNA are displayed.

[0042] FIG. 4(A) shows the amino acid sequence of an exemplary hybrid polymerases that includes a DNA binding domain. FIG. 4(B) shows the amino acid sequence of an exemplary DNA binding domain.

~~WHAT IS CLAIMED IS:~~

1. A method of amplifying a target nucleic acid in a sample, said method comprising

incubating said target nucleic acid in a reaction mixture comprising:

at least one primer,

a hybrid polymerase comprising a polymerase domain and a heterologous DNA binding domain, and

an additive selected from the group consisting of sarcosine and heparin, in a sufficient amount to improve efficiency of the amplification reaction by at least 10% compared to a reaction mixture lacking the additive,

wherein said incubating is under conditions that permit amplification of said target nucleic acid by said hybrid polymerase.

2. The method of claim 1, wherein said hybrid polymerase comprises a Family B-like polymerase that substantially lacks a 3'-5' exonuclease activity.

3. The method of claim 1, wherein the hybrid polymerase comprises SEQ ID NO:2.

4. The method of claim 1, wherein the hybrid polymerase is complexed with one or more antibodies prior to a heating step during the incubating step.

5. The method of claim 4, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the variable regions comprise complementary determining regions (CDRs), wherein:

(a) the heavy chain variable region CDRs comprise SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, and the light chain variable region CDRs comprise SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27; or

(b) the heavy chain variable region CDRs comprise SEQ ID NO:28, SEQ ED NO:29, and SEQ ID NO:30, and the light chain variable region CDRs comprise SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33; or

(c) the heavy chain variable region CDRs comprise SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36, and the light chain variable region CDRs comprise SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39.

6. The method of claim 1, wherein the heterologous DNA binding domain is at least 75% identical to SEQ ID NO:3.

7. The method of claim 1, wherein the reaction mixture further comprises a double-stranded DNA binding dye.

8. A reaction mixture comprising:

a hybrid polymerase, wherein said polymerase comprises a polymerase domain and a heterologous DNA binding domain; and

an additive selected from the group consisting of sarcosine and heparin, in a sufficient amount to improve efficiency of the amplification reaction by at least 10% compared to a reaction mixture lacking the additive.

9. The reaction mixture of claim 8, further comprising at least one oligonucleotide primer.

10. The reaction mixture of claim 8, wherein said hybrid polymerase comprises a Family B-like polymerase that substantially lacks a 3'-5' exonuclease activity.

11. The reaction mixture of claim 8, wherein the polymerase comprises SEQ ID NO:2.

12. The reaction mixture of claim 8, wherein the hybrid polymerase is complexed with an antibody.

13. The reaction mixture of claim 12, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the variable regions comprise complementary determining regions (CDRs), wherein:

(a) the heavy chain variable region CDRs comprise SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, and the light chain variable region CDRs comprise SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27; or

(b) the heavy chain variable region CDRs comprise SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, and the light chain variable region CDRs comprise SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33; or

(c) the heavy chain variable region CDRs comprise SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36, and the light chain variable region CDRs comprise SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39.

14. The reaction mixture of claim 8, wherein the heterologous DNA binding domain is at least 75% identical to SEQ ID NO:3.

15. The reaction mixture of claim 8, wherein the reaction mixture further comprises a double-stranded DNA binding dye.

16. A kit comprising:

a hybrid polymerase comprising a polymerase domain and a heterologous DNA binding domain; and

an additive selected from the group consisting of sarcosine and heparin, in a sufficient amount to improve efficiency of an amplification reaction by at least 10% compared to a reaction mixture lacking the additive.

17. The kit of claim 16, wherein said said hybrid polymerase comprises a Family B-like polymerase that substantially lacks a 3'-5' exonuclease activity.

18. The kit of claim 16, wherein the hybrid polymerase comprise SEQ ID NO:2.

19. The kit of claim 16, further comprising an antibody that is specific for the hybrid polymerase.

20. The kit of claim 19, wherein the antibody comprises a heavy chain variable region and a light chain variable region, wherein the variable regions comprise complementary determining regions (CDRs), wherein:

(a) the heavy chain variable region CDRs comprise SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, and the light chain variable region CDRs comprise SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27; or

(b) the heavy chain variable region CDRs comprise SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, and the light chain variable region CDRs comprise SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33; or

(c) the heavy chain variable region CDRs comprise SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36, and the light chain variable region CDRs comprise SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39.

21. The kit of claim 16, wherein the heterologous DNA binding domain is at least 75% identical to SEQ ID NO:3.

22. ~~The kit of claim 16, further comprising one or more of:~~

one or more different deoxyribonucleotide triphosphates;

a double-stranded DNA binding dye; and

at least one oligonucleotide primer.

23. An isolated antibody having binding specificity for a protein consisting of SEQ ID NO:2, wherein the antibody comprises a heavy chain and a light chain variable region, wherein the variable regions comprise complementary determining regions (CDRs), wherein:

(a) the heavy chain variable region CDRs comprise SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:24, and the light chain variable region CDRs comprise SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27; or

(b) the heavy chain variable region CDRs comprise SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30, and the light chain variable region CDRs comprise SEQ ID NO:31, SEQ ID NO:32, and SEQ ID NO:33; or

(c) the heavy chain variable region CDRs comprise SEQ ID NO:34, SEQ ED NO:35, and SEQ ID NO:36, and the light chain variable region CDRs comprise SEQ ID NO:37, SEQ ID NO:38, and SEQ ID NO:39.

24. The antibody of claim 23, wherein the heavy chain and light chain variable regions comprise:

(a) SEQ ID NO:14 and SEQ ID NO:18, respectively;

(b) SEQ ID NO:15 and SEQ ID NO:19, respectively;

(c) SEQ ID NO:16 and SEQ ID NO:20, respectively; or

(d) SEQ ID NO:17 and SEQ ID NO:21, respectively;

25. An isolated polynucleotide encoding a polypeptide comprising a heavy chain or light chain variable region of claims 23 or claim 24.

26 . An antibody of claim 23 or claim 24 complexed with a polymerase.

WHAT IS CLAIMED IS:

1. A method of amplifying a target nucleic acid in a sample, the method comprising,
incubating said target nucleic acid in a reaction mixture comprising:
at least one primer,
an exonuclease deficient hybrid polymerase comprising a polymerase domain and a heterologous DNA binding domain,
wherein said incubating is under conditions that permit amplification of said target nucleic acid by said hybrid polymerase.

2. The method of claim 1, wherein quantitative polymerase chain reaction (qPCR) detection is used to monitor amplification.

3. The method of claim 1, wherein the reaction mixture comprises at least two primers that can form a primer dimer.

4. The method of claim 1, wherein the reaction is initiated with antibodies bound to the hybrid polymerase.

5. The method of claim 1, wherein the polymerase domain is from a B family polymerase.

6. The method of claim 1, wherein the reaction mixture further comprises an additive selected from the group consisting of sarcosine and heparin, in a sufficient amount to improve efficiency of the amplification reaction by at least 10% compared to a reaction mixture lacking the additive.

7. The method of claim 1, wherein the hybrid polymerase comprises SEQ ID NO:2.

8. The method of claim 1, wherein the heterologous DNA binding domain is at least 75% identical to SEQ ID NO:3.

9. The method of claim 1, wherein the reaction mixture further comprises a double-stranded DNA binding dye.