

CLAIMS

1. A method to amplify nucleic acid, the method comprising:
- 5 (a) forming a reaction mixture comprising:
- (i) a first nucleic acid template comprising a strand having a first nicking agent recognition sequence;
- (ii) a second nucleic acid template comprising a strand having a second nicking agent recognition sequence;
- 10 (iii) at least one primer for a target region on the first or second nucleic acid template;
- (iv) at least one protein having DNA polymerase domain function, wherein the domain function comprises a first domain function capable of strand displacement activity and a second domain function capable of high processivity activity, or
- 15 at least one protein having DNA polymerase domain function capable of strand displacement activity and at least one protein having DNA polymerase domain function capable of high processivity activity;
- 20 (v) at least one deoxynucleoside triphosphate; and
- (vi) a first nicking agent for recognizing the first nicking agent recognition sequence and a second nicking agent for recognizing the second nicking agent recognition sequence;
- (b) incubating the reaction mixture under conditions that amplifies the nucleic acid templates,
- 25 wherein the domain functions capable of strand displacement activity and high processivity activity are separate from each other and capable of carrying out their activities simultaneously.
- 30 2. The method according to claim 1, wherein the first and second domains are separated by a linker to form a chimeric protein.

3. The method according to any one of the preceding claims, wherein the protein having DNA polymerase domain function is selected from the group consisting of *exo⁻* Vent, *exo⁻* Deep Vent, *exo⁻* Bst, *exo⁻* Pfu, *exo⁻* Bca, the Klenow fragment of DNA polymerase I, T5 DNA polymerase, Phi29 DNA polymerase, phage M2 DNA polymerase, phage PhiPRD1 DNA polymerase, Sequenase, PRD1 DNA polymerase, 9°Nm™ DNA polymerase, T4 DNA polymerase, strand displacing Taq polymerase, and combinations thereof.
- 10 4. The method according to any one of the preceding claims, wherein the second template further comprises a region that is not substantially complementary to the at least one primer, and incubating the reaction mixture under conditions to amplify:
- 15 (a) a first single-stranded nucleic acid molecule using the first template nucleic acid as a template; and
- (b) a second single-stranded nucleic acid molecule using the second template nucleic acid as a template.
- 20 5. The method according to any one of the preceding claims, wherein the reaction mixture further comprises a third nucleic acid template comprising:
- (a) a third nicking agent recognition sequence;
- (b) a first region that is substantially identical or complementary to the at least one primer; and
- 25 (c) a second region that is not substantially identical or complementary to the at least one primer.
6. The method according to claim 5, wherein the first, second and third nicking agent recognition sequences are identical.
- 30 7. The method according to any one of the preceding claims, wherein the nicking agent is a nicking endonuclease selected from the group consisting of Nb.BbvCI,

Nb.BsmI, Nb.BsrDI, Nb.BssSI, Nb.BtsI, Nt.AlwI, Nt.BbvCI, Nt.BsmAI, Nt.BspQI, Nt.BstNBI, Nt.CviPII, Zinc Finger nickase, TALE nickase, Cas nickase, and combinations thereof.

5 8. The method according to any one of the preceding claims, wherein the 3' terminus of the first nucleic acid template or the second nucleic acid template is blocked.

9. The method according to any one of the preceding claims, wherein first nucleic acid template and the second nucleic acid template are immobilized.

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10. The method according to any one of the preceding claims, wherein first nucleic acid template or the second nucleic acid template comprises nucleic acid modifications.

11. The method according to any one of the preceding claims, wherein first nucleic acid template or the second nucleic acid template is about 6 to 20,000 nucleotides in length.

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12. The method according to any one of the preceding claims, further comprising the step of characterizing the amplified nucleic acid.

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13. The method according to claim 12, wherein the characterizing step is performed by a technique selected from the group consisting of luminescence spectroscopy or spectrometry, fluorescence spectroscopy or spectrometry, mass spectrometry, liquid chromatography, fluorescence polarization, electrophoresis, mass spectrometry, SYBR I fluorescence, SYBR II fluorescence, SYBR Gold, Pico Green, Evagreen, TOTO-3, intercalating dye detection, FRET, molecular beacon detection, scorpion probe detection, surface capture, capillary electrophoresis, incorporation of labeled nucleotides to allow detection by capture, fluorescence polarization, lateral flow capture, and combinations thereof.

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14. The method according to any one of the preceding claims, wherein the reaction mixture further comprises a template conjugate for linking the first and second nucleic acid templates.
- 5 15. The method according to claim 14, wherein the 3' terminus of the first oligonucleotide template is linked to the 5' terminus of the second oligonucleotide template via the conjugate.
- 10 16. The method according to claim 14, wherein the 3' terminus of the first oligonucleotide template is linked to the 3' terminus of the second oligonucleotide template via the conjugate.
17. The method according to any one of the preceding claims, wherein the first nucleic acid template is identical to the second nucleic acid template.
- 15 18. The method according to any one of the preceding claims, wherein the first nicking agent recognition sequence is identical to the second nicking agent recognition sequence.
19. The method according to any one of the preceding claims, wherein the nucleic acid template further comprises a detectable label.
- 20 20. The method according to claim 19, wherein the detectable label is a fluorescent moiety.
- 25 21. The method according to any one of the preceding claims, wherein the amplification has the kinetic that fits the equation $\sigma \sim \sigma_0 e^{\beta t}$, where σ_0 is an initial concentration of the oligonucleotide, σ is the concentration of the oligonucleotide after the reaction is performed for a period of t , and β is a constant.
- 30 22. The method according to any one of the preceding claims, wherein the incubation step is performed under isothermal conditions.

23. A kit to amplify nucleic acid, the kit comprising:
- (a) at least one primer for a target region on the first or second nucleic acid template;
 - 5 (b) at least one protein having DNA polymerase domain function, wherein the domain function comprises a first domain function capable of strand displacement activity and a second domain function capable of high processivity activity, or
at least one protein having DNA polymerase domain function capable of
10 strand displacement activity and at least one protein having DNA polymerase domain function capable of high processivity activity, wherein the domain functions capable of strand displacement activity and high processivity activity are separate from each other and capable of carrying out their activities simultaneously;
 - 15 (c) at least one deoxynucleoside triphosphate; and
 - (d) at least one nicking agent,
and instructions for using the kit.
24. The kit according to claim 23, wherein the first and second domains are separated
20 by a linker to form a chimeric protein.
25. The kit according to any one of claims 23 or 24, wherein the protein having DNA polymerase domain function is selected from the group consisting of *exo⁻ Vent*, *exo⁻ Deep Vent*, *exo⁻ Bst*, *exo⁻ Pfu*, *exo⁻ Bca*, the Klenow fragment of DNA polymerase I, T5
25 DNA polymerase, Phi29 DNA polymerase, phage M2 DNA polymerase, phage PhiPRD1 DNA polymerase, Sequenase, PRD1 DNA polymerase, 9°Nm™ DNA polymerase, T4 DNA polymerase, strand displacing Taq polymerase, and combinations thereof.
26. The kit according to any one of claims 23 to 25, further comprising a reverse
30 transcriptase.