

thermostable blunt-end ligase as disclosed herein may be useful for many nucleic acid ligation-amplification schemes, including nucleic acid ligation-amplification schemes which operate at a uniform temperature (e.g., at about 60°C or higher), and including nucleic acid ligation-amplification schemes which require temperature cycling such as cycling from, e.g., about 94°C to about 60°C (or higher) for one, two, three, or more cycles. Nucleic acid ligation-amplification schemes which may benefit from the use of thermostable blunt-end DNA ligases disclosed herein may operate at other temperatures as well, e.g., depending on the temperature resistance characteristics of the novel fusion proteins disclosed herein and the requirements of the difference nucleic acid ligation-amplification schemes. Thermostable blunt-end DNA ligases disclosed herein provide the ability to use high temperatures in nucleic acid ligation-amplification schemes and thereby enable higher specificity target amplification, for example, by permitting temperature denaturation of double-stranded DNA templates as well as specific primer binding.

[0009] Accordingly, in a first aspect, the present invention refers to a thermostable blunt-end DNA ligase suitable for use in a blunt-ended DNA ligation reaction performed at about 60 °C or higher, comprising the amino acid sequence (SEQ ID NO: 1). In another aspect, the present invention refers to a method of ligating blunt-end DNA at elevated temperature, comprising using a thermostable blunt-end DNA ligase at an elevated temperature, wherein said thermostable blunt-end DNA ligase is a thermostable blunt-end DNA ligase of the present invention. In still another aspect, the present invention refers to an article of manufacture, comprising a thermostable blunt-end DNA ligase and a container, wherein said thermostable blunt-end DNA ligase is a thermostable blunt-end DNA ligase of the present invention. In a further aspect, the present invention refers to a device for analyzing a sample containing DNA, comprising a thermostable blunt-end DNA ligase, wherein said thermostable blunt-end DNA ligase is a thermostable blunt-end DNA ligase of the present invention. The present invention also refers to a fusion protein comprising an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19. Also, the present invention refers in another aspect to a fusion protein comprising SEQ ID NO: 11 or SEQ ID NO: 12 and a nucleic acid ligase. Furthermore, Applicant also discloses herein a thermostable blunt-end nucleic acid ligase comprising a fusion protein comprising a nucleic acid ligase and a nucleic acid binding protein, wherein said thermostable blunt-end nucleic acid ligase is suitable for use in a blunt-ended nucleic acid ligation reaction performed at an elevated

temperature. In ~~embodiments~~examples, an elevated temperature comprises a temperature of about 60 °C or higher. In ~~examples~~embodiments, an elevated temperature comprises a temperature of about 65 °C, or about 70 °C, or about 75 °C, or about 80 °C, or about 85 °C, or about 90 °C, or about 95 °C, or higher. Applicant further discloses an article of manufacture, comprising such a thermostable blunt-end nucleic acid ligase, and a container.

In ~~examples~~embodiments, the article of manufacture further comprises a buffer. Applicant further discloses a method of ligating blunt-end nucleic acids at an elevated temperature, comprising using a thermostable blunt-end nucleic acid ligase as disclosed herein, wherein the use may be at a temperature of about 60 °C or higher. Applicant further discloses a device for analyzing a sample containing nucleic acids, comprising a thermostable blunt-end nucleic acid ligase comprising a fusion protein comprising a nucleic acid ligase and a nucleic acid binding protein, wherein said thermostable blunt-end nucleic acid ligase is suitable for use in a blunt-ended nucleic acid ligation reaction performed at about 60 °C or higher.

[0010] In ~~examples~~embodiments, Applicant discloses herein a thermostable blunt-end DNA ligase comprising a fusion protein comprising a DNA ligase and a DNA binding protein, wherein said thermostable blunt-end DNA ligase is suitable for use in a blunt-ended DNA ligation

reaction performed at an elevated temperature, for example,, at a temperature of about 60 °C or higher. In exampleembodiments, the thermostable blunt-end DNA ligase comprises a T4 DNA ligase with an N-terminal p50 fusion.

[0011] In a particular exampleembodiment, the thermostable blunt-end DNA ligase comprises the amino acid sequence (SEQ ID NO: 1):

mghhhhhhhhhssghiegrasadgpylqileqpkqrgfrfryvcegpshggplgasseknkksypqvkicny
vgpakvivqlvtngknihlhahslvgkhcedgictvtagpkdmvvgfanlgilhvtkkkvfetlearnatecirgy
npgllvhpdlaylqaegggdrqlgdrekelirqaalqqtkemdlsvvrlmftaflpdstgsftrlepvsdaiyds
apnasnlkivrmrtagcvtggeeiyllcdkvqkddiqrfyeeeenggvwegfgdftsptdvhrqfaivfktkpyk
dinitkpasvfvqlrrksdletsepkpflyypeikdkeevqrkrqkgssgtsgggsgggmtleearkrvnelrdlir
hnyryyvladpeisdaeydrllrelkeleerfelkspdsptlqvgarpleatfrpvrhptmryslndafnldeklaf
erieralgrkgpfaytvehkvdglsvnllyeegvlvygatrgdgevgeevtqnlltiptiprrlkgvperlevrgevy
mpieafirlneelegerifknprnaaagslrqkdpritakgrlatrfyalglgleeveregvatqfallhwlkekghp
vehgyaravgaegveavyqdwllkrralpfeadgvvkldeallwrelgytaraprfaiaiykfpaecketrlldv
fqvgrtgrvtpvgilepvgflegsevsrvtlhnesyieeldirigdwvlvhkaggvipevlrvlkertrgeerpirwpet
cpecghrllkegkvhrpcnplcpakrfearhfasrkamdiqglgeklierllekglvkdvadlyrlrkedlvglerm
geksaqnllrqieeskkrglerllyalglpgvgevlarnlaarfgnmdrileasleelleveevgeltarailetkdpaf
dlvrrlkeagvemeakekggealkglftvitgelsrpreevkallrrlgakvtdsvsrktsylvvgenpgskekaral
gvptlteelyrllcartgkkaeclv.

[0012] A thermostable blunt-end DNA ligase as disclosed herein may comprise a fusion protein which comprises a component selected from a peptide linker, an N-terminal addition, a C-terminal addition, a tag peptide, a D-amino acid, and a peptide mimetic. In exampleembodiments, a thermostable blunt-end DNA ligase as disclosed herein comprises a fusion protein which comprises a component selected from a sugar and a polymer.

[0013] In exampleembodiments, a thermostable blunt-end DNA ligase as disclosed herein is suitable for use in a blunt-ended DNA ligation reaction performed at about 75 °C.

[0014] In exampleembodiments, a thermostable blunt-end DNA ligase as disclosed herein is capable of making concatamers upon multiple ligation events in a blunt-ended DNA ligation reaction performed at about 60 °C or higher.

[0015] In exampleembodiments, the thermostable blunt-end DNA ligase as disclosed herein is suitable for use in a nucleic acid amplification scheme which operates at a uniform temperature of about 60 °C or higher.

[0016] In example embodiments, the thermostable blunt-end DNA ligase as disclosed herein is suitable for use in a nucleic acid amplification scheme which comprises temperature cycling. In example embodiments, such temperature cycling comprises temperature cycling to temperatures of about 60 °C or higher. In example embodiments, such temperature cycling comprises temperature cycling from about 94 °C to about 60 °C. In example embodiments, such temperature cycling comprises two or more cycles of temperature cycling, and, in example embodiments, comprises three or more cycles of temperature cycling.

[0017] Applicant further discloses a method of ligating blunt-end DNA at elevated temperature, comprising using a thermostable blunt-end DNA ligase as disclosed herein, wherein the use may be at an elevated temperature. In example embodiments, an elevated temperature comprises a temperature of about 60 °C or higher. In example embodiments, an elevated temperature comprises a temperature of about 75 °C; and, in further example embodiments, may comprise a temperature higher than about 75 °C.

[0018] Applicant discloses herein an article of manufacture, comprising a thermostable blunt-end DNA ligase and a container, wherein the thermostable blunt-end DNA ligase is a thermostable blunt-end DNA ligase as disclosed herein. In example embodiments, the article of manufacture further comprises a buffer.

[0019] Applicant discloses herein a device for analyzing a sample containing DNA, comprising a thermostable blunt-end DNA ligase, wherein the thermostable blunt-end DNA ligase is a thermostable blunt-end DNA ligase as disclosed herein.

BRIEF DESCRIPTION OF THE FIGURE

[0020] Fig. 1 shows an example of the results of a blunt-ended ligation reaction performed at 75 °C using a thermostable, blunt-end DNA ligase as provided herein. The DNA substrate was a 49-bp duplex DNA made by annealing oligonucleotides EE0139 (SEQ ID NO:20) and EE0140 (SEQ ID NO: 21).

DETAILED DESCRIPTION

[0021] Thermostable, blunt-end ligases are provided herein. As disclosed herein, a thermostable, blunt-end ligase may be prepared by the fusion of a DNA-binding protein to a thermostable ligase. Such ligases produced by these fusions have features and capabilities not provided by their parent compounds alone; the different portions of these fusion proteins provide activities which, when combined, provide new capabilities and unexpectedly

CLAIMS

1 1. A thermostable blunt-end DNA ligase comprising a fusion protein comprising
2 a DNA ligase and a DNA binding protein, wherein said thermostable blunt-end DNA ligase
3 is suitable for use in a blunt-ended DNA ligation reaction performed at about 60 °C or higher.

1 2. The thermostable blunt-end DNA ligase of claim 1, comprising a T4 DNA
2 ligase with an N-terminal p50 fusion.

1 3. The thermostable blunt-end DNA ligase of claim 1, comprising the amino acid
2 sequence (SEQ ID NO: 1):

3 mgghhhhhhhhhssghiegrasadgpylqileqpkqrgfrfryvcegpshggllpgasseknkksypqvkicnyvgpakvivqlvtngkni
4 lhahslvgkhcdgictvtagpkdmvvgfanlgilhvtkkkvfetlearmteacirgynpgllvhpdlaylqaeggdrqlgdrekelirqaalq
5 qtkemdlsvrlmftaflpdstgsfrrlepvvsdaiydkapnasnlkivrmrdrtagcvtggeeiyllcdkvqkddiqirfyeeenggvwegf
6 gdfsptdvhrqfaivfktkpykdinitkpasvfvqlrrksdletsepkpfllyypeikdkeevqrkrqkgsgtsgggsggmlteearkrvnelrd
7 liryhnyryyvladpeisdaeydrllrelkeleerfelkspdsptlqvgarpleatfrpvrhptrmynsldnafnldekafeerieralgrkgpfay
8 tvehkvdglsvnllyeegvlvygatrgdgevgeevtnlltiptiprrlkgvperlevrgevympieaflrlneelegerifknprnaaagslrq
9 kdpritakgrlratfyalglgleeveregvatqfallhwikegfpvehgyaravgaegveavyqdwkkrralpfeadgvvklldelalwrelg
10 ytaraprfaiaykfpaeeketrlldvfvqgrtgrvtpvgilepvflegsevsrvtlhnesyieeldirigdwvlvhkaggvipevlrvlkerntgeer
11 pirwpetcpecghrllkegkvhrpcnplcpakrfeairhfaskamdiqglgeklirlllekglvkdvadlyrirkedlvglermgeksaqnllrqi
12 eeskkrglerllyalglpgvgevlarnlaarfgnmdrleasleelleveevgeltarailetikdpafrdlvrrikeagvemeakekggealkgtfv
13 itgelspreevkallrrigakvtdsvsrktsylvgenpgsklekaralgvptlteelyrllleartgkkaeelv.

1 4. A method of ligating blunt-end DNA at elevated temperature, comprising
2 using a thermostable blunt-end DNA ligase at an elevated temperature, wherein said
3 thermostable blunt-end DNA ligase is selected from a thermostable blunt-end DNA ligase of
4 claim 1, a thermostable blunt-end DNA ligase of claim 2, and a thermostable blunt-end DNA
5 ligase of claim 3.

1 5. An article of manufacture, comprising a thermostable blunt-end DNA ligase
2 and a container, wherein said thermostable blunt-end DNA ligase is selected from a
3 thermostable blunt-end DNA ligase of claim 1, a thermostable blunt-end DNA ligase of claim
4 2, and a thermostable blunt-end DNA ligase of claim 3.

1 6. The article of manufacture of claim 5, further comprising a buffer.

1 7. A device for analyzing a sample containing DNA, comprising a thermostable
2 blunt-end DNA ligase, wherein said thermostable blunt-end DNA ligase is selected from a
3 thermostable blunt-end DNA ligase of claim 1, a thermostable blunt-end DNA ligase of claim
4 2, and a thermostable blunt-end DNA ligase of claim 3.

1 8. The thermostable blunt-end DNA ligase of claim 1, wherein said thermostable
2 blunt-end DNA ligase is suitable for use in a blunt-ended DNA ligation reaction performed at
3 about 75 °C.

1 9. The thermostable blunt-end DNA ligase of claim 1, wherein said thermostable
2 blunt-end DNA ligase is capable of making concatamers upon multiple ligation events in a
3 blunt-ended DNA ligation reaction performed at about 60 °C or higher.

1 10. The thermostable blunt-end DNA ligase of claim 1, wherein said thermostable
2 blunt-end DNA ligase is suitable for use in a nucleic acid amplification scheme which
3 operates at a uniform temperature of about 60 °C or higher.

1 11. The thermostable blunt-end DNA ligase of claim 1, wherein said thermostable
2 blunt-end DNA ligase is suitable for use in a nucleic acid amplification scheme which
3 comprises temperature cycling.

1 12. The thermostable blunt-end DNA ligase of claim 11, wherein said nucleic acid
2 amplification scheme comprises temperature cycling to temperatures of about 60 °C or
3 higher.

1 13. The thermostable blunt-end DNA ligase of claim 11, wherein said nucleic acid
2 amplification scheme comprises temperature cycling from about 94 °C to about 60 °C.

1 14. The thermostable blunt-end DNA ligase of claim 13, wherein said nucleic acid
2 amplification scheme comprises two or more cycles of temperature cycling.

1 15. The thermostable blunt-end DNA ligase of claim 13, wherein said nucleic acid
2 amplification scheme comprises three or more cycles of temperature cycling.

1 16. The thermostable blunt-end DNA ligase of claim 1, wherein said fusion
2 protein comprises a component selected from a peptide linker, an N-terminal addition, a C-
3 terminal addition, a tag peptide, a D-amino acid, and a peptide mimetic.

1 17. The thermostable blunt-end DNA ligase of claim 1, wherein said fusion
2 protein comprises a component selected from a sugar and a polymer.

1 18. The method of claim 4, wherein said elevated temperature comprises a
2 temperature of about 60 °C or higher.

1 19. A thermostable blunt-end nucleic acid ligase comprising a fusion protein
2 comprising a nucleic acid ligase and a nucleic acid binding protein, wherein said
3 thermostable blunt-end nucleic acid ligase is suitable for use in a blunt-ended nucleic acid
4 ligation reaction performed at about 60 °C or higher.

1 20. An article of manufacture, comprising a thermostable blunt-end nucleic acid
2 ligase of claim 19, and a container.

- 1 21. The article of manufacture of claim 20, further comprising a buffer.
- 1 22. A method of ligating blunt-end nucleic acids at an elevated temperature,
2 comprising using a thermostable blunt-end nucleic acid ligase of claim 19 at a temperature of
3 about 60 °C or higher.
- 1 23. A device for analyzing a sample containing nucleic acids, comprising a
2 thermostable blunt-end nucleic acid ligase of claim 19.
- 1 24. A fusion protein comprising an amino acid sequence selected from SEQ ID
2 NO: 1, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17,
3 SEQ ID NO: 18, and SEQ ID NO: 19.
- 1 25. A fusion protein comprising SEQ ID NO: 20 and a nucleic acid ligase.
- 1 26. The fusion protein of claim 25, wherein the nucleic acid ligase is a DNA
2 ligase.
- 1 27. The fusion protein of claim 25, wherein the nucleic acid ligase is a RNA
2 ligase.
- 1 28. The fusion protein of claim 25, wherein the nucleic acid ligase is a
2 thermostable DNA ligase.
- 1 29. The fusion protein of claim 25, wherein the nucleic acid ligase is a
2 thermostable RNA ligase.
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CLAIMS

1. A thermostable blunt-end DNA ligase suitable for use in a blunt-ended DNA
ligation reaction performed at about 60 °C or higher, comprising the amino acid sequence
(SEQ ID NO: 1):

mgghhhhhhhhhssghiegrasadgpylqileqpkqrgfrfryvcegpshgglpgasseknkksypqvkicnyvgpakvivql
vtngknihlhahslvgkhcedgictvtagpkdmvvgfanlgilhvtkkkvfetlearnmteacirgynpgllvhpdlaylqaegggdr
qlgdrekelirqaalqqtkemdlsvvrlmftaflpdstgsftrrlepvsdaiydskapnasnlkivrmrdragcvtggeeiyllcdkvq
kddiqirfyeeeenggvwegfgdfsptdvhrqfaivfktpkykdinitkpasvfvqlrrksdletsepkpflyypeikdkeevqrkrqk
gssgtsgggsgggmtleearkrvnelrdlryhnyryyvladpeisdaeydrllrelkeleerfelkspdsptlqvgarpleatfrpvr
hptmysldnafnldekafeerieralgrkgpfayvehkvdglsvnllyeegvlvygatrgdgevgeevtqnlltiptiprrlkgvpe
rlevrgevympieaflrlneeleergerifknprnaaagslrqkdprtakrglratfyalglgleeveregvatqfallhwlkekfpve
hgyaravgaegveavyqdwlkrralpfeadgvvkldeialwrelgytaraprfaiaaykfpaeeeketrldvvfvqgrtgrvtpvgil
epvflegsevsrvtlhnesyieeldirigdwvlvhkaggvipevlrvlkerrtgeerpirwpetcepcghrllkegkvhrpcnplcpak
rfeairhfaskamdiqglgeklirlekglvkdvadlylrkedlvglermgeksaqnllrqieeskkrglerllyalglpgvgevlarnl
aarfgnmdrleasleelleveevgeltarailtkdpafdrldvrllkeagvemeakekggealkglftvitgelspreevkallrrlga
kvtdsvsrktsylvvgenpgsklekaralgvptlteeelyrllartgkkaeeiv.

2. A method of ligating blunt-end DNA at elevated temperature, comprising
using a thermostable blunt-end DNA ligase at an elevated temperature, wherein said
thermostable blunt-end DNA ligase is a thermostable blunt-end DNA ligase of claim 1.

3. An article of manufacture, comprising a thermostable blunt-end DNA ligase
and a container, wherein said thermostable blunt-end DNA ligase is a thermostable blunt-
end DNA ligase of claim 1.

4. The article of manufacture of claim 3, further comprising a buffer.

5. A device for analyzing a sample containing DNA, comprising a thermostable
blunt-end DNA ligase, wherein said thermostable blunt-end DNA ligase is a thermostable
blunt-end DNA ligase of claim 1.

6. The thermostable blunt-end DNA ligase of claim 1, wherein said thermostable
blunt-end DNA ligase is suitable for use in a blunt-ended DNA ligation reaction performed at
about 75 °C.

7. The thermostable blunt-end DNA ligase of claim 1, wherein said thermostable blunt-end DNA ligase is capable of making concatamers upon multiple ligation events in a blunt-ended DNA ligation reaction performed at about 60 °C or higher.

5 8. The thermostable blunt-end DNA ligase of claim 1, wherein said thermostable blunt-end DNA ligase is suitable for use in a nucleic acid amplification scheme which operates at a uniform temperature of about 60 °C or higher.

10 9. The thermostable blunt-end DNA ligase of claim 1, wherein said thermostable blunt-end DNA ligase is suitable for use in a nucleic acid amplification scheme which comprises temperature cycling.

15 10. The thermostable blunt-end DNA ligase of claim 9, wherein said nucleic acid amplification scheme comprises temperature cycling to temperatures of about 60 °C or higher.

11. The thermostable blunt-end DNA ligase of claim 9, wherein said nucleic acid amplification scheme comprises temperature cycling from about 94 °C to about 60 °C.

20 12. The thermostable blunt-end DNA ligase of claim 11, wherein said nucleic acid amplification scheme comprises two or more cycles of temperature cycling.

25 13. The thermostable blunt-end DNA ligase of claim 11, wherein said nucleic acid amplification scheme comprises three or more cycles of temperature cycling.

14. The thermostable blunt-end DNA ligase of claim 1, wherein said fusion protein further comprises a component selected from a peptide linker, an N-terminal addition, a C-terminal addition, a tag peptide, a D-amino acid, and a peptide mimetic.

30 15. The thermostable blunt-end DNA ligase of claim 1, wherein said fusion protein further comprises a component selected from a sugar and a polymer.

16. The method of claim 2, wherein said elevated temperature comprises a temperature of about 60 °C or higher.

35 17. A fusion protein comprising an amino acid sequence selected from SEQ ID NO: 1, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, and SEQ ID NO: 19.

18. A fusion protein comprising SEQ ID NO: 11 or SEQ ID NO: 12 and a nucleic acid ligase.

5 19. The fusion protein of claim 18, wherein the nucleic acid ligase is a DNA ligase.

20. The fusion protein of claim 18, wherein the nucleic acid ligase is a RNA ligase.

10 21. The fusion protein of claim 18, wherein the nucleic acid ligase is a thermostable DNA ligase.

15 22. The fusion protein of claim 18, wherein the nucleic acid ligase is a thermostable RNA ligase.