

CLAIMS

1.- A DNA polymerase chimera comprising:

- a) an amino acid sequence encoding a ϕ 29 type DNA polymerase, bound by its C-terminal end to
- b) a connecting amino acid sequence bound by its C-terminal end to
- c) an amino acid sequence comprising at least one helix-hairpin-helix (HhH) domain.

2.- The DNA polymerase chimera according to claim 1, wherein the amino acid sequence of (c) comprises at least one HhH domain of a protein which is selected from the list comprising:

- topoisomerase V of *Methanopyrus kandleri*,
- MutY, Nth, MutM/Fpg, Nei, UvrC, DinP, RecR, UmuC, DnaE or DnIJ of *Escherichia coli*,
- RAD1, RAD2, RAD10, RAD27, RAD 55, RAD 57, REV1, OGG1, NTG1, NTG2, DIN-7 or EXO-1 of yeasts, or
- a homologous protein of the above in *Bacillus subtilis*, *Caenorhabditis elegans*, *Haemophilus influenzae*, *Methanococcus jannaschii*, *Micrococcus luteus*, *Methanobacterium thermoformicum* or *Salmonella typhimurium*.

3.- The DNA polymerase chimera according to claim 2, wherein the amino acid sequence of (c) comprises at least one HhH domain derived from the topoisomerase V of *Methanopyrus kandleri*.

4.- The DNA polymerase chimera according to claim 3, wherein the amino acid sequence of (c) is SEQ ID NO: 3.

5.- The DNA polymerase chimera according to claim 3, wherein the amino acid sequence of (c) is SEQ ID NO: 3 bound by its C-terminal end to SEQ ID NO: 4.

6.- The DNA polymerase chimera according to any of claims 1 to 5, wherein the connecting amino acid sequence of

(b) is SEQ ID NO: 5 or SEQ ID NO: 6.

7.- The DNA polymerase chimera according to any of claims 1 to 6, wherein the ϕ 29 type DNA polymerase of (a) is selected from the DNA polymerases isolated from the following phages: ϕ 29, Cp-1, PRD-1, ϕ 15, ϕ 21, PZE, PZA, Nf, MZY, B103, GA-1, SF5, Cp-5, Cp-7, PR4, PR5, PR722, L17 or ABV.

8.- The DNA polymerase chimera according to any of claims 1 to 7, wherein the ϕ 29 type DNA polymerase of (a) has an amino acid sequence having an identity of at least 80% with SEQ ID NO: 1.

9.- The DNA polymerase chimera according to claim 8, wherein the ϕ 29 type DNA polymerase of (a) has an amino acid sequence having an identity of at least 90% with SEQ ID NO: 1.

10.- The DNA polymerase chimera according to claim 9, wherein the ϕ 29 type DNA polymerase of (a) has the amino acid sequence SEQ ID NO: 1.

11.- The DNA polymerase chimera according to any of claims 1 to 10, wherein the ϕ 29 type DNA polymerase of (a) has a modification in the exonuclease domain and wherein said modified DNA polymerase has less than 10% of exonuclease activity than the corresponding naturally occurring DNA polymerase.

12.- The DNA polymerase chimera according to claim 11, wherein the modified ϕ 29 type DNA polymerase of (a) has less than 1% of exonuclease activity than the corresponding naturally occurring DNA polymerase.

13.- The DNA polymerase chimera according to claim 12, wherein the modified ϕ 29 type DNA polymerase of (a) lacks detectable exonuclease activity with respect to the corresponding naturally occurring DNA polymerase.

14.- Use of the DNA polymerase chimera according to any of claims 1 to 13 for replicating, amplifying or sequencing a template DNA.

15.- Method for replicating, amplifying or sequencing a

template DNA which comprises contacting said DNA with a reaction mixture comprising at least:

- a) the DNA polymerase chimera according to any of claims 1 to 12,
- 5 b) a buffer,
- c) magnesium chloride,
- d) a primer, and
- e) nucleoside triphosphates.

10 16.- The method according to claim 15, wherein the reaction mixture further comprises polyoxyethylenated sorbitan monolaurate.

17.- The method according to either of claims 15 or 16, wherein the reaction mixture further comprises an ammonium salt.

15 18.- The method according to any of claims 15 to 17, wherein the reaction mixture further comprises a potassium salt.

19.- The method according to claim 18, wherein the potassium salt is potassium chloride or potassium acetate.

20 20.- The method according to any of claims 16 to 19, wherein the polyoxyethylenated sorbitan monolaurate is in a proportion between 0.003% and 0.1% of the total volume of the reaction.

25 21.- The method according to claim 20, wherein the polyoxyethylenated sorbitan monolaurate is in a proportion between 0.006% and 0.05% of the total volume of the reaction.

22.- The method according to claim 21, wherein the polyoxyethylenated sorbitan monolaurate is in a proportion between 0.01% and 0.03% of the total volume of the reaction.

30 23.- The method according to any of claims 17 to 22, wherein the ammonium salt is selected from the list comprising: ammonium sulfate, ammonium chloride or ammonium acetate.

35 24.- The method according to claim 23, wherein the ammonium salt is ammonium sulfate.

25.- The method according to claim 24, wherein the ammonium sulfate is at a concentration between 30 mM and 60 mM.

5 26.- The method according to claim 25, wherein the ammonium sulfate is at a concentration between 40 mM and 50 mM.

27.- The method according to claim 23, wherein the ammonium salt is ammonium chloride or ammonium acetate.

10 28.- The method according to claim 27, wherein the ammonium chloride or the ammonium acetate is at a concentration between 60 mM and 120 mM.

29.- The method according to claim 28, wherein the ammonium chloride or the ammonium acetate is at a concentration between 80 mM and 100 mM.

15 30.- The method according to any of claims 15 to 29, wherein the buffer is tris-hydrochloric, tris-acetic or HEPES.

31.- The method according to any of claims 15 to 30, wherein the buffer is at a pH between 7.0 and 8.5.

20 32.- The method according to any of claims 15 to 31, wherein the magnesium chloride is at a concentration between 2 mM and 20 mM.

33.- The method according to claim 32, wherein the magnesium chloride is at a concentration between 5 mM and 15 mM.

25 34.- The method according to any of claims 18 to 33, wherein the potassium chloride or the potassium acetate is at a concentration between 30 mM and 70 mM.

30 35.- The method according to claim 34, wherein the potassium chloride or the potassium acetate is at a concentration between 40 mM and 60 mM.

36.- The method according to any of claims 15 to 35, wherein the nucleoside triphosphates are dCTP, dGTP, dTTP and dATP.

35 37.- The method according to claim 36, wherein the dCTP, dGTP, dTTP and dATP nucleoside triphosphates are in equimolar

amounts.

38.- The method according to any of claims 15 to 37, wherein the primer is arbitrary and is protected against the action of exonucleases.

5 39.- The method according to any of claims 15 to 38, wherein the template DNA is plasmid DNA.

40.- The method according to any of claims 15 to 38, wherein the template DNA is genomic DNA.

10 41.- The method according to any of claims 15 to 40, wherein the amplification is performed at an essentially constant temperature between 25 and 40 °C.

42.- The method for amplifying a template DNA according to any of claims 15 to 41, wherein the amplification takes place by means of rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA) or loop mediated amplification (LAMP).

43.- The method according to any of claims 15 to 42, wherein at least one nucleoside triphosphate or one primer is labelled.

20 44.- Kit for carrying out a method according to claims 15 to 43 comprising:

- a) the DNA polymerase chimera according to any of claims 1 to 13,
- b) a buffer, and
- 25 c) magnesium chloride.

45.- Kit according to claim 44, further comprising polyoxyethylenated sorbitan monolaurate.

46.- Kit according to either of claims 44 or 45, further comprising an ammonium salt.

30 47.- Kit according to any of claims 44 to 46, further comprising a potassium salt.

48.- Kit according to either of claims 44 or 47, further comprising a primer.

35 49.- Kit according to any of claims 44 to 48, further comprising the primer according to claim 38.

50.- Kit according to any of claims 44 to 49, further comprising nucleoside triphosphates.

51.- Kit according to claims 48 to 50, wherein at least one nucleoside triphosphate or one primer is labelled.

Claims

1. A DNA polymerase chimera comprising:
 - an amino acid sequence encoding a ϕ 29 type DNA polymerase (a), bound by its C-terminal end to a connecting amino acid sequence (b) bound by its C-terminal end to
 - an amino acid sequence comprising at least one DNA binding helix-hairpin-helix (HhH) domain (c).
2. The DNA polymerase chimera according to claim 1, wherein the amino acid sequence of (c) comprises at least one HhH domain of a protein which is selected from the list comprising:
 - topoisomerase V of *Methanopyrus kandleri*,
 - MutY, Nth, MutM/Fpg, Nei, UvrC, DinP, RecR, UmuC, DnaE or DnIJ of *Escherichia coli*,
 - RAD1, RAD2, RAD10, RAD27, RAD 55, RAD 57, REV1, OGG1, NTG1, NTG2, DIN-7 or EXO-1 of yeasts, or
 - a homologous protein of the above in *Bacillus subtilis*,
 - Caenorhabditis elegans*, *Haemophilus influenzae*, *Methanococcus jannaschii*, *Micrococcus luteus*, *Methanobacterium thermoformicum* or *Salmonella typhimurium*.
3. The DNA polymerase chimera according to claim 2, wherein the amino acid sequence of (c) comprises at least one HhH domain derived from the topoisomerase V of *Methanopyrus kandleri*.
4. The DNA polymerase chimera according to claim 3, wherein the amino acid sequence of (c) is SEQ ID NO: 3.
5. The DNA polymerase chimera according to claim 3, wherein the amino acid sequence of (c) is SEQ ID NO: 3 bound by its C-terminal end to SEQ ID NO: 4.
6. The DNA polymerase chimera according to any of claims 1 to 5, wherein the connecting amino acid sequence of (b) is SEQ ID NO: 5 or SEQ ID NO: 6.
7. The DNA polymerase chimera according to any of claims 1 to 6, wherein the ϕ 29 type DNA polymerase of (a) is selected from the DNA polymerases isolated from the following phages: ϕ 29, Cp-1, PRD-1, ϕ 15, ϕ 21, PZE, PZA, Nf, M2Y, B103, GA-1, SF5, Cp-5, Cp-7, PR4, PR5, PR722, L17 or ABV.
8. The DNA polymerase chimera according to any of claims 1 to 7, wherein the ϕ 29 type DNA polymerase of (a) has an amino acid sequence having an identity of at least 80% with SEQ ID NO: 1.
9. The DNA polymerase chimera according to claim 8, wherein the ϕ 29 type DNA polymerase of (a) has the amino acid sequence SEQ ID NO: 1.
10. The DNA polymerase chimera according to any of claims 1 to 9, wherein the ϕ 29 type DNA polymerase of (a) has a modification in the exonuclease domain and wherein said modified DNA polymerase has less than 10% of exonuclease activity than the corresponding naturally occurring DNA polymerase.
11. The DNA polymerase chimera according to claim 10, wherein the modified ϕ 29 type DNA polymerase of (a) has less than 1% of exonuclease activity than the corresponding naturally occurring DNA polymerase.
12. The DNA polymerase chimera according to claim 11, wherein the modified ϕ 29 type DNA polymerase of (a) lacks detectable exonuclease activity with respect to the corresponding naturally occurring DNA polymerase.
13. Use of the DNA polymerase chimera according to any of claims 1 to 12 for replicating, amplifying or sequencing a template DNA.

14. Method for replicating, amplifying or sequencing a template DNA which comprises contacting said DNA with a reaction mixture comprising at least:
 - a) the DNA polymerase chimera according to any of claims 1 to 11,
 - b) a buffer,
 - c) magnesium chloride,
 - d) a primer, and
 - e) nucleoside triphosphates.
15. The method according to claim 14, wherein the reaction mixture further comprises polyoxyethylenated sorbitan monolaurate.
16. The method according to either of claims 14 or 15, wherein the reaction mixture further comprises an ammonium salt.
17. The method according to any of claims 14 to 16, wherein the reaction mixture further comprises a potassium salt.
18. The method according to claim 17, wherein the potassium salt is potassium chloride or potassium acetate.
19. The method according to any of claims 15 to 18, wherein the polyoxyethylenated sorbitan monolaurate is in a proportion between 0.003% and 0.1% of the total volume of the reaction.
20. The method according to any of claims 16 to 19, wherein the ammonium salt is selected from the list comprising: ammonium sulfate, ammonium chloride or ammonium acetate.
21. The method according to claim 20, wherein the ammonium salt is ammonium sulfate.
22. The method according to claim 21, wherein the ammonium sulfate is at a concentration between 30 mM and 60 mM.
23. The method according to claim 20, wherein the ammonium salt is ammonium chloride or ammonium acetate.
24. The method according to claim 23, wherein the ammonium chloride or the ammonium acetate is at a concentration between 60 mM and 120 mM.
25. The method according to any of claims 14 to 24, wherein the buffer is tris-hydrochloric, tris-acetic or HEPES.
26. The method according to any of claims 14 to 25, wherein the buffer is at a pH between 7.0 and 8.5.
27. The method according to any of claims 14 to 26, wherein the magnesium chloride is at a concentration between 2 mM and 20 mM.
28. The method according to any of claims 17 to 27, wherein the potassium chloride or the potassium acetate is at a concentration between 30 mM and 70 mM.
29. The method according to any of claims 14 to 28, wherein the nucleoside triphosphates are dCTP, dGTP, dTTP and dATP.
30. The method according to claim 29, wherein the dCTP, dGTP, dTTP and dATP nucleoside triphosphates are in equimolar amounts.

31. The method according to any of claims 14 to 30, wherein the primer is arbitrary and is protected against the action of exonucleases.
32. The method according to any of claims 14 to 31, wherein the template DNA is plasmid DNA or genomic DNA.
33. The method according to any of claims 14 to 32, wherein the amplification is performed at an essentially constant temperature between 25 and 40 °C.
34. The method for amplifying a template DNA according to any of claims 14 to 33, wherein the amplification takes place by means of rolling circle amplification (RCA), multiple displacement amplification (MDA), strand displacement amplification (SDA) or loop mediated amplification (LAMP).
35. The method according to any of claims 14 to 34, wherein at least one nucleoside triphosphate or one primer is labelled.
36. Kit for carrying out a method according to claims 14 to 35 comprising:
 - a) the DNA polymerase chimera according to any of claims 1 to 12,
 - b) a buffer, and
 - c) magnesium chloride.
37. Kit according to claim 36, further comprising polyoxyethylenated sorbitan monolaurate.
38. Kit according to either of claims 36 or 37, further comprising an ammonium salt.
39. Kit according to any of claims 36 to 38, further comprising a potassium salt.
40. Kit according to either of claims 36 or 39, further comprising a primer.
41. Kit according to any of claims 36 to 40, further comprising the primer according to claim 31.
42. Kit according to any of claims 36 to 41, further comprising nucleoside triphosphates.
43. Kit according to claims 36 to 42, wherein at least one nucleoside triphosphate or one primer is labelled.