

Single Molecule Real Time (SMRT) DNA sequencing technology and an isothermal amplification method for next generation sequencing published in 2013 by Ma *et al.* (Ma, Z. *et al. Proc Natl Acad Sci U S A* 110(35): 14320-14323).

5 The current toolbox of polymerase enzymes which function well at ambient temperature is, however, very limited. Typically, different isothermal methods require reaction temperatures between 30-65°C which are mainly determined by the working range of the polymerases used in the reactions and are prone to inhibition by salt.

10 A cold-adapted polymerase from a *Psychrobacillus* sp. (PB) belonging to the A-family of DNA polymerases has been characterized. This enzyme possesses high polymerase activity at ambient temperatures but still has good stability at elevated temperatures up to 40 °C. Of particular interest, the marine derived enzyme also possesses good salt tolerance and strong strand-displacement activity (SDA) as well as proficient processivity at 25 °C, and is comparable with the state-
15 of-the art commercial enzymes (WO 2017/162765).

In many IA methods only a polymerase is required and the effectiveness of the method is heavily dependent on the SDA of that polymerase. Therefore anything which served to increase SDA of the PB or other polymerases used in IA would be highly desirable.

20 The present inventors have surprisingly found that a single point mutation in the finger domain of certain polymerases in the A family, in particular replacement of a single Asp residue, leads to significantly enhanced SDA.

Summary

25 Therefore, in a first example, the present disclosure provides a DNA polymerase, said DNA polymerase including the sequence of SEQ ID NO. 1 or a sequence which is at least 70%, preferably at least 75%, 78%, 80%, 82%, 85%, 88%, 90%, 92% or 95%, identical thereto, but wherein the aspartic acid residue at position 18 of SEQ ID NO. 1, or the equivalent aspartic acid residue in other sequences, has been replaced by a non-negatively charged amino acid residue.

30 In one aspect, the present disclosure refers to a DNA polymerase including the sequence of SEQ ID NO. 1 or a variant sequence which is at least 70% identical to SEQ ID NO: 1, but wherein the aspartic acid residue at position 18 of SEQ ID NO. 1, or the equivalent aspartic acid residue in said variant sequence, has been replaced by a alanine.

35 SEQ ID NO. 1 is a fragment of the amino acid sequence of the PB polymerase I, it spans amino acids 405 to 436 in the truncated (lacking the 5'- 3'-exonuclease domain) wild type PB sequence. This region (405-436) within the finger domain is highly conserved amongst some of the DNA polymerase A family (also known as pol I family), see Figures 1 and 5. DNA polymerases of the
40 disclosure would typically be classed as of the DNA polymerase I or A type.

readily identified by using standard sequence alignment techniques such as Clustal X2 (Larkin, M.A. et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics, 23:2947-2948).

5 Of course, SEQ ID NO. 1 does not itself define a fully functional DNA polymerase. In preferred examples the DNA polymerase of the present disclosure is based on the amino acid sequence of PB DNA polymerase I, preferably which lacks the 5'-3'- exonuclease domain that is present in the wild-type *Psychrobacillus* species DNA polymerase I sequence. In preferred examples, the 5'-3'- exonuclease domain is absent from the DNA polymerase enzyme as 5'-3'-
10 exonuclease activity is typically unwanted as it may degrade primers and/or products in an amplification mixture. This truncated wild-type PB sequence is referred to herein as SEQ ID NO. 2.

The disclosure provides a DNA polymerase comprising or consisting of the amino acid sequence of SEQ ID NO:2 or an amino acid sequence which is at least
15 60% identical to SEQ ID NO:2 but wherein the aspartic acid residue at position 422 of SEQ ID NO. 2, or the equivalent aspartic acid residue in other sequences, has been replaced by a non-negatively charged amino acid residue.

In another aspect, the present disclosure refers to a DNA polymerase comprising the amino acid sequence of SEQ ID NO. 2, or a variant sequence which
20 is at least 60% identical to SEQ ID NO. 2, but wherein the aspartic acid residue at position 422 of SEQ ID NO. 2, or the equivalent aspartic acid residue in said variant sequence, has been replaced by alanine.

In preferred examples and examples, the DNA polymerase of the disclosure comprises (or consists of) an amino acid sequence that is at least 70%, or 75%,
25 preferably at least 80%, 85%, 90% or 95%, e.g. at least 98% or 99% or 99.5%, identical to SEQ ID NO:2 but wherein the aspartic acid residue at position 422 of SEQ ID NO. 2, or the equivalent aspartic acid residue in other sequences, has been replaced by a non-negatively charged amino acid residue. It will be understood that position 18 in SEQ ID NO. 1 and 422 in SEQ ID NO. 2 are equivalent, SEQ ID NO.
30 1 is a fragment from position 405 to 436 of SEQ ID NO. 2.

Figure 5 shows an alignment of PB, Bst and Upts DNA polymerases. The key Asp residue is at position 422 in each case.

In further preferred examples, the DNA polymerase of the disclosure comprises (or consists of) an amino acid sequence that is at least 60%, 70% or
35 75%, preferably at least 80%, 85%, 90% or 95%, e.g. at least 98% or 99% or 99.5%, identical to SEQ ID NO. 11 or 12 but wherein the aspartic acid residue at position 422 of SEQ ID NO. 11 or 12, or the equivalent aspartic acid residue in other sequences, has been replaced by a non-negatively charged amino acid residue. Numbering is based on a sequence alignment according to Figure 5. SEQ
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ID NOs. 11 and 12 are the (truncated) variants of the wild type Bst and Ubs polymerase sequences respectively.

In another aspect, the present disclosure refers to a DNA polymerase comprising the amino acid sequence of SEQ ID NO. 11 or 12, or a variant sequence which is at least 60% identical to SEQ ID NO. 11 or 12, but wherein the aspartic acid residue at position 422 of SEQ ID NO. 11, or 12, or the equivalent aspartic acid residue in said variant sequence, has been replaced by alanine.

Preferably, the DNA polymerase of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO:2, 11 or 12 but wherein the aspartic acid residue at position 422 of SEQ ID NO. 2, 11 or 12, or the equivalent aspartic acid residue in other sequences, has been replaced by a non-negatively charged amino acid residue .

In one example, the DNA polymerase comprises (or consists of) the amino acid sequence of SEQ ID NO:4 (incorporating also the 5'→3' exonuclease domain) or a variant or fragment thereof but wherein the aspartic acid residue at position 719 of SEQ ID NO. 4, or the equivalent aspartic acid residue in other sequences, has been replaced by a non-negatively charged amino acid residue. The types of variants and fragments of SEQ ID NO:2 described herein apply, *mutatis mutandis*, to variants and fragments of SEQ ID NO:4, e.g. variants will have at least 70% preferably at least 80% or 90% sequence identity to SEQ ID.NO:4.

DNA polymerases of the disclosure include enzymatically active fragments of native polymerases. Enzymatically active fragments are fragments that have DNA polymerase activity. Enzymatically active fragments may be at least 400, at least 450, at least 475, at least 500, at least 525, at least 550, at least 560, at least 570 or at least 575 amino acids in length. Preferred fragments are at least 525, at least 550, at least 560, at least 570 or at least 575 amino acids in length. The fragments are at least 70%, preferably at least 80%, at least 85% or at least 90%, more preferably at least 95% (e.g. at least 98% or 99% or 99.5%), or 100% identical to the corresponding portion of SEQ ID NO:2, 11 or 12 but wherein the aspartic acid residue at position 422 of SEQ ID NO. 2, 11 or 12, or the equivalent aspartic acid residue in other sequences, has been replaced by a non-negatively charged amino acid residue.

DNA polymerase activity may be assessed using a molecular beacon that bears a loop structure and uses FAM as fluorescence donor and Dabcyl as an acceptor (non-fluorescent quencher) within an 8mer stem. This stem bears a 3'- extension that allows binding of a primer and acts as template for the DNA polymerase. The stem will be opened by the DNA polymerase when the extension proceeds. The following separation of the two labels is recorded by restoration of FAM emission. A suitable assay of this type is described in the Examples.

Preferred DNA polymerases of the present disclosure have useful levels of polymerase activity across a range of salt (NaCl and/or KCl) concentrations. Put another way, preferred DNA polymerases of the present disclosure exhibit across a broad range of salt concentrations a substantial proportion of the DNA polymerase activity observed at the salt concentration at which maximum polymerase activity is observed. Suitable assays for determining DNA polymerase activity are described elsewhere herein. A preferred assay for determining DNA polymerase activity is as described in the Example section.

In some examples, across a concentration range from about 20mM to 200mM NaCl or KCl or a mixture thereof, DNA polymerases of the present disclosure exhibit a substantial proportion (e.g. at least 40%, preferably at least 50%, more preferably at least 60%) of their maximum polymerase activity.

In a further example the present disclosure provides molecules (e.g. proteins, such as fusion proteins) comprising DNA polymerases of the present disclosure.

As used throughout the entire application, the terms "a" and "an" are used in the sense that they mean "at least one", "at least a first", "one or more" or "a plurality" of the referenced components or steps, except in instances wherein an upper limit or exclusion is thereafter specifically stated. The operable limits and parameters of combinations, as with the amounts of any single agent, will be known to those of ordinary skill in the art in light of the present disclosure.

Nucleic acid molecules comprising nucleotide sequences that encode DNA polymerases of the present disclosure as defined herein or fragments thereof, or nucleic acid molecules substantially homologous thereto, form yet further examples of the disclosure. A preferred nucleic acid molecule is a nucleic acid encoding a DNA polymerase I of SEQ ID NO:2, or a sequence substantially homologous thereto (e.g. at least 60%, 70%, 75%, 80%, 85%, 90% or 95% identical thereto), but wherein the aspartic acid residue at position 422 of SEQ ID NO. 2, or the equivalent aspartic acid residue in other sequences, has been replaced by a non-negatively charged amino acid residue.

In another aspect, the present disclosure refers to a nucleic acid molecule comprising a nucleotide sequence that encodes the DNA polymerase as disclosed herein.

A preferred nucleic acid molecule comprises (or consists of) the nucleotide sequence as set forth in SEQ ID NO: 13, 14 or 15, or is a sequence substantially homologous thereto. Optionally, the final three nucleotides of SEQ ID NO: 13, 14 or 15 may be omitted. Nucleic acid sequences of the disclosure include sequences having at least 70% or 75%, preferably at least 80%, and even more preferably at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 99.5%, sequence identity to SEQ

Generally, computer programs will be employed for such calculations. Programs that compare and align pairs of sequences, like ALIGN (Myers and Miller, *CABIOS*, 4:11-17, 1988), FASTA (Pearson and Lipman, *Proc. Natl. Acad. Sci. USA*, 85:2444-2448, 1988; Pearson, *Methods in Enzymology*, 183:63-98, 1990) and gapped BLAST (Altschul *et al.*, *Nucleic Acids Res.*, 25:3389-3402, 1997), BLASTP, BLASTN, or GCG (Devereux, Haeberli, Smithies, *Nucleic Acids Res.*, 12:387, 1984) are also useful for this purpose. Furthermore, the Dali server at the European Bioinformatics institute offers structure-based alignments of protein sequences (Holm, *Trends in Biochemical Sciences*, 20:478-480, 1995; Holm, *J. Mol. Biol.*, 233:123-38, 1993; Holm, *Nucleic Acid Res.*, 26:316-9, 1998).

By way of providing a reference point, sequences according to the present disclosure having 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99%, sequence identity etc. may be determined using the ALIGN program with default parameters (for instance available on Internet at the GENESTREAM network server, IGH, Montpellier, France).

A "conservative amino acid substitution", as used herein, is one in which the amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art.

DNA polymerases of the present disclosure comprise genetically encoded amino acids, but may also contain one or more non-genetically encoded amino acids.

When used in connection with a protein or polypeptide molecule such as a DNA polymerase, the term "isolated" or "purified" typically refers to a protein substantially free of cellular material or other proteins from the source from which it is derived. In some examples, such isolated or purified proteins are substantially free of culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized.

In one further example the present disclosure provides an expression vector (preferably a recombinant expression vector) containing a nucleic acid molecule of the disclosure, or a fragment thereof, and the necessary regulatory sequences for the transcription and translation of the protein sequence encoded by the nucleic acid molecule of the disclosure.

In another aspect, the present disclosure refers to an expression vector containing the nucleic acid molecule as disclosed herein and the necessary regulatory sequences for the transcription and translation of the protein sequence encoded by said nucleic acid molecule.

Possible expression vectors include but are not limited to cosmids or plasmids, so long as the vector is compatible with the host cell used. The

polymerase or product into a composition including at least one additional component, such as an acceptable buffer or carrier.

In another aspect, the present disclosure refers to a method of producing a DNA polymerase as disclosed herein, wherein the method comprises the steps of:

(i) culturing, in a growth medium, a host cell comprising one or more of the recombinant expression vectors as disclosed herein or one or more of the nucleic acid molecules as disclosed herein under conditions suitable for the expression of the encoded DNA polymerase; and optionally

(ii) isolating or obtaining the DNA polymerase from the host cell or from the growth medium or supernatant derived from said growth medium.

The DNA polymerase may be separated, or isolated, from the host cells/culture media using any of the purification techniques for protein known in the art and widely described in the literature or any combination thereof. Such techniques may include for example, precipitation, ultrafiltration, dialysis, various chromatographic techniques, e.g. size exclusion chromatography, ion-exchange chromatography, affinity chromatography, electrophoresis, centrifugation etc. As discussed above, the DNA polymerase of the disclosure may be modified to carry amino acid motifs or other protein or non-protein tags, e.g. polyhistidine tags (e.g. His₆-tag), to assist in isolation, solubilisation and/or purification or identification.

In another example, the present disclosure provides the use of a DNA polymerase of the disclosure for nucleotide (e.g. dNTP) polymerisation. Accordingly, DNA polymerases of the disclosure may be used to extend a nucleic acid (DNA) strand by one or more nucleotides.

In another aspect, the present disclosure refers to use of the DNA polymerase as disclosed herein for nucleotide polymerization.

In another example, the present disclosure provides the use of a DNA polymerase of the disclosure in a nucleic acid (DNA) amplification or sequencing reaction.

In another aspect, the present disclosure refers to use of the DNA polymerase as disclosed herein in a nucleic acid amplification or sequencing reaction.

In another example, the present disclosure provides the use of a DNA polymerase of the disclosure in a molecular beacon assay or in a strand displacement assay, e.g. as described herein.

Preferably, in uses and methods of the present disclosure, DNA polymerases of the present disclosure are used at a constant temperature, i.e. without thermal cycling. Accordingly, the use of DNA polymerases of the disclosure in isothermal reactions is particularly preferred.

The use of DNA polymerases of the disclosure in isothermal amplification reactions is particularly preferred. Isothermal reactions are performed at a constant temperature. Many isothermal amplification techniques are known in the art and include Loop mediated isothermal amplification (LAMP), rolling circle amplification (RCA), strand displacement amplification (SDA), multiple displacement amplification (MDA) and cross priming amplification (CPA).

In another example, the present disclosure provides a method of nucleotide polymerisation using a DNA polymerase of the present disclosure. Preferably, said method comprises providing a reaction mixture comprising a DNA polymerase of the present disclosure, a template nucleic acid molecule, an oligonucleotide primer

which is capable of annealing to a portion of the template nucleic acid molecule and one or more species of nucleotide (e.g. deoxynucleoside triphosphates, dNTPs) and incubating said reaction mixture under conditions whereby the oligonucleotide primer anneals to the template nucleic acid molecule and said DNA polymerase
5 extends said oligonucleotide primer by polymerising one or more nucleotides. Suitable conditions are well known in the art. Preferably a constant temperature is used and preferred temperatures are set out elsewhere herein. Optionally, the generation of the polynucleotide product is detected (e.g. via gel electrophoresis). In another aspect, the present disclosure refers to a method of nucleotide

10 polymerisation using the DNA polymerase as disclosed herein, said method comprising:

(i) providing a reaction mixture comprising the DNA polymerase as disclosed herein, a template nucleic acid molecule, an oligonucleotide primer which is capable of annealing to a portion of the template nucleic acid molecule and one
15 or more species of nucleotide; and

(ii) incubating said reaction mixture under conditions whereby the oligonucleotide primer anneals to the template nucleic acid molecule and said DNA polymerase extends said oligonucleotide primer by polymerising one or more nucleotides.

20 In another example, the present disclosure provides a method of amplifying a nucleic acid (DNA) using a DNA polymerase of the present disclosure. Typically, said method comprises providing a reaction mixture comprising a DNA polymerase of the present disclosure, a template nucleic acid molecule, an oligonucleotide primer(s) (e.g. 2 or more primers such as 2, 3, 4, 5 or 6 primers) which is capable of
25 annealing to a portion of the template nucleic acid molecule, and nucleotides (e.g. deoxynucleoside triphosphates, dNTPs) and incubating said reaction mixture under conditions whereby the oligonucleotide primer(s) anneals to the template nucleic acid molecule and said DNA polymerase extends said oligonucleotide primer(s) by polymerising one or more nucleotides to generate a
30 polynucleotide. Suitable conditions are well known in the art. Preferred methods of nucleic acid amplification are isothermal amplification methods. Isothermal amplification methods of the disclosure are performed at a constant temperature and preferred temperatures are set out elsewhere herein. Optionally, the generation of the polynucleotide product is detected (e.g. via gel electrophoresis).

In another aspect, the present disclosure refers to a method of amplifying a nucleic acid using the DNA polymerase as disclosed herein, said method comprising:

(i) providing a reaction mixture comprising the DNA polymerase as disclosed herein, a template nucleic acid molecule, an oligonucleotide primer(s) which is capable of annealing to a portion of the template nucleic acid molecule, and nucleotides; and

(ii) incubating said reaction mixture under conditions whereby the oligonucleotide primer(s) anneals to the template nucleic acid molecule and said DNA polymerase extends said oligonucleotide primer(s) by polymerising one or more nucleotides to generate a polynucleotide.

Exemplary isothermal amplification methods include Loop mediated isothermal amplification (LAMP), rolling circle amplification (RCA), strand displacement amplification (SDA), multiple displacement amplification (MDA) and cross priming amplification (CPA).

In some examples, particularly those using DNA polymerases based on the PB sequence, the constant temperature used in the methods and uses of the present disclosure is a low-to-moderate temperature, for example, is chosen from within the range 0°C to about 42°C, preferably is chosen from within the range about 10°C to about 40°C, or about 20°C to about 40°C, or about 25°C to about 40°C, or about 30°C to about 40°C or about 35°C to about 40°C, or about 37°C to about 40°C. In some examples, the constant temperature is chosen from within the range about 10°C to about 15°C, or about 10°C to about 20°C. In some

the necessary reagents to carry out a nucleic acid amplification reaction (e.g. an isothermal amplification reaction), e.g. oligonucleotide primers capable of annealing to a region of the template DNA to be amplified and/or nucleotides (e.g. dNTPs). Typically compositions will be aqueous and buffered with a standard buffer such as Tris, HEPES, etc.

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In another aspect, the present disclosure refers to a composition comprising the DNA polymerase as disclosed herein and a buffer.

The disclosure further includes kits comprising one or more of the DNA polymerases of the disclosure, or one or more compositions of the disclosure, or one or more of the nucleic acid molecules of the disclosure, or one or more expression vectors of the disclosure, or one or more host cells or viruses of the disclosure. Preferably said kits are for use in the methods and uses as described herein, e.g., in nucleic acid amplification methods, such as isothermal amplification reactions. Preferably said kits comprise instructions for use of the kit components, for example for nucleic acid amplification.

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CLAIMS

1. A DNA polymerase including the sequence of SEQ ID NO. 1 or a variant sequence which is at least 70% identical to SEQ ID NO: 1, but wherein the aspartic acid residue at position 18 of SEQ ID NO. 1, or the equivalent aspartic acid residue in said variant sequence, has been replaced by alanine.
2. The DNA polymerase according to claim 1, wherein said DNA polymerase includes the sequence of SEQ ID NO: 1 or a variant sequence which is at least 88% identical to SEQ ID NO. 1, but wherein the aspartic acid residue at position 18 of SEQ ID NO. 1, or the equivalent aspartic acid residue in said variant sequence, has been replaced by alanine.
3. The DNA polymerase according to claim 1 or claim 2, wherein said DNA polymerase comprises the amino acid sequence of SEQ ID NO. 6, 7, 8, 9, or 10 but wherein the aspartic acid residue at position 18 of each sequence has been replaced by alanine.
4. A DNA polymerase comprising the amino acid sequence of SEQ ID NO. 2, or a variant sequence which is at least 60% identical to SEQ ID NO. 2, but wherein the aspartic acid residue at position 422 of SEQ ID NO. 2, or the equivalent aspartic acid residue in said variant sequence, has been replaced by alanine.
5. The DNA polymerase according to any one of the preceding claims, wherein said DNA polymerase comprises the amino acid sequence of SEQ ID NO. 2, or a variant sequence which is at least 90% identical to SEQ ID NO. 2, but wherein the aspartic acid residue at position 422 of SEQ ID NO. 2, or the equivalent aspartic acid residue in said variant sequence, has been replaced by alanine.
6. The DNA polymerase according to any one of the preceding claims, wherein said DNA polymerase comprises the amino acid sequence of SEQ ID NO:4 or a variant sequence which is at least 60% identical to SEQ ID NO. 4, but wherein the aspartic acid residue at position 719 of SEQ ID NO. 4, or the

equivalent aspartic acid residue in said variant sequence, has been replaced by alanine.

5 7. A DNA polymerase comprising the amino acid sequence of SEQ ID NO. 11 or 12, or a variant sequence which is at least 60% identical to SEQ ID NO. 11 or 12, but wherein the aspartic acid residue at position 422 of SEQ ID NO. 11, or 12, or the equivalent aspartic acid residue in said variant sequence, has been replaced by alanine.

10 8. The DNA polymerase according to any one of the preceding claims, wherein said DNA polymerase comprises the amino acid sequence of SEQ ID NO: 11 or 12, or a variant sequence which is at least 90% identical to SEQ ID NO. 11 or 12, but wherein the aspartic acid residue at position 422 of SEQ ID NO. 11 or 12, or the equivalent aspartic acid residue in said variant sequence, has been replaced
15 by alanine.

9. The DNA polymerase according to any one of the preceding claims, wherein said DNA polymerase has at least 30% greater strand displacement activity than a DNA polymerase with exactly the same sequence but with aspartic
20 acid at position 18 or 422, relative to SEQ ID NO: 1 or 2 respectively, or at the equivalent position in said variant sequences.

10. The DNA polymerase according to any one of the preceding claims, wherein across a concentration range from 20mM to 200mM NaCl or KCl or a
25 mixture thereof, said DNA polymerase exhibits at least 40% of its maximum polymerase activity.

11. A composition comprising the DNA polymerase according to any one of the preceding claims and a buffer.
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12. A nucleic acid molecule comprising a nucleotide sequence that encodes the DNA polymerase according to any one of claims 1 to 10.

13. The nucleic acid molecule of claim 12, wherein the nucleotide sequence
35 has at least 70% sequence identity to SEQ ID NO: 13, 14 or 15.

14. An expression vector containing the nucleic acid molecule according to claim 12 or claim 13 and the necessary regulatory sequences for the transcription and translation of the protein sequence encoded by said nucleic acid molecule.
- 5 15. A host cell or virus comprising one or more expression vectors according to claim 14 or one or more nucleic acid molecules according to claim 12 or claim 13.
16. A method of producing a DNA polymerase according to any one of claims 1 to 10, wherein the method comprises the steps of:
- 10 (i) culturing, in a growth medium, a host cell comprising one or more of the recombinant expression vectors according to claim 14 or one or more of the nucleic acid molecules according to claim 12 or claim 13 under conditions suitable for the expression of the encoded DNA polymerase; and optionally
- (ii) isolating or obtaining the DNA polymerase from the host cell or from the
- 15 growth medium or supernatant derived from said growth medium.
17. Use of the DNA polymerase according to any one of claims 1 to 10 for nucleotide polymerisation.
- 20 18. Use of the DNA polymerase according to any one of claims 1 to 10 in a nucleic acid amplification or sequencing reaction.
19. The use of claim 18, wherein said reaction is an isothermal nucleic acid amplification reaction.
- 25 20. The use of any one of claims 17 to 19, wherein said DNA polymerase is used at a constant temperature.
21. A method of nucleotide polymerisation using the DNA polymerase
- 30 according to any one of claims 1 to 10, said method comprising:
- (i) providing a reaction mixture comprising the DNA polymerase according to any one of claims 1 to 10, a template nucleic acid molecule, an oligonucleotide primer which is capable of annealing to a portion of the template nucleic acid molecule and one or more species of nucleotide; and

(ii) incubating said reaction mixture under conditions whereby the oligonucleotide primer anneals to the template nucleic acid molecule and said DNA polymerase extends said oligonucleotide primer by polymerising one or more nucleotides.

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22. A method of amplifying a nucleic acid using the DNA polymerase according to any one of claims 1 to 10, said method comprising:

10 (i) providing a reaction mixture comprising the DNA polymerase according to any one of claims 1 to 10, a template nucleic acid molecule, an oligonucleotide primer(s) which is capable of annealing to a portion of the template nucleic acid molecule, and nucleotides; and

15 (ii) incubating said reaction mixture under conditions whereby the oligonucleotide primer(s) anneals to the template nucleic acid molecule and said DNA polymerase extends said oligonucleotide primer(s) by polymerising one or more nucleotides to generate a polynucleotide.

23. The method of claim 21 or claim 22, wherein said method is performed at a constant temperature.

20 24. The use of claim 20 or the method of claim 23, wherein said constant temperature is chosen from within the range 0°C to 42°C.

25. The use or method of claim 24, wherein said constant temperature is chosen from within the range 10°C to 25°C.