



US 20210317422A1

(19) **United States**

(12) **Patent Application Publication**  
**Schafer et al.**

(10) **Pub. No.: US 2021/0317422 A1**

(43) **Pub. Date: Oct. 14, 2021**

(54) **MODIFIED DNA POLYMERASES FOR IMPROVED AMPLIFICATION**

(60) Provisional application No. 61/432,936, filed on Jan. 14, 2011.

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**Publication Classification**

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(51) **Int. Cl.**  
*C12N 9/12* (2006.01)  
*C12P 19/34* (2006.01)  
*C12Q 1/686* (2006.01)

(52) **U.S. Cl.**  
CPC ..... *C12N 9/1252* (2013.01); *C12P 19/34* (2013.01); *Y02P 20/52* (2015.11); *C12Q 1/686* (2013.01); *C12Y 207/07007* (2013.01)

(21) Appl. No.: **17/199,343**

(57) **ABSTRACT**

(22) Filed: **Mar. 11, 2021**

The present invention provides improved DNA polymerases that may be better suited for applications in recombinant DNA technologies, in particular technologies involving plant-derived samples. Among other things, the present invention provides modified DNA polymerases derived from directed evolution experiments designed to select mutations that confer advantageous phenotypes under conditions used in industrial or research applications.

**Related U.S. Application Data**

**Specification includes a Sequence Listing.**

(63) Continuation of application No. 15/130,339, filed on Apr. 15, 2016, now Pat. No. 10,975,361, which is a continuation of application No. 13/979,509, filed on Sep. 3, 2013, now Pat. No. 9,315,787, filed as application No. PCT/US12/21348 on Jan. 13, 2012.

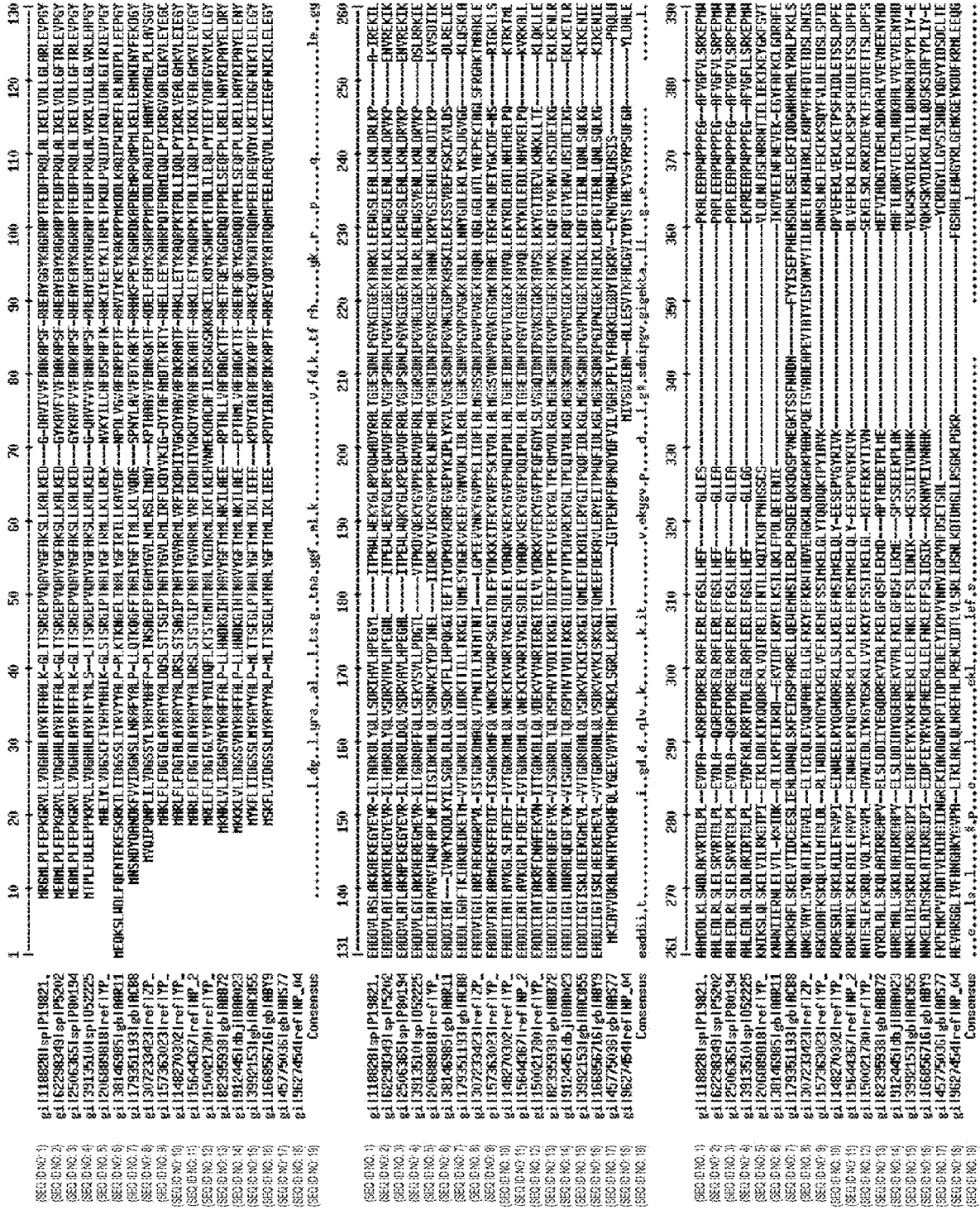


Fig. 1A





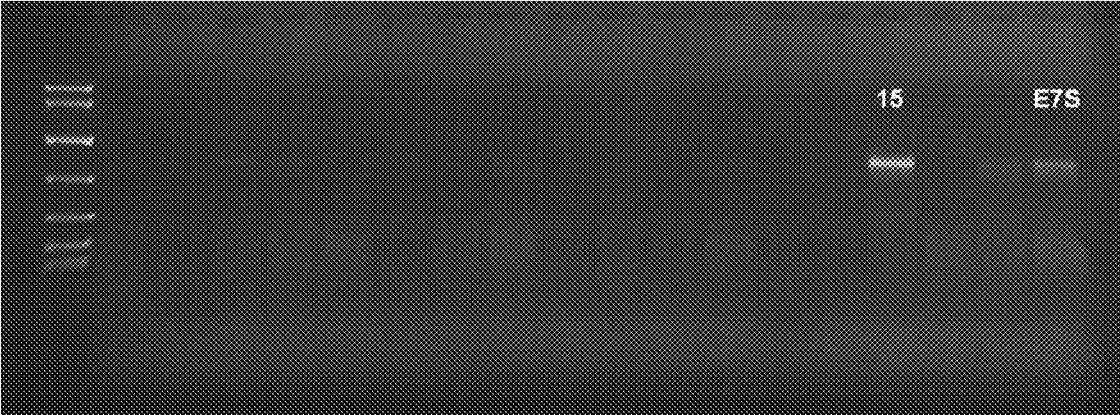


Fig. 2

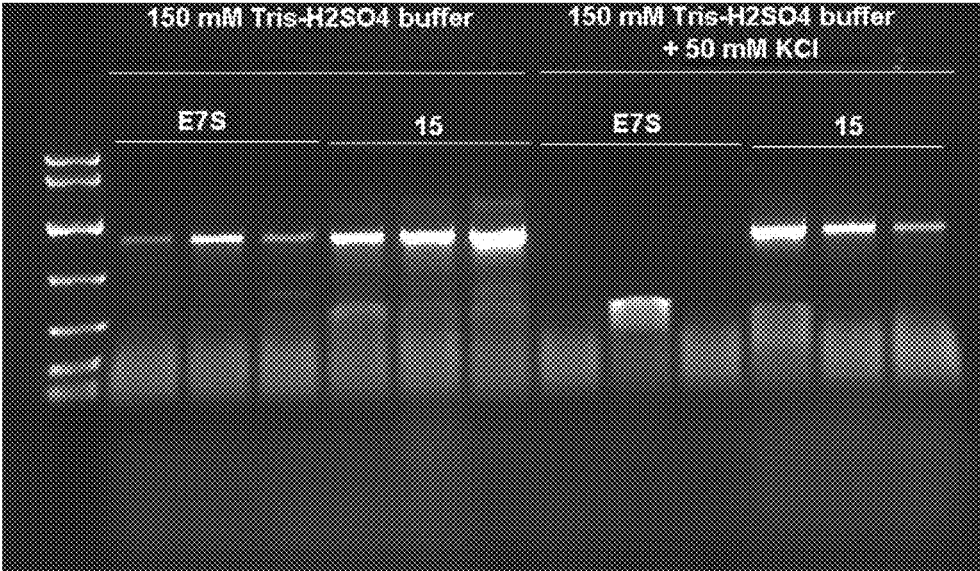


Fig. 3

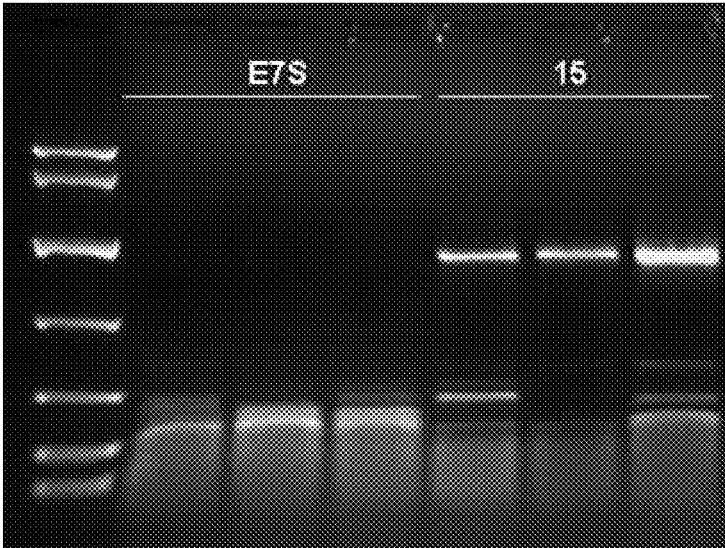


Fig. 4

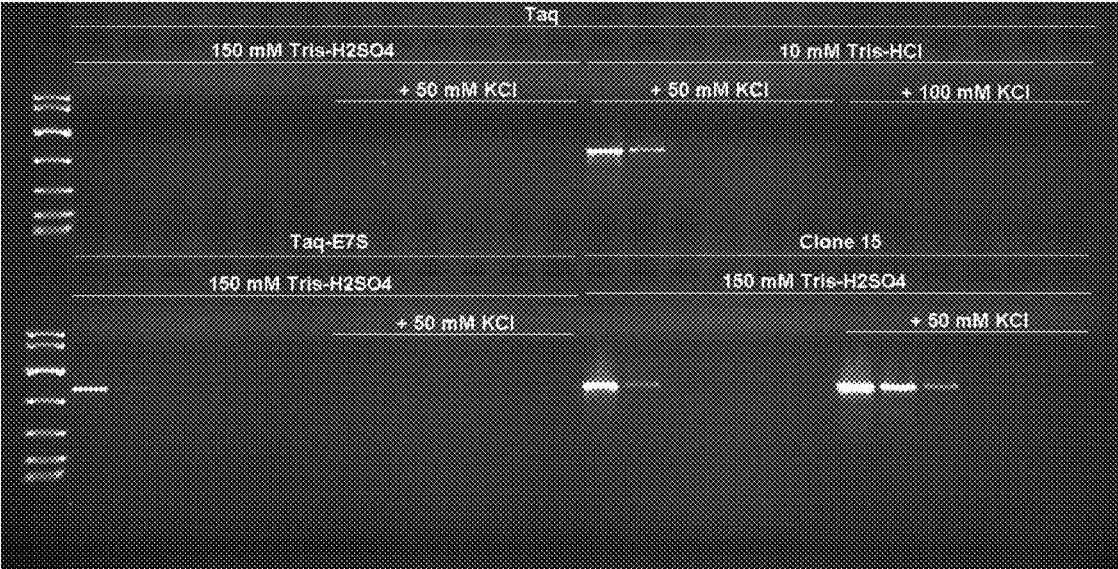


Fig. 5



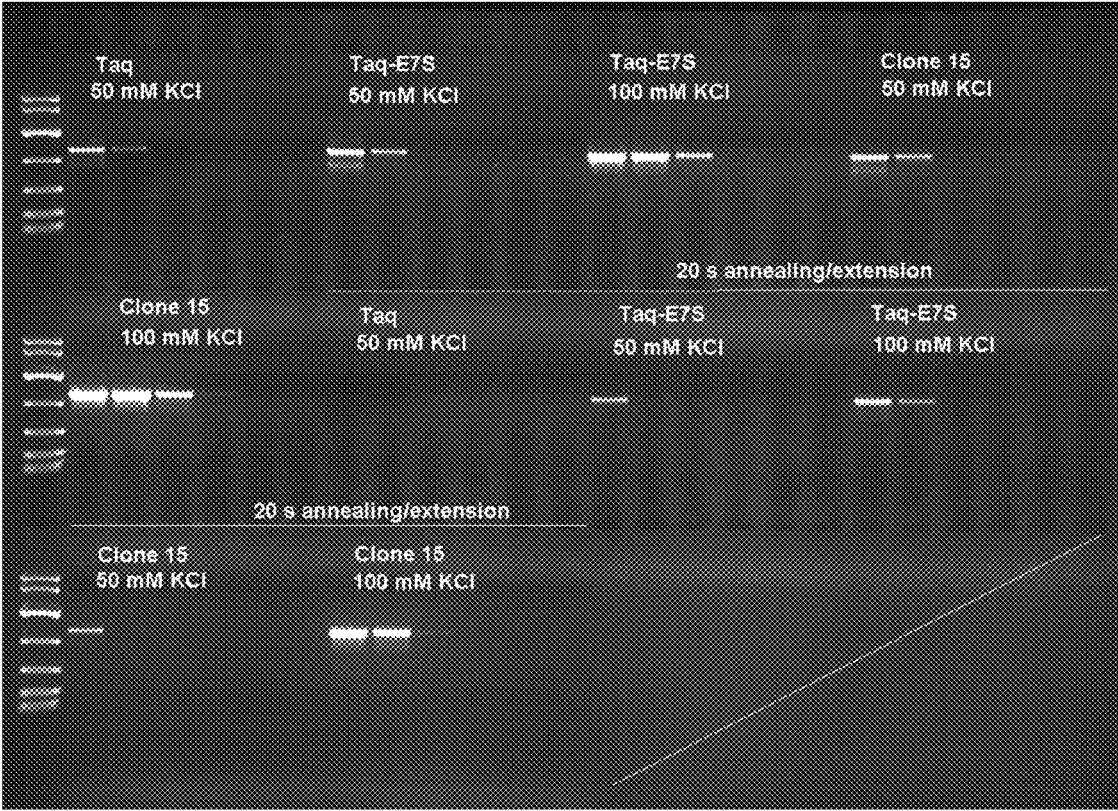


Fig. 6

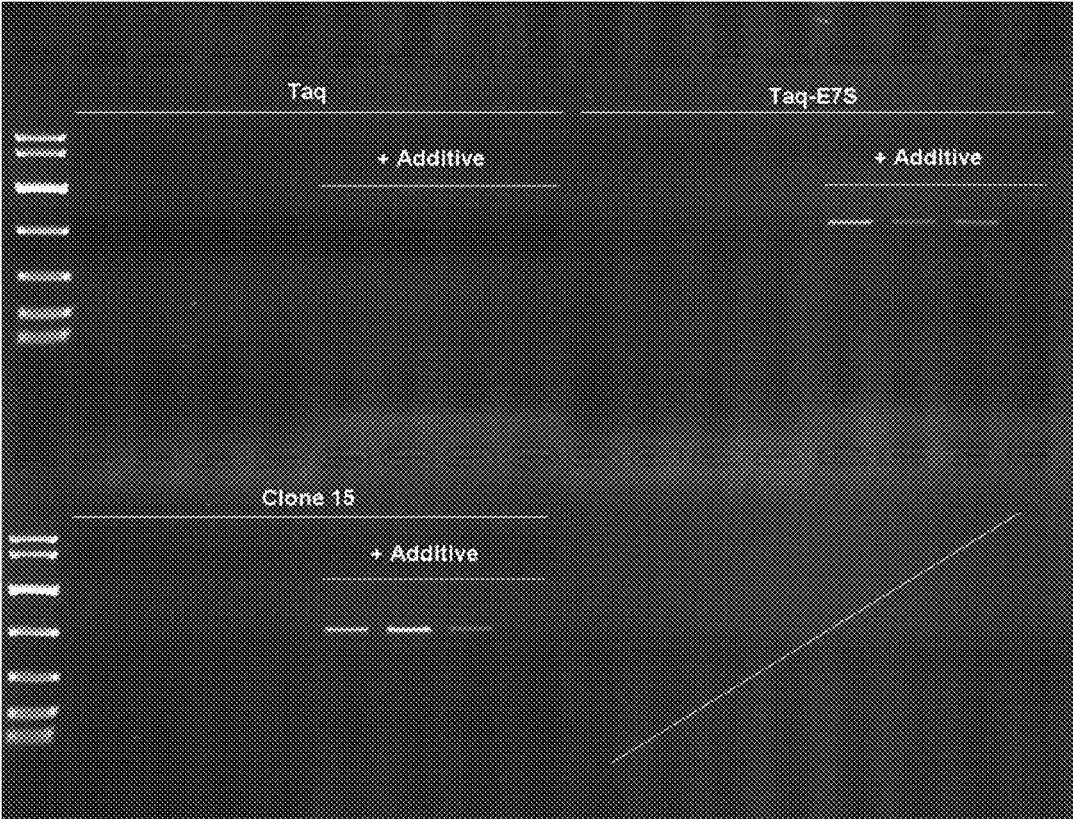


Fig. 7

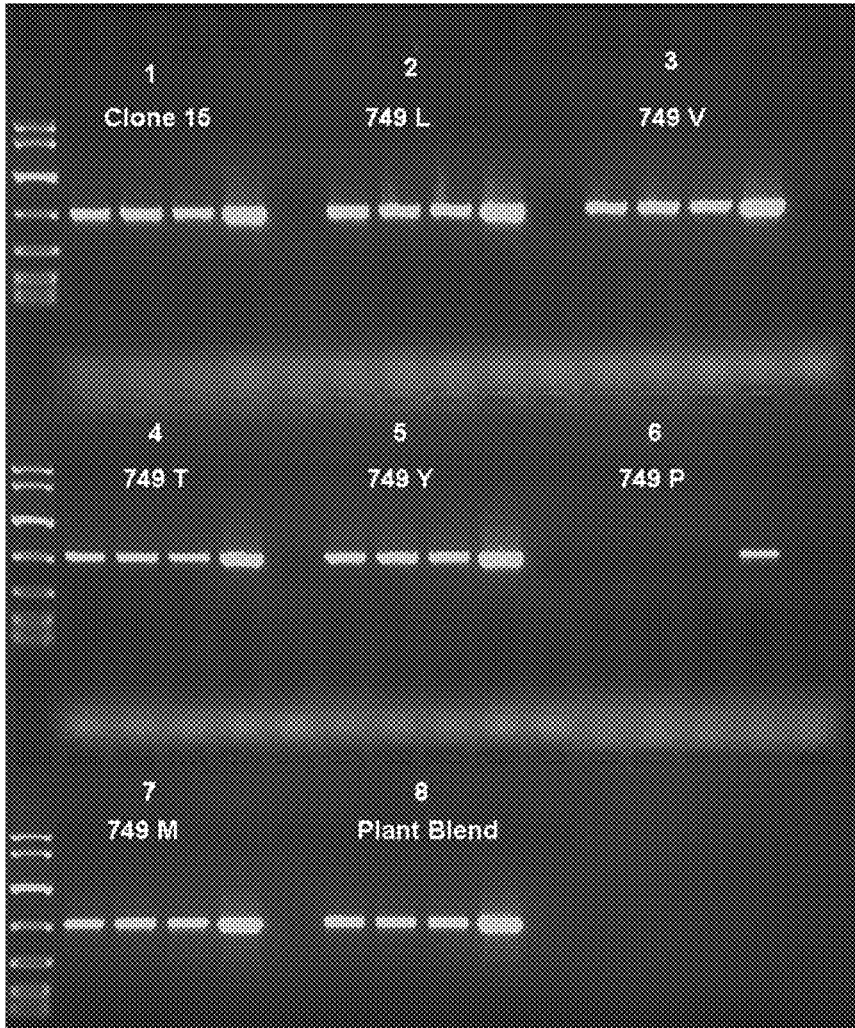


Fig. 8

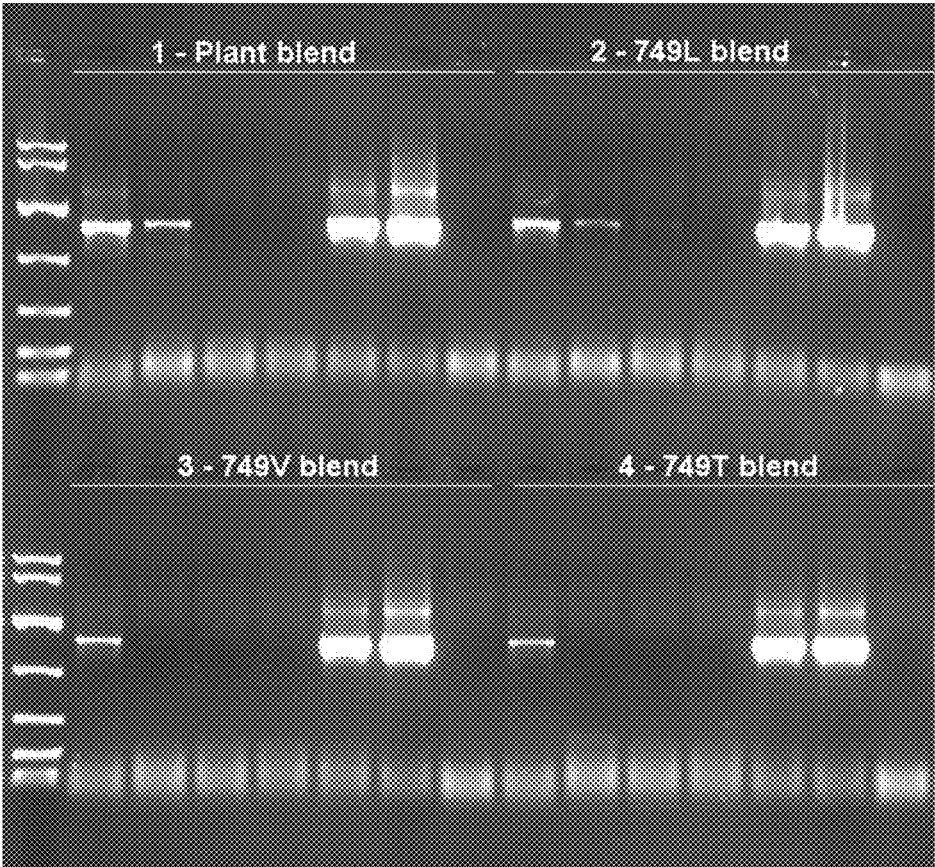


Fig. 9

## MODIFIED DNA POLYMERASES FOR IMPROVED AMPLIFICATION

### CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. patent application Ser. No. 15/130,339 filed Apr. 15, 2016, which is a continuation of U.S. patent application Ser. No. 13/979,509, filed Sep. 3, 2013 (issued as U.S. Pat. No. 9,315,787 on Apr. 19, 2016), which is a National Stage Entry of Patent Cooperation Treaty application number PCT/US2012/021348, filed Jan. 13, 2012, which claims priority to U.S. Provisional Patent Application No. 61/432,936, filed Jan. 14, 2011, the entire disclosure of each of which is incorporated herein by reference.

### BACKGROUND OF THE INVENTION

[0002] DNA polymerases are a family of enzymes that use single-stranded DNA as a template to synthesize the complementary DNA strand. In particular, DNA polymerases can add free nucleotides to the 3' end of a newly-forming strand resulting in elongation of the new strand in a 5'-3' direction. Most DNA polymerases are multifunctional proteins that possess both polymerizing and exonucleolytic activities (e.g., 3'->5' exonuclease or 5'->3' exonuclease activity).

[0003] DNA polymerases, like other natural enzymes, have evolved over millions of years to be efficient in their natural cellular environment. Many of them are almost perfectly adapted to work in that environment. In such an environment, the way that the protein can evolve is constrained by a number of requirements; the protein has to interact with other cellular components, it has to function in the cytoplasm (i.e., particular pH, ionic strength, in the presence of particular compounds, etc.) and it cannot cause lethal or disadvantageous side effects that detract from the fitness of the parent organism as a whole.

[0004] When DNA polymerases are removed from their natural environment and used in industrial or research applications, the environment and conditions under which the enzyme is operating is inevitably vastly different than those in which it evolved. Many of the constraints that limited the evolutionary direction the protein could take fall away. Therefore, there is vast potential for improvement of DNA polymerases for use in industrial or research applications.

### SUMMARY OF THE INVENTION

[0005] The present invention provides improved DNA polymerases that may be better suited for applications in recombinant DNA technologies, in particular technologies involving plant-derived samples. Among other things, the present invention provides modified DNA polymerases derived from directed evolution experiments designed to select mutations that confer advantageous phenotypes under conditions used in industrial or research applications. In particular, the present invention provides modified DNA polymerases that can effectively amplify biological samples containing various PCR inhibitors, especially plant-derived inhibitors. Thus, the present invention represents a significant improvement in recombinant DNA technology.

[0006] Accordingly, in one aspect, the present invention provides a modified DNA polymerase comprising an amino acid alteration (e.g., an amino acid substitution, deletion,

and/or insertion) at a position corresponding to F749 of Taq polymerase (SEQ ID NO: 38) and at least one additional amino acid alteration at a position corresponding to A61, K346, S357, or I707 of Taq polymerase (SEQ ID NO: 38) relative to the corresponding wild-type enzyme. In some embodiments, a modified DNA polymerase according to the present invention contains amino acid alterations (e.g., amino acid substitution(s), deletion(s), and/or insertion(s)) at positions corresponding to A61, K346, S357, I707 and F749 of Taq polymerase (SEQ ID NO: 38) relative to the corresponding wild-type enzyme. In some embodiments, a modified DNA polymerase further contains an amino acid alteration at a position corresponding to E507 of Taq polymerase (SEQ ID NO: 38).

[0007] In some embodiments, the amino acid alteration at position 749 is an amino acid substitution. In some embodiments, the amino acid substitution at position 749 is selected from the group consisting of F749L, F749I, F749V, F749T, F749Y, and F749M. In some embodiments, the amino acid substitution at position 749 is F749L. In some embodiments, the amino acid substitution at position 749 is F749V.

[0008] In another aspect, the present invention provides a modified DNA polymerase comprising one or more amino acid alterations (e.g., amino acid substitution(s), deletion(s), and/or insertion(s)) at one or more positions corresponding to A61, K346 and/or S357 of Taq polymerase (SEQ ID NO: 38) relative to the corresponding wild-type enzyme. In some embodiments, a modified DNA polymerase further comprises one or more additional alterations at one or more additional positions corresponding to E507, I707, and/or F749 of Taq polymerase (SEQ ID NO: 38). In some embodiments, suitable amino acid substitutions are selected from the group consisting of A61T, K346E, S357C, I707M, F749I, E507K and combinations thereof. In some embodiments, a modified DNA polymerase according to the invention contains amino acid substitutions of A61T, K346E, S357C, I707M, F749I, and E507K. In some embodiments, suitable amino acid substitutions are selected from the group consisting of A61T, K346E, S357C, I707M, F749L, E507K and combinations thereof. In some embodiments, a modified DNA polymerase according to the invention contains amino acid substitutions of A61T, K346E, S357C, I707M, F749L, and E507K.

[0009] In a further aspect, the present invention provides modified DNA polymerases containing one or more amino acid alterations (e.g., one or more substitutions, deletions, or insertions) corresponding to one or more positions selected from A61, K346, S357, I707, and/or F749 of Taq polymerase (SEQ ID NO: 38) relative to the corresponding wild-type enzyme. In certain embodiments, an amino acid alteration at I707 is not I707L. In certain embodiments, an amino acid alteration at F749 is not F749Y or F749S. In some embodiments, the modified DNA polymerases contain an additional alteration at a position corresponding to E507 of Taq polymerase (SEQ ID NO: 38).

[0010] In some embodiments, the DNA polymerase is modified from a naturally-occurring polymerase, e.g., a naturally-occurring polymerase isolated from any species of the genus *Thermus*, any species of the genus *Meiothermus*, any species of the genus *Thermotoga*, and/or any species of the genus *Thermomicrobium*. In some embodiments, the naturally-occurring polymerase is isolated from *Bacillus stearothermophilus*, *Sphaerobacter thermophilus*, *Dictoglossus thermophilus*, and/or *Escherichia coli*. In some

embodiments, the naturally-occurring polymerase is isolated from *Thermus aquaticus*, *Thermus thermophilus*, *Thermus caldophilus*, or *Thermus filiformis*. In some embodiments, the naturally-occurring polymerase is isolated from *Thermus aquaticus*.

**[0011]** In some embodiments, the modified DNA polymerase has increased enzyme activity, processivity, resistance to nucleic acid intercalating dyes, and/or salt resistance as compared to the corresponding wild-type enzyme. In some embodiments, the modified DNA polymerase has increased resistance to plant-derived PCR inhibitors as compared to the corresponding wild-type enzyme.

**[0012]** In another aspect, the present invention provides formulations of DNA polymerases containing modified DNA polymerases described herein and at least one DNA polymerase exhibiting 3'-exonuclease activity. In some embodiments, the modified DNA polymerase and the at least one DNA polymerase exhibiting 3'-exonuclease activity are present in a ratio of about 1:1 to about 1:2000 relative units of enzyme. In some embodiments, the modified DNA polymerase and the at least one DNA polymerase exhibiting 3'-exonuclease activity are present in a ratio of about 1:4 to about 1:100 relative units of enzyme. In some embodiments, the at least one DNA polymerase exhibiting 3'-exonuclease activity is selected from the group consisting of *Thermococcus litoralis* (Vent™, GenBank: AAA72101), *Pyrococcus furiosus* (Pfu, GenBank: D12983, BAA02362), *Pyrococcus woessii*, *Pyrococcus* GB-D (Deep Vent™, GenBank: AAA67131), *Thermococcus kodakaraensis* KOD1 (KOD, GenBank: BD175553, BAA06142; *Thermococcus* sp. strain KOD (Pfx, GenBank: AAE68738)), *Thermococcus gorgonarius* (Tgo, Pdb: 4699806), *Sulfolobus solataricus* (GenBank: NC002754, P26811), *Aeropyrum pernix* (GenBank: BAA81109), *Archaeoglobus fulgidus* (GenBank: O29753), *Pyrobaculum aerophilum* (GenBank: AAL63952), *Pyrodictium occultum* (GenBank: BAA07579, BAA07580), *Thermococcus* 9 degree Nm (GenBank: AAA88769, Q56366), *Thermococcus fumicolans* (GenBank: CAA93738, P74918), *Thermococcus hydrothermalis* (GenBank: CAC18555), *Thermococcus* spp. GE8 (GenBank: CAC12850), *Thermococcus* spp. JDF-3 (GenBank: AX135456; WOO132887), *Thermococcus* spp. TY (GenBank: CAA73475), *Pyrococcus abyssi* (GenBank: P77916), *Pyrococcus glycovorans* (GenBank: CAC12849), *Pyrococcus horikoshii* (GenBank: NP 143776), *Pyrococcus* spp. GE23 (GenBank: CAA90887), *Pyrococcus* spp. ST700 (GenBank: CAC12847), *Thermococcus pacificus* (GenBank: AX411312.1), *Thermococcus zilligii* (GenBank: DQ3366890), *Thermococcus aggregans*, *Thermococcus barossii*, *Thermococcus celer* (GenBank: DD259850.1), *Thermococcus profundus* (GenBank: E14137), *Thermococcus siculi* (GenBank: DD259857.1), *Thermococcus thio-reducens*, *Thermococcus onnurineus* NA1, *Sulfolobus acidocaldarium*, *Sulfolobus tokodaii*, *Pyrobaculum calidifontis*, *Pyrobaculum islandicum* (GenBank: AAF27815), *Methanococcus jannaschii* (GenBank: Q58295), *Desulfofrococcus* species TOK, *Desulfofrococcus*, *Pyrolobus*, *Pyrodictium*, *Staphylothermus*, *Vulcanisaetta*, *Methanococcus* (GenBank: P52025), GenBank AAC62712, GenBank P956901, and GenBank BAAA07579.

**[0013]** In other, related aspects, the present invention provides modified Taq polymerases containing one or more, two or more, three or more, four or more, or each of the

amino acid substitutions selected from the group consisting of A61T, K346E, S357C, I707M, E507K and F749I or F749L.

**[0014]** In yet another aspect, the present invention provides modified Taq polymerases containing amino acid substitutions of E507K and F749I and at least one additional amino acid substitution selected from the group consisting of A61T, K346E, S357C, and I707M.

**[0015]** In another aspect, the present invention provides formulations of DNA polymerases containing modified Taq polymerases described herein and at least one DNA polymerase exhibiting 3'-exonuclease activity. In some embodiments, the modified Taq polymerase and the at least one DNA polymerase exhibiting 3'-exonuclease activity are present in a ratio of about 1:1 to about 1:2000 relative units of enzyme. In some embodiments, the modified DNA polymerase and the at least one DNA polymerase exhibiting 3'-exonuclease activity are present in a ratio of about 1:4 to about 1:100 relative units of enzyme. In some embodiments, the at least one DNA polymerase exhibiting 3'-exonuclease activity is selected from the group consisting of *Thermococcus litoralis* (Vent™, GenBank: AAA72101), *Pyrococcus furiosus* (Pfu, GenBank: D12983, BAA02362), *Pyrococcus woessii*, *Pyrococcus* GB-D (Deep Vent™, GenBank: AAA67131), *Thermococcus kodakaraensis* KOD1 (KOD, GenBank: BD175553, BAA06142; *Thermococcus* sp. strain KOD (Pfx, GenBank: AAE68738)), *Thermococcus gorgonarius* (Tgo, Pdb: 4699806), *Sulfolobus solataricus* (GenBank: NC002754, P26811), *Aeropyrum pernix* (GenBank: BAA81109), *Archaeoglobus fulgidus* (GenBank: O29753), *Pyrobaculum aerophilum* (GenBank AAL63952), *Pyrodictium occultum* (GenBank: BAA07579, BAA07580), *Thermococcus* 9 degree Nm (GenBank: AAA88769, Q56366), *Thermococcus fumicolans* (GenBank: CAA93738, P74918), *Thermococcus hydrothermalis* (GenBank: CAC18555), *Thermococcus* spp. GE8 (GenBank: CAC12850), *Thermococcus* spp. JDF-3 (GenBank: AX135456; WOO132887), *Thermococcus* spp. TY (GenBank: CAA73475), *Pyrococcus abyssi* (GenBank: P77916), *Pyrococcus glycovorans* (GenBank: CAC12849), *Pyrococcus horikoshii* (GenBank: NP 143776), *Pyrococcus* spp. GE23 (GenBank: CAA90887), *Pyrococcus* spp. ST700 (GenBank: CAC12847), *Thermococcus pacificus* (GenBank: AX411312.1), *Thermococcus zilligii* (GenBank: DQ3366890), *Thermococcus aggregans*, *Thermococcus barossii*, *Thermococcus celer* (GenBank: DD259850.1), *Thermococcus profundus* (GenBank: E14137), *Thermococcus siculi* (GenBank: DD259857.1), *Thermococcus thio-reducens*, *Thermococcus onnurineus* NA1, *Sulfolobus acidocaldarium*, *Sulfolobus tokodaii*, *Pyrobaculum calidifontis*, *Pyrobaculum islandicum* (GenBank: AAF27815), *Methanococcus jannaschii* (GenBank: Q58295), *Desulfofrococcus* species TOK, *Desulfofrococcus*, *Pyrolobus*, *Pyrodictium*, *Staphylothermus*, *Vulcanisaetta*, *Methanococcus* (GenBank: P52025), GenBank AAC62712, GenBank P956901, and GenBank BAAA07579.

**[0016]** The present invention also features kits containing a modified DNA polymerase described herein and uses thereof. In addition, the present invention provides nucleotide sequences encoding modified DNA polymerases described herein, and vectors and/or cells that include the nucleotide sequences.

**[0017]** The invention further provides methods including amplifying nucleic acids in a biological sample, including

purified DNA and crude DNA extractions, using a modified DNA polymerase (e.g., Taq polymerase) as described herein.

**[0018]** In some embodiments, the biological sample is a plant sample (e.g., a crude plant sample such as leaf tissue, seed tissue, plant tissue, organ tissue, and/or crude plant DNA extracts). In some embodiments, the plant sample is a stored plant sample. In some embodiments, the biological sample is nucleic acid (e.g., DNA) purified from a plant sample.

**[0019]** In some embodiments, the biological sample is a crude non-plant sample (e.g., a sample such as mammalian tissue sample, buccal swabs, forensic samples, blood spots, cell culture samples, stabilized blood samples, microbiological samples, FFPE tissue, Guthrie card blood samples, FTA card blood samples). In some embodiments, the non-plant sample is a stored sample. In some embodiments, the biological sample is nucleic acid (e.g., DNA) purified from a non-plant sample.

**[0020]** In this application, the use of “or” means “and/or” unless stated otherwise. As used in this application, the term “comprise” and variations of the term, such as “comprising” and “comprises,” are not intended to exclude other additives, components, integers or steps. As used herein, the terms “about” and “approximately” are used as equivalents. Any numerals used in this application with or without about/ approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

**[0021]** Other features, objects, and advantages of the present invention are apparent in the detailed description, drawings and claims that follow. It should be understood, however, that the detailed description, the drawings, and the claims, while indicating embodiments of the present invention, are given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**[0022]** The drawings are for illustration purposes only not for limitation.

**[0023]** FIGS. 1A-1C depict an alignment of amino acid sequences of naturally-occurring DNA polymerases from bacterial species. Exemplary amino acid alterations discovered by directed evolution experiments are shown above each alignment. `gil118828|spIP19821`. (SEQ ID NO: 1); `gil62298349|spIP5202` (SEQ ID NO: 2); `gil2506365|spIP80194` (SEQ ID NO: 3); `gil3913510|spI052225` (SEQ ID NO: 4); `gil206889818|reflYP_(SEQ ID NO: 5);` `gil38146985|gb|AAR11` (SEQ ID NO: 6); `gil179351193|gb|ACB8` (SEQ ID NO: 7); `gil307233423|reflIZP_(SEQ ID NO: 8);` `gil157363023|reflYP_(SEQ ID NO: 9);` `gil148270302|reflYP_(SEQ ID NO: 10);` `gil15644367|reflNP_2` (SEQ ID NO: 11); `gil150021780|reflYP_(SEQ ID NO: 12);` `gil82395938|gb|ABB72` (SEQ ID NO: 13);

`gil912445|dbjBAA023` (SEQ ID NO: 14); `gil3992153|gb|AAC855` (SEQ ID NO: 15); `gil166856716|gb|ABY9` (SEQ ID NO: 16); `gil45775036|gb|AAS77` (SEQ ID NO: 17); `gil9627454|reflNP_04` (SEQ ID NO: 18); Consensus (SEQ ID NO: 19).

**[0024]** FIG. 2 depicts an exemplary PCR screening using a mixed plant extract to poison a PCR reaction producing a 1 kb Lambda fragment. Unlabeled lanes are various clones from the selection. Clone 15 (labeled) gave the highest yield compared to test samples and a control sample (Taq-E7S; labeled).

**[0025]** FIG. 3 depicts an exemplary PCR reaction using a control polymerase (Taq-E7S) or Clone 15 to amplify a 1.2 kb amplicon using 0.5 mm diameter grapevine leaf discs under varying KCl conditions. Reactions were performed in triplicate.

**[0026]** FIG. 4 depicts an exemplary PCR reaction using a control polymerase (Taq-E7S) or Clone 15 to amplify a 1.45 kb amplicon using 0.5 mm diameter potato leaf discs. Reactions were performed in triplicate.

**[0027]** FIG. 5 depicts an exemplary PCR reaction using a control polymerase (Taq or Taq-E7S) or Clone 15 to amplify a 1 kb amplicon from various amounts of Lambda template DNA (5 ng, 1 ng, 200 pg, 40 pg, 8 pg, no-template control) in PCR buffer with and without KCl.

**[0028]** FIG. 6 depicts an exemplary PCR reaction using a control polymerase (Taq or Taq-E7S) or Clone 15 to amplify a 1 kb amplicon from various amounts of Lambda template DNA (5 ng, 1 ng, 200 pg, 40 pg, 8 pg, no-template control) in PCR buffer with and without KCl. Two PCR programs, one with a 30 s annealing/extension time and one with a 20 s annealing/extension time were used.

**[0029]** FIG. 7 depicts an exemplary PCR reaction using a control polymerase (Taq or Taq-E7S) or Clone 15 to amplify a 800 bp amplicon using 0.5 mm diameter grapevine leaf discs in the presence or absence of an exemplary additive. Reactions were performed in triplicate.

**[0030]** FIG. 8 depicts an exemplary PCR reaction using Clone 15 polymerase and altered versions of Clone 15 polymerases containing alternative substitutions at position 749, to amplify an 800 bp amplicon from crude extract or purified grapevine leaf DNA extracts.

**[0031]** FIG. 9 depicts an exemplary PCR reaction using blend versions of certain altered versions of Clone 15 from FIG. 8, to amplify a 1221 bp amplicon from crude extract or purified grapevine leaf DNA extracts.

#### DEFINITIONS

**[0032]** In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification.

**[0033]** Amino acid: As used herein, term “amino acid,” in its broadest sense, refers to any compound and/or substance that can be incorporated into a polypeptide chain. In some embodiments, an amino acid has the general structure  $H_2N-C(H)(R)-COOH$ . In some embodiments, an amino acid is a naturally-occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. “Standard amino acid” refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. “Nonstandard amino

acid” refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. As used herein, “synthetic amino acid” encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and/or substitutions. Amino acids, including carboxy- and/or amino-terminal amino acids in peptides, can be modified by methylation, amidation, acetylation, and/or substitution with other chemical without adversely affecting their activity. Amino acids may participate in a disulfide bond. The term “amino acid” is used interchangeably with “amino acid residue,” and may refer to a free amino acid and/or to an amino acid residue of a peptide. It will be apparent from the context in which the term is used whether it refers to a free amino acid or a residue of a peptide. It should be noted that all amino acid residue sequences are represented herein by formulae whose left and right orientation is in the conventional direction of amino-terminus to carboxy-terminus.

**[0034]** Base Pair (bp): As used herein, base pair refers to a partnership of adenine (A) with thymine (T), or of cytosine (C) with guanine (G) in a double stranded DNA molecule. Chimeric polymerase: As used herein, the term “chimeric polymerase” (also referred to as “chimera”) refers to any recombinant polymerase containing at least a first amino acid sequence derived from a first DNA polymerase and a second amino acid sequence derived from a second DNA polymerase. Typically, the first and second DNA polymerases are characterized with at least one distinct functional characteristics (e.g., processivity, elongation rate, fidelity). As used herein, a sequence derived from a DNA polymerase of interest refers to any sequence found in the DNA polymerase of interest, or any sequence having at least 70% (e.g., at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) identical to an amino acid sequence found in the DNA polymerase of interest. A “chimeric polymerase” according to the invention may contain two or more amino acid sequences from related or similar polymerases (e.g., proteins sharing similar sequences and/or structures), joined to form a new functional protein. A “chimeric polymerase” according to the invention may contain two or more amino acid sequences from unrelated polymerases, joined to form a new functional protein. For example, a chimeric polymerase of the invention may be an “interspecies” or “intergenic” fusion of protein structures expressed by different kinds of organisms.

**[0035]** Complementary: As used herein, the term “complementary” refers to the broad concept of sequence complementarity between regions of two polynucleotide strands or between two nucleotides through base-pairing. It is known that an adenine nucleotide is capable of forming specific hydrogen bonds (“base pairing”) with a nucleotide which is thymine or uracil. Similarly, it is known that a cytosine nucleotide is capable of base pairing with a guanine nucleotide.

**[0036]** Corresponding to: As used herein, the term “corresponding to” is often used to designate the position/identity of an amino acid residue in a DNA polymerase or a nucleotide in a polynucleotide encoding a DNA polymerase. Those of ordinary skill will appreciate that, for purposes of simplicity, a canonical numbering system (based on wild type Taq polymerase) is utilized herein (as illustrated, for example, in FIGS. 1A-1C, so that an amino acid “corresponding to” a residue at position 190, for example, need not

actually be the 190<sup>th</sup> amino acid in a particular amino acid chain but rather a residue that plays the same role, structurally or functionally, as the residue found at 190 in wild type Taq polymerase; those of ordinary skill in the art readily appreciate how to identify corresponding amino acids. Exemplary methods for identifying corresponding residues include, but are not limited to, sequence alignment, molecular modeling, and mutagenesis studies.

**[0037]** DNA binding affinity: As used herein, the term “DNA-binding affinity” typically refers to the activity of a DNA polymerase in binding DNA nucleic acid. In some embodiments, DNA binding activity can be measured in a two band-shift assay. For example, in some embodiments (based on the assay of Guagliardi et al. (1997) *J. Mol. Biol.* 267:841-848), double-stranded nucleic acid (the 452-bp HindIII-EcoRV fragment from the *S. solfataricus* lacS gene) is labeled with <sup>32</sup>P to a specific activity of at least about 2.5×10<sup>7</sup> cpm/pg (or at least about 4000 cpm/fmol) using standard methods. See, e.g., Sambrook et al. (2001) *Molecular Cloning: A Laboratory Manual* (3<sup>rd</sup> ed., Cold Spring Harbor Laboratory Press, NY) at 9.63-9.75 (describing end-labeling of nucleic acids). A reaction mixture is prepared containing at least about 0.5 μg of the polypeptide in about 10 μl of binding buffer (50 mM sodium phosphate buffer (pH 8.0), 10% glycerol, 25 mM KCl, 25 mM MgCl<sub>2</sub>). The reaction mixture is heated to 37° C. for 10 min. About 1×10<sup>4</sup> to 5×10<sup>4</sup> cpm (or about 0.5-2 ng) of the labeled double-stranded nucleic acid is added to the reaction mixture and incubated for an additional 10 min. The reaction mixture is loaded onto a native polyacrylamide gel in 0.5× Tris-borate buffer. The reaction mixture is subjected to electrophoresis at room temperature. The gel is dried and subjected to autoradiography using standard methods. Any detectable decrease in the mobility of the labeled double-stranded nucleic acid indicates formation of a binding complex between the polypeptide and the double-stranded nucleic acid. Such nucleic acid binding activity may be quantified using standard densitometric methods to measure the amount of radioactivity in the binding complex relative to the total amount of radioactivity in the initial reaction mixture.

**[0038]** Elongation rate: As used herein, the term “elongation rate” refers to the average speed at which a DNA polymerase extends a polymer chain. As used herein, a high elongation rate refers to an elongation rate higher than 50 nt/s (e.g., higher than 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140 nt/s). As used in this application, the terms “elongation rate” and “speed” are used interchangeably.

**[0039]** Enzyme activity: As used herein, the term “enzyme activity” refers to the specificity and efficiency of a DNA polymerase. Enzyme activity of a DNA polymerase is also referred to as “polymerase activity,” which typically refers to the activity of a DNA polymerase in catalyzing the template-directed synthesis of a polynucleotide. Enzyme activity of a polymerase can be measured using various techniques and methods known in the art. For example, serial dilutions of polymerase can be prepared in dilution buffer (e.g., 20 mM Tris.Cl, pH 8.0, 50 mM KCl, 0.5% NP 40, and 0.5% Tween-20). For each dilution, 5 μl can be removed and added to 45 μl of a reaction mixture containing 25 mM TAPS (pH 9.25), 50 mM KCl, 2 mM MgCl<sub>2</sub>, 0.2 mM dATP, 0.2 mM dGTP, 0.2 mM dTTP, 0.1 mM dCTP, 12.5 μg activated DNA, 100 μM [α-<sup>32</sup>P]dCTP (0.05 μCi/nmol) and



sterile deionized water. The reaction mixtures can be incubated at 37° C. (or 74° C. for thermostable DNA polymerases) for 10 minutes and then stopped by immediately cooling the reaction to 4° C. and adding 10  $\mu$ l of ice-cold 60 mM EDTA. A 25  $\mu$ l aliquot can be removed from each reaction mixture. Unincorporated radioactively labeled dCTP can be removed from each aliquot by gel filtration (Centri-Sep, Princeton Separations, Adelphia, N.J.). The column eluate can be mixed with scintillation fluid (1 ml). Radioactivity in the column eluate is quantified with a scintillation counter to determine the amount of product synthesized by the polymerase. One unit of polymerase activity can be defined as the amount of polymerase necessary to synthesize 10 nmole of product in 30 minutes (Lawyer et al. (1989) *J. Biol. Chem.* 264:6427-647). Other methods of measuring polymerase activity are known in the art (see, e.g. Sambrook et al. (2001) *Molecular Cloning: A Laboratory Manual* (3.sup.rd ed., Cold Spring Harbor Laboratory Press, NY)).

**[0040]** Fidelity: As used herein, the term “fidelity” refers to the accuracy of DNA polymerization by template-dependent DNA polymerase. The fidelity of a DNA polymerase is typically measured by the error rate (the frequency of incorporating an inaccurate nucleotide, i.e., a nucleotide that is not incorporated at a template-dependent manner). The accuracy or fidelity of DNA polymerization is maintained by both the polymerase activity and the exonuclease activity of a DNA polymerase. The term “high fidelity” refers to an error rate less than  $4.45 \times 10^{-6}$  (e.g., less than  $4.0 \times 10^{-6}$ ,  $3.5 \times 10^{-6}$ ,  $3.0 \times 10^{-6}$ ,  $2.5 \times 10^{-6}$ ,  $2.0 \times 10^{-6}$ ,  $1.5 \times 10^{-6}$ ,  $1.0 \times 10^{-6}$ ,  $0.5 \times 10^{-6}$ ) mutations/nt/doubling. The fidelity or error rate of a DNA polymerase may be measured using assays known to the art. For example, the error rates of DNA polymerases can be tested using the lacI PCR fidelity assay described in Cline, J. et al. (96) *NAR* 24: 3546-3551. Briefly, a 1.9 kb fragment encoding the lacIOlacZ $\alpha$  target gene is amplified from pPRIAZ plasmid DNA using 2.5U DNA polymerase (i.e. amount of enzyme necessary to incorporate 25 nmoles of total dNTPs in 30 min. at 72° C.) in the appropriate PCR buffer. The lacI-containing PCR products are then cloned into lambda GT10 arms, and the percentage of lacI mutants (MF, mutation frequency) is determined in a color screening assay, as described (Lundberg, K. S., Shoemaker, D. D., Adams, M. W. W., Short, J. M., Sorge, J. A., and Mathur, E. J. (1991) *Gene* 180: 1-8). Error rates are expressed as mutation frequency per bp per duplication (MF/bp/d), where bp is the number of detectable sites in the lacI gene sequence (349) and d is the number of effective target doublings. Similar to the above, any plasmid containing the lacIOlacZ $\alpha$  target gene can be used as template for the PCR. The PCR product may be cloned into a vector different from lambda GT (e.g., plasmid) that allows for blue/white color screening.

**[0041]** Fusion DNA polymerase: As used herein, the term “fusion DNA polymerase” refers to any DNA polymerase that is combined (e.g., covalently or non-covalently) with one or more protein domains having a desired activity (e.g., DNA-binding, stabilizing template-primer complexes, hydrolyzing dUTP). In some embodiments, the one or more protein domains are derived from a non-polymerase protein. Typically, fusion DNA polymerases are generated to improve certain functional characteristics (e.g., processivity, elongation rate, fidelity, salt-resistance, etc.) of a DNA polymerase.

**[0042]** Joined: As used herein, “joined” refers to any method known in the art for functionally connecting polypeptide domains, including without limitation recombinant fusion with or without intervening domains, inter-mediated fusion, non-covalent association, and covalent bonding, including disulfide bonding, hydrogen bonding, electrostatic bonding, and conformational bonding.

**[0043]** Modified DNA polymerase: As used herein, the term “modified DNA polymerase” refers to a DNA polymerase originated from another (i.e., parental) DNA polymerase and contains one or more amino acid alterations (e.g., amino acid substitution, deletion, or insertion) compared to the parental DNA polymerase. In some embodiments, a modified DNA polymerase of the invention is originated or modified from a naturally-occurring or wild-type DNA polymerase. In some embodiments, a modified DNA polymerase of the invention is originated or modified from a recombinant or engineered DNA polymerase including, but not limited to, chimeric DNA polymerase, fusion DNA polymerase or another modified DNA polymerase. Typically, a modified DNA polymerase has at least one changed phenotype compared to the parental polymerase.

**[0044]** Mutant: As used herein, the term “mutant” refers to a modified protein which displays altered characteristics when compared to the parental protein.

**[0045]** Mutation: As used herein, the term “mutation” refers to a change introduced into a parental sequence, including, but not limited to, substitutions, insertions, deletions (including truncations). The consequences of a mutation include, but are not limited to, the creation of a new character, property, function, phenotype or trait not found in the protein encoded by the parental sequence. Herein, the term “mutation” is used interchangeably with “alteration.”

**[0046]** Nucleotide: As used herein, a monomeric unit of DNA or RNA consisting of a sugar moiety (pentose), a phosphate, and a nitrogenous heterocyclic base. The base is linked to the sugar moiety via the glycosidic carbon (1' carbon of the pentose) and that combination of base and sugar is a nucleoside. When the nucleoside contains a phosphate group bonded to the 3' or 5' position of the pentose it is referred to as a nucleotide. A sequence of operatively linked nucleotides is typically referred to herein as a “base sequence” or “nucleotide sequence,” and is represented herein by a formula whose left to right orientation is in the conventional direction of 5'-terminus to 3'-terminus.

**[0047]** Nucleic acid intercalating dyes: As used herein, the term “nucleic acid intercalating dyes” refers to any molecules that bind to nucleic acids in a reversible, non-covalent fashion, by insertion between the base pairs of the double helix, thereby indicating the presence and amount of nucleic acids. Generally, nucleic acid intercalating dyes are planar, aromatic, ring-shaped chromophore molecules. In some embodiments, intercalating dyes include fluorescent dyes. Numerous intercalating dyes are known in the art. Some non-limiting examples include PICO GREEN (P-7581, Molecular Probes), EB (E-8751, Sigma), propidium iodide (P-4170, Sigma), Acridine orange (A-6014, Sigma), 7-aminoactinomycin D (A-1310, Molecular Probes), cyanine dyes (e.g., TOTO, YOYO, BOBO, and POPO), SYTO, SYBR Green I, SYBR Green II, SYBR DX, OliGreen, CyQuant GR, SYTOX Green, SYTO9, SYTO10, SYTO17, SYBR14, FUN-1, DEAD Red, Hexidium Iodide, Dihydroethidium, Ethidium Homodimer, 9-Amino-6-Chloro-2-Methoxyacri-

dine, DAPI, DIPI, Indole dye, Imidazole dye, Actinomycin D, Hydroxystilbamidine, and LDS 751 (U.S. Pat. No. 6,210,885), BOXTO, LC Green, Evagreen, Bebo.

**[0048]** Oligonucleotide or Polynucleotide: As used herein, the term “oligonucleotide” is defined as a molecule including two or more deoxyribonucleotides and/or ribonucleotides, preferably more than three. Its exact size will depend on many factors, which in turn depend on the ultimate function or use of the oligonucleotide. The oligonucleotide may be derived synthetically or by cloning. As used herein, the term “polynucleotide” refers to a polymer molecule composed of nucleotide monomers covalently bonded in a chain. DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) are examples of polynucleotides.

**[0049]** Polymerase: As used herein, a “polymerase” refers to an enzyme that catalyzes the polymerization of nucleotide (i.e., the polymerase activity). Generally, the enzyme will initiate synthesis at the 3'-end of the primer annealed to a polynucleotide template sequence, and will proceed toward the 5' end of the template strand. A “DNA polymerase” catalyzes the polymerization of deoxynucleotides.

**[0050]** Primer: As used herein, the term “primer” refers to an oligonucleotide, whether occurring naturally or produced synthetically, which is capable of acting as a point of initiation of nucleic acid synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is induced, e.g., in the presence of four different nucleotide triphosphates and thermostable enzyme in an appropriate buffer (“buffer” includes pH, ionic strength, cofactors, etc.) and at a suitable temperature. The primer is preferably single-stranded for maximum efficiency in amplification, but may alternatively be double-stranded. If double-stranded, the primer is first treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the thermostable enzyme. The exact lengths of the primers will depend on many factors, including temperature, source of primer and use of the method. For example, depending on the complexity of the target sequence, the oligonucleotide primer typically contains 15-25 nucleotides, although it may contain more or few nucleotides. Short primer molecules generally require colder temperatures to form sufficiently stable hybrid complexes with template.

**[0051]** Processivity: As used herein, “processivity” refers to the ability of a polymerase to remain attached to the template and perform multiple modification reactions. “Modification reactions” include but are not limited to polymerization, and exonucleolytic cleavage. In some embodiments, “processivity” refers to the ability of a DNA polymerase to perform a sequence of polymerization steps without intervening dissociation of the enzyme from the growing DNA chains. Typically, “processivity” of a DNA polymerase is measured by the length of nucleotides (for example 20 nts, 300 nts, 0.5-1 kb, or more) that are polymerized or modified without intervening dissociation of the DNA polymerase from the growing DNA chain. “Processivity” can depend on the nature of the polymerase, the sequence of a DNA template, and reaction conditions, for example, salt concentration, temperature or the presence of specific proteins. As used herein, the term “high processivity” refers to a processivity higher than 20 nts (e.g., higher

than 40 nts, 60 nts, 80 nts, 100 nts, 120 nts, 140 nts, 160 nts, 180 nts, 200 nts, 220 nts, 240 nts, 260 nts, 280 nts, 300 nts, 320 nts, 340 nts, 360 nts, 380 nts, 400 nts, or higher) per association/disassociation with the template. Processivity can be measured according the methods defined herein and in WO 01/92501 A1.

**[0052]** Synthesis: As used herein, the term “synthesis” refers to any in vitro method for making new strand of polynucleotide or elongating existing polynucleotide (i.e., DNA or RNA) in a template dependent manner. Synthesis, according to the invention, includes amplification, which increases the number of copies of a polynucleotide template sequence with the use of a polymerase. Polynucleotide synthesis (e.g., amplification) results in the incorporation of nucleotides into a polynucleotide (i.e., a primer), thereby forming a new polynucleotide molecule complementary to the polynucleotide template. The formed polynucleotide molecule and its template can be used as templates to synthesize additional polynucleotide molecules. “DNA synthesis,” as used herein, includes, but is not limited to, PCR, the labeling of polynucleotide (i.e., for probes and oligonucleotide primers), polynucleotide sequencing.

**[0053]** Template DNA molecule: As used herein, the term “template DNA molecule” refers to a strand of a nucleic acid from which a complementary nucleic acid strand is synthesized by a DNA polymerase, for example, in a primer extension reaction.

**[0054]** Template dependent manner: As used herein, the term “template dependent manner” refers to a process that involves the template dependent extension of a primer molecule (e.g., DNA synthesis by DNA polymerase). The term “template dependent manner” typically refers to polynucleotide synthesis of RNA or DNA wherein the sequence of the newly synthesized strand of polynucleotide is dictated by the well-known rules of complementary base pairing (see, for example, Watson, J. D. et al., In: *Molecular Biology of the Gene*, 4th Ed., W. A. Benjamin, Inc., Menlo Park, Calif. (1987)).

**[0055]** Thermostable enzyme: As used herein, the term “thermostable enzyme” refers to an enzyme which is stable to heat (also referred to as heat-resistant) and catalyzes (facilitates) polymerization of nucleotides to form primer extension products that are complementary to a polynucleotide template sequence. Typically, thermostable stable polymerases are preferred in a thermocycling process wherein double stranded nucleic acids are denatured by exposure to a high temperature (e.g., about 95° C.) during the PCR cycle. A thermostable enzyme described herein effective for a PCR amplification reaction satisfies at least one criteria, i.e., the enzyme does not become irreversibly denatured (inactivated) when subjected to the elevated temperatures for the time necessary to effect denaturation of double-stranded nucleic acids. Irreversible denaturation for purposes herein refers to permanent and complete loss of enzymatic activity. The heating conditions necessary for denaturation will depend, e.g., on the buffer salt concentration and the length and nucleotide composition of the nucleic acids being denatured, but typically range from about 90° C. to about 96° C. for a time depending mainly on the temperature and the nucleic acid length, typically about 0.5 to ten minutes. Higher temperatures may be tolerated as the buffer salt concentration and/or GC composition of the nucleic acid is increased. In some embodiments, thermostable enzymes will not become irreversibly denatured at

about 90° C.-100° C. Typically, a thermostable enzyme suitable for the invention has an optimum temperature at which it functions that is higher than about 40° C., which is the temperature below which hybridization of primer to template is promoted, although, depending on (1) magnesium and salt, concentrations and (2) composition and length of primer, hybridization can occur at higher temperature (e.g., 45° C.-70° C.). The higher the temperature optimum for the enzyme, the greater the specificity and/or selectivity of the primer-directed extension process. However, enzymes that are active below 40° C. (e.g., at 37° C.) are also within the scope of this invention provided they are heat-stable. In some embodiments, the optimum temperature ranges from about 50° C. to 90° C. (e.g., 60° C.-80° C.).

**[0056]** Wild-type: As used herein, the term “wild-type” refers to a gene or gene product which has the characteristics of that gene or gene product when isolated from a naturally-occurring source.

#### DETAILED DESCRIPTION OF THE INVENTION

**[0057]** The present invention provides, among other things, modified DNA polymerases containing amino acid alterations based on mutations identified in directed evolution experiments designed to select enzymes that are better suited for applications in recombinant DNA technologies. In particular, the present invention provides modified DNA polymerases that have superior activity in amplifying biological samples containing various PCR inhibitors (e.g., plant-derived inhibitors).

**[0058]** Traditionally, inhibitors are a major obstacle for efficient amplification in PCR, for example in PCR reactions containing plant-derived samples. It was known that polysaccharides, secondary metabolites, polyphenolics and the like co-isolate with nucleic acids from plant tissues resulting in inhibition of amplification. See, Koonjul P. K. et al. “Inclusion of polyvinylpyrrolidone in the polymerase chain reaction reverses the inhibitory effects of polyphenolic contamination of RNA,” *Nucleic Acids Research*, 1999, 27(3): 915-916; Demeke T. and Jenkins G. R., “Influence of DNA extraction methods, PCR inhibitors and quantification methods on real-time PCR assay of biotechnology-derived traits,” *Anal Bioanal Chem*, 2010, 396:1977-1990. As described in the Examples section, the present inventors have, through directed DNA polymerase evolution screening, successfully discovered mutations (see e.g., Table 2) that renders a modified DNA polymerase containing such mutations able to effectively amplify inhibitor-containing samples with higher yield and sensitivity as compared to a wild-type or unmodified parental polymerase control. In some cases, a modified DNA polymerase provided by the present invention may amplify inhibitor-containing samples where a wild-type or unmodified parental polymerase control fails completely. Thus, the present invention provides an effective solution to overcome this major obstacle for efficient PCR amplification.

**[0059]** As can be appreciated by one skilled in the art, Taq polymerase was used as an exemplary polymerase. One or more modifications described herein may be introduced to various DNA polymerases to achieve the same effects.

**[0060]** Various aspects of the invention are described in detail in the following sections. The use of sections is not meant to limit the invention. Each section can apply to any

aspect of the invention. In this application, the use of “or” means “and/or” unless stated otherwise.

#### Directed DNA Polymerase Evolution Screening

**[0061]** As described in the Examples section, the present inventors have successfully developed directed DNA polymerase evolution experiments by mimicking the typical or less-than typical environments and conditions under which an enzyme is usually used or expected to be used in real-life industrial or research applications.

**[0062]** Various mutations have been observed during the selection process. Many mutations confer advantages relating to enzyme characteristics including, but not limited to, expression efficiency, solubility and folding robustness, thermostability, polymerization activity, processivity, speed (elongation rate), concentration robustness, resistance to impurities, resistance to chemical additives, fidelity, avoidance of primer-dimers, strand-displacement activity, altered nuclease activity, nucleotide selectivity, and other properties and characteristics involved in the process of DNA polymerization.

**[0063]** It is contemplated that the mutations identified herein confer a variety of phenotypes that can make DNA polymerases better suited for applications in recombinant DNA technologies. For example, mutations identified in accordance with the present invention may render modified DNA polymerases containing one or more of such mutations that are resistant to PCR inhibitors (e.g., plant-derived PCR inhibitors), salt, PCR additives (e.g., PCR enhancers). In some embodiments, a modified DNA polymerase is resistant to a PCR inhibitor (e.g., a plant-derived inhibitor) if the modified DNA polymerase amplifies a sample containing the PCR inhibitor (e.g., a plant-derived inhibitor) with higher yield (e.g., with more than 1.2-fold, 1.5-fold, 2-fold, 2.5-fold, 3-fold, 3.5-fold, 4-fold, 4.5-fold, 5-fold or more in yield) as compared to a wild-type or an unmodified parental polymerase control under otherwise identical conditions. In some embodiments, a modified DNA polymerase is resistant to a PCR inhibitor (e.g., a plant-derived inhibitor) if the modified DNA polymerase amplifies a sample containing the PCR inhibitor (e.g., a plant-derived inhibitor) where a wild-type or an unmodified parental polymerase control fails to amplify the sample under otherwise identical conditions. As used herein, an unmodified parental polymerase control refers to a polymerase from which a modified DNA polymerase of the invention is derived. An unmodified parental polymerase control may have a sequence of a wild-type polymerase. In some embodiments, an unmodified parental polymerase control may also contain one or more mutations as compared to a wild-type polymerase, or a chimeric polymerase, fusion polymerase or any type of DNA polymerases described in the DNA polymerases section below. As used herein, PCR inhibitors include, but are not limited to, polysaccharides, secondary metabolites, polyphenolics, ionic detergents, organic solvents, heavy metals, salts, pigments, alcohols, urea, DMSO, betaine, heparin, fluorescent dyes, humic acid, heme, immunoglobulins. Typical plant-derived PCR inhibitors include, but are not limited to, polysaccharides, secondary metabolites, polyphenolics, polytic acid, tannins, dextran sulfate, pigments, plant oils, plant waxes.

**[0064]** Mutations identified in accordance with the present invention may also confer enzymatic phenotypes related to the selective advantages described herein. Indeed, the pres-

ent inventors have identified or expect to identify mutant polymerases that express well, are more soluble, that display higher activity, processivity and/or speed, that are active over a wide range of concentrations, that have a higher fidelity, and other phenotypes that may not be immediately measurable. Since many of these phenotypes may depend on the manner in which the DNA and polymerase interact, it is contemplated that many of the mutations identified in accordance with the present invention may affect DNA-polymerase binding characteristics.

**[0065]** In addition, it is contemplated that mutations identified according to the present invention may confer enzymatic phenotypes not directly related to the selective advantages described herein. For example, some phenotypes may confer no advantage, but merely be a side effect of the advantageous mutation. In addition, some mutants may display phenotypes that could be considered disadvantageous. For example, some mutations confer an advantage (for example, high activity), but this advantage comes at a cost (for example, high error-rate). If the advantage outweighs the disadvantage, the mutation will still be selected for. Such mutations may have commercial uses. For example, a low fidelity enzyme could be used in error prone PCR (e.g., for mutagenesis).

**[0066]** Exemplary mutations and mutant clones containing combinations of mutations associated with specific phenotypes are discussed in the Examples section and are shown at least in Tables 3, 4, 5, 8, 12, and 15.

**[0067]** It is further contemplated that, since many DNA polymerases have similar sequences, structures and functional domains, mutations and/or the positions where mutations occur identified herein can serve as bases for modification of DNA polymerases in general. For example, same or similar mutations, as well as other alterations, may be introduced at the corresponding positions in various DNA polymerases to generate modified enzymes that are better adapted for recombinant use.

#### DNA Polymerases

**[0068]** Modified DNA polymerases in accordance with the present invention may be modified from any types of DNA polymerases including, but not limited to, naturally-occurring wild-type DNA polymerases, recombinant DNA polymerase or engineered DNA polymerases such as chimeric DNA polymerases, fusion DNA polymerases, or other modified DNA polymerases. In particular embodiments, DNA polymerases suitable for the invention are thermostable DNA polymerases (PCR-able).

#### **[0069]** Naturally-Occurring DNA Polymerases

**[0070]** In some embodiments, naturally-occurring DNA polymerases suitable for the invention are type A DNA polymerases (also known as family A DNA polymerases). Type A DNA polymerases are classified based on amino acid sequence homology to *E. coli* polymerase I (Braithwaite and Ito, *Nuc. Acids. Res.* 21:787-802, 1993), and include *E. coli* pol I, *Thermus aquaticus* DNA pol I (Taq polymerase), *Thermus flavus* DNA pol I, *Streptococcus pneumoniae* DNA pol I, *Bacillus stearothermophilus* pol I, phage polymerase T5, phage polymerase T7, mitochondrial DNA polymerase pol gamma, as well as additional polymerases discussed below.

**[0071]** Family A DNA polymerases are commercially available, including Taq polymerase (New England BioLabs), *E. coli* pol I (New England BioLabs), *E. coli* pol I

Klenow fragment (New England BioLabs), and T7 DNA polymerase (New England BioLabs), and *Bacillus stearothermophilus* (Bst) DNA polymerase (New England BioLabs).

**[0072]** Suitable DNA polymerases can also be derived from bacteria or other organisms with optimal growth temperatures that are similar to the desired assay temperatures. For example, such suitable bacteria or other organisms may exhibit maximal growth temperatures of >80-85° C. or optimal growth temperatures of >70-80° C.

**[0073]** Sequence information of many type A DNA polymerases are publicly available. Table 1 provides a list of GenBank Accession numbers and other GenBank Accession information for exemplary type A DNA polymerases, including species from which they are derived.

TABLE 1

Sequence Accession Information for  
Certain Type A DNA Polymerases

<i>Geobacillus stearothermophilus</i>
ACCESSION 3BDP_A
VERSION 3BDP_A GI:4389065
DBSOURCE pdb: molecule 3BDP, chain 65, release Aug. 27, 2007.
<i>Natranaerobius thermophilus</i> JW/NM-WN-LF
ACCESSION ACB85463
VERSION ACB85463.1 GI:179351193
DBSOURCE accession CP001034.1
<i>Thermus thermophilus</i> HB8
ACCESSION P52028
VERSION P52028.2 GI:62298349
DBSOURCE swissprot: locus DPO1T_THET8, accession P52028
<i>Thermus thermophilus</i>
ACCESSION P30313
VERSION P30313.1 GI:232010
DBSOURCE swissprot: locus DPO1F_THETH, accession P30313
<i>Thermus caldophilus</i>
ACCESSION P80194
VERSION P80194.2 GI:2506365
DBSOURCE swissprot: locus DPO1_THECA, accession P80194
<i>Thermus filiformis</i>
ACCESSION O52225
VERSION O52225.1 GI:3913510
DBSOURCE swissprot: locus DPO1_THEFI, accession O52225
<i>Thermus filiformis</i>
ACCESSION AAR11876
VERSION AAR11876.1 GI:38146983
DBSOURCE accession AY247645.1
<i>Thermus aquaticus</i>
ACCESSION P19821
VERSION P19821.1 GI:118828
DBSOURCE swissprot: locus DPO1_THEAQ, accession P19821
<i>Thermotoga lettingae</i> TMO
ACCESSION YP_001469790
VERSION YP_001469790.1 GI:157363023
DBSOURCE REFSEQ: accession NC_009828.1
<i>Thermosipho melanesiensis</i> BI429
ACCESSION YP_001307134
VERSION YP_001307134.1 GI:150021780
DBSOURCE REFSEQ: accession NC_009616.1
<i>Thermotoga petrophila</i> RKU-1
ACCESSION YP_001244762
VERSION YP_001244762.1 GI:148270302
DBSOURCE REFSEQ: accession NC_009486.1
<i>Thermotoga maritima</i> MSB8
ACCESSION NP_229419
VERSION NP_229419.1 GI:15644367
DBSOURCE REFSEQ: accession NC_000853.1
<i>Thermodesulfovibrio yellowstonii</i> DSM 11347
ACCESSION YP_002249284
VERSION YP_002249284.1 GI:206889818
DBSOURCE REFSEQ: accession NC_011296.1

TABLE 1-continued

Sequence Accession Information for Certain Type A DNA Polymerases
<i>Dictyoglomus thermophilum</i> ACCESSION AAR11877 VERSION AAR11877.1 GI:38146985 DBSOURCE accession AY247646.1 <i>Geobacillus</i> sp. MKK-2005 ACCESSION ABB72056 VERSION ABB72056.1 GI:82395938 DBSOURCE accession DQ244056.1 <i>Bacillus caldotenax</i> ACCESSION BAA02361 VERSION BAA02361.1 GI:912445 DBSOURCE locus BACPOLYTG accession D12982.1 <i>Thermoanaerobacter thermohydrosulfuricus</i> ACCESSION AAC85580 VERSION AAC85580.1 GI:3992153 DBSOURCE locus AR003995 accession AAC85580.1 <i>Thermoanaerobacter pseudethanolicus</i> ATCC 33223 ACCESSION ABY95124 VERSION ABY95124.1 GI:166856716 DBSOURCE accession CP000924.1 <i>Enterobacteria</i> phage T5 ACCESSION AAS77168 CAA04580 VERSION AAS77168.1 GI:45775036 DBSOURCE accession AY543070.1 <i>Enterobacteria</i> phage T7 (T7) ACCESSION NP_041982 VERSION NP_041982.1 GI:9627454 DBSOURCE REFSEQ: accession NC_001604.1 <i>Escherichia coli</i> str. K-12 substr. MG1655 ACCESSION AAB02998 VERSION AAB02998.1 GI:304969 DBSOURCE locus ECOUW87 accession L19201.1

[0074] Additional DNA polymerases are shown in FIGS. 1A-1C. DNA polymerases suitable for the present invention include DNA polymerases that have not yet been isolated.

[0075] In some embodiments, a naturally-occurring DNA polymerase suitable for the present invention is isolated from any species of the genus *Thermus*, any species of the genus *Meiothermus*, any species of the genus *Thermotoga*, and/or any species of the genus *Thermomicrobium*. In some embodiments, a naturally-occurring polymerase suitable for the present invention is isolated from *Bacillus stearothermophilus*, *Sphaerobacter thermophilus*, *Dictyoglomus thermophilum*, and/or *Escherichia coli*. In some embodiments, a naturally-occurring polymerase suitable for the present invention is isolated from *Thermus quaticus*, *Thermus thermophilus*, *Thermus caldophilus*, or *Thermus filiformis*. In some embodiments, the naturally-occurring polymerase is isolated from *Thermus aquaticus*.

[0076] Truncated DNA Polymerases

[0077] In some embodiments, DNA polymerases suitable for the present invention include truncated versions of naturally-occurring polymerases (e.g., a fragment of a DNA polymerase resulted from an N-terminal, C-terminal or internal deletion that retains polymerase activity). One exemplary truncated DNA polymerase suitable for the invention is KlenTaq which contains a deletion of a portion of the 5' to 3' exonuclease domain (see, Barnes W. M. (1992) *Gene* 112:29-35; and Lawyer F. C. et al. (1993) *PCR Methods and Applications*, 2:275-287).

[0078] In some embodiments, DNA polymerases in accordance with the present invention are defined by or comprise the consensus sequence

(SEQ ID NO: 20)

XXXXXXXXXXXXXXXXXXXXXXXXXLDGXXLYRAXXALXXXLXTSXGXXTN  
 AXYGFXXMLKXXXXXXXXXXXXXXXXVFXDKXXTFXRHXXXXXYKXXRXXX  
 PXXXXXQXXXXXXXXXXXXXXXXLEXXGYEADDIIXTXXXXXXXXXXXXXXXXXI  
 XXGDXDXQLVXXKXXXXXXXXKXITXXXXXXXXVXEKYGVPXXXXDXXX  
 LXGXSDNIPGVXGIGKXTAXXLLXXXGXEXXXXXXXXXXXXXXXXXXXXX  
 XXXLXXXXEXXLSXXLXXXXXXXXPXXXEXXXXXLXXXXXXXXXKXXXXXXXX  
 LEFXX  
 XXXXXLXX  
 XXXXXXXXXXXXXXXXXXXXXXXXXXXLXXXXXXXXXXXXXXXXXXXXLXXGX  
 LXXXXFXDXDXAYLLXPXXXXXXXXDXAXXVXXXXXXXXXXXXXXXXXXXX  
 XXXXXXXXXXXXXXXXXXXAXXAXXXXXLXXXXXXXXXXXXEXLXXLXXIEX  
 PLXXVLXXMEXGXDXDXLXKLKLSXXXXXXXXLXXXIXXXXXXXXXXXXX  
 AGXXFNXNSXKQLXXXLFXLXLPXXXKTXTXGXSTXXEVLXXLXXXHPX  
 XXIXXILXXYRXLKLSKYDXLXXXXXXXXPXTPGRXHTXFNQTXATGR  
 LSSXPXNLQXIPXXRXEXGXIXRAXFVXXXXXXXXIXXADYSQIELRXLAX  
 HLSXDXNLIXAFXGXGXDXDXDHTXTASXIFVXXEXXXXXXXXXVXXMR  
 RXAKXVNXGIYGXSSXGLSXXLXXXXXXXXXXXXXXXXXXXXIXXEXAXXIE  
 XYFXXXPXXXXIXXXXXAKXXGVVXLPFGRRRXXXPIXSRNXVXXXXE  
 RXAXNXP IQGTAADI I KLAMXXXXXXXXLXXXXXXXXXXXXXXXXLQXHDLELV  
 XEVXXEEXXVXXXXKXMXEXVXLVPPXXXXLXVXXXXGXWXXXXXXXXXX  
 XXX  
 XXXXXXXXXXXXXXXXXXXXXXX,  
 wherein X is any amino acid or a peptide bond.

[0079] In some embodiments, DNA polymerases in accordance with the present invention are defined by or comprise the consensus sequence

(SEQ ID NO: 21)

LXDGXXLYRAXXALXXXLXTSXGXXTNAXYGFXXMLKXXXXXXXXXXXX  
 XXXVFXDXKXTFXRHXXXXXYKXXRXXXPXXXXXQXXXXXXXXXXXXXXXX  
 EXXGYEADDIIXTXXXXXXXXXXXXXXXXIXXGDXDXQLVXXKXXXXXXXXKX  
 ITTXXXXXXXXXXVXEKYGVPXXXXDXLXGXSDNIPGVXGIGKXTAXX  
 LLXXXGXEXXXXXXXXXXXXXXXXXXXXXXXXXLXXXEXXLSXXLXXXX  
 XXPXXEXXXXXLXXXXXXXXXKXXXXXXXXLEFXXSXXXXXXXXXXXXXXXX  
 XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXLXXXXXXXXXXXXXXXX  
 XXX  
 XXXLXXXXXXXXXXXXXXXXLXXGXXLXXXXFXDXDXAYLLXPXXX  
 XXXDXAXXVYXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXAXXAX  
 XXXXXLXXXXXXXXXXEXLXXLXXXIEXPLXXVLXXMEXGXDXDXLXK  
 XLXXXXXXXXLXXXIXXXXXXXXXXXAGXKXENXNSXKQLXXXLXXXXLX  
 LPXXXKTXTGXSTXXEVLXXLXXXHPXXXIXXIXLXXYRXLKLSKYTX

-continued

DXLXXXXXXXXPTGRXHTXFNQXTATGRLSSSSXPXNLQXIPXXRXEXGXX  
 IRXAFVXXXXXXXXIXXADYSQIELRLXAXHLSXDNLIXAFXXGXXXXXXXX  
 XDHTXTASXIFVXXEXXXXXXVTTXMMRXAKXVNXGIXYGXSSXXGLSXX  
 LXXXXXXXXXXXXXXXXXXIXXEAAXXIEXYFXXXPVXXXIXXXXXAKX  
 XGVVXTLFGRRRXXPXIXSRNXVVRXXXERXAXNXP IQGTAADII KLAMXX  
 XXXXLXXXXXXXXXXXXXXXXLQXHDELVXEVEEXXVXXXXXXXXMEXX  
 VXLXVPXXXXLXVXXXXGXW,  
 wherein X is any amino acid or a peptide bond.

[0080] In some embodiments, DNA polymerases in accordance with the present invention are defined by or comprise the consensus sequence

(SEQ ID NO: 22)  
 LXDGXXLYRAXXALXXXLXTSGXXTNAXYGFXXMLKXXXXXXXXXXXXX  
 XXVFDXKXTFXRXHXXXXYKXRXXPXXXXXQXXXXXXXXXXXXXXXXL  
 EXXGYEADDIXTXXXXXXXXXXXXXIXXGDXXQLVXXX,  
 wherein X is any amino acid or a peptide bond.

[0081] In some embodiments, DNA polymerases in accordance with the present invention are defined by or comprise the consensus sequence

(SEQ ID NO: 23)  
 EXXLXLXXIXELPXXVLXXMEXXGXDXDXXLFXLSXXXXXXXXXXLXXX  
 IXXXXXXXXXXXXAGXXFNXNSXKQLXXXLFXLXLPXXXXTXXTGXXSTX  
 XEVLXLXHPXXXIXXIXLXXYRXLXKLKSTYDXLXXXXXXXXPTGRX  
 HTXFNQXTATGRLSSSSXPXNLQXIPXXRXEXGXXIRXAFVXXXXXXXXIXX  
 ADYSQIELRLXAXHLSXDNLIXAFXXGXXXXXXXXXDHTXTASXIFVXX  
 EXXXXXVTTXMMRXAKXVNXGIXYGXSSXXGLSXXLXXXXXXXXXXXXX  
 XXIXXXEAAXXIEXYFXXXPVXXXIXXXXXAKXXGVVXTLFGRRRXXPX  
 IXSRNXVVRXXXERXAXNXP IQGTAADII KLAMXXXXXLXXXXXXXXXX  
 XXXXLQXHDELVXEVEEXXVXXXXKXMXVXLXVPXXXXLXVXXX  
 XGXXW,  
 wherein X is any amino acid or a peptide bond.

[0082] Chimeric DNA Polymerases

[0083] In some embodiments, chimeric DNA polymerases suitable for the invention include any DNA polymerases containing sequences derived from two or more different DNA polymerases. In some embodiments, chimeric DNA polymerases suitable for the invention include chimeric DNA polymerases as described in co-pending application entitled "Chimeric DNA polymerases" filed on even date, the disclosures of which are hereby incorporated by reference.

[0084] Chimeric DNA polymerases suitable for the invention also include the chimeric DNA polymerases described in U.S. Publication No. 20020119461, U.S. Pat. Nos. 6,228, 628 and 7,244,602, herein incorporated by reference.

[0085] Fusion DNA Polymerases

[0086] Suitable fusion DNA polymerases include any DNA polymerases that are combined (e.g., covalently or

non-covalently) with one or more protein domains having a desired activity (e.g., DNA-binding, dUTP hydrolysis or stabilizing template-primer complexes). In some embodiments, the one or more protein domains having the desired activity are derived from a non-polymerase protein. Typically, fusion DNA polymerases are generated to improve certain functional characteristics (e.g., processivity, elongation rate, fidelity, salt-resistance, dUTP tolerance etc.) of a DNA polymerase. For example, DNA polymerase has been fused in frame to the helix-hairpin-helix DNA binding motifs from DNA topoisomerase V and shown to increase processivity, salt resistance and thermostability of the fusion DNA polymerase as described in Pavlov et al., 2002, *Proc. Natl. Acad. Sci USA*, 99:13510-13515. Fusion of the thioredoxin binding domain to 17 DNA polymerase enhances the processivity of the DNA polymerase fusion in the presence of thioredoxin as described in WO 97/29209, U.S. Pat. No. 5,972,603 and Bedford et al. *Proc. Natl. Acad. Sci. USA* 94: 479-484 (1997). Fusion of the archaeal PCNA binding domain to Taq DNA polymerase results in a DNA polymerase fusion that has enhanced processivity and produces higher yields of PCR amplified DNA in the presence, of PCNA (Motz, M., et al., *J. Biol. Chem. May* 3, 2002; 277 (18); 16179-88). Also, fusion of the sequence non-specific DNA binding protein Sso7d or Sac7d from *Sulfolobus sulfataricus* to a DNA polymerase, such as Pfu or Taq DNA polymerase, was shown to greatly increase the processivity of these DNA polymerases as disclosed in WO 01/92501 A1, which is hereby incorporated by reference. Additional fusion polymerases are described in US Publication No. 20070190538A1, which is incorporated herein by reference.

[0087] Commercially available exemplary fusion polymerases include, but are not limited to, TopoTaq™ (Fidelity Systems) which is a hybrid of Taq polymerase fused to a sequence non-specific Helix-hairpin-helix (HhH) motif from DNA topoisomerase V (Topo V) (see, U.S. Pat. Nos. 5,427, 928; 5,656,463; 5,902,879; 6,548,251; Pavlov et al., 2002, *Proc. Natl. Acad. Sci USA*, 99:13510-13515, all of which are incorporated herein by references); Phusion™ (Finnzymes and NEB, sold by BioRad as iProof) which is a chimeric Deep Vent™/Pfu DNA polymerase fused to a small basic chromatin-like Sso7d protein (see, U.S. Pat. No. 6,627,424, U.S. Application Publication Nos. 20040191825, 20040081963, 20040002076, 20030162173, 20030148330, and Wang et al. 2004, *Nucleic Acids Research*, 32(3), 1197-1207, all of which are hereby incorporated by reference); PfuUltra™ II Fusion (Agilent) which is a Pfu-based DNA polymerase fused to a double stranded DNA binding protein (U.S. Application No. 20070148671, which is incorporated by reference); Herculase II Fusion (Agilent) which is a Herculase II enzyme fused to a DNA-binding domain; and Pfx50 (Invitrogen) which is a DNA polymerase from *T. zilligii* fused to an accessory protein that stabilizes primer-template complexes.

Generation of Modified DNA Polymerases of the Invention

[0088] Modified DNA polymerases can be generated by introducing one or more amino acid alterations into a DNA polymerase at the positions corresponding to the positions described herein (e.g., positions identified in Table 2).

TABLE 2

Mutations in Taq polymerase.	
Position	Mutation
61	A61T
346	K346E
357	S357C
507	E507K
707	I707M
749	F749I

**[0089]** Typically, corresponding positions in various DNA polymerases can be determined by alignment of amino acid sequences. Alignment of amino acid sequences can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Preferably, the WU-BLAST-2 software is used to determine amino acid sequence identity (Altschul et al., *Methods in Enzymology* 266, 460-489 (1996); URL://blast.wustl/edu/blast/RE-ADME.html). WU-BLAST-2 uses several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span=1, overlap fraction=0.125, word threshold (T)=11. HSP score (S) and HSP S2 parameters are dynamic values and are established by the program itself, depending upon the composition of the particular sequence, however, the minimum values may be adjusted and are set as indicated above. An example of an alignment is shown in FIGS. 1A-1C. Exemplary amino acid alterations (e.g., corresponding to those alterations in Taq polymerase described above) in DNA polymerases from various organisms are shown in Table. 3.

**[0090]** Alterations may be a substitution, deletion or insertion of one or more amino acid residues. Appropriate alteration for each position can be determined by examining the nature and the range of mutations at the corresponding position described herein. In some embodiments, appropriate amino acid alterations can be determined by evaluating a three-dimensional structure of a DNA polymerase of interest (e.g., parental DNA polymerase). For example, amino acid substitutions identical or similar to those described in Table 2 can be introduced to a DNA polymerase. Alternative amino acid substitutions can be made using any of the techniques and guidelines for conservative and non-conservative amino acids as set forth, for example, by a standard Dayhoff frequency exchange matrix or BLO-SUM matrix. Six general classes of amino acid side chains have been categorized and include: Class I (Cys); Class II (Ser, Thr, Pro, Ala, Gly); Class III (Asn, Asp, Gln, Glu); Class IV (His, Arg, Lys); Class V (Ile, Leu, Val, Met); and Class VI (Phe, Tyr, Trp). For example, substitution of an Asp for another class III residue such as Asn, Gln, or Glu, is a conservative substitution. As used herein, "non-conservative substitution" refers to the substitution of an amino acid in one class with an amino acid from another class; for example, substitution of an Ala, a class II residue, with a class III residue such as Asp, Asn, Glu, or Gln. Insertions or deletions may optionally be in the range of 1 to 5 amino acids.

**[0091]** Appropriate amino acid alterations allowed in relevant positions may be confirmed by testing the resulting modified DNA polymerases for activity in the in vitro assays known in the art or as described in the Examples below.

**[0092]** The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, and PCR mutagenesis. Site-directed mutagenesis (Carter et al., *Nucl. Acids Res.*, 13:4331 (1986); Zoller

TABLE 3

Exemplary Amino Acid Alterations in DNA Polymerases (pairwise alignments were performed to the Taq DNA polymerase protein sequence using the ClustalW algorithm, with default settings).				
Position	Residue in Taq Polymerase (Exemplary Mutation)	Corresponding Residue in <i>Thermus</i> <i>thermophilus</i> DNA Polymerase I (Exemplary Mutation)	Corresponding Residue in <i>Geobacillus</i> <i>staerothermophilus</i> DNA Polymerase I (Exemplary Mutation)	Corresponding Residue in <i>E.coli</i> DNA Polymerase I (Exemplary Mutation)
61	DAVIVVFDA (A61T)	KAVFVVFDA (A62T)	THLLVAFDA (H55T)	THAAVVFDA (H57T)
346	LKEARGLLA (K346E)	LKEVRGLLA (K348E)	ETKKKSMFD (T367E)	LELLKPLLE (E403)
357	LSVLALREG (S357C)	LAVLASREG (A359C)	RAVVALKWK (A378C)	KALKVGQNL (A414C)
507	TEKTGKRST (E507K)	TQKTGKRST Q509K	TKTGYSTSA K553	TPGGAPSTS P603K
707	WIEKTLEEG (I707M)	WIEKTLEEG (I709M)	YMENIVQEA (M752)	YMERTRAQA (M802)
749	AFNMPVQGT (F749I)	AFNMPVQGT (F751I)	AMNTPIQGS (M794I)	AINAPMQGT (I844)

et al., *Nucl. Acids Res.*, 10:6487 (1987)), cassette mutagenesis (Wells et al., *Gene*, 34:315 (1985)), restriction selection mutagenesis (Wells et al., *Philos. Trans. R. Soc. London SerA*, 317:415 (1986)), inverse PCR with mutations included in the primer sequence, or other known techniques can be performed on the cloned DNA to produce desired modified DNA polymerases.

**[0093]** In some embodiments, alterations suitable for the invention also include chemical modification including acetylation, acylation, amidation, ADP-ribosylation, glycosylation, GPI anchor formation, covalent attachment of a lipid or lipid derivative, methylation, myristylation, pegylation, prenylation, phosphorylation, ubiquitination, or any similar process.

**[0094]** Modified DNA polymerases according to the invention may contain one or more (e.g., one, two, three, four, or five) of the amino acid alterations at one or more (e.g., one, two, three, four, or five) positions corresponding to those described in Table 2. Modified DNA polymerases according to the invention may also contain additional substitutions, insertions and/or deletions independent of the mutations observed or selected in the directed evolution experiments. Thus, in some embodiments, a modified DNA polymerase according to the invention has an amino acid sequence at least 70%, e.g., at least 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99%, identical to a corresponding wild-type (or naturally-occurring) DNA polymerase. In some embodiments, a modified DNA polymerase has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acid substitutions, deletions, insertions, or a combination thereof, relative to a wild type form of the polymerase.

**[0095]** “Percent (%) amino acid sequence identity” is defined as the percentage of amino acid residues in a modified sequence that are identical with the amino acid residues in the corresponding parental sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity are similar to the alignment for purposes of determining corresponding positions as described above.

Methods well known in the art may be applied to express and isolate modified DNA polymerases. Many bacterial expression vectors contain sequence elements or combinations of sequence elements allowing high level inducible expression of the protein encoded by a foreign sequence. For example, expression vectors are commercially available from, for example, Novagen (<http://www.emdbiosciences.com/html/NVG/AllTables.html#>).

**[0096]** As an example, bacteria expressing an integrated inducible form of the T7 RNA polymerase gene may be transformed with an expression vector bearing a modified DNA polymerase gene linked to the T7 promoter. Induction of the T7 RNA polymerase by addition of an appropriate inducer, for example, isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) for a lac-inducible promoter, induces the high level expression of the chimeric gene from the T7 promoter.

**[0097]** Appropriate host strains of bacteria may be selected from those available in the art by one of skill in the art. As a non-limiting example, *E. coli* strain BL-21 is commonly used for expression of exogenous proteins since it is protease deficient relative to other strains of *E. coli*. For

situations in which codon usage for the particular polymerase gene differs from that normally seen in *E. coli* genes, there are strains of BL-21 that are modified to carry tRNA genes encoding tRNAs with rarer anticodons (for example, argU, ileY, leuW, and proL tRNA genes), allowing high efficiency expression of cloned genes (several BL21-CODON PLUS™ cell strains carrying rare-codon tRNAs are available from Agilent, for example). Additionally or alternatively, genes encoding DNA polymerases may be codon optimized to facilitate expression in *E. coli*. Codon optimized sequences can be chemically synthesized.

**[0098]** There are many methods known to those of skill in the art that are suitable for the purification of a modified DNA polymerase of the invention. For example, the method of Lawyer et al. (1993, *PCR Meth. & App.* 2: 275) is well suited for the isolation of DNA polymerases expressed in *E. coli*, as it was designed originally for the isolation of Taq polymerase. Alternatively, the method of Kong et al. (1993, *J. Biol. Chem.* 268: 1965, incorporated herein by reference) may be used, which employs a heat denaturation step to destroy host proteins, and two column purification steps (over DEAE-Sepharose and heparin-Sepharose columns) to isolate highly active and approximately 80% pure DNA polymerase.

Further, modified DNA polymerase may be isolated by an ammonium sulfate fractionation, followed by Q Sepharose and DNA cellulose columns, or by adsorption of contaminants on a HiTrap Q column, followed by gradient elution from a HiTrap heparin column.

#### Applications of Modified DNA Polymerases of the Invention

**[0099]** Modified DNA polymerases of the present invention may be used for any methods involving polynucleotide synthesis. Polynucleotide synthesis methods are well known to a person of ordinary skill in the art and can be found, for example, in *Molecular Cloning* second edition, Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). For example, modified DNA polymerases of the present invention have a variety of uses in recombinant DNA technology including, but not limited to, labeling of DNA by nick translation, second-strand cDNA synthesis in cDNA cloning, DNA sequencing, whole-genome amplification and amplifying, detecting, and/or cloning nucleic acid sequences using polymerase chain reaction (PCR).

**[0100]** In some embodiments, the invention provides enzymes that are better suited for PCR used in industrial or research applications. PCR refers to an in vitro method for amplifying a specific polynucleotide template sequence. The technique of PCR is described in numerous publications, including, PCR: A Practical Approach, M. J. McPherson, et al., IRL Press (1991), PCR Protocols: A Guide to Methods and Applications, by Innis, et al., Academic Press (1990), and PCR Technology: Principals and Applications for DNA Amplification, H. A. Erlich, Stockton Press (1989). PCR is also described in many U. S. patents, including U.S. Pat. Nos. 4,683,195; 4,683,202; 4,800,159; 4,965,188; 4,889,818; 5,075,216; 5,079,352; 5,104,792; 5,023,171; 5,091,310; and 5,066,584, each of which is herein incorporated by reference.

**[0101]** In some embodiments, the invention provides enzymes that are better suited for PCR amplification of plant-derived samples. Inhibitors, such as polysaccharides,



secondary metabolites, and polyphenolics, among others, can co-isolate with nucleic acids from plant tissues, leading to an inhibition of downstream molecular manipulations, such as PCR (see, for example, Koonjul, 1998, *Nucleic Acids Research*, 27(3):915. Such inhibitors may act in a variety of ways, such as by causing precipitation of DNA, denaturation of DNA, decreasing the ability of a polymerase enzyme to bind to magnesium ions (e.g., kinetically modifying the PCR reaction by chelating cofactors such as magnesium), binding to target DNA or DNA polymerase, etc. (see, for example, Demeke, 2010, *Anal Bioanal Chem.*, 396:1977. PCR inhibitors may originate from plant tissue (e.g., leaves, bark, fruit, etc.) or from reagents used for DNA isolation. Modified DNA polymerases in accordance with the present invention may be more resistant to PCR inhibitors, in particular PCR inhibitors present in plant-derived samples, as compared to their wild-type counterparts or unmodified parental polymerases.

**[0102]** Modified DNA polymerases with higher processivity, elongation rate, salt resistance, and/or fidelity are expected to improve efficiency and success rate of long-range amplification (higher yield, longer targets amplified) and reduce the amount of required DNA template.

**[0103]** Various specific PCR amplification applications are available in the art (for reviews, see for example, Erlich, 1999, *Rev Immunogenet.*, 1: 127-34; Prediger 2001, *Methods Mol. Biol.* 160: 49-63; Jurecic et al., 2000, *Curr. Opin. Microbiol.* 3: 316-21; Triglia, 2000, *Methods Mol. Biol.* 130: 79-83; MacClelland et al., 1994, *PCR Methods Appl.* 4: S66-81; Abramson and Myers, 1993, *Current Opinion in Biotechnology* 4: 41-47; each of which is incorporated herein by references).

**[0104]** As non-limiting examples, modified DNA polymerases described herein can be used in PCR applications including, but are not limited to, i) hot-start PCR which reduces non-specific amplification; ii) touch-down PCR which starts at high annealing temperature, then decreases annealing temperature in steps to reduce non-specific PCR product; iii) nested PCR which synthesizes more reliable product using an outer set of primers and an inner set of primers; iv) inverse PCR for amplification of regions flanking a known sequence. In this method, DNA is digested, the desired fragment is circularized by ligation, then PCR using primer complementary to the known sequence extending outwards; v) AP-PCR (arbitrary primed)/RAPD (random amplified polymorphic DNA). These methods create genomic fingerprints from species with little-known target sequences by amplifying using arbitrary oligonucleotides; vi) RT-PCR which uses RNA-directed DNA polymerase (e.g., reverse transcriptase) to synthesize cDNAs which is then used for PCR. This method is extremely sensitive for detecting the expression of a specific sequence in a tissue or cells. It may also be used to quantify mRNA transcripts; vii) RACE (rapid amplification of cDNA ends). This is used where information about DNA/protein sequence is limited. The method amplifies 3' or 5' ends of cDNAs generating fragments of cDNA with only one specific primer each (plus one adaptor primer). Overlapping RACE products can then be combined to produce full length cDNA; viii) DD-PCR (differential display PCR) which is used to identify differentially expressed genes in different tissues. A first step in DD-PCR involves RT-PCR, then amplification is performed using short, intentionally nonspecific primers; ix) Multiplex-PCR in which two or more unique targets of DNA sequences

in the same specimen are amplified simultaneously. One DNA sequence can be used as control to verify the quality of PCR; x) Q/C—PCR (Quantitative comparative) which uses an internal control DNA sequence (but of different size) which compete with the target DNA (competitive PCR) for the same set of primers; xi) Recursive PCR which is used to synthesize genes. Oligonucleotides used in this method are complementary to stretches of a gene (>80 bases), alternately to the sense and to the antisense strands with ends overlapping (–20 bases); xii) Asymmetric PCR; xiii) In Situ PCR; xiv) Site-directed PCR Mutagenesis; xv) DOP-PCR that uses partially degenerate primers for whole-genome amplification; xvi) quantitative PCR using SYBR green or oligonucleotide probes to detect amplification; and xvii) error-prone PCR in which conditions are optimized to give an increased number of mutations in the PCR product.

**[0105]** It should be understood that this invention is not limited to any particular amplification system. As other systems are developed, those systems may benefit by practice of this invention.

**[0106]** In some embodiments, modified DNA polymerases are blended with other DNA polymerases in order to further increase processivity, elongation rate, salt resistance, and/or fidelity and reduce the amount of required DNA for PCR applications. For examples, in some embodiments, modified DNA polymerases in accordance with the present invention may be used with DNA polymerases exhibiting 3'-exonuclease activity (e.g., proofreading activity). In some embodiments, DNA polymerases exhibiting 3'-exonuclease activity are type B DNA polymerases (also known as family B DNA polymerases). Family B polymerases include, but are not limited to, *E. coli* pol II, archaeal polymerases, PRD1, phi29, M2, T4 bacteriophage DNA polymerases, eukaryotic polymerases  $\alpha$ ,  $\Delta$ ,  $\epsilon$ , and many viral polymerases. In some embodiments, DNA polymerases suitable for the invention are archaeal polymerases (e.g., euryarchaeal polymerases). Suitable exemplary archaeal polymerases include, but are not limited to, DNA polymerases from archaea (e.g., *Thermococcus litoralis* (Vent™, GenBank: AAA72101), *Pyrococcus furiosus* (Pfu, GenBank: D12983, BAA02362), *Pyrococcus woessii*, *Pyrococcus* GB-D (Deep Vent™, GenBank: AAA67131), *Thermococcus kodakaraensis* KOD1 (KOD, GenBank: BD175553, BAA06142; *Thermococcus* sp. strain KOD (Pfx, GenBank: AAE68738)), *Thermococcus gorgonarius* (Tgo, Pdb: 4699806), *Sulfolobus solataricus* (GenBank: NC002754, P26811), *Aeropyrum pernix* (GenBank: BAA81109), *Archaeoglobus fulgidus* (GenBank: O29753), *Pyrobaculum aerophilum* (GenBank: AAL63952), *Pyrodicticum occultum* (GenBank: BAA07579, BAA07580), *Thermococcus* 9 degree Nm (GenBank: AAA88769, Q56366), *Thermococcus funicolans* (GenBank: CAA93738, P74918), *Thermococcus hydrothermalis* (GenBank: CAC18555), *Thermococcus* spp. GE8 (GenBank: CAC12850), *Thermococcus* spp. JDF-3 (GenBank: AX135456; WO0132887), *Thermococcus* spp. TY (GenBank: CAA73475), *Pyrococcus abyssi* (GenBank: P77916), *Pyrococcus glycovorans* (GenBank: CAC12849), *Pyrococcus horikoshii* (GenBank: NP 143776), *Pyrococcus* spp. GE23 (GenBank: CAA90887), *Pyrococcus* spp. ST700 (GenBank: CAC12847), *Thermococcus pacificus* (GenBank: AX411312.1), *Thermococcus zilligii* (GenBank: DQ3366890), *Thermococcus* aggregans, *Thermococcus barossii*, *Thermococcus celer* (GenBank: DD259850.1), *Thermococcus profundus* (GenBank: E14137), *Thermococ-*

*cus siculi* (GenBank: DD259857.1), *Thermococcus thioreducens*, *Thermococcus onnurineus* NA1, *Sulfolobus acidocaldarius*, *Sulfolobus tokodaii*, *Pyrobaculum calidifontis*, *Pyrobaculum islandicum* (GenBank: AAF27815), *Methanococcus jannaschii* (GenBank: Q58295), *Desulfurococcus* species TOK, *Desulfurococcus*, *Pyrolobus*, *Pyrodictium*, *Staphylothermus*, *Vulcanisaetta*, *Methanococcus* (GenBank: P52025) and other archaeal B polymerases, such as GenBank AAC62712, P956901, BAAA07579)). See, for example, International Patent Application PCT/US09/063166 (WO2010/062776), entitled "CHIMERIC DNA POLYMERASES", the entire contents of which is incorporated herein by reference. Type B DNA polymerases suitable for the present invention also include modified type B DNA polymerases such as those described in International Patent Application PCT/US2009/063169 (WO2010/062779), entitled "MODIFIED DNA POLYMERASES", the entire contents of which is herein incorporated by reference.

[0107] It will be appreciated that polymerase blends in accordance with the present disclosure may contain a ratio of modified DNA polymerases to other types of DNA polymerases exhibiting 3'-exonuclease activity (e.g., Type B polymerases) in any appropriate range, for example, from about 1:1 to about 1:2000 relative units of modified DNA polymerase to DNA polymerases exhibiting 3'-exonuclease activity; 1:2 to about 1:1000 relative units of modified DNA polymerase to DNA polymerases exhibiting 3'-exonuclease activity; 1:4 to about 1:500 relative units of modified DNA polymerase to DNA polymerases exhibiting 3'-exonuclease activity; and from about 1:1 to about 1:100 relative units of modified DNA polymerase to DNA polymerases exhibiting 3'-exonuclease activity. See, for example, suitable exemplary ratios between various polymerases and methods and formulations for making polymerase blends are described in U.S. Pat. No. 5,436,149, the entire contents of which are herein incorporated by reference.

[0108] In some embodiments, modified DNA polymerases are used with PCR additives in order to further increase processivity, elongation rate, salt resistance, and/or fidelity and reduce the amount of required DNA for PCR applications. In some embodiments, additives provide improved enzyme thermostability, modified primer annealing characteristics, improved melting characteristics of DNA, sequestration of PCR inhibitors. Exemplary additives that may be used in accordance with the present disclosure include, but are not limited to, bovine serum albumin, tetramethyl ammonium chloride, dimethylsulfoxide, beta-mercaptoethanol, tris (2-carboxyethyl) phosphine, sodium metabisulfite, povidone, Tween 20, Triton X-100, Nonidet P-40, polyethylene glycol, betaine, formamide, 7-deaza dGTP, spermidine, thermostable RecA, glycerol, gelatin, low-fat milk powder, and combinations thereof.

#### Kits

[0109] The invention also contemplates kit formats which include a package unit having one or more containers containing modified DNA polymerases of the invention and compositions thereof. In some embodiments, the present invention provides kits further including containers of various reagents used for polynucleotide synthesis, including synthesis in PCR.

[0110] Inventive kits in accordance with the present invention may also contain one or more of the following items: polynucleotide precursors, primers, buffers, instructions,

PCR additives and controls. Kits may include containers of reagents mixed together in suitable proportions for performing the methods in accordance with the invention. Reagent containers preferably contain reagents in unit quantities that obviate measuring steps when performing the subject methods.

#### EXAMPLES

##### Example 1. Directed Evolution Experiments Using Taq Polymerase

[0111] To select mutated enzymes that would better be suited for recombinant DNA technologies, a directed evolution experiment is designed by simply mimicking the normal conditions under which the enzyme is usually used, or possibly under less than perfect conditions such as are expected in real-life applications. After conducting enough rounds of selection, an enzyme (or multiple enzymes) that is better suited for typical applications in recombinant DNA technologies should appear. Details of directed evolution experiments and exemplary advantages of associated with selected mutations are described in the co-pending application entitled "Modified DNA Polymerases (WO2010/062779), which is incorporated by reference herein.

[0112] In particular, we have performed directed evolution experiments using a mutant type A DNA polymerase, Taq-E507K. Directed evolution experiments were conducted on Taq-E507K mutant libraries created by error-prone PCR.

[0113] Several rounds of selection were conducted. During the course of the ongoing selection, it is likely that many different mutations will confer different types of advantage, to different degrees, either alone or in combination. Typically, during the first rounds of selection, there are no obvious dominant clones, while the huge numbers of neutral or disadvantageous mutants are likely to be eliminated. Thereafter, a number of particular mutations typically appear in higher than expected numbers. These mutations are there because they have some advantages.

[0114] Typically, the selections are considered to have worked when the vast pool of mutants that are in the starting material have been eliminated and the pool is dominated by a remaining few types or families of mutants that have out-competed the other mutants and the wild type. At this stage, it is not necessary to define exactly the nature of the improvement that the mutations confer. The fact that it was selected for is sufficient proof, especially if the same mutation becomes dominant in independently run selections.

[0115] Further selection results in the number of some of these mutations increasing in the pool, while others may be eliminated possibly because they have some advantages but they are not sufficient to compete with better-adapted clones. At the same time, some previously unnoticed mutants may appear. The late appearance of these mutants might be due to the fact that these specific mutations were low in number in the starting pool, or that the mutation required another (or more than one) mutation in the same clone for the advantage to manifest. If selections continue even further, eventually, a few clones will likely to dominate substantially. Typically, it is important to isolate clones before this final point if it is desirable to isolate a wide range of beneficial mutations.

[0116] In particular experiments, DNA polymerase mutants of Taq-E507K were generated and screened for resistance to plant-derived PCR inhibitors. Several rounds of selection were conducted on Taq-E507K. During the course

of the ongoing selections, many different mutations were observed either alone or in combination at various positions. Clones that exhibited higher tolerance than wild-type or its parental clone Taq-E507K to plant-derived PCR inhibitors were selected and sequenced. Clone 15 is an exemplary clone which demonstrated high yield amplification of samples containing plant-derived PCR inhibitors (FIG. 2). Sequence analysis of Clone 15 revealed mutations shown in Table 4. A general phenotype of Clone 15 is higher resistance to plant-derived PCR inhibitors than wild-type Taq or parental clone Taq-E507K. Clone 15 is further characterized for a variety of phenotypes, as described in further Examples below.

TABLE 4

Mutations Observed in Taq Mutant Clone 15 Selected for Resistance to Plant-derived PCR Inhibitors	
Position	Mutation
61	A61T
346	K346E
357	S357C
707	I707M
749	F749I

[0117] It was contemplated a subset (e.g., one, two, three, four) of the five mutations shown in Table 4 may be sufficient to render the beneficial phenotypes of clone 5. Taq-E507K DNA polymerase and other mutant Taq-E507K polymerases were used as an example in the screen. It was contemplated that one or more mutations similar to those described in Table 4 may be introduced to various other DNA polymerases, in particular Type A DNA polymerases including those described herein.

#### Example 2. Types of Selective Advantage

[0118] There are a wide range of advantages that may have been selected for, some of which are listed and discussed below:

##### 1) Expression Efficiency:

[0119] The clones that express higher levels of the enzyme will have an advantage over those that express less. The specific activity of the mutated enzyme may not have been improved but the total activity will have. This characteristics is particularly valuable to a manufacture of enzymes because this will allow increased production levels and/or reduced production costs.

##### 2) Solubility and Folding Robustness:

[0120] When solubility increases, the probability of inclusion bodies forming decreases. Therefore, in these clones, a higher proportion of useful, correctly folded enzyme product is expressed.

##### 3) Thermostability:

[0121] It is well known that, during the thermocycling required for PCR, a certain fraction of the enzyme is inactivated due to the heating. An enzyme that is resistant to heat-inactivation will maintain activity longer. Therefore, less enzyme can be used and/or more cycles can be conducted.

##### 4) Activity:

[0122] Mutants with increased enzymatic activity provide more efficient polymerization.

##### 5) Processivity:

[0123] Mutants with increased processivity are able to synthesize long PCR products and synthesize sequences with complexed secondary structure. Mutant enzymes that can incorporate more nucleotides/extension step are likely to operate efficiently at lower concentrations.

##### 6) Speed:

[0124] Mutants with increased elongation rate provide more efficient polymerization. Enzymes that are fast can also be used with shorter extension times. This is particularly valuable for a high-throughput system.

##### 7) Concentration Robustness:

[0125] It is known that PCR reactions may not be carried out appropriately if too much or too little enzyme is used. Under the selection conditions we used, a polymerase that can generate appropriate products whether it is supplied in excess or at low levels will have an advantage.

##### 8) Resistance to Salts, PCR Additives and Other Inhibitors:

[0126] The selection was conducted in the presence of salts, PCR additives (e.g., intercalating dyes), and other impurities (e.g., plant derived inhibitors). The presence of salts may reduce the DNA binding affinity of polymerases. The presence of impurities may interfere with formation of a desired PCR product. A polymerase that is resistant to salts and inhibitors and can synthesize desired products is advantageous and will be selected for. The characteristic is particularly suited for applications in which PCR is used in crude samples.

##### 9) Fidelity:

[0127] All polymerases make mistakes during replication, either by incorporating the wrong dNTP or by stuttering which causes deletions and insertions. Such mistakes can eliminate functional genes during selection, so there is a pressure for mistakes not to be made. A polymerase with higher fidelity is advantageous and will be selected for.

##### 10) Strand-Displacement Activity:

[0128] Secondary structure in the DNA due to intramolecular self annealing may inhibit DNA strand-elongation catalyzed by the polymerase. Similarly, partial re-annealing of the complementary DNA in addition to the primer will inhibit PCR. Any enzyme with improved strand-displacement activity will have an advantage in the selection.

##### 11) Pyrophosphate Tolerance:

[0129] Pyrophosphate is released during incorporation of nucleotides into the nascent strand by polymerases. Accumulation of pyrophosphate may lead to inhibition of the polymerase activity. Polymerases that were selected for in the Directed evolution example may have evolved to become less affected by pyrophosphate inhibition.

12) Unknown:

**[0130]** There many other factors involved in the process of PCR. Enzymes that are better adapted to PCR for any reason may be selected under our selection conditions.

Clone 15 is further characterized for a variety of phenotypes. So far, we have conducted tests for a few different phenotypes: tolerance to inhibitors, tolerance to salt, performance in various buffers, and speed. The tests to examine phenotypes are described in the following examples.

#### Example 3. Tolerance to Plant Inhibitors and High Salt

**[0131]** Clone 15 was tested for the ability to amplify a 1.2 kb PCR amplicon using a 0.5 mm diameter grapevine leaf discs directly in PCR reactions in the absence or presence of high salt. Reactions were performed in a buffer containing 150 mM Tris-H<sub>2</sub>SO<sub>4</sub> (pH 8.5) and optionally 50 mM KCl. Exemplary reaction components are shown in Table 5 and Table 6. Exemplary cycling profile for this assay is shown in Table 7.

**[0132]** Exemplary primers include:

Forward primer: (SEQ ID NO: 24)  
GATCAACCCCGCTGCCCCAC

Reverse primer: (SEQ ID NO: 25)  
CGAAGCCCATCCCCGCTCAG

TABLE 5

Exemplary Reaction Components for Assays without KCl Reaction volume = 50		
Reaction component	Concentration	In 50 uL
PCR water	— —	29.3
Tris- H <sub>2</sub> SO <sub>4</sub> (pH 8.5)	2 M	3.8
MgCl <sub>2</sub> (supplement to 2.0 mM)	25 mM	4.0
Povidone	20% m/v	7.5
dNTPs	10 mM each	1.0
Primer	10 uM	1.5
Primer	10 uM	1.5
0.5 mm dia grapevine leaf disc	— ng/uL	—
Taq DNA polymerase	20 ng/uL	1.5
TOTAL		50.0

TABLE 6

Exemplary Reaction Components for Assays with 50 mM KCl Reaction volume = 50		
Reaction component	Concentration	In 50 uL
PCR water	— —	26.8
Tris- H <sub>2</sub> SO <sub>4</sub> (pH 8.5)	2 M	3.8
MgCl <sub>2</sub> (supplement to 2.0 mM)	25 mM	4.0
KCl	50 mM	2.5
Povidone	20% m/v	7.5
dNTPs	10 mM each	1.0
Primer	10 uM	1.5
Primer	10 uM	1.5
0.5 mm dia grapevine leaf disc	— ng/uL	—
Taq DNA polymerase	20 ng/uL	1.5
TOTAL		50.0

TABLE 7

Exemplary Cycling Profile Cycling profile:			
Cycle	No.	Temp (° C.)	Time
Initial denaturation	1	95	10 min
Denaturation	45	95	20 sec
Annealing/Extension	45	72	40 sec
Final elongation	1	72	1 min
HOLD	1	4	Indefinite

**[0133]** Reaction products were run on an agarose gel and scored for a presence or absence, as well as intensity of a band at the appropriate fragment size. Exemplary results are shown in Table 8 and FIG. 3. Clone 15 gave a higher yield than control polymerase Taq-E7S. Furthermore, Taq-E7S failed when an additional 50 mM KCl was added to the buffer, indicating that Clone 15 is more salt-tolerant than Taq-E7S. Taq-E7S was previously shown to be tolerant to a host of PCR inhibitors compared to wild-type Taq (See, for example, WO2010/062777, the entire contents of which is herein incorporated by reference).

TABLE 8

Fragments Produced by Clone 15		
Clone Name:	-50 mM KCl	+50 mM KCl
Taq-E7S	+	-
Clone 15	++	+

#### Example 4. Tolerance to Plant Inhibitors

**[0134]** Clone 15 was tested for the ability to amplify a 1.45 kb PCR amplicon using 0.5 mm diameter potato leaf discs directly in PCR reactions. Reactions were performed in a buffer containing 150 mM Tris-H<sub>2</sub>SO<sub>4</sub> (pH 8.5). Exemplary reaction components are shown in Table 9. Exemplary cycling profile for this assay is shown in Table 10.

**[0135]** Exemplary Primers:

Forward primer: (SEQ ID NO: 26)  
GCGCATGCAAGCTGACCTGG

Reverse primer: (SEQ ID NO: 27)  
TCACGCTCCAAGCGGGAAAC

TABLE 9

Exemplary Reaction Components for Assays Reaction volume = 50		
Reaction component	Concentration	In 50 uL
PCR water	— —	29.3
Tris- H <sub>2</sub> SO <sub>4</sub> (pH 8.5)	2 M	3.8
MgCl <sub>2</sub> (supplement to 2.0 mM)	25 mM	4.0
Povidone	20% m/v	7.5
dNTPs	10 mM each	1.0
Primer	10 uM	1.5
Primer	10 uM	1.5
0.5 mm dia potato leaf disc	— ng/uL	—
Taq DNA polymerase	20 ng/uL	1.5
TOTAL		50.0

TABLE 10

Exemplary Cycling Profile Cycling profile:				
Cycle	No.	Temp (° C.)	Time	
Initial denaturation	1	95	3	min
Denaturation	40	95	20	sec
Annealing/Extension	40	72	45	sec
Final elongation	1	72	1	min
HOLD	1	4	Indefinite	

**[0136]** Reaction products were run on an agarose gel and scored for either a presence or absence of a band at the appropriate fragment size. Exemplary results are shown in Table 11 and FIG. 4. Clone 15 exhibited positive amplification from the leaf discs whereas clone Taq-E7S did not exhibit positive amplification.

TABLE 11

Fragments Produced by Clone 15	
Clone Name:	Amplicon
Taq-E7S	-
Clone 15	+

#### Example 5. Tolerance to Buffer Conditions

**[0137]** Taq, Taq-E7S, and Clone 15 were tested for the ability to amplify a 1 kb PCR amplicon using  $\lambda$  DNA in PCR reactions. Reactions were performed in a buffer containing 150 mM Tris-H<sub>2</sub>SO<sub>4</sub> (pH 8.5; with and without 50 mM KCl) or 10 mM Tris-HCl (pH 8.3; with either 50 mM or 100 mM KCl). Exemplary reaction components are shown in Table 12. Exemplary cycling profile for this assay is shown in Table 13.

**[0138]** Exemplary Primers:

Forward primer: (SEQ ID NO: 28)  
CCTGCTCTGCCGCTTCACGC

Reverse primer: (SEQ ID NO: 29)  
GATGACGCATCCTCAGATAATATCCGG

TABLE 12

Exemplary Reaction Components for Assays	
Reaction component	Concentration
Buffer (150 mM Tris-H <sub>2</sub> SO <sub>4</sub> (pH 8.5) or 10 mM Tris-HCl (pH 8.3))	1X
KCl	0 mM, 50 mM, or 100 mM
MgCl <sub>2</sub>	1.5 mM
dNTPs	0.2 mM each
Primer	0.3 $\mu$ M
Primer	0.3 $\mu$ M
Template ( $\lambda$ DNA)	5 ng, 1 ng, 200 pg, 40 pg, 8 pg or 0 pg
DNA polymerase	1 unit

TABLE 13

Exemplary Cycling Profile Cycling profile:				
Cycle	No.	Temp (° C.)	Time	
Initial denaturation	1	95	3	min
Denaturation	30	95	20	sec
Annealing/Extension	30	72	30	sec
Final elongation	1	72	1	min
HOLD	1	4	Indefinite	

**[0139]** Reaction products were run on an agarose gel and scored for either a presence or absence of a band at the appropriate fragment size. Exemplary results are shown in Table 14 and FIG. 5. Taq did not amplify in 150 mM Tris-H<sub>2</sub>SO<sub>4</sub> buffer, whereas both Taq-E7S and Clone 15 did. Taq-E7S did not amplify when 50 mM KCl was added, whereas the performance of Clone 15 actually improved upon addition of 50 mM KCl. Taq amplified in 10 mM Tris-HCl buffer with 50 mM KCl, but not with 100 mM KCl.

TABLE 14

Fragments Produced by Taq, Taq-E7S and Clone 15						
		Amount of Template DNA				
		5 ng	1 ng	200 pg	40 pg	8 pg
Taq	150 mM Tris H <sub>2</sub> SO <sub>4</sub>					
	-50 mM KCl	no	no	no	no	no
	+50 mM KCl	no	no	no	no	no
	10 mM Tris HCl					
	+50 mM KCl	yes	yes	no	no	no
Taq-E7S	+100 mM KCl	no	no	no	no	no
	150 mM Tris H <sub>2</sub> SO <sub>4</sub>					
	-50 mM KCl	yes	yes	no	no	no
	+50 mM KCl	no	no	no	no	no
	Clone 5	150 mM Tris H <sub>2</sub> SO <sub>4</sub>				
-50 mM KCl		yes	yes	no	no	no
+50 mM KCl		yes	yes	yes	no	no

#### Example 6. Tolerance to Buffer Conditions and Annealing/Extension Times

**[0140]** Taq, Taq-E7S, and Clone 15 were tested for the ability to amplify a 1 kb PCR amplicon using  $\lambda$  DNA in PCR reactions. Reactions were performed in a buffer containing 10 mM Tris-HCl (pH 8.3; with either 50 mM or 100 mM KCl). Exemplary reaction components are shown in Table 15. Exemplary cycling profile for this assay is shown in Tables 16 and 17.

**[0141]** Exemplary Primers:

Forward primer: (SEQ ID NO: 30)  
CCTGCTCTGCCGCTTCACGC

Reverse primer: (SEQ ID NO: 31)  
GATGACGCATCCTCAGATAATATCCGG

TABLE 15

Exemplary Reaction Components for Assays	
Reaction component	Concentration
Buffer (10 mM Tris-HCl (pH 8.3))	1X
KCl	50 mM or 100 mM
MgCl <sub>2</sub>	1.5 mM
dNTPs	0.2 mM each
Primer	0.3 uM
Primer	0.3 uM
Template (λ DNA)	5 ng, 1 ng, 200 pg, 40 pg, 8 pg or 0 pg
DNA polymerase	1 unit

TABLE 16

Exemplary Cycling Profile Cycling profile:			
Cycle	No.	Temp (° C.)	Time
Initial denaturation	1	95	3 min
Denaturation	30	95	20 sec
Annealing/Extension	30	72	30 sec
Final elongation	1	72	1 min
HOLD	1	4	Indefinite

TABLE 17

Exemplary Cycling Profile Cycling profile:			
Cycle	No.	Temp (° C.)	Time
Initial denaturation	1	95	3 min
Denaturation	30	95	20 sec
Annealing/Extension	30	72	20 sec
Final elongation	1	72	1 min
HOLD	1	4	Indefinite

[0142] Reaction products were run on an agarose gel and scored for either a presence or absence of a band at the appropriate fragment size. Exemplary results are shown in Table 18 and FIG. 6. Clone 15 outperforms both Taq and Taq-E7S, including when KCl is added to 100 mM. The annealing/extension time can be decreased to 20 s, with a concomitant decrease in sensitivity, but the advantage of Clone 15 over Taq and Taq-E7S is nonetheless maintained. Clone 15 is able to tolerate higher-salt buffer environments compared to Taq and Taq-E7S polymerases, which may translate, for example, into better performance with crude sample types.

TABLE 18

Fragments Produced by Taq, Taq-E7S and Clone 15						
		Amount of Template DNA				
		5 ng	1 ng	200 pg	40 pg	8 pg
Taq	30s					
	50 mM KCl	yes	yes	no	no	no
	20s					
Taq-E7S	+50 mM KCl	yes/no	no	no	no	no
	30s					
	+50 mM KCl	yes	yes	no	no	no
	+100 mM KCl	yes	yes	yes	no	no

TABLE 18-continued

Fragments Produced by Taq, Taq-E7S and Clone 15						
		Amount of Template DNA				
		5 ng	1 ng	200 pg	40 pg	8 pg
Clone 15	20s					
	+50 mM KCl	yes	yes/no	no	no	no
	+100 mM KCl	yes	yes	no	no	no
	30s					
	+50 mM KCl	yes	yes	no	no	no
	+100 mM KCl	yes	yes	yes	yes/no	no
	20s					
	+50 mM KCl	yes	yes/no	no	no	no
	+100 mM KCl	yes	yes	yes	no	no

Example 7. Tolerance to Sample and Buffer Conditions

[0143] Taq, Taq-E7S, and Clone 15 were tested for the ability to amplify an 800 bp fragment using 0.5 mm diameter grapevine leaf discs as template in PCR reactions. Reactions were performed in a buffer containing 10 mM Tris-HCl (pH 8.3; with either 50 mM or 100 mM KCl). Exemplary reaction components are shown in Table 19. Exemplary cycling profile for this assay is shown in Tables 20 and 21.

[0144] Exemplary Primers:

Forward primer: (SEQ ID NO: 32)  
 ATGTCACCAACAGAGACTAAAG  
 Reverse primer: (SEQ ID NO: 33)  
 TGCATTACGATCGGAACGCCCA

TABLE 19

Exemplary Reaction Components for Assays	
Reaction component	Concentration
Buffer (10 mM Tris-HCl (pH 8.3))	1X
KCl	50 mM or 100 mM
MgCl <sub>2</sub>	2.0 mM
dNTPs	0.2 mM each
Primer	0.3 uM
Primer	0.3 uM
Template (grapevine leaf disc)	0.5 mm diameter
DNA polymerase	1 unit
Additive (povidone)	3% m/v

TABLE 20

Exemplary Cycling Profile for Taq polymerase Cycling profile:			
Cycle	No.	Temp (° C.)	Time
Initial denaturation	1	95	10 min
Denaturation	30	95	20 sec
	30	55	15 sec
Annealing/Extension	30	72	50 sec
Final elongation	1	72	1 min
HOLD	1	4	Indefinite

TABLE 21

Exemplary Cycling Profile for Taq-E7S and Clone 15 Cycling profile:			
Cycle	No.	Temp (' C.)	Time
Initial denaturation	1	95	10 min
Denaturation	30	95	20 sec
	30	55	15 sec
Annealing/Extension	30	72	25 sec
Final elongation	1	72	1 min
HOLD	1	4	Indefinite

**[0145]** Reaction products were run on an agarose gel and scored for either a presence or absence of a band at the appropriate fragment size. Exemplary results are shown in FIG. 7. None of the enzymes are capable of amplifying from this difficult sample without povidone additive, but Clone 15 provides higher average yield than Taq-E7S in the presence of the additive. Taq failed to amplify. Exemplary additives that may be used in accordance with the present disclosure include, but are not limited to, bovine serum albumin, tetramethyl ammonium chloride, dimethylsulfoxide, beta-mercaptoethanol, sodium metabisulfite, povidone, Tween 20, Triton X-100, Nonidet P-40, polyethylene glycol, betaine, formamide, 7-deaza dGTP, spermidine, thermostable RecA, glycerol, gelatin, low-fat milk powder, and combinations thereof.

Example 8. Alternative Substitutions at Position 749 in Clone 15

**[0146]** Clone 15 polymerase, and altered versions of Clone 15 polymerases, containing alternative substitutions at position 749 (e.g., F749L, F749V, F749T, F749Y, F749P, F749M), were tested for their ability to amplify an 800 bp amplicon from the plant chloroplast genome using crude extract or purified genomic DNA. Reactions were performed in a buffer containing reaction components as shown in Table 22. Exemplary cycling profile for this assay is shown in Table 23.

**[0147]** Exemplary Primers Include:

Forward primer: (SEQ ID NO: 34)  
ATGTCACCACAAACAGAGACTAAAG

Reverse primer: (SEQ ID NO: 35)  
TGCATTACGATCGGAACGCCCA

TABLE 22

Exemplary Reaction Components Reaction volume = 50		
Reaction component	Concentration	In 50 uL
PCR water	— —	21.3-25.8
Tris- HCl (pH 8.5)	1 M	6.75
MgCl <sub>2</sub> (supplement to 2.0 mM)	25 mM	4.0
Povidone	20% m/v	7.5
dNTPs	10 mM each	1.0
Primer F	10 uM	1.5
Primer R	10 uM	1.5

TABLE 22-continued

Exemplary Reaction Components Reaction volume = 50		
Reaction component	Concentration	In 50 uL
Grapevine leaf extract or purified DNA	— —	0.5-5
DNA polymerase	20 ng/uL	1.5
TOTAL		50.0

TABLE 23

Exemplary Cycling Profile Cycling profile:			
Cycle	No.	Temp (' C.)	Time
Initial denaturation	1	95	10 min
Denaturation	35	95	20 sec
Annealing	35	55	15 sec
Extension	35	72	30 sec
Final elongation	1	72	1 min
HOLD	1	4	Indefinite

**[0148]** Reaction products were run on an agarose gel and scored for a presence or absence, as well as intensity of a band at the appropriate fragment size. Exemplary results are shown in Table 24 and FIG. 8. As shown in FIG. 8, from left to right for each series, results were obtained from crude template in the form of 0.5 µl, 1.0 µl, and 5.0 µl of a crude grapevine leaf extract, followed by a reaction with 2.5 ng purified grapevine genomic DNA and a no-template control. "Plant Blend" indicates a blend of Clone 15 with a proof-reading DNA polymerase. A substitution of a P at position 749 resulted in a detrimental effect on the performance of the enzyme, producing a lower yield of PCR product from purified DNA, and no product from crude extract. All other substitutions generated an 800 bp PCR product from both the crude extract and purified DNA, with sub situations of L or V at position 749 seemed to be most promising.

TABLE 24

	Fragments Produced				
	Crude Extract			Purified DNA	Neg. Control
	0.5 µl	1.0 µl	5.0 µl	2.5 ng	-
Clone 15	yes	yes	yes	yes	no
749 L	yes	yes	yes	yes	no
749 V	yes	yes	yes	yes	no
749 T	yes	yes	yes	yes	no
749 Y	yes	yes	yes	yes	no
749 P	no	no	no	yes	no
749 M	yes	yes	yes	yes	no
Plant Blend	yes	yes	yes	yes	no

Example 9. Amplification of Long PCR Fragment from Plant Extract

**[0149]** For this experiment, some the altered versions of Clone 15 polymerases from Example 7, blended with a small percentage of proofreading DNA Polymerase, were used to amplify a 1221 bp fragment of the grapevine chromosomal genome. The following primer set was used, at 50 PCR cycles (other conditions the same as in Example 7 above):

[0150] Exemplary Primers Include:

Forward primer: (SEQ ID NO: 36)  
 GATCAACCCCGCTGCCCCAC

Reverse primer: (SEQ ID NO: 37)  
 CGAAGCCCATCCCGCTCAG

[0151] Reaction products were run on an agarose gel and scored for a presence or absence, as well as intensity of a band at the appropriate fragment size. Exemplary results are shown in Table 25 and FIG. 9. As shown in FIG. 9, from left to right for each series, results were obtained from crude template in the form of a 0.5 mm diameter grapevine leaf disc, 0.5 µl, 1.0 µl, and 5.0 µl of a crude grapevine leaf

extract, followed by a reaction with 7 ng purified grapevine genomic DNA spiked with 1.0 µl crude extract, 7 ng purified grapevine genomic DNA alone and a no-template control. “Plant Blend” indicates a blend of Clone 15 with a proofreading DNA polymerase. “Blend” versions of altered Clone 15, for example, those containing substitutions of Leucine, Valine and Threonine, indicates a blend of altered Clone 15 with a proofreading DNA polymerase.

[0152] These results indicate that blend versions of altered Clone 15, for example, those containing substitutions of Leucine, Valine and Threonine at position 749, are capable of amplifying long PCR fragments from plant materials (e.g., leaf disc or crude extract). In particular, modified Clone 15 containing a leucine residue at position 749 provides similar performance as a modified Clone 15 containing an isoleucine residue at position 749.

TABLE 25

	Fragments Produced						
	Crude Template 0.5 mm leaf disk	Crude Extract			Purified DNA spiked with Crude Extract 7 ng DNA + 1.0 ul Extract	Purified DNA 7 ng	Neg. Control
		0.5 ul	1.0 ul	5.0 ul	1.0 ul	7 ng	—
Plant Blend	yes	yes	no	no	yes	yes	no
749 L Blend	yes	yes	yes	no	yes	yes	no
749 V Blend	yes	no	no	no	yes	yes	no
749 T Blend	yes	no	no	no	yes	yes	no

TABLE 26

Amino acid sequences of wildtype and modified DNA polymerases

>Wild-type Taq (SEQ ID NO: 38)  
 MRGMLPLFEPKGRVLLVDGHHLAYRTFHALKGLTTSRGEVPQAVYGFAKSLKALKEDGDAVIVVFDKAPKAPSRHEA  
 YGGYKAGRPTPEDFPRQLALIKELVDLLGLARLEVPGYEADDVLASLAKKAEKEGYEVRIILTADKLDLYQLLSDRIH  
 VLHPEGYLITPAWLWEKYGLRDPQWADYRALTGDESDNLPGVKGI GEKTARKLLEEWGSLEALLKNLDRPKPAIREK  
 ILAHMDDLKLSWDLAKVRTDLPLEVDFAKRREPDRELRRAFLEERLEFGSLLHEFGLES SPKALEEAPWPPPEGAFV  
 FVLSRKEPMWADLLALAAARGGRVHRAPEPYKALRDLKEARGLLAKDLSVLAALREGLGLPPGDDPMLLAYLLDPSNT  
 TPEGVARRYGGEWTEEAGERAALSERLFPANLWGRLEGEERLLWLYREVERPLSAVLAHMEATGVRDVAYLRAALSLE  
 VAEETARLEAEVFLAGHPFNLSRDQLERLVFDELGLPAIGKTEKTGKRSTSAAVLEALREAHPIVEKILQYRELT  
 KLKSTYIDPLPDLIHPRTGRLHTRFNQTATATGRLLSSDPNLQNI PVRTPLGQIRRAFIAEEGWLLVALDYSQIEL  
 RVLAHLSGDENLIRVFQEGRD IHTETASWMFGVPREAVDPLMRAAKTINFGVLYGMSAHRLSQELAI PYEEAQAFI  
 ERYFQSF PKVRAWI EKTLEEGRRRGYVETLFGRRRYVPDLEARVKS VREAAERMAFNMPVQGTAAADLMKMLAMVKLFP  
 RLEEMGARMLLQVHDELVLEAPKERAEAVARLAKEVMEGVYPLAVPLEVEVGI GEDWLSAKE\*

Taq-E507K (SEQ ID NO: 39)  
 MRGMLPLFEPKGRVLLVDGHHLAYRTFHALKGLTTSRGEVPQAVYGFAKSLKALKEDGDAVIVVFDKAPKAPSRHEA  
 YGGYKAGRPTPEDFPRQLALIKELVDLLGLARLEVPGYEADDVLASLAKKAEKEGYEVRIILTADKLDLYQLLSDRIH  
 VLHPEGYLITPAWLWEKYGLRDPQWADYRALTGDESDNLPGVKGI GEKTARKLLEEWGSLEALLKNLDRPKPAIREK  
 ILAHMDDLKLSWDLAKVRTDLPLEVDFAKRREPDRELRRAFLEERLEFGSLLHEFGLES SPKALEEAPWPPPEGAFV  
 FVLSRKEPMWADLLALAAARGGRVHRAPEPYKALRDLKEARGLLAKDLSVLAALREGLGLPPGDDPMLLAYLLDPSNT  
 TPEGVARRYGGEWTEEAGERAALSERLFPANLWGRLEGEERLLWLYREVERPLSAVLAHMEATGVRDVAYLRAALSLE  
 VAEETARLEAEVFLAGHPFNLSRDQLERLVFDELGLPAIGKTKTKTKRSTSAAVLEALREAHPIVEKILQYRELT  
 KLKSTYIDPLPDLIHPRTGRLHTRFNQTATATGRLLSSDPNLQNI PVRTPLGQIRRAFIAEEGWLLVALDYSQIEL  
 RVLAHLSGDENLIRVFQEGRD IHTETASWMFGVPREAVDPLMRAAKTINFGVLYGMSAHRLSQELAI PYEEAQAFI  
 ERYFQSF PKVRAWI EKTLEEGRRRGYVETLFGRRRYVPDLEARVKS VREAAERMAFNMPVQGTAAADLMKMLAMVKLFP  
 RLEEMGARMLLQVHDELVLEAPKERAEAVARLAKEVMEGVYPLAVPLEVEVGI GEDWLSAKE\*

Taq-E75 (SEQ ID NO: 40)  
 MRGMLPLFEPKGRVLLVDGHHLAYRTFHALKGLTTSRGEVPQAVYGFAKSLNLAQDDGDAVIVVFDKAPKAPSRHEA  
 YGGYKAGRPTPEDFPRQLALIKELVDLLGLARLEVPGYEADDVLASLAKKAEKEGYEVRIILTADKLDLYQLLSDRIH  
 VLHPEGYLITPAWLWEKYGLRDPQWADYRALTGDESDNLPGVKGI GEKTARKLLEEWGSLEALLKNLDRPKPAIREK  
 ILAHMDDLKLSWDLAKVRTDLPLEVDFAKRREPDRELRRAFLEERLEFGSLLHEFGLES SPKALEEAPWPPPEGAFV  
 FVLSRKEPMWADLLALAAARGGRVHRAPEPYKALRDLKEARGLLAKDLSVLAALREGLGLPPGDDPMLLAYLLDPSNT



TABLE 26-continued

Amino acid sequences of wildtype and modified DNA polymerases
<p>TPEGVARRYGGEWTEEEAGERAALSERLFLANLWGRLEGEERLLWLYREVERPLSAVLAHMEATGVRLDVAYLRALSLEVAEEIARLEAEVFRLAGHPFNLSRDQLERVLFDLGLPAIGKTKTKTKRSTSAVLEALREAHPIVEKILQYRELT KLKSTYIDPLPDLIHPRTGRLHTRFNQTATATGRLLSSSDPNLQNI PVRTPLGQRIRRAFI AEEGWLLVALDYSQIEL RVLAHLSGDENLIRVQEGRD IHTETASWMPGVPREAVDPLMRRRAKTINFGVLYGMSAHRLSQELAI PYEEAQAFI ERYFQSFPKVRAWIEKTL EEGRRRGYVETLFGRRRYVPDLNARVKSREAAERMAFNMPVQGT AADLMKMLAMVKL FPRLEMGARMLLQVHDELLEAPKERAEAVARLAKAEVMEGVYPLAVPLEVEVIGEDWLSAKE*</p> <p>&gt;gi 55981023 ref YP_144320.1  DNA polymerase I [<i>Thermus thermophilus</i> HB8] (SEQ ID NO: 41)                      MEAMLPLEPEKGRVLLVDGHHLAYRTFFALKGLTTSRGEVPQAVYGFAKSLLKALKEDGYKAVFVFDKAPSPFRHE AYEAYKAGRAPTPEDFPRQLALI KELVDLLGFTRLEVPGYEADDVLA TLAKKAEKEGYEVRI LTADRDLVQLVSDRV AVLHPEGLHI TPEWLWEKYGLRPEQWVDFRALVGDPSDNLPGVKGI GEKTKALKLKEWGSLENLLKNLDRVKPENVR EKIKAHLEDLRLSLELSRVRTDLPLEVDLAQGREPDREGLRAFLEERLEFGSLLHEFGLEAPAPLEEAPWPPPEGAF VGFVLSRPEPMWAEALKAACRDGRVHRAADPLAGLKDLEVRGGLLAKDLAVLASREGLDLVPGDDPMLLALYLLDPS NTPTEGVARRYGGEWTEEDAHRALLSERLHRNLLKRLLEGEKLLWLYHEVEKPLSRVLAHMEATGVRLDVAYLQALS LELAEIIRLREEVEVFRLAGHPFNLSRDQLERVLFDLRLPALGKTKTKTKRSTSAVLEALREAHPIVEKILQHRE LTKLKNTYVDPLPSLVHPRTRGLHTRFNQTATATGRLLSSSDPNLQNI PVRTPLGQRIRRAFVAEAGWALVALDYSQI ELRVLAHLSGDENLIRVQEGKD IHTQTASWMPGVPEAVDPLMRRRAKTINFGVLYGMSAHRLSQELAI PYEEAVA FIERVYFQSFPKVRAWIEKTL EEGRRRGYVETLFGRRRYVPDLNARVKSREAAERMAFNMPVQGT AADLMKMLAMVKL FPRLEMGARMLLQVHDELLEAPQARAEVAALAKEAMEKAYPLAVPLEVEVGMGEDWLSAKG</p> <p><i>Thermus thermophilus</i> HB8 with Clone 15 mutations in corresponding positions (SEQ ID NO: 42)                      MEAMLPLEPEKGRVLLVDGHHLAYRTFFALKGLTTSRGEVPQAVYGFAKSLLKALKEDGYKAVFVFDKAPSPFRHE AYEAYKAGRAPTPEDFPRQLALI KELVDLLGFTRLEVPGYEADDVLA TLAKKAEKEGYEVRI LTADRDLVQLVSDRV AVLHPEGLHI TPEWLWEKYGLRPEQWVDFRALVGDPSDNLPGVKGI GEKTKALKLKEWGSLENLLKNLDRVKPENVR EKIKAHLEDLRLSLELSRVRTDLPLEVDLAQGREPDREGLRAFLEERLEFGSLLHEFGLEAPAPLEEAPWPPPEGAF VGFVLSRPEPMWAEALKAACRDGRVHRAADPLAGLKDLEVRGGLLAKDLAVLASREGLDLVPGDDPMLLALYLLDPS NTPTEGVARRYGGEWTEEDAHRALLSERLHRNLLKRLLEGEKLLWLYHEVEKPLSRVLAHMEATGVRLDVAYLQALS LELAEIIRLREEVEVFRLAGHPFNLSRDQLERVLFDLRLPALGKTKTKTKRSTSAVLEALREAHPIVEKILQHRE LTKLKNTYVDPLPSLVHPRTRGLHTRFNQTATATGRLLSSSDPNLQNI PVRTPLGQRIRRAFVAEAGWALVALDYSQI ELRVLAHLSGDENLIRVQEGKD IHTQTASWMPGVPEAVDPLMRRRAKTINFGVLYGMSAHRLSQELAI PYEEAVA FIERVYFQSFPKVRAWIEKTL EEGRRRGYVETLFGRRRYVPDLNARVKSREAAERMAFNMPVQGT AADLMKMLAMVKL FPRLEMGARMLLQVHDELLEAPQARAEVAALAKEAMEKAYPLAVPLEVEVGMGEDWLSAKG</p> <p>&gt;gi 2231821 gb AAB62092.1  DNA polymerase I [<i>Geobacillus stearothermophilus</i>] (SEQ ID NO: 43)                      MRLKKLVLIDGNSVAYRAFFAPLPLHNDKGIHTNAVYGFMTMLNKI LAEEQPTLLVAFDAGKTTFRHETPQYKGR QQQTPPELS EQPFLRLRELLKTYRI PAYELYI YEADDI IGTLAARAEQEGFEVKI I SGDRDLTQLASRHVTVDI TTK GITDIEPYTPETVREKYGLTPEQIVDLKGLMGDKSDNIPGVPGIGEKTAVKLLKQFGTVENVLASIDEVKGEKVKEL LRQHRDLALLSKQLASI CRDAPVELSLDALVYEGDREKVI ALFKELGFSFLEKMAAPAEGRKPLEEMEF AIVDV I TEENLADKALLVVMEENYHDAP IVGIALVNEHGRFFMRPETALADSQFLAWLADETKKKSMPDAKRAVVALKWK GIDVRGVAFDLLLAAYLLNPAQDAGDIAAVAKMKQYEAVRSDAEVYKGVKRS LPDEQTLAEHLVRKAAAIWALEQP FMDDLRRNNEQQDQLLTKLEQPLAAI LAEMEFTGVNVDTKRLEBQMGSELAELRAIEQRI YEHAGQEFNINSPKQLGVI LFEKQLQPLVLLKTKTGYST SADVLEKLAHPHE IVENILHYRQLGKLQSTYI EGLLKVVRPDTGKVHTMNFQTLTQTG RLSSAEPNLQNIPIRLEBGRKIRQAFVPSPEPDL IFAADYSQIELRVLAHI ADDDNLI EAPQRDLDIHTKTAMDIFH VSEEEVTANMRRQAKAVNFGI VYGISDYGLAQNLI TRKEAAEFIER YFASFPQVRRY MENI VQEAQKQGVV TLLH RRRYL PDI TSNRNFVRS FAERTAMNTP IQGSAADI I KKAMIDLAAARLKEEQQLARLLQVHDELILEAPKEIERLC ELVPEVMEQAVSSVPLKVDYHYGPTWYDAK</p> <p><i>Bacillus stearothermophilus</i> with Clone 15 mutations in corresponding positions (SEQ ID NO: 44)                      MRLKKLVLIDGNSVAYRAFFAPLPLHNDKGIHTNAVYGFMTMLNKI LAEEQPTLLVAFDAGKTTFRHETPQYKGR QQQTPPELS EQPFLRLRELLKTYRI PAYELYI YEADDI IGTLAARAEQEGFEVKI I SGDRDLTQLASRHVTVDI TTK GITDIEPYTPETVREKYGLTPEQIVDLKGLMGDKSDNIPGVPGIGEKTAVKLLKQFGTVENVLASIDEVKGEKVKEL LRQHRDLALLSKQLASI CRDAPVELSLDALVYEGDREKVI ALFKELGFSFLEKMAAPAEGRKPLEEMEF AIVDV I TEENLADKALLVVMEENYHDAP IVGIALVNEHGRFFMRPETALADSQFLAWLADETKKKSMPDAKRAVVALKWK GIDVRGVAFDLLLAAYLLNPAQDAGDIAAVAKMKQYEAVRSDAEVYKGVKRS LPDEQTLAEHLVRKAAAIWALEQP FMDDLRRNNEQQDQLLTKLEQPLAAI LAEMEFTGVNVDTKRLEBQMGSELAELRAIEQRI YEHAGQEFNINSPKQLGVI LFEKQLQPLVLLKTKTGYST SADVLEKLAHPHE IVENILHYRQLGKLQSTYI EGLLKVVRPDTGKVHTMNFQTLTQTG RLSSAEPNLQNIPIRLEBGRKIRQAFVPSPEPDL IFAADYSQIELRVLAHI ADDDNLI EAPQRDLDIHTKTAMDIFH VSEEEVTANMRRQAKAVNFGI VYGISDYGLAQNLI TRKEAAEFIER YFASFPQVRRY MENI VQEAQKQGVV TLLH RRRYL PDI TSNRNFVRS FAERTAMNTP IQGSAADI I KKAMIDLAAARLKEEQQLARLLQVHDELILEAPKEIERLC ELVPEVMEQAVSSVPLKVDYHYGPTWYDAK</p> <p>&gt;gi 307233423 ref ZP_07519834.1  DNA polymerase I [<i>Escherichia coli</i> TA143] (SEQ ID NO: 45)                      MVQIPQNPLI LVDGSSYLYRAYHAPPLTNSAGEPTGAMYGVNMLRSLIMQYKPTHAAVVFDAKGGKTRDELFEHY KSHRPPMPDDLRAQIEPLHAMVKAMGLPLLAVSGVEADDVIGTLAREAEKAGRPVLI STGDKDMAQLVTPNITLINT MTNTILGPEEVVKNYGVPELIIIDFLALMGDSSDNI PGVPGVGEKTAQALLQGLGGLD TL YAEPKIAGLSFRGAKT MAAKLEQNKVAYLSYQLATI KTDVLELTC EQLEVQQPAABEELGLPKYEFKRWTADVEAGKWLQAKGAKPAKP QETSVADEAPEVATVVI SYDNVVTI LDEETLKAWIAKLEKAPVFADF TETDSL DNI SANLVGLSFAIEPGVAAYI PV AHYLDADPQISRERALELLKPLLEDEKALKVGNLKYDRGILANYGIELRGIAFDTMLESYILNSVAGRHDMSLA ERWLKHTITPEEIIAGKGNQLTFNQIAL EEAAGR YAAEDADVTLQLHLKMPD LQKHGKLN VFNENI EMLVVPVLSR IERNGVKIDPKVLHNHSEBLT LRLALEKKAHEI AGEFNLSSTKQLQTLIFEKQGIKPLKKTTPGAPSTS BEVLEE</p>

TABLE 26-continued

Amino acid sequences of wildtype and modified DNA polymerases	
LALDYPLPKVILEYRGLAKLKSTYTDKLPMLNPKTGRVHTSYHQAVTATGRLSSTDPNLQNI PVRNEEGRRIRQAF IAPEDYVIVSADYSQIELRIMAHLSRDKGLLTAF AEGKDIHRATAAEVFG LPLETVTSEQRRS AKAINFGLIYGMSA FGLARQLNI PRKEAQKYMDLY FERYPGVLQYMER TRAQAKEQGYVETLDGRRLYLPDI KSSNGARRAAERAAINAP MQGTAADI IKRAMI AVDAWLQAEQPRVRMIQVHDEL VFEVHKDDVDAVAKQIHQLMENC TRLDVPLLVEVSGENW DQAH	
<i>Escherichia coli</i> with Clone 15 mutations in corresponding positions (SEQ ID NO: 46)	
MVQIQPNPLI LDVGDSSYLYRAYHAFPLTNSAGEPTGAMYGVNMLRSLIMQYKPTTAAVVFDAKGGKTRFDELFEHY KSHRPPMPDDLRAQIEPLHAMVKAMGLPLLAVSGVEADDVIGTLAREAEKAGRPVLI STGDKDMAQLVTPNITLINT MTNTILGPEEVNKYGVPP ELIIDFLALMGDS SDNIPGVPGVGEKTAQALLQGLGGLD TLYAEPEKIAGLSFRGAKT MAAKLEQNKEVAYLSYQLATI KTDVLELTC EQLEVQQPAEELLGLPKKYEFKRWTADV EAGKWLQAKGAKPAKP QETSVADEAPEVTATVISYDNYVTI LDEETLKWIAKLEKAPVFAFD TETDSDLNI SANLVGLSFAIEPGVAAYIPV AHDYLDAPDQISRERALEL LKPLLEDEKCLKVGNLKYDRGILANYGIELRGIAFD TMLSEYI LNSVAGRHDMSLA ERWLKHKTIITFE EIA GKGKNQ LTFNQI ALEEAGR YAAEDADVTLQLHLKMWPD LQKHKGPLNVFENI EMPLVPLSR IERNGVKIDPKVLHNHSEBELT LRLAELEKKAHEIAGEEFNLSSTKQLQTI LFEKQGIKPLKKT KGGAPSTSEEVLEE LALDYPLPKVILEYRGLAKLKSTYTDKLPMLNPKTGRVHTSYHQAVTATGRLSSTDPNLQNI PVRNEEGRRIRQAF IAPEDYVIVSADYSQIELRIMAHLSRDKGLLTAF AEGKDIHRATAAEVFG LPLETVTSEQRRS AKAINFGLIYGMSA FGLARQLNI PRKEAQKYMDLY FERYPGVLQYMER TRAQAKEQGYVETLDGRRLYLPDI KSSNGARRAAERAAINAP MQGTAADI IKRAMI AVDAWLQAEQPRVRMIQVHDEL VFEVHKDDVDAVAKQIHQLMENC TRLDVPLLVEVSGENW DQAH	
>Clone 15 (SEQ ID NO: 47)	
MRGMLPLFEPKGRVLLVDGHHLAYRTFHALKGLTTSRGEVPQAVYGF AKSLLKALKEDGDTVI VVFDKAP SFRHEA YGGYKAGRAPT PEDFPRQLALIKELVDLLGLARLEVPGYEADDV LASLAKKAEKEGYEVRILTADKDYQLLSDRIH VLNHP EGYLITPAWLWEKYGLR PDQWADYRALTGDES DNLPGVKGI GEKTARKLLEEWGSLEALLKNLDR LKPAIR EK I LAHMDDLKLSWDLAKVRTD LPLEVDFAKRREPDRERLRAFLE RLEFGSLLHEFG LLES PKALEEAPWPPPEGAFVG FVLSRKEPMWADLLALAAARGGRVHRAPEPYKALRDLEBARGLLAKDLCV LALREGLGLPPGDDPMLLAYLLDPSNT TPEGVARRYGGEWTEEAGERAALSERL PANLWGRLEGEERLLWLYREVERPLSAVLAHMEATGVRLDVAYLRALSLE VAEEIARLEAEVFR LAGHPFNLSRDQLERVLFDELGLPAIGTKTKTKRSTSAAVLEALREAHPIVEKILQYRELT KLKSTYIDPLPDLIHPRTGRHLTRFNQTATATGR LSSSDPNLQNI PVRTPLGQRIRRAFI AEEGWLLVALDYSQIEL RVL AHS G DENLIRV FQEGRD IHTETASWMPGVPREAVDPLMRRAAKTINFGVLYGMSAHRLSQELAI P YEEAAQAFI ERYFQSPKVRAWMEK TLEEGRRRGYVETLFGRRRYVPDLEARVKS VREAAERMAINMPVQGTAA DLMK LAMVKLFP RLEEMGARMLLQVHDELVLEAPKERAEAVARLAK EVM EGVYPLAVPLEVEVGI GEDWLSAKE*	
>Clone 15 (F749L) (SEQ ID NO: 48)	
MRGMLPLFEPKGRVLLVDGHHLAYRTFHALKGLTTSRGEVPQAVYGF AKSLLKALKEDGDTVI VVFDKAP SFRHEA YGGYKAGRAPT PEDFPRQLALIKELVDLLGLARLEVPGYEADDV LASLAKKAEKEGYEVRILTADKDYQLLSDRIH VLNHP EGYLITPAWLWEKYGLR PDQWADYRALTGDES DNLPGVKGI GEKTARKLLEEWGSLEALLKNLDR LKPAIR EK I LAHMDDLKLSWDLAKVRTD LPLEVDFAKRREPDRERLRAFLE RLEFGSLLHEFG LLES PKALEEAPWPPPEGAFVG FVLSRKEPMWADLLALAAARGGRVHRAPEPYKALRDLEBARGLLAKDLCV LALREGLGLPPGDDPMLLAYLLDPSNT TPEGVARRYGGEWTEEAGERAALSERL PANLWGRLEGEERLLWLYREVERPLSAVLAHMEATGVRLDVAYLRALSLE VAEEIARLEAEVFR LAGHPFNLSRDQLERVLFDELGLPAIGTKTKTKRSTSAAVLEALREAHPIVEKILQYRELT KLKSTYIDPLPDLIHPRTGRHLTRFNQTATATGR LSSSDPNLQNI PVRTPLGQRIRRAFI AEEGWLLVALDYSQIEL RVL AHS G DENLIRV FQEGRD IHTETASWMPGVPREAVDPLMRRAAKTINFGVLYGMSAHRLSQELAI P YEEAAQAFI ERYFQSPKVRAWMEK TLEEGRRRGYVETLFGRRRYVPDLEARVKS VREAAERMAINMPVQGTAA DLMK LAMVKLFP RLEEMGARMLLQVHDELVLEAPKERAEAVARLAK EVM EGVYPLAVPLEVEVGI GEDWLSAKE	

TABLE 27

Exemplary alignments of DNA polymerase amino acid sequences	
<i>Thermus aquaticus/Thermus thermophilus</i> alignment	
1	MRGMLPLFEPKGRVLLVDGHHLAYRTFHALKGLTTSRGEVPQAVYGF AKSLLKALKEDG-
1	MEAMLPLFEPKGRVLLVDGHHLAYRTFFALKGLTTSRGEVPQAVYGF AKSLLKALKEDGY
60	DAVIVVFDKAP SFRHEAYGGYKAGRAPT PEDFPRQLALIKELVDLLGLARLEVPGYEAD
61	KAVFVVFDAKAP SFRHEAYEAYKAGRAPT PEDFPRQLALIKELVDLLGFTRLEVPGYEAD
120	DVLSLAKKAEKEGYEVRILTADKDYQLLSDRIHVLNHP EGYLITPAWLWEKYGLR PDQW
121	DVLATLAKKAEKEGYEVRILTADRDL YQLVSDRVAVLNHP EGH LITPEWLWEKYGLRPEQW
180	ADYRALTGDES DNLPGVKGI GEKTARKLLEEWGSLEALLKNLDR LK P - AIREKILAHMDD
181	VDFRALVGDPSDNLPGVKGI GEKTALKLLKEWGSLENLKNLDRVKPENVREKIKAHLED
239	LKLSWDLAKVRTD LPLEVDFAKRREPDRERLRAFLE RLEFGSLLHEFG LLES PKALEEAP
241	LRLSLELSRVRTD LPLEVDLAQGREPDREGLRAFLE RLEFGSLLHEFG LLEAPALEEAP
299	WPPPEGAFVGFVLSRKEPMWADLLALAAARGGRVHRAPEPYKALRD LK EARGLLAKDLSV
301	WPPPEGAFVGFVLSRPEPMWAE LKALAACRDGRVHRAADPLAGKDLKEVRGLLAKDLAV
359	LALREGLGLPPGDDPMLLAYLLDPSNT TPEGVARRYGGEWTEEAGERAALSERL PANLW
361	LASREGLDLVPGDDPMLLAYLLDPSNT TPEGVARRYGGEWTEEAHRALLSERLHRNLK
419	RLEGEERLLWLYREVERPLSAVLAHMEATGVRLDVAYLRALSLEVAEEIARLEAEVFR LA
421	RLEGEERLLWLYHEVEKPLSRVLAHMEATGVRLDVAYLQALSLELAEEIRRLEEEVFR LA
479	GHPFNLSRDQLERVLFDELGLPAIGKTEKTGKRSTSAAVLEALREAHPIVEKILQYREL
481	GHPFNLSRDQLERVLFDELRLPALGKTQKTGKRSTSAAVLEALREAHPIVEKILQHREL

TABLE 27-continued

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Exemplary alignments of DNA polymerase amino acid sequences

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539 TKLKSTYIDPLPDLIHPRTGRLHTRFNQTATATGRLSSSDPNLQNI PVRTPLGQRIIRRAF  
 541 TKLKNTYVDPLPSLVHPRTRGLHTRFNQTATATGRLSSSDPNLQNI PVRTPLGQRIIRRAF  
 599 IAEAGWLLVALDYSQI ELRVLVAHLSGDENLIRVFQEGRDIHTETASWFMFGVPREAVDPLM  
 601 VAEAGWALVALDYSQI ELRVLVAHLSGDENLIRVFQEGKDIHTQASWFMFGVPEAVDPLM  
 659 RRAAKTINFGVLYGMSAHRLSQELAI PYEEAQAFI ERYFQSF PKVRAWI EKTLEEGRRRG  
 661 RRAAKTVNFGVLYGMSAHRLSQELAI PYEEAVAFI ERYFQSF PKVRAWI EKTLEEGRRRG  
 719 YVETLFGRRRYVPDLEARVKS VREAERMAFNMPVQGTAAADLMKLMAMV KLFPRLEEMGAR  
 721 YVETLFGRRRYVPDLNARVKS VREAERMAFNMPVQGTAAADLMKLMAMV KLFPRLEEMGAR  
 779 MLLQVHDELVEAPKERAEAVARLAKEVM EGVYPLAVPLEVEVIGEDWLSAKE (SEQ ID NO: 49)  
 781 MLLQVHDELLEAPQARABEVAALAKEAMEKAYPLAVPLEVEVIGEDWLSAKG (SEQ ID NO: 50)

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*Thermus aquaticus/Bacillus stearothermophilus* alignment

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1 MRGMLPLPEFKGRVLLVDGHHLAYRTFHALKGLTTSRGEVPQAVYGFSAKSLKALKEDG-  
 1 MR-----LKKKLVLDIGNSVAYRAFFALPLLHNDKGIHTNAVYFTMMLNKLILAEEQP  
 60 DAVIVVFDKAPKAPSRHEAYGGYKAGRAPT PEDFPRLQALI KELVDLLGLARLEVPGYEAD  
 54 THLLVAFDAGKTFRHETFQYKGGRQQTPELSEQFP LLRELLKTYRIPAYELYIY EAD  
 120 DVLASLAKKAEKEGYEVRI LTADKDYQLLSDR IHVLPHEG-----YLI TPAWLWEKYGL  
 114 DIIGTLAARAEQEGFEVKI I SGDRDLTQLASRHVTVDI TKKGI TDI EPYTPETVREKYGL  
 175 RPDQWADYRALTGDESNDLPGVKIG EKTARKLLEEWGSL EALLKNLDR LKP-AIREKIL  
 174 TPEQIVDLKGLMGDKSDNIPGVPGI GEKTA V KLLKQFGT VENVLAS IDEVKGEKVK EKL R  
 234 AHMDDLKLSWDLAKVRTDLP LEVDFAKRRE--PDRERLRAF LERLEFGSLLHEFG--LLE  
 234 QHRDLALLS KQLAS ICRDAPVELSLDALV YEGQDR EKVIALF KELGFGQS FLEKMAAPAAE  
 290 SPKALEEAPWPPPE-----GAFVGFVLSRKEPMWADLLALAAARG--GRVHRAPEPY  
 294 GRKPLEEMEF AIVDVI TEEMLADKAA LVVEEMEENYHDAP I VGI ALVNEHGRPFMRPETA  
 340 KA-----LRDLKEARGLLAKDLSV LALR-EG LGLP-PGDDPMLL AYLLDPSNTT----  
 354 LADSQFLAWLADETKKKSMF DAKRAVVALKWKGI D V RGVAFD LLLAAYLLNPAQDAGDIA  
 387 PEGVARRYGGEWTEEAG-----ERAALSERLF---ANLWGRLE-----GEERLL  
 414 AVAKMKQYEA VRSD EAVYKGVKRS LPDEQTLAEHLVRKAAA I WALEQFPMDLDRNNEQD  
 428 WLRYREVERPLSAVLAHMEATGVRLD VAYLRALSLEVAEETARLEAEVFR LAGHPFNLSR  
 474 QLLTKLEQLAAI LAEMEFTGVNVDTKRLEQMGSELAEQ LRAIEQR IYEHAGQEFNINS P  
 488 DQLERVLFDEBLGPAI GKTEKTKRSTSAAVLEALREAHPI VEKILQYRELTKLKSTYID  
 534 KQLGVILFEKLQLPV LKKTGTG--YSTADVLEK LAPHHEI V ENI LHYRQLGKLQSTYIE  
 548 PLPDLIHPRTGRLHTRFNQTATATGRLSSSDPNLQNI PVRTPLGQRIIRRAFI AEEG-WLL  
 592 GLLKVVPRDPTGKVHTM FNQTLTQTGRLSSAEPNLQNI PIRLEEGR KIRQAFVPS EPDWLI  
 607 VALDYSQI ELRVLVAHLSGDENLIRVFQEGRDIHTETASWFMFGVPREAVDPLM RRAAKTIN  
 652 FAADYSQI ELRVLVAH IADDDNLI EAFQRDLDIHTKTAMD I FHVSEEV TANMRRQAKAVN  
 667 FGVLYGMSAHRLSQELAI PYEEAQAFI ERYFQSF PKVRAWI EKTLEEGRRRGYVETLFG R  
 712 FGI VYGISD YGLAQNLMI TRKEAAEPI ERYFASFP GVRRYM ENI VQ EAKQKGVYTTLLHR  
 727 RRYVPDLEARVKS VREAERMAFNMPVQGTAAADLMKLMAMV KLFPR L--EEMGARMLLQVH  
 772 RRYLPDI TSNRNFI VRSFAERTAMNTPI QGSAADI I KKAMIDLAA RLKEEQ LQARLLQVH  
 785 DELVLEAPKERAEAVARLAKEVM EGVYPLAVPLEVEVIGEDWLSAKE (SEQ ID NO: 51)  
 832 DELILEAPKEEIERLCELVEVMEQAVS-SVPLKVDYHYGPTWYDAK- (SEQ ID NO: 52)

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*Thermus aquaticus/Escherichia coli* alignment

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1 MRGMLPLPEFKGRVLLVDGHHLAYRTFHALKGLTTSRGEVPQAVYGFSAKSLKALKEDG-  
 1 ----MVQIPQNP LILVDGSSYLYRAYHAFPPLTNSAGEPTGAMYGV LNMRLS LIMOYKP  
 60 DAVIVVFDKAPKAPSRHEAYGGYKAGRAPT PEDFPRLQALI KELVDLLGLARLEVPGYEAD  
 56 THAAVVFDAKGTFRDELFEHYKSHRPPMPDDLRAQI EPLHAMVKAMGLPLLAVS GVEAD  
 120 DVLASLAKKAEKEGYEVRI LTADKDYQLLSDR IHVLPHEG-YLITPAWLWEKYGLRDPDQ  
 116 DVIGTLAREAEKAGRPVLISTGDKDMAQLVTPNITLINTMTNT I LGPEEVVNKYGVPPPEL  
 179 WADYRALTGDESNDLPGVKIG EKTARKLLEEWGSL EALLKNLDR LK-----PAIREK  
 176 I IDFLALMGDSSDNIPGVPGVGEKTAQALLQGLGGDLTLYAEPEKIAGLSFRGAKTMAAK  
 232 I LAHMDDLKLSWDLAKVRTDLP LEVDFAK--RREPRERLRAF LERLEFG-----S  
 236 LEQNKEVAYLSYQLATI KTDVELELTC EQLEVQQAEEELLGLFKKYEPRKRW TADVEAGK  
 281 LLHEFGLLLESPK-----ALEEAPWPPP-----EGAFVGFVLSRKEP-----  
 296 WLQAKGAKPAKQPQET SVADEAPEVTATV I SYDNYVTI LDEETLKAWI AKLEKAPVFAPD  
 316 -----MWADLLALAAARGGRVHR-----APEPKALRDLKEARGLLAKDLSV  
 356 TETDSLDNI SANLVGLSFAI EPGVAAYI PVAHDYLDAPDQISRRERALELLKPLLEDEKAL  
 359 LA---LREGLGLPPGD-----DPMLLAYLLDPSN-----TTPEG  
 416 KVQGNLKYDRGI LANYGI ELRGI AFDTMLESYI LNSVAGRHDMS LAERWLKHKTITFEE  
 390 VARR-----YGGEWTEEAG---ERAALSERLFANLWGRLEGEERLLWLYREVERPLSA  
 476 IAGKGNQLTFNQI ALEEAGRYAAEDADVTLQLHLKMWDP LQKHGKPLNVFENIEMPLVP  
 440 VLAHMEATGVRLDVA YLRALSLEVAEETARLEAEVFR LAGHPFNLSRDQ LERVLDELG  
 536 VLSRIERNGVKIDPKVLHNNSEELTLRLAEL EKKAH E IAGEEFNLSSTKQLQTLILFEKQG  
 500 LPAIGKTEKTKRSTSAAVLEALREAHPI VEKILQYRELTKLKSTYIDPLPDLIHPRTG R  
 596 IKPLKKT PG-GAPTS EEVLEELALDYPLPKVI LEYRGLAKL KSTYTDKLP LMINPKTGR  
 560 LHIRFNQTATATGRLSSSDPNLQNI PVRTPLGQRIIRRAFI AEEGWLLVALDYSQI ELRVL  
 655 VHSYHQAVTATGRLSSIDPNLQNI PVRNEEGRRIRQAFI APEDYVIVSADYSQI ELRIM  
 620 AHLSGDENLIRVFQEGRDIHTETASWFMFGVPREAVDPLM RRAAKTINFGVLYGMSAHRLS  
 715 AHLSRDKGLLTAF AEGKDIHRATAAEVFG LPLETVI SEQRRSAKAINFG LIYGMSAFGLA  
 680 QELAI PYEEAQAFI ERYFQSF PKVRAWI EKTLEEGRRRGYVETLFGRRRYVPDLEARVKS  
 775 RQLNI PRKEAQKMDLYFERYPGVLYMERTRAQAK EGGYVETLDGRRLLYLPDI KSSNGA  
 740 VREAERMAFNMPVQGTAAADLMKLMAMV KLFPRLEEMG--ARMLLQVHDELVEAPKERAE

TABLE 27-continued

Exemplary alignments of DNA polymerase amino acid sequences	
835	RRAAAERAAINAPMQGTAADIIKRAMIAVDAWLQAEQPRVRMIMQVHDELVFEVHKDDVD
798	AVARLAKEVMEGVYPLAVPLEVEVGIGEDWLSAKE (SEQ ID NO: 53)
895	AVAKQIHQLMENCNTLRDVLPLLEVEVGSGENWDQAH- (SEQ ID NO: 54)

## EQUIVALENTS

**[0153]** Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the appended claims. The articles “a”, “an”, and “the” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to include the plural referents. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where elements are presented as lists, e.g., in Markush group or similar format, it is to be understood that each subgroup of the elements is also

disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc. For purposes of simplicity those embodiments have not in every case been specifically set forth herein. It should also be understood that any embodiment of the invention, e.g., any embodiment found within the prior art, can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification.

**[0154]** It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one act, the order of the acts of the method is not necessarily limited to the order in which the acts of the method are recited, but the invention includes embodiments in which the order is so limited. Furthermore, where the claims recite a composition, the invention encompasses methods of using the composition and methods of making the composition. Where the claims recite a composition, it should be understood that the invention encompasses methods of using the composition and methods of making the composition.

## INCORPORATION OF REFERENCES

**[0155]** All publications and patent documents cited in this application are incorporated by reference in their entirety to the same extent as if the contents of each individual publication or patent document were incorporated herein. All sequence information associated with sequence accession numbers publically available as of the filing date of the present application is hereby incorporated by reference.

## SEQUENCE LISTING

```

<160> NUMBER OF SEQ ID NOS: 54

<210> SEQ ID NO 1
<211> LENGTH: 832
<212> TYPE: PRT
<213> ORGANISM: Thermus aquaticus

<400> SEQUENCE: 1

Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
1                5                10                15

Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly
20                25                30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
35                40                45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val
50                55                60

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly
65                70                75                80

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Tyr	Lys	Ala	Gly	Arg	Ala	Pro	Thr	Pro	Glu	Asp	Phe	Pro	Arg	Gln	Leu
				85					90					95	
Ala	Leu	Ile	Lys	Glu	Leu	Val	Asp	Leu	Leu	Gly	Leu	Ala	Arg	Leu	Glu
			100					105					110		
Val	Pro	Gly	Tyr	Glu	Ala	Asp	Asp	Val	Leu	Ala	Ser	Leu	Ala	Lys	Lys
		115					120					125			
Ala	Glu	Lys	Glu	Gly	Tyr	Glu	Val	Arg	Ile	Leu	Thr	Ala	Asp	Lys	Asp
	130					135					140				
Leu	Tyr	Gln	Leu	Leu	Ser	Asp	Arg	Ile	His	Val	Leu	His	Pro	Glu	Gly
145					150					155					160
Tyr	Leu	Ile	Thr	Pro	Ala	Trp	Leu	Trp	Glu	Lys	Tyr	Gly	Leu	Arg	Pro
				165					170					175	
Asp	Gln	Trp	Ala	Asp	Tyr	Arg	Ala	Leu	Thr	Gly	Asp	Glu	Ser	Asp	Asn
			180					185					190		
Leu	Pro	Gly	Val	Lys	Gly	Ile	Gly	Glu	Lys	Thr	Ala	Arg	Lys	Leu	Leu
		195					200					205			
Glu	Glu	Trp	Gly	Ser	Leu	Glu	Ala	Leu	Leu	Lys	Asn	Leu	Asp	Arg	Leu
	210					215					220				
Lys	Pro	Ala	Ile	Arg	Glu	Lys	Ile	Leu	Ala	His	Met	Asp	Asp	Leu	Lys
225				230						235					240
Leu	Ser	Trp	Asp	Leu	Ala	Lys	Val	Arg	Thr	Asp	Leu	Pro	Leu	Glu	Val
				245					250					255	
Asp	Phe	Ala	Lys	Arg	Arg	Glu	Pro	Asp	Arg	Glu	Arg	Leu	Arg	Ala	Phe
			260					265					270		
Leu	Glu	Arg	Leu	Glu	Phe	Gly	Ser	Leu	Leu	His	Glu	Phe	Gly	Leu	Leu
		275					280					285			
Glu	Ser	Pro	Lys	Ala	Leu	Glu	Glu	Ala	Pro	Trp	Pro	Pro	Pro	Glu	Gly
		290				295					300				
Ala	Phe	Val	Gly	Phe	Val	Leu	Ser	Arg	Lys	Glu	Pro	Met	Trp	Ala	Asp
305					310					315					320
Leu	Leu	Ala	Leu	Ala	Ala	Ala	Arg	Gly	Gly	Arg	Val	His	Arg	Ala	Pro
				325					330					335	
Glu	Pro	Tyr	Lys	Ala	Leu	Arg	Asp	Leu	Lys	Glu	Ala	Arg	Gly	Leu	Leu
			340					345					350		
Ala	Lys	Asp	Leu	Ser	Val	Leu	Ala	Leu	Arg	Glu	Gly	Leu	Gly	Leu	Pro
		355					360					365			
Pro	Gly	Asp	Asp	Pro	Met	Leu	Leu	Ala	Tyr	Leu	Leu	Asp	Pro	Ser	Asn
	370					375						380			
Thr	Thr	Pro	Glu	Gly	Val	Ala	Arg	Arg	Tyr	Gly	Gly	Glu	Trp	Thr	Glu
385					390					395					400
Glu	Ala	Gly	Glu	Arg	Ala	Ala	Leu	Ser	Glu	Arg	Leu	Phe	Ala	Asn	Leu
				405					410					415	
Trp	Gly	Arg	Leu	Glu	Gly	Glu	Glu	Arg	Leu	Leu	Trp	Leu	Tyr	Arg	Glu
			420					425					430		
Val	Glu	Arg	Pro	Leu	Ser	Ala	Val	Leu	Ala	His	Met	Glu	Ala	Thr	Gly
			435				440					445			
Val	Arg	Leu	Asp	Val	Ala	Tyr	Leu	Arg	Ala	Leu	Ser	Leu	Glu	Val	Ala
	450					455					460				
Glu	Glu	Ile	Ala	Arg	Leu	Glu	Ala	Glu	Val	Phe	Arg	Leu	Ala	Gly	His
465				470						475					480
Pro	Phe	Asn	Leu	Asn	Ser	Arg	Asp	Gln	Leu	Glu	Arg	Val	Leu	Phe	Asp

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485					490					495					
Glu	Leu	Gly	Leu	Pro	Ala	Ile	Gly	Lys	Thr	Glu	Lys	Thr	Gly	Lys	Arg
			500					505					510		
Ser	Thr	Ser	Ala	Ala	Val	Leu	Glu	Ala	Leu	Arg	Glu	Ala	His	Pro	Ile
			515					520					525		
Val	Glu	Lys	Ile	Leu	Gln	Tyr	Arg	Glu	Leu	Thr	Lys	Leu	Lys	Ser	Thr
			530					535					540		
Tyr	Ile	Asp	Pro	Leu	Pro	Asp	Leu	Ile	His	Pro	Arg	Thr	Gly	Arg	Leu
			545					550					555		
His	Thr	Arg	Phe	Asn	Gln	Thr	Ala	Thr	Ala	Thr	Gly	Arg	Leu	Ser	Ser
				565					570					575	
Ser	Asp	Pro	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Thr	Pro	Leu	Gly	Gln
			580					585						590	
Arg	Ile	Arg	Arg	Ala	Phe	Ile	Ala	Glu	Glu	Gly	Trp	Leu	Leu	Val	Ala
			595					600						605	
Leu	Asp	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala	His	Leu	Ser	Gly
			610					615						620	
Asp	Glu	Asn	Leu	Ile	Arg	Val	Phe	Gln	Glu	Gly	Arg	Asp	Ile	His	Thr
			625					630						635	
Glu	Thr	Ala	Ser	Trp	Met	Phe	Gly	Val	Pro	Arg	Glu	Ala	Val	Asp	Pro
				645					650					655	
Leu	Met	Arg	Arg	Ala	Ala	Lys	Thr	Ile	Asn	Phe	Gly	Val	Leu	Tyr	Gly
			660					665						670	
Met	Ser	Ala	His	Arg	Leu	Ser	Gln	Glu	Leu	Ala	Ile	Pro	Tyr	Glu	Glu
			675					680						685	
Ala	Gln	Ala	Phe	Ile	Glu	Arg	Tyr	Phe	Gln	Ser	Phe	Pro	Lys	Val	Arg
			690					695						700	
Ala	Trp	Ile	Glu	Lys	Thr	Leu	Glu	Glu	Gly	Arg	Arg	Arg	Gly	Tyr	Val
			705					710						715	
Glu	Thr	Leu	Phe	Gly	Arg	Arg	Arg	Tyr	Val	Pro	Asp	Leu	Glu	Ala	Arg
				725					730					735	
Val	Lys	Ser	Val	Arg	Glu	Ala	Ala	Glu	Arg	Met	Ala	Phe	Asn	Met	Pro
			740					745						750	
Val	Gln	Gly	Thr	Ala	Ala	Asp	Leu	Met	Lys	Leu	Ala	Met	Val	Lys	Leu
			755					760						765	
Phe	Pro	Arg	Leu	Glu	Glu	Met	Gly	Ala	Arg	Met	Leu	Leu	Gln	Val	His
			770					775						780	
Asp	Glu	Leu	Val	Leu	Glu	Ala	Pro	Lys	Glu	Arg	Ala	Glu	Ala	Val	Ala
			785					790						795	
Arg	Leu	Ala	Lys	Glu	Val	Met	Glu	Gly	Val	Tyr	Pro	Leu	Ala	Val	Pro
				805					810					815	
Leu	Glu	Val	Glu	Val	Gly	Ile	Gly	Glu	Asp	Trp	Leu	Ser	Ala	Lys	Glu
				820					825					830	

&lt;210&gt; SEQ ID NO 2

&lt;211&gt; LENGTH: 834

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Thermus thermophilus HB8

&lt;400&gt; SEQUENCE: 2

Met	Glu	Ala	Met	Leu	Pro	Leu	Phe	Glu	Pro	Lys	Gly	Arg	Val	Leu	Leu
1				5						10				15	

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Val Asp Gly His His Leu Ala Tyr Arg Thr Phe Phe Ala Leu Lys Gly  
                   20                          25                          30  
 Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala  
                   35                          40                          45  
 Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Tyr Lys Ala Val Phe  
                   50                          55                          60  
 Val Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Glu  
                   65                          70                          75                          80  
 Ala Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln  
                   85                          90                          95  
 Leu Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Phe Thr Arg Leu  
                   100                          105                          110  
 Glu Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Thr Leu Ala Lys  
                   115                          120                          125  
 Lys Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Arg  
                   130                          135                          140  
 Asp Leu Tyr Gln Leu Val Ser Asp Arg Val Ala Val Leu His Pro Glu  
                   145                          150                          155                          160  
 Gly His Leu Ile Thr Pro Glu Trp Leu Trp Glu Lys Tyr Gly Leu Arg  
                   165                          170                          175  
 Pro Glu Gln Trp Val Asp Phe Arg Ala Leu Val Gly Asp Pro Ser Asp  
                   180                          185                          190  
 Asn Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Leu Lys Leu  
                   195                          200                          205  
 Leu Lys Glu Trp Gly Ser Leu Glu Asn Leu Leu Lys Asn Leu Asp Arg  
                   210                          215                          220  
 Val Lys Pro Glu Asn Val Arg Glu Lys Ile Lys Ala His Leu Glu Asp  
                   225                          230                          235                          240  
 Leu Arg Leu Ser Leu Glu Leu Ser Arg Val Arg Thr Asp Leu Pro Leu  
                   245                          250                          255  
 Glu Val Asp Leu Ala Gln Gly Arg Glu Pro Asp Arg Glu Gly Leu Arg  
                   260                          265                          270  
 Ala Phe Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly  
                   275                          280                          285  
 Leu Leu Glu Ala Pro Ala Pro Leu Glu Glu Ala Pro Trp Pro Pro Pro  
                   290                          295                          300  
 Glu Gly Ala Phe Val Gly Phe Val Leu Ser Arg Pro Glu Pro Met Trp  
                   305                          310                          315                          320  
 Ala Glu Leu Lys Ala Leu Ala Ala Cys Arg Asp Gly Arg Val His Arg  
                   325                          330                          335  
 Ala Ala Asp Pro Leu Ala Gly Leu Lys Asp Leu Lys Glu Val Arg Gly  
                   340                          345                          350  
 Leu Leu Ala Lys Asp Leu Ala Val Leu Ala Ser Arg Glu Gly Leu Asp  
                   355                          360                          365  
 Leu Val Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro  
                   370                          375                          380  
 Ser Asn Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp  
                   385                          390                          395                          400  
 Thr Glu Asp Ala Ala His Arg Ala Leu Leu Ser Glu Arg Leu His Arg  
                   405                          410                          415  
 Asn Leu Leu Lys Arg Leu Glu Gly Glu Glu Lys Leu Leu Trp Leu Tyr

-continued

420					425					430					
His	Glu	Val	Glu	Lys	Pro	Leu	Ser	Arg	Val	Leu	Ala	His	Met	Glu	Ala
	435						440					445			
Thr	Gly	Val	Arg	Leu	Asp	Val	Ala	Tyr	Leu	Gln	Ala	Leu	Ser	Leu	Glu
	450					455					460				
Leu	Ala	Glu	Glu	Ile	Arg	Arg	Leu	Glu	Glu	Glu	Val	Phe	Arg	Leu	Ala
465						470					475				480
Gly	His	Pro	Phe	Asn	Leu	Asn	Ser	Arg	Asp	Gln	Leu	Glu	Arg	Val	Leu
				485					490					495	
Phe	Asp	Glu	Leu	Arg	Leu	Pro	Ala	Leu	Gly	Lys	Thr	Gln	Lys	Thr	Gly
		500						505					510		
Lys	Arg	Ser	Thr	Ser	Ala	Ala	Val	Leu	Glu	Ala	Leu	Arg	Glu	Ala	His
		515					520						525		
Pro	Ile	Val	Glu	Lys	Ile	Leu	Gln	His	Arg	Glu	Leu	Thr	Lys	Leu	Lys
	530					535					540				
Asn	Thr	Tyr	Val	Asp	Pro	Leu	Pro	Ser	Leu	Val	His	Pro	Arg	Thr	Gly
545						550					555				560
Arg	Leu	His	Thr	Arg	Phe	Asn	Gln	Thr	Ala	Thr	Ala	Thr	Gly	Arg	Leu
				565					570					575	
Ser	Ser	Ser	Asp	Pro	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Thr	Pro	Leu
			580					585					590		
Gly	Gln	Arg	Ile	Arg	Arg	Ala	Phe	Val	Ala	Glu	Ala	Gly	Trp	Ala	Leu
		595					600						605		
Val	Ala	Leu	Asp	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala	His	Leu
	610					615					620				
Ser	Gly	Asp	Glu	Asn	Leu	Ile	Arg	Val	Phe	Gln	Glu	Gly	Lys	Asp	Ile
625				630							635				640
His	Thr	Gln	Thr	Ala	Ser	Trp	Met	Phe	Gly	Val	Pro	Pro	Glu	Ala	Val
				645					650					655	
Asp	Pro	Leu	Met	Arg	Arg	Ala	Ala	Lys	Thr	Val	Asn	Phe	Gly	Val	Leu
			660					665					670		
Tyr	Gly	Met	Ser	Ala	His	Arg	Leu	Ser	Gln	Glu	Leu	Ala	Ile	Pro	Tyr
		675					680						685		
Glu	Glu	Ala	Val	Ala	Phe	Ile	Glu	Arg	Tyr	Phe	Gln	Ser	Phe	Pro	Lys
	690					695					700				
Val	Arg	Ala	Trp	Ile	Glu	Lys	Thr	Leu	Glu	Glu	Gly	Arg	Lys	Arg	Gly
	705					710					715				720
Tyr	Val	Glu	Thr	Leu	Phe	Gly	Arg	Arg	Arg	Tyr	Val	Pro	Asp	Leu	Asn
				725					730					735	
Ala	Arg	Val	Lys	Ser	Val	Arg	Glu	Ala	Ala	Glu	Arg	Met	Ala	Phe	Asn
			740					745					750		
Met	Pro	Val	Gln	Gly	Thr	Ala	Ala	Asp	Leu	Met	Lys	Leu	Ala	Met	Val
		755					760						765		
Lys	Leu	Phe	Pro	Arg	Leu	Arg	Glu	Met	Gly	Ala	Arg	Met	Leu	Leu	Gln
	770					775					780				
Val	His	Asp	Glu	Leu	Leu	Leu	Glu	Ala	Pro	Gln	Ala	Arg	Ala	Glu	Glu
	785					790					795				800
Val	Ala	Ala	Leu	Ala	Lys	Glu	Ala	Met	Glu	Lys	Ala	Tyr	Pro	Leu	Ala
				805					810					815	
Val	Pro	Leu	Glu	Val	Glu	Val	Gly	Met	Gly	Glu	Asp	Trp	Leu	Ser	Ala
			820					825					830		



-continued

Lys Gly

&lt;210&gt; SEQ ID NO 3

&lt;211&gt; LENGTH: 834

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Thermus caldophilus

&lt;400&gt; SEQUENCE: 3

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Met Glu Ala Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu
1           5           10           15

Val Asp Gly His His Leu Ala Tyr Arg Thr Phe Phe Ala Leu Lys Gly
20           25           30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala
35           40           45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Tyr Lys Ala Val Phe
50           55           60

Val Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Glu
65           70           75           80

Ala Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln
85           90           95

Leu Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Phe Thr Arg Leu
100          105          110

Glu Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Thr Leu Ala Lys
115          120          125

Asn Pro Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Arg
130          135          140

Asp Leu Asp Gln Leu Val Ser Asp Arg Val Ala Val Leu His Pro Glu
145          150          155          160

Gly His Leu Ile Thr Pro Glu Trp Leu Trp Gln Lys Tyr Gly Leu Lys
165          170          175

Pro Glu Gln Trp Val Asp Phe Arg Ala Leu Val Gly Asp Pro Ser Asp
180          185          190

Asn Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Leu Lys Leu
195          200          205

Leu Lys Glu Trp Gly Ser Leu Glu Asn Leu Leu Lys Asn Leu Asp Arg
210          215          220

Val Lys Pro Glu Asn Val Arg Glu Lys Ile Lys Ala His Leu Glu Asp
225          230          235          240

Leu Arg Leu Ser Leu Glu Leu Ser Arg Val Arg Thr Asp Leu Pro Leu
245          250          255

Glu Val Asp Leu Ala Gln Gly Arg Glu Pro Asp Arg Glu Gly Leu Arg
260          265          270

Ala Phe Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly
275          280          285

Leu Leu Glu Ala Pro Ala Pro Leu Glu Glu Ala Pro Trp Pro Pro Pro
290          295          300

Glu Gly Ala Phe Val Gly Phe Val Leu Ser Arg Pro Glu Pro Met Trp
305          310          315          320

Ala Glu Leu Lys Ala Leu Ala Ala Cys Arg Asp Gly Arg Val His Arg
325          330          335

Ala Ala Asp Pro Leu Ala Gly Leu Lys Asp Leu Lys Glu Val Arg Gly
340          345          350

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Leu Leu Ala Lys Asp Leu Ala Val Leu Ala Ser Arg Glu Gly Leu Asp  
355 360 365

Leu Val Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro  
370 375 380

Ser Asn Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp  
385 390 395 400

Thr Glu Asp Ala Ala His Arg Ala Leu Leu Ser Glu Arg Leu His Arg  
405 410 415

Asn Leu Leu Lys Arg Leu Gln Gly Glu Glu Lys Leu Leu Trp Leu Tyr  
420 425 430

His Glu Val Glu Lys Pro Leu Ser Arg Val Leu Ala His Met Glu Ala  
435 440 445

Thr Gly Val Arg Leu Asp Val Ala Tyr Leu Gln Ala Leu Ser Leu Glu  
450 455 460

Leu Ala Glu Glu Ile Arg Arg Leu Glu Glu Glu Val Phe Arg Leu Ala  
465 470 475 480

Gly His Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu  
485 490 495

Phe Asp Glu Leu Arg Leu Pro Ala Leu Gly Lys Thr Gln Lys Thr Gly  
500 505 510

Lys Arg Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His  
515 520 525

Pro Ile Val Glu Lys Ile Leu Gln His Arg Glu Leu Thr Lys Leu Lys  
530 535 540

Asn Thr Tyr Val Asp Pro Leu Pro Ser Leu Val His Pro Asn Thr Gly  
545 550 555 560

Arg Leu His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu  
565 570 575

Ser Ser Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu  
580 585 590

Gly Gln Arg Ile Arg Arg Ala Phe Val Ala Glu Ala Gly Trp Ala Leu  
595 600 605

Val Ala Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu  
610 615 620

Ser Gly Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Lys Asp Ile  
625 630 635 640

His Thr Gln Thr Ala Ser Trp Met Phe Gly Val Pro Pro Glu Ala Val  
645 650 655

Asp Pro Leu Met Arg Arg Ala Ala Lys Thr Val Asn Phe Gly Val Leu  
660 665 670

Tyr Gly Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr  
675 680 685

Glu Glu Ala Val Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys  
690 695 700

Val Arg Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Lys Arg Gly  
705 710 715 720

Tyr Val Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Asn  
725 730 735

Ala Arg Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn  
740 745 750



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Ala Phe Leu Glu Glu Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly  
275 280 285

Leu Leu Gly Gly Glu Lys Pro Arg Glu Glu Ala Pro Trp Pro Pro Pro  
290 295 300

Glu Gly Ala Phe Val Gly Phe Leu Leu Ser Arg Lys Glu Pro Met Trp  
305 310 315 320

Ala Glu Leu Leu Ala Leu Ala Ala Ala Ser Glu Gly Arg Val His Arg  
325 330 335

Ala Thr Ser Pro Val Glu Ala Leu Ala Asp Leu Lys Glu Ala Arg Gly  
340 345 350

Phe Leu Ala Lys Asp Leu Ala Val Leu Ala Leu Arg Glu Gly Val Ala  
355 360 365

Leu Asp Pro Thr Asp Asp Pro Leu Leu Val Ala Tyr Leu Leu Asp Pro  
370 375 380

Ala Asn Thr His Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Phe  
385 390 395 400

Thr Glu Asp Ala Ala Glu Arg Ala Leu Leu Ser Glu Arg Leu Phe Gln  
405 410 415

Asn Leu Phe Pro Arg Leu Ser Glu Lys Leu Leu Trp Leu Tyr Gln Glu  
420 425 430

Val Glu Arg Pro Leu Ser Arg Val Leu Ala His Met Glu Ala Arg Gly  
435 440 445

Val Arg Leu Asp Val Pro Leu Leu Glu Ala Leu Ser Phe Glu Leu Glu  
450 455 460

Lys Glu Met Glu Arg Leu Glu Gly Glu Val Phe Arg Leu Ala Gly His  
465 470 475 480

Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp  
485 490 495

Glu Leu Gly Leu Thr Pro Val Gly Arg Thr Glu Lys Thr Gly Lys Arg  
500 505 510

Ser Thr Ala Gln Gly Ala Leu Glu Ala Leu Arg Gly Ala His Pro Ile  
515 520 525

Val Glu Leu Ile Leu Gln Tyr Arg Glu Leu Ser Lys Leu Lys Ser Thr  
530 535 540

Tyr Leu Asp Pro Leu Pro Arg Leu Val His Pro Arg Thr Gly Arg Leu  
545 550 555 560

His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser  
565 570 575

Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln  
580 585 590

Arg Ile Arg Lys Ala Phe Val Ala Glu Glu Gly Trp Leu Leu Leu Ala  
595 600 605

Ala Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly  
610 615 620

Asp Glu Asn Leu Lys Arg Val Phe Arg Glu Gly Lys Asp Ile His Thr  
625 630 635 640

Glu Thr Ala Ala Trp Met Phe Gly Leu Asp Pro Ala Leu Val Asp Pro  
645 650 655

Lys Met Arg Arg Ala Ala Lys Thr Val Asn Phe Gly Val Leu Tyr Gly  
660 665 670

Met Ser Ala His Arg Leu Ser Gln Glu Leu Gly Ile Asp Tyr Lys Glu

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675	680	685
Ala Glu Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg		
690	695	700
Ala Trp Ile Glu Arg Thr Leu Glu Glu Gly Arg Thr Arg Gly Tyr Val		
705	710	715
Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Ala Ser Arg		
	725	730
Val Arg Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro		
	740	745
Val Gln Gly Thr Ala Ala Asp Leu Met Lys Ile Ala Met Val Lys Leu		
	755	760
Phe Pro Arg Leu Lys Pro Leu Gly Ala His Leu Leu Leu Gln Val His		
	770	775
Asp Glu Leu Val Leu Glu Val Pro Glu Asp Arg Ala Glu Glu Ala Lys		
	785	790
Ala Leu Val Lys Glu Val Met Glu Asn Ala Tyr Pro Leu Asp Val Pro		
	805	810
Leu Glu Val Glu Val Gly Val Gly Arg Asp Trp Leu Glu Ala Lys Gln		
	820	825
		830

Asp

&lt;210&gt; SEQ ID NO 5

&lt;211&gt; LENGTH: 838

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Thermodesulfovibrio yellowstonii DSM 11347

&lt;400&gt; SEQUENCE: 5

Met His Glu Ile Tyr Leu Val Asp Gly Ser Cys Phe Ile Tyr Arg Ala		
1	5	10
Tyr His Ala Ile Lys Gly Leu Ser Thr Ser Arg Gly Ile Pro Thr Asn		
	20	25
Ala Ile Tyr Gly Phe Thr Arg Met Leu Leu Lys Leu Leu Arg Glu Lys		
	35	40
Asn Val Lys Tyr Ile Leu Cys Ala Phe Asp Ser Pro His Pro Thr Lys		
	50	55
Arg His Lys Ile Tyr Glu Glu Tyr Lys Ile Thr Arg Pro Glu Thr Pro		
	65	70
Lys Asp Leu Pro Val Gln Ile Asp Tyr Ile Lys Gln Ile Ile Asp Ala		
	85	90
Leu Gly Ile Thr Arg Ile Glu Val Pro Gly Tyr Glu Ala Asp Asp Ile		
	100	105
Ile Ala Thr Ala Val Gly Val Ile Asn Gln Phe Ala Pro Leu Asn Phe		
	115	120
Ile Ile Ile Ser Ile Asp Lys Asp Met Leu Gln Leu Val Ser Asp Asn		
	130	135
Val Lys Ile Tyr Asp Pro Ile Asn Glu Leu Ile Ile Asp Arg Glu Tyr		
	145	150
Val Ile Lys Lys Tyr Gly Val Pro Pro Glu Lys Leu Asn Asp Phe Met		
	165	170
Ala Leu Val Gly Asp Ala Ile Asp Asn Ile Pro Gly Val Lys Gly Ile		
	180	185
		190
Gly Glu Lys Thr Ala Ala Asn Leu Ile Lys Arg Tyr Gly Ser Ile Glu		

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195		200		205											
Asn	Ile	Leu	Lys	Asn	Leu	Asp	Ile	Ile	Lys	Pro	Leu	Lys	Val	Ser	Asp
210					215						220				
Ile	Ile	Lys	Lys	Asn	Ile	Lys	Ser	Leu	Gln	Leu	Ser	Lys	Glu	Leu	Val
225					230						235				240
Ile	Leu	Arg	Lys	Asp	Thr	Pro	Ile	Glu	Ile	Lys	Leu	Asp	Asp	Leu	Lys
				245					250					255	
Ile	Lys	Gln	Gln	Asp	Arg	Glu	Lys	Leu	Val	Gln	Ile	Phe	Arg	Glu	Leu
				260					265					270	
Glu	Phe	Asn	Thr	Leu	Leu	Lys	Gln	Ile	Ile	Lys	Asp	Phe	Pro	Asn	His
		275					280						285		
Ser	Ser	Cys	Ser	Val	Leu	Gln	Leu	Asn	Leu	Ala	Ser	Glu	Asn	Arg	Arg
290						295					300				
Asn	Thr	Ile	Glu	Leu	Ile	Glu	Lys	Ile	Lys	Glu	Tyr	Gly	Lys	Phe	Ser
305					310						315				320
Val	Thr	Phe	Asn	Lys	Asp	Ser	Ile	Ile	Ala	Gly	Val	Asn	Gly	Thr	Leu
				325					330						335
Tyr	Glu	Ile	Ala	Phe	Asn	Asp	Thr	Arg	Val	Asn	Glu	Ile	Leu	Ser	Ser
			340						345						350
Glu	Ile	Leu	Lys	Ile	Ile	Tyr	Asn	Ala	Lys	Glu	Ala	Leu	Lys	Lys	Leu
		355					360						365		
Lys	Asn	Ser	Gly	Leu	Lys	Leu	Ser	Pro	Pro	Tyr	Phe	Asp	Leu	Met	Ile
370							375				380				
Val	Ala	Tyr	Leu	Ile	Asn	Pro	Asn	Arg	Gly	Lys	Tyr	Asn	Ile	Asp	Glu
385					390						395				400
Leu	Ile	Leu	Glu	Tyr	Thr	Gly	Lys	Phe	Tyr	Glu	Asn	Ala	Glu	Asn	Ile
				405					410						415
Asn	Phe	Tyr	Met	Phe	Glu	Leu	Tyr	Glu	Lys	Leu	Asn	Lys	Glu	Leu	Lys
			420						425						430
Glu	Lys	Glu	Leu	Glu	Asn	Leu	Tyr	Phe	Asp	Ile	Glu	Met	Pro	Leu	Ile
		435					440						445		
Glu	Val	Leu	Phe	Asp	Met	Glu	Glu	Thr	Gly	Ile	Lys	Val	Asn	Ile	Glu
450					455						460				
Lys	Leu	Glu	Thr	Leu	Thr	Lys	His	Ile	Ser	Met	Glu	Leu	Asp	Lys	Ile
465					470						475				480
Lys	Glu	Lys	Ile	Tyr	Thr	Ile	Ala	Gly	Thr	Glu	Phe	Asn	Ile	Asn	Ser
				485					490						495
Pro	Lys	Gln	Leu	Ala	Glu	Val	Leu	Tyr	Asp	Arg	Leu	Gly	Leu	Lys	Thr
			500						505						510
Arg	Lys	Arg	Gly	Lys	Lys	Ala	Arg	Ser	Thr	Glu	Met	Glu	Val	Leu	Glu
		515					520								525
Glu	Leu	Ala	Ile	Gln	His	Glu	Leu	Pro	His	Glu	Val	Ile	Asn	Tyr	Arg
530					535										540
Thr	Leu	Asn	Lys	Leu	Leu	Thr	Gly	Tyr	Leu	Ile	Pro	Leu	Arg	Asp	Tyr
545					550						555				560
Ile	Asn	Pro	Glu	Thr	Lys	Arg	Ile	His	Thr	Lys	Trp	Ser	Gln	Thr	Val
				565					570						575
Ala	Gly	Thr	Gly	Arg	Ile	Val	Ser	Ser	Glu	Pro	Asn	Leu	Gln	Asn	Ile
			580						585						590
Pro	Val	Lys	Gly	Glu	Trp	Ala	Glu	Phe	Leu	Arg	Glu	Val	Phe	Ile	Pro
		595					600								605

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Glu Asn Gly Tyr Met Phe Leu Ser Ala Asp Tyr Ser Gln Ile Glu Leu  
 610 615 620  
 Arg Leu Leu Ala His Met Ser Glu Asp Pro Ala Leu Ile Lys Ala Phe  
 625 630 635 640  
 Leu Asp Gly Lys Asp Ile His Thr Ala Thr Ala Ser Glu Ile Phe Ser  
 645 650 655  
 Ile Pro Glu Asn Ala Val Thr Asp Glu His Arg Arg Ile Ala Lys Thr  
 660 665 670  
 Val Asn Phe Gly Ile Ser Tyr Gly Ile Ser Pro Phe Gly Leu Ser Glu  
 675 680 685  
 Ser Ile Lys Ile Pro Tyr Glu Lys Ala Glu Glu Leu Ile Glu Leu Tyr  
 690 695 700  
 Phe Leu Arg Tyr Pro Met Val Arg Lys Phe Ile Glu Glu Thr Ile Ser  
 705 710 715 720  
 Phe Ala Gln Lys Asn Gly Tyr Val Arg Thr Leu Phe Gly Arg Ile Arg  
 725 730 735  
 Pro Leu Pro Glu Ile Asn Ser Pro Asn Gln Phe Leu Arg Met Gln Ser  
 740 745 750  
 Glu Arg Met Ala Val Asn Ala Arg Val Gln Gly Thr Ala Ala Asp Ile  
 755 760 765  
 Ile Lys Ile Ala Met Ile Arg Ile Tyr Asn Arg Leu Lys Lys Glu Lys  
 770 775 780  
 Leu Asn Ala Lys Ile Ile Leu Gln Ile His Asp Glu Ile Val Leu Glu  
 785 790 795 800  
 Val Glu Gln Lys Val Ile Glu Lys Val Ser Glu Ile Val Gln Asn Glu  
 805 810 815  
 Met Lys Asp Phe Ser Leu Ser Val Pro Leu Glu Val Asn Val Phe Ser  
 820 825 830  
 Gly Asn Ser Leu Asn Leu  
 835  
  
 <210> SEQ ID NO 6  
 <211> LENGTH: 856  
 <212> TYPE: PRT  
 <213> ORGANISM: Dictyoglomus thermophilum  
  
 <400> SEQUENCE: 6  
  
 Met Glu Gln Lys Ser Leu Trp Asp Leu Phe Gln Glu Asn Thr Glu Lys  
 1 5 10 15  
 Glu Ser Lys Arg Lys Ile Leu Ile Ile Asp Gly Ser Ser Leu Ile Tyr  
 20 25 30  
 Arg Val Tyr Tyr Ala Leu Pro Pro Leu Lys Thr Lys Asn Gly Glu Leu  
 35 40 45  
 Thr Asn Ala Leu Tyr Gly Phe Ile Arg Ile Leu Leu Lys Ala Val Glu  
 50 55 60  
 Asp Phe Asn Pro Asp Leu Val Gly Val Ala Phe Asp Arg Pro Glu Pro  
 65 70 75 80  
 Thr Phe Arg His Val Ile Tyr Lys Glu Tyr Lys Ala Lys Arg Pro Pro  
 85 90 95  
 Met Lys Asp Asp Leu Lys Ala Gln Ile Pro Trp Ile Arg Glu Phe Leu  
 100 105 110  
 Arg Leu Asn Asp Ile Pro Leu Leu Glu Glu Pro Gly Tyr Glu Ala Asp





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Lys Lys Gly Lys Thr Gly Tyr Ser Thr Ser Ser Ser Val Leu Gln Asn  
 530 535 540

Leu Ile Asn Ala His Pro Ile Val Arg Lys Ile Leu Gln Tyr Arg Glu  
 545 550 555 560

Leu Tyr Lys Leu Lys Ser Thr Tyr Val Asp Ala Ile Pro Asn Leu Val  
 565 570 575

Asn Pro Gln Thr Gly Arg Val His Thr Lys Phe Asn Pro Thr Gly Thr  
 580 585 590

Ala Thr Gly Arg Ile Ser Ser Ser Glu Pro Asn Leu Gln Asn Ile Pro  
 595 600 605

Ile Lys Ser Glu Glu Gly Arg Lys Ile Arg Arg Ala Phe Val Ser Glu  
 610 615 620

Asp Gly Tyr Phe Leu Val Ser Leu Asp Tyr Ser Gln Ile Glu Leu Arg  
 625 630 635 640

Ile Met Ala His Leu Ser Gln Glu Pro Lys Leu Ile Ser Ala Phe Gln  
 645 650 655

Lys Gly Glu Asp Ile His Arg Arg Thr Ala Ser Glu Ile Phe Gly Val  
 660 665 670

Pro Glu Glu Glu Val Asp Asp Leu Leu Arg Ser Arg Ala Lys Ala Val  
 675 680 685

Asn Phe Gly Ile Ile Tyr Gly Ile Ser Ser Phe Gly Leu Ser Glu Thr  
 690 695 700

Val Ser Ile Thr Pro Glu Glu Ala Glu Lys Phe Ile Asp Ser Tyr Phe  
 705 710 715 720

Lys His Tyr Pro Arg Val Lys Leu Phe Ile Asp Lys Thr Ile His Glu  
 725 730 735

Ala Arg Glu Lys Leu Tyr Val Lys Thr Leu Phe Gly Arg Lys Arg Tyr  
 740 745 750

Ile Pro Glu Ile Lys Ser Ile Asn Lys Gln Val Arg Asn Ala Tyr Glu  
 755 760 765

Arg Ile Ala Ile Asn Ala Pro Ile Gln Gly Thr Ala Ala Asp Ile Ile  
 770 775 780

Lys Leu Ala Met Ile Glu Ile Tyr Lys Glu Ile Glu Asn Lys Asn Leu  
 785 790 795 800

Lys Ser Arg Ile Leu Leu Gln Ile His Asp Glu Leu Ile Leu Glu Val  
 805 810 815

Pro Glu Glu Glu Met Glu Phe Thr Pro Leu Met Ala Lys Glu Lys Met  
 820 825 830

Glu Lys Val Val Glu Leu Ser Val Pro Leu Val Val Glu Ile Ser Val  
 835 840 845

Gly Lys Asn Leu Ala Glu Leu Lys  
 850 855

<210> SEQ ID NO 7  
 <211> LENGTH: 930  
 <212> TYPE: PRT  
 <213> ORGANISM: Natranaerobius thermophilus

<400> SEQUENCE: 7

Met Asn Ser Asn Asp Tyr Gln Ala Asn Asp Lys Phe Val Val Ile Asp  
 1 5 10 15

Gly Asn Ser Leu Leu Asn Arg Ala Phe Tyr Ala Leu Pro Leu Leu Gln

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	20					25						30			
Thr	Lys	Gln	Gly	Phe	Phe	Thr	Asn	Ala	Ile	Tyr	Gly	Phe	Thr	Thr	Met
	35						40					45			
Leu	Leu	Lys	Leu	Val	Gln	Asp	Glu	Ser	Pro	Asn	Tyr	Leu	Ala	Val	Val
	50					55					60				
Phe	Asp	Thr	Lys	Ala	Lys	Thr	Phe	Arg	His	His	Lys	Phe	Pro	Glu	Tyr
65					70					75					80
Lys	Gly	His	Arg	Asp	Lys	Ala	Pro	Asp	Glu	Met	Arg	Pro	Gln	Met	Pro
				85					90					95	
Met	Leu	Lys	Glu	Leu	Leu	Glu	Ala	Met	Asn	Ile	Asn	Tyr	Phe	Glu	Lys
			100					105					110		
Asp	Gly	Tyr	Glu	Ala	Asp	Asp	Leu	Ile	Gly	Ala	Phe	Thr	Lys	Ile	Ala
		115					120					125			
Lys	Gln	Glu	Asp	Lys	Glu	Thr	Met	Val	Val	Thr	Gly	Asp	Lys	Asp	Leu
	130					135					140				
Leu	Gln	Leu	Leu	Asp	Asp	Lys	Thr	Thr	Ile	Leu	Leu	Thr	Lys	Lys	Gly
145					150					155					160
Ile	Thr	Gln	Met	Glu	Ser	Tyr	Asp	Gly	Glu	Lys	Val	Lys	Glu	Glu	Phe
				165					170						175
Gly	Val	Asn	Val	Asp	Lys	Leu	Ile	Asp	Leu	Lys	Ala	Leu	Thr	Gly	Asp
			180					185						190	
Lys	Ser	Asp	Asn	Val	Pro	Gly	Val	Pro	Gly	Val	Gly	Lys	Lys	Thr	Ala
		195				200						205			
Leu	Lys	Leu	Leu	Asn	Asn	Tyr	Gly	Asp	Leu	Glu	Lys	Leu	Tyr	Lys	Ser
	210					215					220				
Leu	Asp	Gly	Val	Gly	Gly	Lys	Leu	Gln	Ser	Lys	Leu	Ala	Asp	Asn	Lys
225					230					235					240
Asp	Lys	Ala	Phe	Leu	Ser	Lys	Glu	Leu	Val	Thr	Ile	Asp	Cys	Glu	Glu
			245						250						255
Ser	Leu	Ile	Glu	Asn	Leu	Asp	Trp	Asn	Gln	Leu	Ser	Lys	Phe	Glu	Ile
		260						265						270	
Ala	Ser	Pro	Lys	Ala	Arg	Glu	Leu	Leu	Gln	Glu	Trp	Glu	Met	Asn	Ser
		275					280						285		
Ile	Leu	Glu	Arg	Leu	Pro	Ala	Ser	Asp	Glu	Glu	Gln	Lys	Lys	Asp	Gln
	290					295					300				
Ser	Pro	Val	Asn	Glu	Gly	Lys	Thr	Ser	Ser	Phe	Asn	Trp	Asp	Asn	Phe
305					310					315					320
Tyr	Tyr	Ile	Ser	Glu	Phe	Pro	His	Glu	Asn	Ser	Asp	Asn	Leu	Glu	Ser
				325					330						335
Glu	Leu	Glu	Lys	Phe	Ile	Gln	Asp	Gly	Asn	His	Lys	Met	Ala	Leu	Tyr
			340					345					350		
Arg	His	Leu	Pro	Lys	Lys	Leu	Ser	Thr	Ala	Lys	Gln	Lys	Asp	Ser	Tyr
		355					360						365		
Pro	Glu	Pro	Glu	Gly	Gly	Leu	Val	Val	Ser	Ile	Asn	Asp	Leu	Ile	Phe
	370					375					380				
Tyr	Val	Pro	Glu	Lys	Leu	Leu	Ser	Gln	Val	Leu	Ala	Glu	Thr	Ile	Ala
385					390					395					400
Pro	Lys	Leu	Ile	Lys	Gly	Asn	Asp	Lys	Gly	Thr	Glu	Thr	Glu	Asp	Ala
				405					410						415
Pro	Lys	Leu	Lys	Ile	Ala	Ser	Tyr	Asn	Ile	Lys	Arg	Ile	Trp	His	Leu
			420					425							430

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Phe Lys Asn Asn Thr Glu Leu Asp Leu Tyr Asp Leu Asp Thr Asn Lys  
435 440 445  
Phe Leu Phe Tyr Asp Thr Glu Leu Met Ala Tyr Leu Leu Glu Pro Thr  
450 455 460  
Glu Ala Pro His Ser Ile Glu Asp Met Met Asn Arg Tyr Tyr Gly Gln  
465 470 475 480  
Phe Asp Leu Thr Pro Tyr Gly Gln Asp Trp Gln Ala Val Cys Glu Arg  
485 490 495  
Gly Ala Ile Leu Leu Asp Leu Ile Ser Pro Leu Glu Asp Ile Leu Gln  
500 505 510  
Glu Arg Asn Gln Trp Gln Leu Tyr Lys Asn Ile Glu Leu Pro Leu Ala  
515 520 525  
Phe Ile Leu Ala Arg Met Glu Phe Arg Gly Ile Lys Val Asp Ala Arg  
530 535 540  
Val Leu Thr Glu Met Glu Ala Asn Ile Asp His Arg Leu Ser Glu Ile  
545 550 555 560  
Ser Thr Lys Ile Phe Glu Ile Ala Gly Glu Glu Phe Asn Leu Asn Ser  
565 570 575  
Pro Lys Gln Leu Gly Tyr Ile Leu Phe Glu Lys Leu Gln Leu Pro Val  
580 585 590  
Val Lys Lys Thr Lys Thr Gly Tyr Ser Thr Asp Ala Lys Thr Leu Glu  
595 600 605  
Thr Leu Ser His Asp Tyr Glu Ile Cys Lys Leu Leu Leu Asp Tyr Arg  
610 615 620  
Gln Leu His Lys Leu Lys Thr Thr Tyr Leu Val Gly Leu Lys Asp Leu  
625 630 635 640  
Ile Ser Lys Thr Thr Gly Lys Ile His Thr Thr Tyr Asn Gln Thr Ile  
645 650 655  
Thr Ala Thr Gly Arg Leu Ser Ser Thr Asp Pro Asn Leu Gln Asn Ile  
660 665 670  
Pro Ile Lys Leu Glu Glu Gly Arg Lys Ile Arg Lys Gly Phe Val Ile  
675 680 685  
Gln Asn Ser Asp Gln Leu Phe Leu Ala Ala Asp Tyr Ser Gln Ile Glu  
690 695 700  
Leu Arg Ile Leu Ala His Val Ser Glu Asp Thr Asn Leu Ile Gln Ala  
705 710 715 720  
Phe Gln Glu Gln Gln Asp Ile His Thr Gln Thr Ala Ala Gln Val Phe  
725 730 735  
Glu Val Glu Ser Thr Gln Val Thr Arg Glu Met Arg Ser His Ala Lys  
740 745 750  
Ala Val Asn Phe Gly Ile Val Tyr Gly Ile Ser Asp Tyr Gly Leu Ser  
755 760 765  
Arg Gln Leu Gly Ile Ser Arg Lys Gln Ala Lys Thr Tyr Ile Asp Asn  
770 775 780  
Tyr Leu Thr Arg Phe Ser Gly Val Lys Glu Tyr Met Asp Gln Ile Val  
785 790 795 800  
Asn Gln Ala Lys Met Asn Gly Tyr Val Glu Thr Leu Tyr Asn Arg Arg  
805 810 815  
Arg Asn Leu Pro Asp Ile Ser His Arg Asn Phe Asn Ile Arg Ser Ala  
820 825 830

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Ala Glu Arg Thr Ala Ile Asn Thr Pro Ile Gln Gly Thr Ala Ala Asp  
835 840 845

Ile Ile Lys Asp Ala Met Val Lys Val Glu Lys Glu Leu Glu Lys Gln  
850 855 860

Asp Leu Leu Asp Lys Ala Ala Leu Leu Leu Gln Val His Asp Glu Leu  
865 870 875 880

Ile Leu Glu Ile Asn Lys Glu Val Leu Ser Asp Val Ala Thr Lys Val  
885 890 895

Lys Glu Ile Met Glu Asn Ile Ile Glu Leu Lys Val Pro Leu Thr Val  
900 905 910

Asp Leu Lys Thr Gly Pro Asn Trp Tyr Asp Leu Asn Pro Tyr Gln Ser  
915 920 925

Gly Glu  
930

<210> SEQ ID NO 8  
<211> LENGTH: 928  
<212> TYPE: PRT  
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 8

Met Val Gln Ile Pro Gln Asn Pro Leu Ile Leu Val Asp Gly Ser Ser  
1 5 10 15

Tyr Leu Tyr Arg Ala Tyr His Ala Phe Pro Pro Leu Thr Asn Ser Ala  
20 25 30

Gly Glu Pro Thr Gly Ala Met Tyr Gly Val Leu Asn Met Leu Arg Ser  
35 40 45

Leu Ile Met Gln Tyr Lys Pro Thr His Ala Ala Val Val Phe Asp Ala  
50 55 60

Lys Gly Lys Thr Phe Arg Asp Glu Leu Phe Glu His Tyr Lys Ser His  
65 70 75 80

Arg Pro Pro Met Pro Asp Asp Leu Arg Ala Gln Ile Glu Pro Leu His  
85 90 95

Ala Met Val Lys Ala Met Gly Leu Pro Leu Leu Ala Val Ser Gly Val  
100 105 110

Glu Ala Asp Asp Val Ile Gly Thr Leu Ala Arg Glu Ala Glu Lys Ala  
115 120 125

Gly Arg Pro Val Leu Ile Ser Thr Gly Asp Lys Asp Met Ala Gln Leu  
130 135 140

Val Thr Pro Asn Ile Thr Leu Ile Asn Thr Met Thr Asn Thr Ile Leu  
145 150 155 160

Gly Pro Glu Glu Val Val Asn Lys Tyr Gly Val Pro Pro Glu Leu Ile  
165 170 175

Ile Asp Phe Leu Ala Leu Met Gly Asp Ser Ser Asp Asn Ile Pro Gly  
180 185 190

Val Pro Gly Val Gly Glu Lys Thr Ala Gln Ala Leu Leu Gln Gly Leu  
195 200 205

Gly Gly Leu Asp Thr Leu Tyr Ala Glu Pro Glu Lys Ile Ala Gly Leu  
210 215 220

Ser Phe Arg Gly Ala Lys Thr Met Ala Ala Lys Leu Glu Gln Asn Lys  
225 230 235 240

Glu Val Ala Tyr Leu Ser Tyr Gln Leu Ala Thr Ile Lys Thr Asp Val  
245 250 255

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Glu Leu Glu Leu Thr Cys Glu Gln Leu Glu Val Gln Gln Pro Ala Ala  
260 265 270

Glu Glu Leu Leu Gly Leu Phe Lys Lys Tyr Glu Phe Lys Arg Trp Thr  
275 280 285

Ala Asp Val Glu Ala Gly Lys Trp Leu Gln Ala Lys Gly Ala Lys Pro  
290 295 300

Ala Ala Lys Pro Gln Glu Thr Ser Val Ala Asp Glu Ala Pro Glu Val  
305 310 315 320

Thr Ala Thr Val Ile Ser Tyr Asp Asn Tyr Val Thr Ile Leu Asp Glu  
325 330 335

Glu Thr Leu Lys Ala Trp Ile Ala Lys Leu Glu Lys Ala Pro Val Phe  
340 345 350

Ala Phe Asp Thr Glu Thr Asp Ser Leu Asp Asn Ile Ser Ala Asn Leu  
355 360 365

Val Gly Leu Ser Phe Ala Ile Glu Pro Gly Val Ala Ala Tyr Ile Pro  
370 375 380

Val Ala His Asp Tyr Leu Asp Ala Pro Asp Gln Ile Ser Arg Glu Arg  
385 390 395 400

Ala Leu Glu Leu Leu Lys Pro Leu Leu Glu Asp Glu Lys Ala Leu Lys  
405 410 415

Val Gly Gln Asn Leu Lys Tyr Asp Arg Gly Ile Leu Ala Asn Tyr Gly  
420 425 430

Ile Glu Leu Arg Gly Ile Ala Phe Asp Thr Met Leu Glu Ser Tyr Ile  
435 440 445

Leu Asn Ser Val Ala Gly Arg His Asp Met Asp Ser Leu Ala Glu Arg  
450 455 460

Trp Leu Lys His Lys Thr Ile Thr Phe Glu Glu Ile Ala Gly Lys Gly  
465 470 475 480

Lys Asn Gln Leu Thr Phe Asn Gln Ile Ala Leu Glu Glu Ala Gly Arg  
485 490 495

Tyr Ala Ala Glu Asp Ala Asp Val Thr Leu Gln Leu His Leu Lys Met  
500 505 510

Trp Pro Asp Leu Gln Lys His Lys Gly Pro Leu Asn Val Phe Glu Asn  
515 520 525

Ile Glu Met Pro Leu Val Pro Val Leu Ser Arg Ile Glu Arg Asn Gly  
530 535 540

Val Lys Ile Asp Pro Lys Val Leu His Asn His Ser Glu Glu Leu Thr  
545 550 555 560

Leu Arg Leu Ala Glu Leu Glu Lys Lys Ala His Glu Ile Ala Gly Glu  
565 570 575

Glu Phe Asn Leu Ser Ser Thr Lys Gln Leu Gln Thr Ile Leu Phe Glu  
580 585 590

Lys Gln Gly Ile Lys Pro Leu Lys Lys Thr Pro Gly Gly Ala Pro Ser  
595 600 605

Thr Ser Glu Glu Val Leu Glu Glu Leu Ala Leu Asp Tyr Pro Leu Pro  
610 615 620

Lys Val Ile Leu Glu Tyr Arg Gly Leu Ala Lys Leu Lys Ser Thr Tyr  
625 630 635 640

Thr Asp Lys Leu Pro Leu Met Ile Asn Pro Lys Thr Gly Arg Val His  
645 650 655

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Thr Ser Tyr His Gln Ala Val Thr Ala Thr Gly Arg Leu Ser Ser Thr
      660                               665                               670

Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Asn Glu Glu Gly Arg Arg
      675                               680                               685

Ile Arg Gln Ala Phe Ile Ala Pro Glu Asp Tyr Val Ile Val Ser Ala
      690                               695                               700

Asp Tyr Ser Gln Ile Glu Leu Arg Ile Met Ala His Leu Ser Arg Asp
      705                               710                               715                               720

Lys Gly Leu Leu Thr Ala Phe Ala Glu Gly Lys Asp Ile His Arg Ala
      725                               730                               735

Thr Ala Ala Glu Val Phe Gly Leu Pro Leu Glu Thr Val Thr Ser Glu
      740                               745                               750

Gln Arg Arg Ser Ala Lys Ala Ile Asn Phe Gly Leu Ile Tyr Gly Met
      755                               760                               765

Ser Ala Phe Gly Leu Ala Arg Gln Leu Asn Ile Pro Arg Lys Glu Ala
      770                               775                               780

Gln Lys Tyr Met Asp Leu Tyr Phe Glu Arg Tyr Pro Gly Val Leu Gln
      785                               790                               795                               800

Tyr Met Glu Arg Thr Arg Ala Gln Ala Lys Glu Gln Gly Tyr Val Glu
      805                               810                               815

Thr Leu Asp Gly Arg Arg Leu Tyr Leu Pro Asp Ile Lys Ser Ser Asn
      820                               825                               830

Gly Ala Arg Arg Ala Ala Ala Glu Arg Ala Ala Ile Asn Ala Pro Met
      835                               840                               845

Gln Gly Thr Ala Ala Asp Ile Ile Lys Arg Ala Met Ile Ala Val Asp
      850                               855                               860

Ala Trp Leu Gln Ala Glu Gln Pro Arg Val Arg Met Ile Met Gln Val
      865                               870                               875                               880

His Asp Glu Leu Val Phe Glu Val His Lys Asp Asp Val Asp Ala Val
      885                               890                               895

Ala Lys Gln Ile His Gln Leu Met Glu Asn Cys Thr Arg Leu Asp Val
      900                               905                               910

Pro Leu Leu Val Glu Val Gly Ser Gly Glu Asn Trp Asp Gln Ala His
      915                               920                               925
    
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<210> SEQ ID NO 9
<211> LENGTH: 892
<212> TYPE: PRT
<213> ORGANISM: Thermotoga lettingae TMO
    
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<400> SEQUENCE: 9

Met Ala Lys Leu Phe Leu Phe Asp Gly Thr Gly Leu Ala Tyr Arg Ala
 1      5      10      15

Tyr Tyr Ala Leu Asp Gln Ser Leu Ser Thr Thr Ser Gly Ile Pro Thr
 20     25     30

Asn Ala Thr Tyr Gly Val Leu Arg Met Leu Ile Arg Phe Leu Lys Asp
 35     40     45

Tyr Val Lys Ile Gly Asp Tyr Thr Ala Phe Ala Met Asp Thr Lys Thr
 50     55     60

Arg Thr Tyr Arg His Glu Leu Leu Glu Glu Tyr Lys Ala His Arg Pro
 65     70     75     80

Gln Thr Pro Asp Ala Met Ile Gln Gln Leu Pro Tyr Ile Lys Arg Gly
 85     90     95
    
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Val Gln Ala Leu Gly Ile Lys Val Leu Glu Tyr Glu Gly Cys Glu Ala  
 100 105 110

Asp Asp Val Ile Ala Thr Leu Ala Arg Met Gly Glu Lys Glu Phe Glu  
 115 120 125

Asp Ile Phe Ile Ile Ser Gly Asp Lys Asp Met Phe Gln Leu Val Asn  
 130 135 140

Asp Lys Ile Lys Val Trp Arg Pro Ser Lys Gly Ile Thr Asp Leu Glu  
 145 150 155 160

Phe Tyr Asp Lys Lys Lys Ile Ile Glu Lys Tyr Arg Val Glu Pro Ser  
 165 170 175

Lys Ile Val Asp Leu Leu Ala Leu Met Gly Asp Ser Val Asp Asn Val  
 180 185 190

Pro Gly Val Lys Gly Ile Gly Met Lys Thr Ala Ala Glu Leu Ile Glu  
 195 200 205

Lys Phe Gly Asn Leu Asp Glu Ile Tyr Gly Lys Ile Asp Glu Asn Ser  
 210 215 220

Arg Ile Gly Lys Leu Leu Ser Arg Gly Lys Asp Asp Ala Phe Lys Ser  
 225 230 235 240

Lys Gln Leu Val Thr Leu Met Thr Asp Leu Asp Leu Arg Leu Thr Trp  
 245 250 255

Asp Asp Leu Lys Tyr Ala Gly Tyr Lys Glu Lys Glu Leu Val Glu Phe  
 260 265 270

Leu Arg Glu Met Glu Phe Ser Ser Ile Met Lys Glu Leu Gly Leu Tyr  
 275 280 285

Thr Gln Gln Asp Gln Lys Thr Pro Tyr Ile Ala Val Lys Asp Asn Asn  
 290 295 300

Ser Leu Asn Glu Leu Phe Glu Lys Ile Lys Lys Ser Gln Tyr Phe Val  
 305 310 315 320

Leu Asp Leu Glu Thr Asp Ser Leu Ser Pro Ile Asp Ala Glu Ile Ile  
 325 330 335

Gly Phe Ser Ile Ser Leu Pro Ser Lys Glu Ser Tyr Tyr Val Pro Leu  
 340 345 350

Ala His Lys Asn Gly Pro Asn Val Asp Lys Lys Ser Ala Leu Asn Asn  
 355 360 365

Leu Lys Ser Ile Leu Glu Asn Gln Ser Ala Lys Ile Ile Gly Gln Asn  
 370 375 380

Leu Lys Tyr Asp Tyr Ser Val Leu Lys Met His Gly Ile Glu Pro Val  
 385 390 395 400

Arg Pro Ser Phe Asp Thr Met Ile Ala Ala Tyr Leu Leu Asn Pro Asp  
 405 410 415

Glu Lys Arg Phe Asn Leu Asp Glu Leu Ala Met Lys Phe Leu Asn Tyr  
 420 425 430

Lys Met Ile Ser Phe Glu Glu Leu Phe Lys Asp Thr Ser Pro Leu Phe  
 435 440 445

Gly Ala Val Thr Phe Ala Asp Val Ser Val Glu Asp Ala Thr Lys Tyr  
 450 455 460

Ser Ala Glu Asp Ala Asp Ile Thr Arg Arg Leu Tyr Glu Ile Leu Asn  
 465 470 475 480

Ile Lys Leu His Glu Ala Asp Leu Leu Glu Val Leu Glu Lys Ile Glu  
 485 490 495

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Met	Pro	Leu	Ile	Pro	Val	Leu	Val	His	Met	Glu	Leu	Glu	Gly	Val	Tyr	500	505	510	
Met	Asn	Ile	Asp	Tyr	Leu	Lys	Asp	Leu	Ser	Ser	Lys	Tyr	Ala	Ala	Arg	515	520	525	
Met	Asn	Glu	Leu	Ser	Gln	Gln	Ile	Tyr	Asn	His	Ala	Gly	Glu	Ala	Phe	530	535	540	
Asn	Leu	Asn	Ser	Pro	Lys	Gln	Val	Ala	His	Ile	Leu	Phe	Asp	Lys	Leu	545	550	555	560
Lys	Ile	Gln	Pro	Thr	Lys	Lys	Thr	Ser	Thr	Gly	Glu	Pro	Ser	Thr	Arg	565	570	575	
Ala	Asp	Val	Leu	Glu	Glu	Leu	Ser	Glu	Glu	His	Pro	Ile	Val	Arg	Leu	580	585	590	
Ile	Leu	Glu	Tyr	Arg	Lys	Tyr	Gln	Lys	Ile	Lys	Ser	Thr	Tyr	Leu	Asp	595	600	605	
Val	Leu	Pro	Lys	Leu	Val	His	Pro	Glu	Thr	Gly	Arg	Ile	His	Ser	Ser	610	615	620	
Phe	His	Gln	Thr	Gly	Thr	Ala	Thr	Gly	Arg	Leu	Ser	Ser	Ser	Asp	Pro	625	630	635	640
Asn	Leu	Gln	Asn	Leu	Pro	Ser	Lys	Gln	Glu	Glu	Ser	Arg	Glu	Ile	Arg	645	650	655	
Lys	Ala	Val	Val	Pro	Gln	Arg	Asp	Ser	Trp	Lys	Ile	Leu	Ser	Ala	Asp	660	665	670	
Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala	His	Leu	Ser	Asn	Asp	Gln	675	680	685	
Asn	Leu	Ile	Glu	Ala	Phe	Lys	Lys	Asp	Glu	Asp	Ile	His	Asn	Phe	Thr	690	695	700	
Ser	Ser	Arg	Ile	Phe	Gln	Val	Pro	Glu	Asn	Gln	Val	Thr	Pro	Gln	Met	705	710	715	720
Arg	Ser	Ile	Gly	Lys	Met	Val	Asn	Phe	Ser	Val	Ile	Tyr	Gly	Val	Ser	725	730	735	
Pro	Tyr	Gly	Leu	Ser	Gln	Arg	Thr	Gly	Leu	Ser	Tyr	Asp	Gln	Ala	Gln	740	745	750	
Lys	Phe	Ile	Gly	Glu	Tyr	Phe	Ser	Leu	Tyr	Pro	Ser	Val	Lys	Glu	Tyr	755	760	765	
Phe	Ala	Lys	Ile	Val	Ser	Tyr	Ala	Lys	Thr	His	Gly	Tyr	Val	Arg	Thr	770	775	780	
Met	Phe	Gly	Arg	Arg	Arg	Glu	Val	Pro	Gln	Leu	Arg	Ser	Lys	Asn	Ala	785	790	795	800
Ser	Val	Arg	Gln	Glu	Gly	Glu	Arg	Ile	Ala	Ile	Asn	Thr	Pro	Ile	Gln	805	810	815	
Gly	Thr	Ala	Ala	Asp	Ile	Met	Lys	Leu	Ala	Met	Ile	Asn	Leu	Tyr	Asp	820	825	830	
Lys	Ile	Lys	Gln	Met	Arg	Leu	Gln	Ser	Lys	Met	Ile	Leu	Gln	Val	His	835	840	845	
Asp	Glu	Leu	Val	Phe	Glu	Val	Pro	Asp	Glu	Glu	Val	Glu	Ile	Val	Lys	850	855	860	
Asn	Leu	Val	Arg	Asp	Ser	Met	Glu	Asn	Val	Val	Arg	Leu	Ser	Val	Pro	865	870	875	880
Leu	Lys	Val	Asp	Leu	Lys	Ile	Ser	Asp	Ser	Trp	Glu	885	890						



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<210> SEQ ID NO 10
<211> LENGTH: 893
<212> TYPE: PRT
<213> ORGANISM: Thermotoga petrophila RKU-1

<400> SEQUENCE: 10

Met Ala Arg Leu Phe Leu Phe Asp Gly Thr Ala Leu Ala Tyr Arg Ala
1           5           10           15
Tyr Tyr Ala Leu Asp Arg Ser Leu Ser Thr Ser Ala Gly Ile Pro Thr
20           25           30
Asn Ala Thr Tyr Gly Val Ala Arg Met Leu Val Arg Phe Ile Lys Asp
35           40           45
His Ile Ile Val Gly Lys Asp Tyr Ala Ala Val Ala Phe Asp Lys Arg
50           55           60
Ala Ala Thr Phe Arg His Lys Leu Leu Glu Thr Tyr Lys Ala Gln Arg
65           70           75           80
Pro Lys Thr Pro Asp Leu Leu Ile Gln Gln Leu Pro Tyr Ile Lys Arg
85           90           95
Leu Val Glu Ala Leu Gly Met Lys Val Leu Glu Ile Glu Gly Tyr Glu
100          105          110
Ala Asp Asp Ile Ile Ala Thr Leu Ala Val Lys Gly Leu Ser Leu Phe
115          120          125
Asp Glu Ile Phe Ile Val Thr Gly Asp Lys Asp Met Leu Gln Leu Val
130          135          140
Asn Glu Lys Ile Lys Val Trp Arg Ile Val Lys Gly Ile Ser Asp Leu
145          150          155          160
Glu Leu Tyr Asp Ala Gln Lys Val Lys Glu Lys Tyr Gly Val Glu Pro
165          170          175
His Gln Ile Pro Asp Leu Leu Ala Leu Thr Gly Asp Glu Ile Asp Asn
180          185          190
Ile Pro Gly Val Thr Gly Ile Gly Glu Lys Thr Ala Val Gln Leu Leu
195          200          205
Glu Lys Tyr Arg Asp Leu Glu Asp Ile Leu Asn His Ile His Glu Leu
210          215          220
Pro Gln Lys Thr Arg Lys Thr Met Leu Arg Asp Arg Glu Ser Ala Ile
225          230          235          240
Leu Ser Lys Lys Leu Ala Ile Leu Glu Thr Asn Val Pro Ile Glu Ile
245          250          255
Asn Trp Glu Glu Leu Arg Tyr Gln Gly His Asp Arg Glu Lys Leu Leu
260          265          270
Ser Leu Leu Lys Glu Leu Glu Phe Ala Ser Ile Met Lys Glu Leu Gln
275          280          285
Leu Tyr Glu Glu Ser Glu Pro Val Gly Tyr Arg Ile Val Lys Asp Pro
290          295          300
Val Glu Phe Glu Lys Leu Val Glu Lys Leu Lys Glu Thr Pro Ser Phe
305          310          315          320
Ala Ile Asp Leu Glu Thr Ser Ser Leu Asp Pro Phe Glu Cys Asp Ile
325          330          335
Ala Gly Ile Ser Leu Ser Phe Lys Pro Lys Glu Ala Tyr Tyr Ile Pro
340          345          350
Leu His His Arg Asn Ala Gln Asn Leu Asp Glu Lys Glu Val Leu Lys
355          360          365

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Lys Leu Lys Glu Ile Leu Glu Asp Pro Gly Ala Lys Ile Val Gly Gln  
 370 375 380

Asn Leu Lys Phe Asp Tyr Lys Val Leu Met Val Lys Gly Ile Glu Pro  
 385 390 395 400

Val Pro Pro His Phe Asp Thr Met Ile Ala Ala Tyr Leu Ile Glu Pro  
 405 410 415

Asn Glu Lys Lys Phe Asn Leu Asp Asp Leu Ala Leu Lys Phe Leu Gly  
 420 425 430

Tyr Lys Met Thr Ser Tyr Gln Glu Leu Met Ser Phe Ser Ser Pro Leu  
 435 440 445

Phe Gly Phe Ser Phe Val Asp Val Pro Leu Glu Lys Ala Ala Asn Tyr  
 450 455 460

Ser Cys Glu Asp Ala Asp Ile Thr Tyr Arg Leu Tyr Lys Thr Leu Ser  
 465 470 475 480

Leu Lys Leu His Glu Ala Asp Leu Glu Asn Val Phe Tyr Lys Ile Glu  
 485 490 495

Met Pro Leu Val Ser Val Leu Ala Arg Met Glu Leu Asn Gly Val Tyr  
 500 505 510

Val Asp Thr Glu Phe Leu Lys Lys Leu Ser Glu Glu Tyr Gly Lys Lys  
 515 520 525

Leu Glu Glu Leu Ala Glu Glu Ile Tyr Arg Ile Ala Gly Glu Pro Phe  
 530 535 540

Asn Ile Asn Ser Pro Lys Gln Val Ser Arg Ile Leu Phe Glu Lys Leu  
 545 550 555 560

Gly Ile Lys Pro Arg Gly Lys Thr Thr Lys Thr Gly Asp Tyr Ser Thr  
 565 570 575

Arg Ile Glu Val Leu Glu Glu Leu Ala Gly Glu His Glu Ile Ile Pro  
 580 585 590

Leu Ile Leu Glu Tyr Arg Lys Ile Gln Lys Leu Lys Ser Thr Tyr Ile  
 595 600 605

Asp Ala Leu Pro Lys Met Val Asn Pro Lys Thr Gly Arg Ile His Ala  
 610 615 620

Ser Phe Asn Gln Thr Gly Thr Ala Thr Gly Arg Leu Ser Ser Ser Asp  
 625 630 635 640

Pro Asn Leu Gln Asn Leu Pro Thr Lys Ser Glu Glu Gly Lys Glu Ile  
 645 650 655

Arg Lys Ala Ile Val Pro Gln Asp Pro Asn Trp Trp Ile Val Ser Ala  
 660 665 670

Asp Tyr Ser Gln Ile Glu Leu Arg Ile Leu Ala His Leu Ser Gly Asp  
 675 680 685

Glu Asn Leu Leu Arg Ala Phe Glu Glu Gly Ile Asp Val His Thr Leu  
 690 695 700

Thr Ala Ser Arg Ile Phe Asn Val Lys Pro Glu Glu Val Thr Glu Glu  
 705 710 715 720

Met Arg Arg Ala Gly Lys Met Val Asn Phe Ser Ile Ile Tyr Gly Val  
 725 730 735

Thr Pro Tyr Gly Leu Ser Val Arg Leu Gly Val Pro Val Lys Glu Ala  
 740 745 750

Glu Lys Met Ile Val Asn Tyr Phe Val Leu Tyr Pro Lys Val Arg Asp  
 755 760 765

Tyr Ile Gln Arg Val Val Ser Glu Ala Lys Glu Lys Gly Tyr Val Arg

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770				775				780							
Thr	Leu	Phe	Gly	Arg	Lys	Arg	Asp	Ile	Pro	Gln	Leu	Met	Ala	Arg	Asp
785				790						795					800
Arg	Asn	Thr	Gln	Ala	Glu	Gly	Glu	Arg	Ile	Ala	Ile	Asn	Thr	Pro	Ile
			805							810					815
Gln	Gly	Thr	Ala	Ala	Asp	Ile	Ile	Lys	Leu	Ala	Met	Ile	Glu	Ile	Asp
			820							825					830
Arg	Glu	Leu	Lys	Glu	Arg	Lys	Met	Arg	Ser	Lys	Met	Ile	Ile	Gln	Val
			835				840								845
His	Asp	Glu	Leu	Val	Phe	Glu	Val	Pro	Asn	Glu	Glu	Lys	Asp	Ala	Leu
			850			855					860				
Val	Glu	Leu	Val	Lys	Asp	Arg	Met	Thr	Asn	Val	Val	Lys	Leu	Ser	Val
			865			870					875				880
Pro	Leu	Glu	Val	Asp	Val	Thr	Ile	Gly	Lys	Thr	Trp	Ser			
			885								890				

<210> SEQ ID NO 11  
 <211> LENGTH: 893  
 <212> TYPE: PRT  
 <213> ORGANISM: Thermotoga maritima MSB8

<400> SEQUENCE: 11

Met	Ala	Arg	Leu	Phe	Leu	Phe	Asp	Gly	Thr	Ala	Leu	Ala	Tyr	Arg	Ala
1				5						10					15
Tyr	Tyr	Ala	Leu	Asp	Arg	Ser	Leu	Ser	Thr	Ser	Thr	Gly	Ile	Pro	Thr
			20							25					30
Asn	Ala	Thr	Tyr	Gly	Val	Ala	Arg	Met	Leu	Val	Arg	Phe	Ile	Lys	Asp
			35				40								45
His	Ile	Ile	Val	Gly	Lys	Asp	Tyr	Val	Ala	Val	Ala	Phe	Asp	Lys	Lys
			50			55					60				
Ala	Ala	Thr	Phe	Arg	His	Lys	Leu	Leu	Glu	Thr	Tyr	Lys	Ala	Gln	Arg
			65			70					75				80
Pro	Lys	Thr	Pro	Asp	Leu	Leu	Ile	Gln	Gln	Leu	Pro	Tyr	Ile	Lys	Lys
			85							90					95
Leu	Val	Glu	Ala	Leu	Gly	Met	Lys	Val	Leu	Glu	Val	Glu	Gly	Tyr	Glu
			100							105					110
Ala	Asp	Asp	Ile	Ile	Ala	Thr	Leu	Ala	Val	Lys	Gly	Leu	Pro	Leu	Phe
			115				120								125
Asp	Glu	Ile	Phe	Ile	Val	Thr	Gly	Asp	Lys	Asp	Met	Leu	Gln	Leu	Val
			130			135					140				
Asn	Glu	Lys	Ile	Lys	Val	Trp	Arg	Ile	Val	Lys	Gly	Ile	Ser	Asp	Leu
			145			150					155				160
Glu	Leu	Tyr	Asp	Ala	Gln	Lys	Val	Lys	Glu	Lys	Tyr	Gly	Val	Glu	Pro
			165							170					175
Gln	Gln	Ile	Pro	Asp	Leu	Leu	Ala	Leu	Thr	Gly	Asp	Glu	Ile	Asp	Asn
			180								185				190
Ile	Pro	Gly	Val	Thr	Gly	Ile	Gly	Glu	Lys	Thr	Ala	Val	Gln	Leu	Leu
			195								200				205
Glu	Lys	Tyr	Lys	Asp	Leu	Glu	Asp	Ile	Leu	Asn	His	Val	Arg	Glu	Leu
			210				215								220
Pro	Gln	Lys	Val	Arg	Lys	Ala	Leu	Leu	Arg	Asp	Arg	Glu	Asn	Ala	Ile
			225			230					235				240

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Leu Ser Lys Lys Leu Ala Ile Leu Glu Thr Asn Val Pro Ile Glu Ile  
 245 250 255

Asn Trp Glu Glu Leu Arg Tyr Gln Gly Tyr Asp Arg Glu Lys Leu Leu  
 260 265 270

Pro Leu Leu Lys Glu Leu Glu Phe Ala Ser Ile Met Lys Glu Leu Gln  
 275 280 285

Leu Tyr Glu Glu Ser Glu Pro Val Gly Tyr Arg Ile Val Lys Asp Leu  
 290 295 300

Val Glu Phe Glu Lys Leu Ile Glu Lys Leu Arg Glu Ser Pro Ser Phe  
 305 310 315 320

Ala Ile Asp Leu Glu Thr Ser Ser Leu Asp Pro Phe Asp Cys Asp Ile  
 325 330 335

Val Gly Ile Ser Val Ser Phe Lys Pro Lys Glu Ala Tyr Tyr Ile Pro  
 340 345 350

Leu His His Arg Asn Ala Gln Asn Leu Asp Glu Lys Glu Val Leu Lys  
 355 360 365

Lys Leu Lys Glu Ile Leu Glu Asp Pro Gly Ala Lys Ile Val Gly Gln  
 370 375 380

Asn Leu Lys Phe Asp Tyr Lys Val Leu Met Val Lys Gly Val Glu Pro  
 385 390 395 400

Val Pro Pro Tyr Phe Asp Thr Met Ile Ala Ala Tyr Leu Leu Glu Pro  
 405 410 415

Asn Glu Lys Lys Phe Asn Leu Asp Asp Leu Ala Leu Lys Phe Leu Gly  
 420 425 430

Tyr Lys Met Thr Ser Tyr Gln Glu Leu Met Ser Phe Ser Phe Pro Leu  
 435 440 445

Phe Gly Phe Ser Phe Ala Asp Val Pro Val Glu Lys Ala Ala Asn Tyr  
 450 455 460

Ser Cys Glu Asp Ala Asp Ile Thr Tyr Arg Leu Tyr Lys Thr Leu Ser  
 465 470 475 480

Leu Lys Leu His Glu Ala Asp Leu Glu Asn Val Phe Tyr Lys Ile Glu  
 485 490 495

Met Pro Leu Val Asn Val Leu Ala Arg Met Glu Leu Asn Gly Val Tyr  
 500 505 510

Val Asp Thr Glu Phe Leu Lys Lys Leu Ser Glu Glu Tyr Gly Lys Lys  
 515 520 525

Leu Glu Glu Leu Ala Glu Glu Ile Tyr Arg Ile Ala Gly Glu Pro Phe  
 530 535 540

Asn Ile Asn Ser Pro Lys Gln Val Ser Arg Ile Leu Phe Glu Lys Leu  
 545 550 555 560

Gly Ile Lys Pro Arg Gly Lys Thr Thr Lys Thr Gly Asp Tyr Ser Thr  
 565 570 575

Arg Ile Glu Val Leu Glu Glu Leu Ala Gly Glu His Glu Ile Ile Pro  
 580 585 590

Leu Ile Leu Glu Tyr Arg Lys Ile Gln Lys Leu Lys Ser Thr Tyr Ile  
 595 600 605

Asp Ala Leu Pro Lys Met Val Asn Pro Lys Thr Gly Arg Ile His Ala  
 610 615 620

Ser Phe Asn Gln Thr Gly Thr Ala Thr Gly Arg Leu Ser Ser Ser Asp  
 625 630 635 640

Pro Asn Leu Gln Asn Leu Pro Thr Lys Ser Glu Glu Gly Lys Glu Ile

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645					650					655					
Arg	Lys	Ala	Ile	Val	Pro	Gln	Asp	Pro	Asn	Trp	Trp	Ile	Val	Ser	Ala
			660					665					670		
Asp	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Ile	Leu	Ala	His	Leu	Ser	Gly	Asp
		675					680					685			
Glu	Asn	Leu	Leu	Arg	Ala	Phe	Glu	Glu	Gly	Ile	Asp	Val	His	Thr	Leu
	690					695					700				
Thr	Ala	Ser	Arg	Ile	Phe	Asn	Val	Lys	Pro	Glu	Glu	Val	Thr	Glu	Glu
	705					710					715				720
Met	Arg	Arg	Ala	Gly	Lys	Met	Val	Asn	Phe	Ser	Ile	Ile	Tyr	Gly	Val
			725						730					735	
Thr	Pro	Tyr	Gly	Leu	Ser	Val	Arg	Leu	Gly	Val	Pro	Val	Lys	Glu	Ala
			740					745					750		
Glu	Lys	Met	Ile	Val	Asn	Tyr	Phe	Val	Leu	Tyr	Pro	Lys	Val	Arg	Asp
		755					760					765			
Tyr	Ile	Gln	Arg	Val	Val	Ser	Glu	Ala	Lys	Glu	Lys	Gly	Tyr	Val	Arg
	770					775					780				
Thr	Leu	Phe	Gly	Arg	Lys	Arg	Asp	Ile	Pro	Gln	Leu	Met	Ala	Arg	Asp
	785					790					795				800
Arg	Asn	Thr	Gln	Ala	Glu	Gly	Glu	Arg	Ile	Ala	Ile	Asn	Thr	Pro	Ile
			805						810					815	
Gln	Gly	Thr	Ala	Ala	Asp	Ile	Ile	Lys	Leu	Ala	Met	Ile	Glu	Ile	Asp
			820					825					830		
Arg	Glu	Leu	Lys	Glu	Arg	Lys	Met	Arg	Ser	Lys	Met	Ile	Ile	Gln	Val
		835					840					845			
His	Asp	Glu	Leu	Val	Phe	Glu	Val	Pro	Asn	Glu	Glu	Lys	Asp	Ala	Leu
	850					855					860				
Val	Glu	Leu	Val	Lys	Asp	Arg	Met	Thr	Asn	Val	Val	Lys	Leu	Ser	Val
	865					870					875				880
Pro	Leu	Glu	Val	Asp	Val	Thr	Ile	Gly	Lys	Thr	Trp	Ser			
			885						890						

&lt;210&gt; SEQ ID NO 12

&lt;211&gt; LENGTH: 890

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Thermosipho melanesiensis* BI429

&lt;400&gt; SEQUENCE: 12

Met	Arg	Glu	Leu	Phe	Leu	Phe	Asp	Gly	Thr	Gly	Leu	Val	Tyr	Arg	Ala
1				5					10					15	
Phe	Tyr	Ala	Ile	Asp	Gln	Phe	Leu	Lys	Thr	Ser	Thr	Gly	Met	His	Thr
			20					25					30		
Asn	Ala	Leu	Tyr	Gly	Ile	Ala	Lys	Met	Leu	Ile	Lys	Phe	Leu	Lys	Glu
			35				40					45			
His	Val	Asn	Met	Glu	Lys	Asp	Ala	Cys	Ala	Phe	Ile	Leu	Asp	Ser	Lys
	50					55					60				
Gly	Gly	Ser	Lys	Lys	Arg	Lys	Glu	Ile	Leu	Lys	Asp	Tyr	Lys	Ser	Asn
	65					70					75				80
Arg	Pro	Glu	Thr	Pro	Asp	Leu	Ile	Leu	Glu	Gln	Leu	Pro	Tyr	Ile	Glu
				85					90					95	
Glu	Phe	Val	Asp	Ala	Phe	Gly	Val	Lys	Val	Leu	Lys	Leu	Leu	Gly	Tyr
			100					105						110	

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Glu Ala Asp Asp Ile Ile Ala Thr Ile Ala Lys Arg Phe Cys Asn Ala  
 115 120 125  
 Phe Glu Lys Val Asn Ile Ile Thr Gly Asp Lys Asp Leu Leu Gln Leu  
 130 135 140  
 Val Asp Glu Lys Val Tyr Val Trp Arg Ile Glu Arg Gly Ile Thr Glu  
 145 150 155 160  
 Leu Val Leu Tyr Asp Arg Lys Lys Val Phe Glu Lys Tyr Gly Val Phe  
 165 170 175  
 Pro Glu Gln Phe Gly Asp Tyr Leu Ser Leu Val Gly Asp Gln Ile Asp  
 180 185 190  
 Asn Ile Pro Gly Val Lys Gly Ile Gly Lys Lys Thr Ala Val Ser Leu  
 195 200 205  
 Leu Lys Lys Tyr Gly Thr Ile Asp Glu Val Leu Lys Asn Lys Lys Leu  
 210 215 220  
 Leu Thr Glu Lys Leu Gln Lys Leu Leu Glu Asn Ala Thr Glu Ser Leu  
 225 230 235 240  
 Glu Lys Ser Arg Gln Leu Val Gln Leu Ile Tyr Asp Val Pro Leu Asp  
 245 250 255  
 Val Asn Ile Glu Asp Leu Ile Tyr Lys Gly Tyr Asp Ser Lys Lys Leu  
 260 265 270  
 Leu Val Val Leu Lys Lys Tyr Glu Phe Ser Ser Ile Ile Lys Glu Leu  
 275 280 285  
 Gly Leu Lys Glu Glu Phe Glu Lys Lys Tyr Thr Ile Val Asn Ser Glu  
 290 295 300  
 Lys Glu Leu Ser Lys Leu Arg Lys Arg Ile Asp Glu Val Lys Thr Phe  
 305 310 315 320  
 Ser Ile Asp Thr Glu Thr Thr Ser Leu Asp Pro Phe Ser Ala Lys Leu  
 325 330 335  
 Val Gly Val Ser Ile Ser Thr Asn Glu Gly Glu Ala Tyr Tyr Ile Pro  
 340 345 350  
 Ile Ser His Val Ser Glu Asn Asn Leu Thr Lys Glu Ile Val Leu Lys  
 355 360 365  
 Phe Leu Lys Glu Ile Leu Glu Cys Glu Arg Tyr Asn Ile Val Gly Gln  
 370 375 380  
 Asn Leu Lys Phe Asp Tyr Lys Val Phe Met Val Asn Gly Ile Glu Pro  
 385 390 395 400  
 Gln Ile Pro His Phe Asp Thr Met Val Ala Ala Tyr Leu Ile Asn Pro  
 405 410 415  
 Glu Glu Arg Arg Tyr Asn Leu Glu Glu Leu Ala Leu Lys Tyr Leu Gly  
 420 425 430  
 Tyr Lys Met Ile Ser Phe Glu Glu Leu Val Asp Asn Asn Met Pro Leu  
 435 440 445  
 Phe Gly Asn Asp Phe Ser Phe Ile Ser Ile Glu Lys Ala Ala Glu Tyr  
 450 455 460  
 Ser Cys Glu Asp Val Asp Ile Thr Phe Arg Leu Tyr Ser Tyr Leu Ser  
 465 470 475 480  
 Lys Tyr Ile Gly Glu Met Lys Glu Leu Phe Tyr Asn Ile Glu Met Pro  
 485 490 495  
 Leu Ile Asn Val Leu Ala Gln Met Glu Leu Asn Gly Val Tyr Phe Asp  
 500 505 510  
 Val Asp Tyr Leu Lys Glu Leu Ser Lys Arg Tyr Glu Glu Glu Met Lys

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515	520	525
Lys Leu Glu Glu Lys Ile Phe Glu Ile Ser Gly Glu Gln Phe Asn Ile		
530	535	540
Asn Ser Ser Lys Gln Val Ala Glu Ile Leu Phe Glu Lys Leu Lys Leu		
545	550	555
Pro Ile Val Lys Lys Thr Ala Thr Gly Arg Asn Ser Thr Asn Ala Glu		
	565	570
		575
Val Leu Glu Glu Leu Ala Lys Asp Tyr Glu Ile Ala Arg Leu Ile Leu		
	580	585
		590
Glu Tyr Arg Lys Phe Gln Lys Leu Lys Ser Thr Tyr Val Asp Ser Ile		
	595	600
		605
Pro Ser Ser Val Asn Ile Thr Thr Asn Arg Val His Ser Ser Phe His		
	610	615
		620
Gln Thr Gly Thr Ser Thr Gly Arg Leu Ser Ser Ser Ala Pro Asn Leu		
	625	630
		635
Gln Asn Leu Pro Thr Arg Ser Glu Glu Gly Lys Glu Ile Arg His Ala		
	645	650
		655
Val Lys Pro Gln Phe Glu Asn Trp Tyr Ile Val Gly Ala Asp Tyr Ser		
	660	665
		670
Gln Ile Glu Leu Arg Val Leu Ala His Met Ser Glu Asp Glu Lys Leu		
	675	680
		685
Leu Asp Ala Phe Glu Asn Asp Tyr Asp Ile His Thr Ile Thr Ala Ser		
	690	695
		700
Lys Ile Phe Asn Val Ser Glu Leu Met Val Thr Glu Asp Met Arg Arg		
	705	710
		715
Ile Gly Lys Met Ile Asn Phe Ala Ile Ile Tyr Gly Ile Ser Pro Tyr		
	725	730
		735
Gly Leu Ser Arg Arg Ile Gly Leu Asn Val Asn Glu Thr Lys Lys Ile		
	740	745
		750
Ile Asp Asn Tyr Phe Lys Tyr Tyr Gln Gly Val Phe Glu Phe Ile Lys		
	755	760
		765
Lys Thr Ile Asp Phe Ala Lys Lys Asn Gly Phe Val Lys Thr Leu Phe		
	770	775
		780
Gly Arg Lys Arg Phe Ile Pro Gln Leu Lys Leu Lys Asn Lys Asn Leu		
	785	790
		795
Ile Gln Glu Gly Glu Arg Ile Ala Ile Asn Thr Pro Val Gln Gly Thr		
	805	810
		815
Ala Ala Asp Ile Ile Lys Ile Ala Met Val Lys Val His Asn Glu Leu		
	820	825
		830
Lys Arg Asn Ser Leu Lys Thr Lys Leu Ile Leu Gln Val His Asp Glu		
	835	840
		845
Leu Val Phe Glu Val Pro Phe Asp Glu Leu Gln Ile Val Lys Glu Ile		
	850	855
		860
Ile Lys Asp Lys Met Glu Asn Ala Val Lys Leu Lys Val Pro Leu Lys		
	865	870
		875
Val Asp Leu Tyr Glu Gly Arg Glu Trp Glu		
	885	890

<210> SEQ ID NO 13  
 <211> LENGTH: 876  
 <212> TYPE: PRT  
 <213> ORGANISM: Geobacillus sp. MKK-2005

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&lt;400&gt; SEQUENCE: 13

Met Lys Asn Lys Leu Val Leu Ile Asp Gly Asn Ser Val Ala Tyr Arg  
 1 5 10 15  
 Ala Phe Phe Ala Leu Pro Leu Leu His Asn Asp Lys Gly Ile His Thr  
 20 25 30  
 Asn Ala Val Tyr Gly Phe Thr Met Met Leu Asn Lys Ile Leu Ala Glu  
 35 40 45  
 Glu Arg Pro Thr His Leu Leu Val Ala Phe Asp Ala Gly Lys Thr Thr  
 50 55 60  
 Phe Arg His Glu Thr Phe Gln Glu Tyr Lys Gly Gly Arg Gln Gln Thr  
 65 70 75 80  
 Pro Pro Glu Leu Ser Glu Gln Phe Pro Leu Leu Arg Glu Leu Leu Asn  
 85 90 95  
 Ala Tyr Arg Ile Pro Ala Tyr Glu Leu Asp Arg Tyr Glu Ala Asp Asp  
 100 105 110  
 Ile Ile Gly Thr Leu Ala Ala Arg Ala Glu Gln Glu Gly Phe Glu Val  
 115 120 125  
 Lys Val Ile Ser Gly Asp Arg Asp Leu Thr Gln Leu Ala Ser Pro His  
 130 135 140  
 Val Thr Val Asp Ile Thr Lys Lys Gly Ile Thr Asp Ile Glu Pro Tyr  
 145 150 155 160  
 Thr Pro Glu Thr Val Glu Glu Lys Tyr Gly Leu Thr Pro Glu Gln Met  
 165 170 175  
 Val Asp Leu Lys Gly Leu Met Gly Asp Lys Ser Asp Asn Ile Pro Gly  
 180 185 190  
 Val Pro Gly Ile Gly Glu Lys Thr Ala Val Lys Leu Leu Lys Gln Phe  
 195 200 205  
 Gly Thr Val Glu Asn Val Leu Ala Ser Ile Asp Glu Ile Lys Gly Glu  
 210 215 220  
 Lys Leu Lys Glu Asn Leu Arg Gln Tyr Arg Asp Leu Ala Leu Leu Ser  
 225 230 235 240  
 Lys Gln Leu Ala Ala Ile Arg Arg Asp Ala Pro Val Glu Leu Ser Leu  
 245 250 255  
 Asp Asp Ile Ile Tyr Glu Gly Gln Asp Arg Glu Lys Val Ile Ala Leu  
 260 265 270  
 Phe Lys Glu Leu Gly Phe Gln Ser Phe Leu Glu Lys Met Asp Ala Pro  
 275 280 285  
 Thr Ala Glu Asp Glu Thr Pro Leu Met Glu Met Glu Phe Val Ile Ala  
 290 295 300  
 Asp Gly Ile Thr Asp Glu Met Leu Ala Asp Lys Ala Ala Leu Val Val  
 305 310 315 320  
 Glu Val Met Glu Glu Asn Tyr His Asp Ala Pro Ile Val Gly Ile Ala  
 325 330 335  
 Leu Val Asn Glu His Gly Arg Phe Phe Leu Arg Ala Glu Met Ala Leu  
 340 345 350  
 Ala Asp Ser Gln Phe Leu Ala Trp Leu Ala Asp Glu Thr Lys Lys Lys  
 355 360 365  
 Ser Met Phe Asp Ala Lys Arg Ala Ala Val Ala Leu Lys Trp Lys Gly  
 370 375 380  
 Ile Glu Leu Arg Gly Val Ala Phe Asp Leu Leu Leu Ala Ala Tyr Leu



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385	390	395	400
Leu Asn Pro Ala Gln Asp Ala Gly Asp Val Ala Ala Val Ala Lys Met	405	410	415
Lys Gln Tyr Glu Ala Val Arg Pro Asp Glu Ala Val Tyr Gly Lys Gly	420	425	430
Ala Lys Arg Ser Leu Pro Asp Glu Pro Thr Leu Ala Glu His Leu Val	435	440	445
Arg Lys Ala Ala Ala Ile Trp Ala Leu Glu Arg Pro Phe Leu Asp Glu	450	455	460
Leu Arg Ser Asn Glu Gln Asp Gly Leu Leu Ile Lys Leu Glu Gln Pro	465	470	475
Leu Ala Thr Ile Leu Ala Glu Met Glu Phe Thr Gly Ile Lys Val Asp	485	490	495
Thr Lys Arg Leu Glu Gln Met Gly Ser Glu Leu Ala Glu Gln Leu Arg	500	505	510
Ala Val Glu Gln Arg Ile Tyr Glu Leu Ala Gly Gln Glu Phe Asn Ile	515	520	525
Asn Ser Pro Lys Gln Leu Gly Ile Ile Leu Phe Glu Lys Leu Gln Leu	530	535	540
Pro Val Leu Lys Lys Thr Lys Thr Gly Tyr Ser Thr Ser Ala Asp Val	545	550	555
Leu Glu Lys Leu Ala Pro His His Glu Ile Val Glu Asn Ile Leu His	565	570	575
Tyr Arg Gln Leu Gly Lys Leu Gln Ser Thr Tyr Ile Glu Gly Leu Leu	580	585	590
Lys Val Val His Pro Asp Thr Gly Lys Val His Thr Met Phe Asn Gln	595	600	605
Ala Leu Thr Gln Thr Gly Arg Leu Ser Ser Ala Glu Pro Asn Leu Gln	610	615	620
Asn Ile Pro Ile Arg Leu Glu Glu Gly Arg Lys Ile Arg Gln Ala Phe	625	630	635
Val Pro Ser Glu Pro Asp Trp Leu Ile Phe Ala Ala Asp Tyr Ser Gln	645	650	655
Ile Glu Leu Arg Val Leu Ala His Ile Ala Asp Asp Asp Asn Leu Ile	660	665	670
Glu Ala Phe Arg Arg Asp Leu Asp Ile His Thr Lys Thr Ala Met Asp	675	680	685
Ile Phe His Val Ser Glu Glu Glu Val Thr Ala Thr Met Arg Arg Gln	690	695	700
Ala Lys Ala Val Asn Phe Gly Ile Val Tyr Gly Ile Ser Asp Tyr Gly	705	710	715
Leu Ala Gln Asn Leu Asn Ile Thr Arg Lys Glu Ala Ala Glu Phe Ile	725	730	735
Glu Arg Tyr Phe Ala Ser Phe Pro Gly Val Lys Arg Tyr Met Glu Thr	740	745	750
Ile Val Gln Glu Ala Lys Gln Lys Gly Tyr Val Thr Thr Leu Leu His	755	760	765
Arg Arg Arg Tyr Leu Pro Asp Ile Thr Ser Arg Asn Phe Asn Val Arg	770	775	780
Ser Phe Ala Glu Arg Thr Ala Met Asn Thr Pro Ile Gln Gly Ser Ala	785	790	795
			800

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Ala Asp Ile Ile Lys Lys Ala Met Ile Asp Leu Ala Ala Arg Leu Lys  
805 810 815

Glu Glu Arg Leu Gln Ala Arg Leu Leu Leu Gln Val His Asp Glu Leu  
820 825 830

Ile Leu Glu Ala Pro Lys Glu Glu Met Glu Arg Leu Cys Gln Leu Val  
835 840 845

Pro Glu Val Met Glu Gln Ala Val Ala Leu Arg Val Pro Leu Lys Val  
850 855 860

Asp Tyr His Tyr Gly Pro Thr Trp Tyr Asp Ala Lys  
865 870 875

<210> SEQ ID NO 14  
<211> LENGTH: 877  
<212> TYPE: PRT  
<213> ORGANISM: Bacillus caldotenax

<400> SEQUENCE: 14

Met Lys Lys Lys Leu Val Leu Ile Asp Gly Ser Ser Val Ala Tyr Arg  
1 5 10 15

Ala Phe Phe Ala Leu Pro Leu Leu His Asn Asp Lys Gly Ile His Thr  
20 25 30

Asn Ala Val Tyr Gly Phe Thr Met Met Leu Asn Lys Ile Leu Ala Glu  
35 40 45

Glu Glu Pro Thr His Met Leu Val Ala Phe Asp Ala Gly Lys Thr Thr  
50 55 60

Phe Arg His Glu Ala Phe Gln Glu Tyr Lys Gly Gly Arg Gln Gln Thr  
65 70 75 80

Pro Pro Glu Leu Ser Glu Gln Phe Pro Leu Leu Arg Glu Leu Leu Arg  
85 90 95

Ala Tyr Arg Ile Pro Ala Tyr Glu Leu Glu Asn Tyr Glu Ala Asp Asp  
100 105 110

Ile Ile Gly Thr Leu Ala Ala Arg Ala Glu Gln Glu Gly Phe Glu Val  
115 120 125

Lys Val Ile Ser Gly Asp Arg Asp Leu Thr Gln Leu Ala Ser Pro His  
130 135 140

Val Thr Val Asp Ile Thr Lys Lys Gly Ile Thr Asp Ile Glu Pro Tyr  
145 150 155 160

Thr Pro Glu Ala Val Arg Glu Lys Tyr Gly Leu Thr Pro Glu Gln Ile  
165 170 175

Val Asp Leu Lys Gly Leu Met Gly Asp Lys Ser Asp Asn Ile Pro Gly  
180 185 190

Val Pro Gly Ile Gly Glu Lys Thr Ala Val Lys Leu Leu Arg Gln Phe  
195 200 205

Gly Thr Val Glu Asn Val Leu Ala Ser Ile Asp Glu Ile Lys Gly Glu  
210 215 220

Lys Leu Lys Glu Thr Leu Arg Gln His Arg Glu Met Ala Leu Leu Ser  
225 230 235 240

Lys Lys Leu Ala Ala Ile Arg Arg Asp Ala Pro Val Glu Leu Ser Leu  
245 250 255

Asp Asp Ile Ala Tyr Gln Gly Glu Asp Arg Glu Lys Val Val Ala Leu  
260 265 270

Phe Lys Glu Leu Gly Phe Gln Ser Phe Leu Glu Lys Met Glu Ser Pro

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275				280				285							
Ser	Ser	Glu	Glu	Glu	Lys	Pro	Leu	Ala	Lys	Met	Ala	Phe	Thr	Leu	Ala
290						295					300				
Asp	Arg	Val	Thr	Glu	Glu	Met	Leu	Ala	Asp	Lys	Ala	Ala	Leu	Val	Val
305					310					315					320
Glu	Val	Val	Glu	Glu	Asn	Tyr	His	Asp	Ala	Pro	Ile	Val	Gly	Ile	Ala
				325					330					335	
Val	Val	Asn	Glu	His	Gly	Arg	Phe	Phe	Leu	Arg	Pro	Glu	Thr	Ala	Leu
			340					345						350	
Ala	Asp	Pro	Gln	Phe	Val	Ala	Trp	Leu	Gly	Asp	Glu	Thr	Lys	Lys	Lys
		355					360					365			
Ser	Met	Phe	Asp	Ser	Lys	Arg	Ala	Ala	Val	Ala	Leu	Lys	Trp	Lys	Gly
370						375					380				
Ile	Glu	Leu	Cys	Gly	Val	Ser	Phe	Asp	Leu	Leu	Leu	Ala	Ala	Tyr	Leu
385					390					395					400
Leu	Asp	Pro	Ala	Gln	Gly	Val	Asp	Asp	Val	Ala	Ala	Ala	Ala	Lys	Met
				405					410						415
Lys	Gln	Tyr	Glu	Ala	Val	Arg	Pro	Asp	Glu	Ala	Val	Tyr	Gly	Lys	Gly
			420					425						430	
Ala	Lys	Arg	Ala	Val	Pro	Asp	Glu	Pro	Val	Leu	Ala	Glu	His	Leu	Val
		435					440						445		
Arg	Lys	Ala	Ala	Ala	Ile	Trp	Ala	Leu	Glu	Arg	Pro	Phe	Leu	Asp	Glu
450						455					460				
Leu	Arg	Arg	Asn	Glu	Gln	Asp	Arg	Leu	Leu	Val	Glu	Leu	Glu	Gln	Pro
465					470					475					480
Leu	Ser	Ser	Ile	Leu	Ala	Glu	Met	Glu	Phe	Ala	Gly	Val	Lys	Val	Asp
				485					490						495
Thr	Lys	Arg	Leu	Glu	Gln	Met	Gly	Glu	Glu	Leu	Ala	Glu	Gln	Leu	Arg
			500					505						510	
Thr	Val	Glu	Gln	Arg	Ile	Tyr	Glu	Leu	Ala	Gly	Gln	Glu	Phe	Asn	Ile
		515					520						525		
Asn	Ser	Pro	Lys	Gln	Leu	Gly	Val	Ile	Leu	Phe	Glu	Lys	Leu	Gln	Leu
530						535					540				
Pro	Val	Leu	Lys	Lys	Ser	Lys	Thr	Gly	Tyr	Ser	Thr	Ser	Ala	Asp	Val
545					550					555					560
Leu	Glu	Lys	Leu	Ala	Pro	Tyr	His	Glu	Ile	Val	Glu	Asn	Ile	Leu	Gln
				565					570						575
His	Tyr	Arg	Gln	Leu	Gly	Lys	Leu	Gln	Ser	Thr	Tyr	Ile	Glu	Gly	Leu
			580					585						590	
Leu	Lys	Val	Val	Arg	Pro	Asp	Thr	Lys	Lys	Val	His	Thr	Ile	Phe	Asn
		595					600						605		
Gln	Ala	Leu	Thr	Gln	Thr	Gly	Arg	Leu	Ser	Ser	Thr	Glu	Pro	Asn	Leu
						615							620		
Gln	Asn	Ile	Pro	Ile	Arg	Leu	Glu	Glu	Gly	Arg	Lys	Ile	Arg	Gln	Ala
625					630						635				640
Phe	Val	Pro	Ser	Glu	Ser	Asp	Trp	Leu	Ile	Phe	Ala	Ala	Asp	Tyr	Ser
				645						650					655
Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala	His	Ile	Ala	Glu	Asp	Asp	Asn	Leu
				660					665						670
Met	Glu	Ala	Phe	Arg	Arg	Asp	Leu	Asp	Ile	His	Thr	Lys	Thr	Ala	Met
				675				680							685

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Asp Ile Phe Gln Val Ser Glu Asp Glu Val Thr Pro Asn Met Arg Arg  
 690 695 700  
 Gln Ala Lys Ala Val Asn Phe Gly Ile Val Tyr Gly Ile Ser Asp Tyr  
 705 710 715 720  
 Gly Leu Ala Gln Asn Leu Asn Ile Ser Arg Lys Glu Ala Ala Glu Phe  
 725 730 735  
 Ile Glu Arg Tyr Phe Glu Ser Phe Pro Gly Val Lys Arg Tyr Met Glu  
 740 745 750  
 Asn Ile Val Gln Glu Ala Lys Gln Lys Gly Tyr Val Thr Thr Leu Leu  
 755 760 765  
 His Arg Arg Arg Tyr Leu Pro Asp Ile Thr Ser Arg Asn Phe Asn Val  
 770 775 780  
 Arg Ser Phe Ala Glu Arg Met Ala Met Asn Thr Pro Ile Gln Gly Ser  
 785 790 795 800  
 Ala Ala Asp Ile Ile Lys Lys Ala Met Ile Asp Leu Asn Ala Arg Leu  
 805 810 815  
 Lys Glu Glu Arg Leu Gln Ala Arg Leu Leu Leu Gln Val His Asp Glu  
 820 825 830  
 Leu Ile Leu Glu Ala Pro Lys Glu Glu Met Glu Arg Leu Cys Arg Leu  
 835 840 845  
 Val Pro Glu Val Met Glu Gln Ala Val Thr Leu Arg Val Pro Leu Lys  
 850 855 860  
 Val Asp Tyr His Tyr Gly Ser Thr Trp Tyr Asp Ala Lys  
 865 870 875

&lt;210&gt; SEQ ID NO 15

&lt;211&gt; LENGTH: 872

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Bacillus caldondenax

&lt;400&gt; SEQUENCE: 15

Met Tyr Lys Phe Leu Ile Ile Asp Gly Ser Ser Leu Met Tyr Arg Ala  
 1 5 10 15  
 Tyr Tyr Ala Leu Pro Met Leu Thr Thr Ser Glu Gly Leu Pro Thr Asn  
 20 25 30  
 Ala Leu Tyr Gly Phe Thr Met Met Leu Ile Lys Leu Ile Glu Glu Glu  
 35 40 45  
 Lys Pro Asp Tyr Ile Ala Ile Ala Phe Asp Lys Lys Ala Pro Thr Phe  
 50 55 60  
 Arg His Lys Glu Tyr Gln Asp Tyr Lys Ala Thr Arg Gln Ala Met Pro  
 65 70 75 80  
 Glu Glu Leu Ala Glu Gln Val Asp Tyr Leu Lys Glu Ile Ile Asp Gly  
 85 90 95  
 Phe Asn Ile Lys Thr Leu Glu Leu Glu Gly Tyr Glu Ala Asp Asp Ile  
 100 105 110  
 Ile Gly Thr Ile Ser Lys Leu Ala Glu Glu Lys Gly Met Glu Val Leu  
 115 120 125  
 Val Val Thr Gly Asp Arg Asp Ala Leu Gln Leu Val Ser Asp Lys Val  
 130 135 140  
 Lys Ile Lys Ile Ser Lys Lys Gly Ile Thr Gln Met Glu Glu Phe Asp  
 145 150 155 160  
 Glu Lys Ala Ile Leu Glu Arg Tyr Gly Ile Thr Pro Gln Gln Phe Ile

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165				170				175							
Asp	Leu	Lys	Gly	Leu	Met	Gly	Asp	Lys	Ser	Asp	Asn	Ile	Pro	Gly	Val
			180							185				190	
Pro	Asn	Ile	Gly	Glu	Lys	Thr	Ala	Ile	Lys	Leu	Leu	Lys	Asp	Phe	Gly
			195				200							205	
Thr	Ile	Glu	Asn	Leu	Ile	Gln	Asn	Leu	Ser	Gln	Leu	Lys	Gly	Lys	Ile
			210				215							220	
Lys	Glu	Asn	Ile	Glu	Asn	Asn	Lys	Glu	Leu	Ala	Ile	Met	Ser	Lys	Arg
			225				230							235	240
Leu	Ala	Thr	Ile	Lys	Arg	Asp	Ile	Pro	Ile	Glu	Ile	Asp	Phe	Glu	Glu
			245							250				255	
Tyr	Lys	Val	Lys	Lys	Phe	Asn	Glu	Glu	Lys	Leu	Leu	Glu	Leu	Phe	Asn
			260							265				270	
Lys	Leu	Glu	Phe	Phe	Ser	Leu	Ile	Asp	Asn	Ile	Lys	Lys	Glu	Ser	Ser
			275							280				285	
Ile	Glu	Ile	Val	Asp	Asn	His	Lys	Val	Glu	Lys	Trp	Ser	Lys	Val	Asp
			290				295							300	
Ile	Lys	Glu	Leu	Val	Thr	Leu	Leu	Gln	Asp	Asn	Arg	Asn	Ile	Ala	Phe
			305								315				320
Tyr	Pro	Leu	Ile	Tyr	Glu	Gly	Glu	Ile	Lys	Lys	Ile	Ala	Phe	Ser	Phe
			325								330				335
Gly	Lys	Asp	Thr	Val	Tyr	Ile	Asp	Val	Phe	Gln	Thr	Glu	Asp	Leu	Lys
			340							345				350	
Glu	Ile	Phe	Glu	Lys	Glu	Asp	Phe	Glu	Phe	Thr	Thr	His	Glu	Ile	Lys
			355							360				365	
Asp	Phe	Leu	Val	Arg	Leu	Ser	Tyr	Lys	Gly	Ile	Glu	Cys	Lys	Ser	Lys
			370				375							380	
Tyr	Ile	Asp	Thr	Ala	Val	Met	Ala	Tyr	Leu	Leu	Asn	Pro	Ser	Glu	Ser
			385				390							395	400
Asn	Tyr	Asp	Leu	Asp	Arg	Val	Leu	Lys	Lys	Tyr	Leu	Lys	Val	Asp	Val
			405								410				415
Pro	Ser	Tyr	Glu	Gly	Ile	Phe	Gly	Lys	Gly	Arg	Asp	Lys	Lys	Lys	Ile
			420								425				430
Glu	Glu	Ile	Asp	Glu	Asn	Ile	Leu	Ala	Asp	Tyr	Ile	Cys	Ser	Arg	Cys
			435											445	
Val	Tyr	Leu	Phe	Asp	Leu	Lys	Glu	Lys	Leu	Met	Asn	Phe	Ile	Glu	Glu
			450				455							460	
Met	Asp	Met	Lys	Lys	Leu	Leu	Leu	Glu	Ile	Glu	Met	Pro	Leu	Val	Glu
			465				470				475				480
Val	Leu	Lys	Ser	Met	Glu	Val	Ser	Gly	Phe	Thr	Leu	Asp	Lys	Glu	Val
			485								490				495
Leu	Lys	Glu	Leu	Ser	Gln	Lys	Ile	Asp	Asp	Arg	Ile	Gly	Glu	Ile	Leu
			500							505				510	
Asp	Lys	Ile	Tyr	Lys	Glu	Ala	Gly	Tyr	Gln	Phe	Asn	Val	Asn	Ser	Pro
			515							520				525	
Lys	Gln	Leu	Ser	Glu	Phe	Leu	Phe	Glu	Lys	Leu	Asn	Leu	Pro	Val	Ile
			530				535							540	
Lys	Lys	Thr	Lys	Thr	Gly	Tyr	Ser	Thr	Asp	Ser	Glu	Val	Leu	Glu	Gln
			545				550				555				560
Leu	Val	Pro	Tyr	Asn	Asp	Ile	Val	Ser	Asp	Ile	Ile	Glu	Tyr	Arg	Gln
			565							570					575

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Leu Thr Lys Leu Lys Ser Thr Tyr Ile Asp Gly Phe Leu Pro Leu Met  
 580 585 590

Asp Glu Asn Asn Arg Val His Ser Asn Phe Lys Gln Met Val Thr Ala  
 595 600 605

Thr Gly Arg Ile Ser Ser Thr Glu Pro Asn Leu Gln Asn Ile Pro Ile  
 610 615 620

Arg Glu Glu Phe Gly Arg Gln Ile Arg Arg Ala Phe Ile Pro Arg Ser  
 625 630 635 640

Arg Asp Gly Tyr Ile Val Ser Ala Asp Tyr Ser Gln Ile Glu Leu Arg  
 645 650 655

Val Leu Ala His Val Ser Gly Asp Glu Lys Leu Ile Glu Ser Phe Met  
 660 665 670

Asn Asn Glu Asp Ile His Leu Arg Thr Ala Ser Glu Val Phe Lys Val  
 675 680 685

Pro Met Glu Lys Val Thr Pro Glu Met Arg Arg Ala Ala Lys Ala Val  
 690 695 700

Asn Phe Gly Ile Ile Tyr Gly Ile Ser Asp Tyr Gly Leu Ser Arg Asp  
 705 710 715 720

Leu Lys Ile Ser Arg Lys Glu Ala Lys Glu Tyr Ile Asn Asn Tyr Phe  
 725 730 735

Glu Arg Tyr Lys Gly Val Lys Asp Tyr Ile Glu Lys Ile Val Arg Phe  
 740 745 750

Ala Lys Glu Asn Gly Tyr Val Thr Thr Ile Met Asn Arg Arg Arg Tyr  
 755 760 765

Ile Pro Glu Ile Asn Ser Arg Asn Phe Thr Gln Arg Ser Gln Ala Glu  
 770 775 780

Arg Leu Ala Met Asn Ala Pro Ile Gln Gly Ser Ala Ala Asp Ile Ile  
 785 790 795 800

Lys Met Ala Met Val Lys Val Tyr Asn Asp Leu Lys Lys Leu Lys Leu  
 805 810 815

Lys Ser Lys Leu Ile Leu Gln Val His Asp Glu Leu Val Val Asp Thr  
 820 825 830

Tyr Lys Asp Glu Val Asp Ile Ile Lys Lys Ile Leu Lys Glu Asn Met  
 835 840 845

Glu Asn Val Val Gln Leu Lys Val Pro Leu Val Val Glu Ile Gly Val  
 850 855 860

Gly Pro Asn Trp Phe Leu Ala Lys  
 865 870

<210> SEQ ID NO 16  
 <211> LENGTH: 872  
 <212> TYPE: PRT  
 <213> ORGANISM: Thermoanaerobacter pseudethanolicus ATCC 33223

<400> SEQUENCE: 16

Met Ser Lys Phe Leu Val Ile Asp Gly Ser Ser Leu Met Tyr Arg Ala  
 1 5 10 15

Tyr Tyr Ala Leu Pro Met Leu Thr Thr Ser Glu Gly Leu His Thr Asn  
 20 25 30

Ala Leu Tyr Gly Phe Thr Met Met Leu Ile Lys Leu Ile Glu Glu Glu  
 35 40 45

Lys Pro Asp Tyr Ile Ala Ile Ala Phe Asp Lys Lys Ala Pro Thr Phe



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Met Asp Met Lys Arg Leu Leu Leu Glu Ile Glu Met Pro Leu Val Glu  
 465 470 475 480  
 Val Leu Lys Ser Met Glu Val Ser Gly Phe Thr Leu Asp Lys Glu Val  
 485 490 495  
 Leu Lys Glu Leu Ser Gln Lys Ile Asn Asp Arg Ile Ala Glu Ile Leu  
 500 505 510  
 Asp Lys Ile Tyr Lys Glu Ala Gly Tyr Gln Phe Asn Val Asn Ser Pro  
 515 520 525  
 Lys Gln Leu Ser Glu Phe Leu Phe Glu Lys Leu Asn Leu Pro Val Ile  
 530 535 540  
 Lys Lys Thr Lys Thr Gly Tyr Ser Thr Asp Ser Glu Val Leu Glu Gln  
 545 550 555 560  
 Leu Val Pro Tyr Asn Asn Ile Val Asn Asp Ile Ile Glu Tyr Arg Gln  
 565 570 575  
 Leu Thr Lys Leu Lys Ser Thr Tyr Ile Asn Gly Phe Leu Pro Leu Met  
 580 585 590  
 Asp Glu Asn Asn Arg Val His Ser Asn Phe Lys Gln Met Val Thr Ser  
 595 600 605  
 Thr Gly Arg Ile Ser Ser Thr Glu Pro Asn Leu Gln Asn Ile Pro Ile  
 610 615 620  
 Arg Glu Glu Phe Gly Arg Gln Ile Arg Arg Ala Phe Ile Pro Arg Thr  
 625 630 635 640  
 Lys Asp Gly Tyr Ile Val Ser Ala Asp Tyr Ser Gln Ile Glu Leu Arg  
 645 650 655  
 Val Leu Ala His Val Ser Gly Asp Glu Lys Leu Ile Glu Ser Phe Met  
 660 665 670  
 Asn Asn Glu Asp Ile His Leu Arg Thr Ala Ser Glu Val Phe Lys Val  
 675 680 685  
 Pro Met Glu Lys Val Thr Pro Glu Met Arg Arg Ala Ala Lys Ala Val  
 690 695 700  
 Asn Phe Gly Ile Ile Tyr Gly Ile Ser Asp Tyr Gly Leu Ser Arg Asp  
 705 710 715 720  
 Leu Lys Ile Ser Arg Lys Glu Ala Lys Glu Tyr Ile Asn Asn Tyr Phe  
 725 730 735  
 Glu Arg Tyr Lys Gly Val Lys Glu Tyr Ile Glu Lys Ile Val Arg Phe  
 740 745 750  
 Ala Lys Glu Asn Gly Tyr Val Ile Thr Ile Met Asn Arg Arg Arg Tyr  
 755 760 765  
 Ile Pro Glu Ile Asn Ser Arg Asn Phe Thr Gln Arg Ser Gln Ala Glu  
 770 775 780  
 Arg Leu Ala Met Asn Ala Pro Ile Gln Gly Ser Ala Ala Asp Ile Ile  
 785 790 795 800  
 Lys Met Ala Met Val Arg Val Tyr Asn Asp Leu Glu Lys Leu Lys Leu  
 805 810 815  
 Lys Ser Lys Leu Ile Leu Gln Val His Asp Glu Leu Val Val Asp Thr  
 820 825 830  
 Tyr Lys Asp Glu Val Glu Ile Val Lys Lys Ile Leu Lys Asp Asn Met  
 835 840 845  
 Glu Asn Val Val Gln Leu Lys Val Pro Leu Val Val Glu Ile Gly Val  
 850 855 860



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Gly Pro Asn Trp Phe Leu Ala Lys  
865 870

<210> SEQ ID NO 17  
<211> LENGTH: 855  
<212> TYPE: PRT  
<213> ORGANISM: Enterobacteria phage T5

<400> SEQUENCE: 17

Met Lys Ile Ala Val Val Asp Lys Ala Leu Asn Asn Thr Arg Tyr Asp  
1 5 10 15  
Lys His Phe Gln Leu Tyr Gly Glu Glu Val Asp Val Phe His Met Cys  
20 25 30  
Asn Glu Lys Leu Ser Gly Arg Leu Leu Lys Lys His Ile Thr Ile Gly  
35 40 45  
Thr Pro Glu Asn Pro Phe Asp Pro Asn Asp Tyr Asp Phe Val Ile Leu  
50 55 60  
Val Gly Ala Glu Pro Phe Leu Tyr Phe Ala Gly Lys Lys Gly Ile Gly  
65 70 75 80  
Asp Tyr Thr Gly Lys Arg Val Glu Tyr Asn Gly Tyr Ala Asn Trp Ile  
85 90 95  
Ala Ser Ile Ser Pro Ala Gln Leu His Phe Lys Pro Glu Met Lys Pro  
100 105 110  
Val Phe Asp Ala Thr Val Glu Asn Ile His Asp Ile Ile Asn Gly Arg  
115 120 125  
Glu Lys Ile Ala Lys Ala Gly Asp Tyr Arg Pro Ile Thr Asp Pro Asp  
130 135 140  
Glu Ala Glu Glu Tyr Ile Lys Met Val Tyr Asn Met Val Ile Gly Pro  
145 150 155 160  
Val Ala Phe Asp Ser Glu Thr Ser Ala Leu Tyr Cys Arg Asp Gly Tyr  
165 170 175  
Leu Leu Gly Val Ser Ile Ser His Gln Glu Tyr Gln Gly Val Tyr Ile  
180 185 190  
Asp Ser Asp Cys Leu Thr Glu Val Ala Val Tyr Tyr Leu Gln Lys Ile  
195 200 205  
Leu Asp Ser Glu Asn His Thr Ile Val Phe His Asn Leu Lys Phe Asp  
210 215 220  
Met His Phe Tyr Lys Tyr His Leu Gly Leu Thr Phe Asp Lys Ala His  
225 230 235 240  
Lys Glu Arg Arg Leu His Asp Thr Met Leu Gln His Tyr Val Leu Asp  
245 250 255  
Glu Arg Arg Gly Thr His Gly Leu Lys Ser Leu Ala Met Lys Tyr Thr  
260 265 270  
Asp Met Gly Asp Tyr Asp Phe Glu Leu Asp Lys Phe Lys Asp Asp Tyr  
275 280 285  
Cys Lys Ala His Lys Ile Lys Lys Glu Asp Phe Thr Tyr Asp Leu Ile  
290 295 300  
Pro Phe Asp Ile Met Trp Pro Tyr Ala Ala Lys Asp Thr Asp Ala Thr  
305 310 315 320  
Ile Arg Leu His Asn Phe Phe Leu Pro Lys Ile Glu Lys Asn Glu Lys  
325 330 335  
Leu Cys Ser Leu Tyr Tyr Asp Val Leu Met Pro Gly Cys Val Phe Leu  
340 345 350

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Gln Arg Val Glu Asp Arg Gly Val Pro Ile Ser Ile Asp Arg Leu Lys  
 355 360 365  
 Glu Ala Gln Tyr Gln Leu Thr His Asn Leu Asn Lys Ala Arg Glu Lys  
 370 375 380  
 Leu Tyr Thr Tyr Pro Glu Val Lys Gln Leu Glu Gln Asp Gln Asn Glu  
 385 390 395 400  
 Ala Phe Asn Pro Asn Ser Val Lys Gln Leu Arg Val Leu Leu Phe Asp  
 405 410 415  
 Tyr Val Gly Leu Thr Pro Thr Gly Lys Leu Thr Asp Thr Gly Ala Asp  
 420 425 430  
 Ser Thr Asp Ala Glu Ala Leu Asn Glu Leu Ala Thr Gln His Pro Ile  
 435 440 445  
 Ala Lys Thr Leu Leu Glu Ile Arg Lys Leu Thr Lys Leu Ile Ser Thr  
 450 455 460  
 Tyr Val Glu Lys Ile Leu Leu Ser Ile Asp Ala Asp Gly Cys Ile Arg  
 465 470 475 480  
 Thr Gly Phe His Glu His Met Thr Thr Ser Gly Arg Leu Ser Ser Ser  
 485 490 495  
 Gly Lys Leu Asn Leu Gln Gln Leu Pro Arg Asp Glu Ser Ile Ile Lys  
 500 505 510  
 Gly Cys Val Val Ala Pro Pro Gly Tyr Arg Val Ile Ala Trp Asp Leu  
 515 520 525  
 Thr Thr Ala Glu Val Tyr Tyr Ala Ala Val Leu Ser Gly Asp Arg Asn  
 530 535 540  
 Met Gln Gln Val Phe Ile Asn Met Arg Asn Glu Pro Asp Lys Tyr Pro  
 545 550 555 560  
 Asp Phe His Ser Asn Ile Ala His Met Val Phe Lys Leu Gln Cys Glu  
 565 570 575  
 Pro Arg Asp Val Lys Lys Leu Phe Pro Ala Leu Arg Gln Ala Ala Lys  
 580 585 590  
 Ala Ile Thr Phe Gly Ile Leu Tyr Gly Ser Gly Pro Ala Lys Val Ala  
 595 600 605  
 His Ser Val Asn Glu Ala Leu Leu Glu Gln Ala Ala Lys Thr Gly Glu  
 610 615 620  
 Pro Phe Val Glu Cys Thr Val Ala Asp Ala Lys Glu Tyr Ile Glu Thr  
 625 630 635 640  
 Tyr Phe Gly Gln Phe Pro Gln Leu Lys Arg Trp Ile Asp Lys Cys His  
 645 650 655  
 Asp Gln Ile Lys Asn His Gly Phe Ile Tyr Ser His Phe Gly Arg Lys  
 660 665 670  
 Arg Arg Leu His Asn Ile His Ser Glu Asp Arg Gly Val Gln Gly Glu  
 675 680 685  
 Glu Ile Arg Ser Gly Phe Asn Ala Ile Ile Gln Ser Ala Ser Ser Asp  
 690 695 700  
 Ser Leu Leu Leu Gly Ala Val Asp Ala Asp Asn Glu Ile Ile Ser Leu  
 705 710 715 720  
 Gly Leu Glu Gln Glu Met Lys Ile Val Met Leu Val His Asp Ser Val  
 725 730 735  
 Val Ala Ile Val Arg Glu Asp Leu Ile Asp Gln Tyr Asn Glu Ile Leu  
 740 745 750

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Ile Arg Asn Ile Gln Lys Asp Arg Gly Ile Ser Ile Pro Gly Cys Pro
      755                               760       765
Ile Gly Ile Asp Ser Asp Ser Glu Ala Gly Gly Ser Arg Asp Tyr Ser
      770                               775       780
Cys Gly Lys Met Lys Lys Gln His Pro Ser Ile Ala Cys Ile Asp Asp
      785                               790       795                               800
Asp Glu Tyr Thr Arg Tyr Val Lys Gly Val Leu Leu Asp Ala Glu Phe
      805                               810                               815
Glu Tyr Lys Lys Leu Ala Ala Met Asp Lys Glu His Pro Asp His Ser
      820                               825                               830
Lys Tyr Lys Asp Asp Lys Phe Ile Ala Val Cys Lys Asp Leu Asp Asn
      835                               840       845
Val Lys Arg Ile Leu Gly Ala
      850                               855
    
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<210> SEQ ID NO 18
<211> LENGTH: 704
<212> TYPE: PRT
<213> ORGANISM: Enterobacteria phage T7
    
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<400> SEQUENCE: 18

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Met Ile Val Ser Asp Ile Glu Ala Asn Ala Leu Leu Glu Ser Val Thr
 1      5      10      15
Lys Phe His Cys Gly Val Ile Tyr Asp Tyr Ser Thr Ala Glu Tyr Val
 20     25     30
Ser Tyr Arg Pro Ser Asp Phe Gly Ala Tyr Leu Asp Ala Leu Glu Ala
 35     40     45
Glu Val Ala Arg Gly Gly Leu Ile Val Phe His Asn Gly His Lys Tyr
 50     55     60
Asp Val Pro Ala Leu Thr Lys Leu Ala Lys Leu Gln Leu Asn Arg Glu
 65     70     75     80
Phe His Leu Pro Arg Glu Asn Cys Ile Asp Thr Leu Val Leu Ser Arg
 85     90     95
Leu Ile His Ser Asn Leu Lys Asp Thr Asp Met Gly Leu Leu Arg Ser
100    105    110
Gly Lys Leu Pro Gly Lys Arg Phe Gly Ser His Ala Leu Glu Ala Trp
115    120    125
Gly Tyr Arg Leu Gly Glu Met Lys Gly Glu Tyr Lys Asp Asp Phe Lys
130    135    140
Arg Met Leu Glu Glu Gln Gly Glu Glu Tyr Val Asp Gly Met Glu Trp
145    150    155    160
Trp Asn Phe Asn Glu Glu Met Met Asp Tyr Asn Val Gln Asp Val Val
165    170    175
Val Thr Lys Ala Leu Leu Glu Lys Leu Leu Ser Asp Lys His Tyr Phe
180    185    190
Pro Pro Glu Ile Asp Phe Thr Asp Val Gly Tyr Thr Thr Phe Trp Ser
195    200    205
Glu Ser Leu Glu Ala Val Asp Ile Glu His Arg Ala Ala Trp Leu Leu
210    215    220
Ala Lys Gln Glu Arg Asn Gly Phe Pro Phe Asp Thr Lys Ala Ile Glu
225    230    235    240
Glu Leu Tyr Val Glu Leu Ala Ala Arg Arg Ser Glu Leu Leu Arg Lys
245    250    255
    
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Leu	Thr	Glu	Thr	Phe	Gly	Ser	Trp	Tyr	Gln	Pro	Lys	Gly	Gly	Thr	Glu			
			260					265						270				
Met	Phe	Cys	His	Pro	Arg	Thr	Gly	Lys	Pro	Leu	Pro	Lys	Tyr	Pro	Arg			
		275						280					285					
Ile	Lys	Thr	Pro	Lys	Val	Gly	Gly	Ile	Phe	Lys	Lys	Pro	Lys	Asn	Lys			
	290					295						300						
Ala	Gln	Arg	Glu	Gly	Arg	Glu	Pro	Cys	Glu	Leu	Asp	Thr	Arg	Glu	Tyr			
305					310						315				320			
Val	Ala	Gly	Ala	Pro	Tyr	Thr	Pro	Val	Glu	His	Val	Val	Phe	Asn	Pro			
				325					330					335				
Ser	Ser	Arg	Asp	His	Ile	Gln	Lys	Lys	Leu	Gln	Glu	Ala	Gly	Trp	Val			
			340					345						350				
Pro	Thr	Lys	Tyr	Thr	Asp	Lys	Gly	Ala	Pro	Val	Val	Asp	Asp	Glu	Val			
		355					360						365					
Leu	Glu	Gly	Val	Arg	Val	Asp	Asp	Pro	Glu	Lys	Gln	Ala	Ala	Ile	Asp			
	370					375						380						
Leu	Ile	Lys	Glu	Tyr	Leu	Met	Ile	Gln	Lys	Arg	Ile	Gly	Gln	Ser	Ala			
385					390					395					400			
Glu	Gly	Asp	Lys	Ala	Trp	Leu	Arg	Tyr	Val	Ala	Glu	Asp	Gly	Lys	Ile			
				405					410					415				
His	Gly	Ser	Val	Asn	Pro	Asn	Gly	Ala	Val	Thr	Gly	Arg	Ala	Thr	His			
			420					425					430					
Ala	Phe	Pro	Asn	Leu	Ala	Gln	Ile	Pro	Gly	Val	Arg	Ser	Pro	Tyr	Gly			
		435					440						445					
Glu	Gln	Cys	Arg	Ala	Ala	Phe	Gly	Ala	Glu	His	His	Leu	Asp	Gly	Ile			
	450					455					460							
Thr	Gly	Lys	Pro	Trp	Val	Gln	Ala	Gly	Ile	Asp	Ala	Ser	Gly	Leu	Glu			
465					470					475					480			
Leu	Arg	Cys	Leu	Ala	His	Phe	Met	Ala	Arg	Phe	Asp	Asn	Gly	Glu	Tyr			
			485					490						495				
Ala	His	Glu	Ile	Leu	Asn	Gly	Asp	Ile	His	Thr	Lys	Asn	Gln	Ile	Ala			
			500					505					510					
Ala	Glu	Leu	Pro	Thr	Arg	Asp	Asn	Ala	Lys	Thr	Phe	Ile	Tyr	Gly	Phe			
		515					520					525						
Leu	Tyr	Gly	Ala	Gly	Asp	Glu	Lys	Ile	Gly	Gln	Ile	Val	Gly	Ala	Gly			
	530					535					540							
Lys	Glu	Arg	Gly	Lys	Glu	Leu	Lys	Lys	Lys	Phe	Leu	Glu	Asn	Thr	Pro			
545					550					555					560			
Ala	Ile	Ala	Ala	Leu	Arg	Glu	Ser	Ile	Gln	Gln	Thr	Leu	Val	Glu	Ser			
				565					570					575				
Ser	Gln	Trp	Val	Ala	Gly	Glu	Gln	Gln	Val	Lys	Trp	Lys	Arg	Arg	Trp			
			580						585					590				
Ile	Lys	Gly	Leu	Asp	Gly	Arg	Lys	Val	His	Val	Arg	Ser	Pro	His	Ala			
		595					600						605					
Ala	Leu	Asn	Thr	Leu	Leu	Gln	Ser	Ala	Gly	Ala	Leu	Ile	Cys	Lys	Leu			
	610					615							620					
Trp	Ile	Ile	Lys	Thr	Glu	Glu	Met	Leu	Val	Glu	Lys	Gly	Leu	Lys	His			
625					630					635					640			
Gly	Trp	Asp	Gly	Asp	Phe	Ala	Tyr	Met	Ala	Trp	Val	His	Asp	Glu	Ile			
				645					650						655			

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Gln Val Gly Cys Arg Thr Glu Glu Ile Ala Gln Val Val Ile Glu Thr  
660 665 670

Ala Gln Glu Ala Met Arg Trp Val Gly Asp His Trp Asn Phe Arg Cys  
675 680 685

Leu Leu Asp Thr Glu Gly Lys Met Gly Pro Asn Trp Ala Ile Cys His  
690 695 700

<210> SEQ ID NO 19  
<211> LENGTH: 1090  
<212> TYPE: PRT  
<213> ORGANISM: artificial sequence  
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<223> OTHER INFORMATION: consensus sequence  
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<222> LOCATION: (1)..(1)  
<223> OTHER INFORMATION: X= is M  
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<222> LOCATION: (2)..(2)  
<223> OTHER INFORMATION: X= is E  
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<222> LOCATION: (3)..(3)  
<223> OTHER INFORMATION: X= is Q  
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<223> OTHER INFORMATION: X= is K  
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<223> OTHER INFORMATION: X= is S  
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<223> OTHER INFORMATION: X= is L  
<220> FEATURE:  
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<222> LOCATION: (7)..(7)  
<223> OTHER INFORMATION: X= is W  
<220> FEATURE:  
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<222> LOCATION: (8)..(8)  
<223> OTHER INFORMATION: X= is D  
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<222> LOCATION: (9)..(9)  
<223> OTHER INFORMATION: X= is M or L  
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<223> OTHER INFORMATION: X= is R, E, T or F  
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<223> OTHER INFORMATION: X= is M, L, E or N  
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<222> LOCATION: (13)..(13)  
<223> OTHER INFORMATION: X= is L, F, N or S  
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<222> LOCATION: (14)..(14)  
<223> OTHER INFORMATION: X= is P, D, T, N or M  
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<223> OTHER INFORMATION: X= is L, E, D or V

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<220> FEATURE:  
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<222> LOCATION: (19)..(19)  
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<223> OTHER INFORMATION: X= is R  
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<220> FEATURE:  
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<223> OTHER INFORMATION: X= is F or Y  
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<223> OTHER INFORMATION: X= is H, F or Y  
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<223> OTHER INFORMATION: X= is A  
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<223> OTHER INFORMATION: X= is L  
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<223> OTHER INFORMATION: X= is Q or R  
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<223> OTHER INFORMATION: X= is L  
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<223> OTHER INFORMATION: X= is K, T, S, Q or H  
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<223> OTHER INFORMATION: X= is N  
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<223> OTHER INFORMATION: X= is A  
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<223> OTHER INFORMATION: X= is V, I, L, M or T

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<220> FEATURE:  
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<223> OTHER INFORMATION: X= is Y  
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<223> OTHER INFORMATION: X= is G  
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<223> OTHER INFORMATION: X= is F  
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<223> OTHER INFORMATION: X= is K, R, T, N or M  
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<223> OTHER INFORMATION: X= is M  
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<223> OTHER INFORMATION: X= is L  
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<223> OTHER INFORMATION: X= is V, I, N, R or L  
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<223> OTHER INFORMATION: X= is I or Y

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20     25     30

Arg Ala Xaa Xaa Ala Leu Xaa Xaa Leu Xaa Thr Ser Xaa Gly Xaa
35     40     45

Xaa Thr Asn Ala Xaa Tyr Gly Phe Xaa Xaa Met Leu Xaa Lys Xaa Xaa
50     55     60

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Val Xaa Phe Asp
65     70     75     80

Xaa Lys Xaa Xaa Thr Phe Xaa Arg His Xaa Xaa Xaa Xaa Xaa Tyr Lys
85     90     95

Xaa Xaa Arg Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Gln Xaa Xaa Xaa
100    105    110

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Glu Xaa Xaa
115    120    125

Gly Tyr Glu Ala Asp Asp Ile Ile Xaa Thr Xaa Xaa Xaa Xaa Xaa
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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ile Xaa Xaa Gly Asp Xaa Asp Xaa
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180 185 190  
Gly Val Xaa Pro Xaa Xaa Xaa Xaa Asp Xaa Xaa Xaa Leu Xaa Gly Xaa  
195 200 205  
Xaa Ser Asp Asn Ile Pro Gly Val Xaa Gly Ile Gly Glu Lys Thr Ala  
210 215 220  
Xaa Xaa Leu Leu Xaa Xaa Xaa Gly Xaa Xaa Glu Xaa Xaa Xaa Xaa Xaa  
225 230 235 240  
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245 250 255  
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530 535 540  
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				580				585						590	
Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Ile	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
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				610			615					620			
Leu	Xaa	Xaa	Xaa	Leu	Phe	Xaa	Xaa	Leu	Xaa	Leu	Pro	Xaa	Xaa	Xaa	Lys
				625			630				635				640
Thr	Xaa	Xaa	Thr	Gly	Xaa	Xaa	Ser	Thr	Xaa	Xaa	Glu	Val	Leu	Xaa	Xaa
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Leu	Xaa	Xaa	Xaa	His	Pro	Xaa	Xaa	Xaa	Ile	Xaa	Xaa	Xaa	Ile	Leu	Xaa
				660				665						670	
Xaa	Tyr	Arg	Xaa	Leu	Xaa	Lys	Leu	Lys	Ser	Thr	Tyr	Xaa	Asp	Xaa	Leu
				675				680					685		
Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Pro	Xaa	Thr	Gly	Arg	Xaa	His	Thr	Xaa
							695					700			
Phe	Asn	Gln	Thr	Xaa	Thr	Ala	Thr	Gly	Arg	Leu	Ser	Ser	Ser	Xaa	Pro
						710					715				720
Xaa	Asn	Leu	Gln	Xaa	Ile	Pro	Xaa	Xaa	Arg	Xaa	Glu	Xaa	Gly	Xaa	Xaa
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Ile	Arg	Xaa	Ala	Phe	Val	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Ile	Xaa	Xaa
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Ala	Asp	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Xaa	Leu	Ala	Xaa	His	Leu	Ser
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Xaa	Asp	Xaa	Asn	Leu	Ile	Xaa	Ala	Phe	Xaa	Xaa	Gly	Xaa	Xaa	Xaa	Xaa
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Val	Xaa	Xaa	Glu	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Val	Thr	Xaa	Xaa	Met	Arg
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Arg	Xaa	Ala	Lys	Xaa	Val	Asn	Xaa	Gly	Ile	Xaa	Tyr	Gly	Xaa	Ser	Xaa
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Phe	Gly	Arg	Arg	Arg	Xaa	Xaa	Pro	Xaa	Ile	Xaa	Ser	Arg	Asn	Xaa	Xaa
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Gly Xaa Xaa Trp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa	1010	1015	1020
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa	1025	1030	1035
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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa	1070	1075	1080
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		275					280					285			
Xaa	Leu	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Glu	Lys	Leu	Xaa	Xaa	Xaa	Xaa
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	305				310						315				320
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Val Xaa Xaa Glu Xaa Xaa Xaa Xaa Xaa	Xaa Val Thr Xaa Xaa Met Arg		805	810	815			
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420 425 430  
Xaa Gly Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Phe Xaa Xaa Asp Xaa Xaa Xaa  
435 440 445  
Xaa Ala Tyr Leu Leu Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Xaa  
450 455 460  
Xaa Ala Xaa Xaa Tyr Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
465 470 475 480  
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
485 490 495  
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala Xaa Xaa Xaa Ala Xaa Xaa Xaa  
500 505 510  
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Glu Xaa Xaa  
515 520 525  
Leu Xaa Xaa Leu Xaa Xaa Xaa Xaa Ile Glu Xaa Pro Leu Xaa Xaa Val Leu  
530 535 540  
Xaa Xaa Met Glu Xaa Xaa Gly Xaa Xaa Xaa Asp Xaa Xaa Xaa Leu Lys  
545 550 555 560  
Xaa Leu Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa  
565 570 575  
Ile Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala Gly Xaa  
580 585 590  
Xaa Phe Asn Xaa Asn Ser Xaa Lys Gln Leu Xaa Xaa Xaa Leu Phe Xaa  
595 600 605  
Xaa Leu Xaa Leu Pro Xaa Xaa Xaa Xaa Lys Thr Xaa Xaa Thr Gly Xaa Xaa  
610 615 620  
Ser Thr Xaa Xaa Glu Val Leu Xaa Xaa Leu Xaa Xaa Xaa His Pro Xaa  
625 630 635 640  
Xaa Xaa Ile Xaa Xaa Xaa Ile Leu Xaa Xaa Tyr Arg Xaa Leu Xaa Lys  
645 650 655  
Leu Lys Ser Thr Tyr Xaa Asp Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
660 665 670  
Pro Xaa Thr Gly Arg Xaa His Thr Xaa Phe Asn Gln Thr Xaa Thr Ala  
675 680 685  
Thr Gly Arg Leu Ser Ser Ser Xaa Pro Xaa Asn Leu Gln Xaa Ile Pro  
690 695 700  
Xaa Xaa Arg Xaa Glu Xaa Gly Xaa Xaa Ile Arg Xaa Ala Phe Val Xaa  
705 710 715 720  
Xaa Xaa Xaa Xaa Xaa Xaa Ile Xaa Xaa Ala Asp Tyr Ser Gln Ile Glu  
725 730 735  
Leu Arg Xaa Leu Ala Xaa His Leu Ser Xaa Asp Xaa Asn Leu Ile Xaa  
740 745 750  
Ala Phe Xaa Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Ile His  
755 760 765  
Thr Xaa Thr Ala Ser Xaa Ile Phe Xaa Val Xaa Xaa Glu Xaa Xaa Xaa  
770 775 780  
Xaa Xaa Xaa Val Thr Xaa Xaa Met Arg Arg Xaa Ala Lys Xaa Val Asn  
785 790 795 800  
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805	810	815
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa		
820	825	830
Xaa Ile Xaa Xaa Xaa Glu Ala Xaa Xaa Xaa Ile Glu Xaa Tyr Phe Xaa		
835	840	845
Xaa Xaa Pro Xaa Val Xaa Xaa Xaa Ile Xaa Xaa Xaa Xaa Xaa Xaa Ala		
850	855	860
Lys Xaa Xaa Gly Tyr Val Xaa Thr Leu Phe Gly Arg Arg Arg Xaa Xaa		
865	870	875
Pro Xaa Ile Xaa Ser Arg Asn Xaa Xaa Val Arg Xaa Xaa Xaa Glu Arg		
885	890	895
Xaa Ala Xaa Asn Xaa Pro Ile Gln Gly Thr Ala Ala Asp Ile Ile Lys		
900	905	910
Leu Ala Met Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa		
915	920	925
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa		
930	935	940
Leu Val Xaa Glu Val Xaa Xaa Glu Glu Xaa Xaa Xaa Val Xaa Xaa Xaa		
945	950	955
Xaa Lys Xaa Xaa Met Glu Xaa Xaa Val Xaa Leu Xaa Val Pro Xaa Xaa		
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Xaa Xaa Leu Xaa Val Xaa Xaa Xaa Xaa Xaa Gly Xaa Xaa Trp		
980	985	

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20           25           30
Phe Xaa Xaa Met Leu Xaa Lys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
35           40           45
Xaa Xaa Xaa Xaa Xaa Val Xaa Phe Asp Xaa Lys Xaa Xaa Thr Phe Xaa
50           55           60
Arg His Xaa Xaa Xaa Xaa Xaa Tyr Lys Xaa Xaa Arg Xaa Xaa Xaa Pro
65           70           75           80
Xaa Xaa Xaa Xaa Xaa Gln Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
85           90           95
Xaa Xaa Xaa Xaa Xaa Leu Glu Xaa Xaa Gly Tyr Glu Ala Asp Asp Ile
100          105          110
Ile Xaa Thr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
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1          5          10          15

Xaa Val Leu Xaa Xaa Met Glu Xaa Xaa Gly Xaa Xaa Xaa Asp Xaa Xaa
20          25          30

Xaa Leu Lys Xaa Leu Ser Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu
35          40          45

Xaa Xaa Xaa Ile Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa

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50	55	60
Ala Gly Xaa Xaa Phe Asn Xaa Asn Ser Xaa Lys Gln Leu Xaa Xaa Xaa 65 70 75 80		
Leu Phe Xaa Xaa Leu Xaa Leu Pro Xaa Xaa Xaa Lys Thr Xaa Xaa Thr 85 90 95		
Gly Xaa Xaa Ser Thr Xaa Xaa Glu Val Leu Xaa Xaa Leu Xaa Xaa Xaa 100 105 110		
His Pro Xaa Xaa Xaa Ile Xaa Xaa Xaa Ile Leu Xaa Xaa Tyr Arg Xaa 115 120 125		
Leu Xaa Lys Leu Lys Ser Thr Tyr Xaa Asp Xaa Leu Xaa Xaa Xaa Xaa 130 135 140		
Xaa Xaa Xaa Pro Xaa Thr Gly Arg Xaa His Thr Xaa Phe Asn Gln Thr 145 150 155 160		
Xaa Thr Ala Thr Gly Arg Leu Ser Ser Ser Xaa Pro Xaa Asn Leu Gln 165 170 175		
Xaa Ile Pro Xaa Xaa Arg Xaa Glu Xaa Gly Xaa Xaa Ile Arg Xaa Ala 180 185 190		
Phe Val Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ile Xaa Xaa Ala Asp Tyr Ser 195 200 205		
Gln Ile Glu Leu Arg Xaa Leu Ala Xaa His Leu Ser Xaa Asp Xaa Asn 210 215 220		
Leu Ile Xaa Ala Phe Xaa Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Xaa 225 230 235 240		
Asp Ile His Thr Xaa Thr Ala Ser Xaa Ile Phe Xaa Val Xaa Xaa Glu 245 250 255		
Xaa Xaa Xaa Xaa Xaa Xaa Val Thr Xaa Xaa Met Arg Arg Xaa Ala Lys 260 265 270		
Xaa Val Asn Xaa Gly Ile Xaa Tyr Gly Xaa Ser Xaa Xaa Gly Leu Ser 275 280 285		
Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa 290 295 300		
Xaa Xaa Xaa Xaa Ile Xaa Xaa Xaa Glu Ala Xaa Xaa Xaa Ile Glu Xaa 305 310 315 320		
Tyr Phe Xaa Xaa Xaa Pro Xaa Val Xaa Xaa Xaa Ile Xaa Xaa Xaa Xaa 325 330 335		
Xaa Xaa Ala Lys Xaa Xaa Gly Tyr Val Xaa Thr Leu Phe Gly Arg Arg 340 345 350		
Arg Xaa Xaa Pro Xaa Ile Xaa Ser Arg Asn Xaa Xaa Val Arg Xaa Xaa 355 360 365		
Xaa Glu Arg Xaa Ala Xaa Asn Xaa Pro Ile Gln Gly Thr Ala Ala Asp 370 375 380		
Ile Ile Lys Leu Ala Met Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa 385 390 395 400		
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Gln Xaa 405 410 415		
His Asp Glu Leu Val Xaa Glu Val Xaa Xaa Glu Glu Xaa Xaa Xaa Val 420 425 430		
Xaa Xaa Xaa Xaa Lys Xaa Xaa Met Glu Xaa Xaa Val Xaa Leu Xaa Val 435 440 445		
Pro Xaa Xaa Xaa Xaa Leu Xaa Val Xaa Xaa Xaa Xaa Gly Xaa Xaa Trp 450 455 460		



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gatcaacccc gctgccccac 20

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cgaagcccat ccccgctcag 20

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gcgcatgcaa gctgacctgg 20

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tcacgctcca aggcgggaac 20

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cctgctctgc cgcttcacgc 20

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gatgacgcat cctcagata atatccgg 28

<210> SEQ ID NO 30  
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cctgctctgc cgtttcacgc 20

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tgcattacga tcggaacgcc ca 22

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tgcattacga tcggaacgcc ca 22

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20

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&lt;220&gt; FEATURE:

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cgaagcccat ccccgctcag

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&lt;213&gt; ORGANISM: Thermus aquaticus

&lt;400&gt; SEQUENCE: 38

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1 5 10 15Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly  
20 25 30Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala  
35 40 45Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val  
50 55 60Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly  
65 70 75 80Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu  
85 90 95Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu  
100 105 110Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys  
115 120 125Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp  
130 135 140Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly  
145 150 155 160Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro  
165 170 175Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn  
180 185 190Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu  
195 200 205Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu  
210 215 220Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys  
225 230 235 240Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val  
245 250 255Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe  
260 265 270

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Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu  
 275 280 285

Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly  
 290 295 300

Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp  
 305 310 315 320

Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro  
 325 330 335

Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu  
 340 345 350

Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro  
 355 360 365

Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn  
 370 375 380

Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu  
 385 390 395 400

Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu  
 405 410 415

Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu  
 420 425 430

Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly  
 435 440 445

Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala  
 450 455 460

Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His  
 465 470 475 480

Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp  
 485 490 495

Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg  
 500 505 510

Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile  
 515 520 525

Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr  
 530 535 540

Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu  
 545 550 555 560

His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser  
 565 570 575

Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln  
 580 585 590

Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala  
 595 600 605

Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly  
 610 615 620

Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr  
 625 630 635 640

Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro  
 645 650 655

Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly  
 660 665 670

Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu



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Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu  
 210 215 220  
 Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys  
 225 230 235 240  
 Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val  
 245 250 255  
 Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe  
 260 265 270  
 Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu  
 275 280 285  
 Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly  
 290 295 300  
 Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp  
 305 310 315 320  
 Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro  
 325 330 335  
 Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu  
 340 345 350  
 Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro  
 355 360 365  
 Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn  
 370 375 380  
 Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu  
 385 390 395 400  
 Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu  
 405 410 415  
 Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu  
 420 425 430  
 Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly  
 435 440 445  
 Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala  
 450 455 460  
 Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His  
 465 470 475 480  
 Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp  
 485 490 495  
 Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Lys Lys Thr Gly Lys Arg  
 500 505 510  
 Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile  
 515 520 525  
 Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr  
 530 535 540  
 Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu  
 545 550 555 560  
 His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser  
 565 570 575  
 Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln  
 580 585 590  
 Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala  
 595 600 605  
 Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly



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Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly  
 145 150 155 160

Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro  
 165 170 175

Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn  
 180 185 190

Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu  
 195 200 205

Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu  
 210 215 220

Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys  
 225 230 235 240

Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val  
 245 250 255

Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe  
 260 265 270

Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu  
 275 280 285

Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly  
 290 295 300

Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp  
 305 310 315 320

Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro  
 325 330 335

Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu  
 340 345 350

Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro  
 355 360 365

Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn  
 370 375 380

Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu  
 385 390 395 400

Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu  
 405 410 415

Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu  
 420 425 430

Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly  
 435 440 445

Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala  
 450 455 460

Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His  
 465 470 475 480

Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp  
 485 490 495

Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Lys Lys Thr Gly Lys Arg  
 500 505 510

Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile  
 515 520 525

Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr  
 530 535 540

Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu





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Ala	Tyr	Lys	Ala	Gly	Arg	Ala	Pro	Thr	Pro	Glu	Asp	Phe	Pro	Arg	Gln
				85					90					95	
Leu	Ala	Leu	Ile	Lys	Glu	Leu	Val	Asp	Leu	Leu	Gly	Phe	Thr	Arg	Leu
			100					105					110		
Glu	Val	Pro	Gly	Tyr	Glu	Ala	Asp	Asp	Val	Leu	Ala	Thr	Leu	Ala	Lys
		115					120					125			
Lys	Ala	Glu	Lys	Glu	Gly	Tyr	Glu	Val	Arg	Ile	Leu	Thr	Ala	Asp	Arg
	130					135					140				
Asp	Leu	Tyr	Gln	Leu	Val	Ser	Asp	Arg	Val	Ala	Val	Leu	His	Pro	Glu
145					150					155					160
Gly	His	Leu	Ile	Thr	Pro	Glu	Trp	Leu	Trp	Glu	Lys	Tyr	Gly	Leu	Arg
				165					170						175
Pro	Glu	Gln	Trp	Val	Asp	Phe	Arg	Ala	Leu	Val	Gly	Asp	Pro	Ser	Asp
			180					185					190		
Asn	Leu	Pro	Gly	Val	Lys	Gly	Ile	Gly	Glu	Lys	Thr	Ala	Leu	Lys	Leu
		195					200					205			
Leu	Lys	Glu	Trp	Gly	Ser	Leu	Glu	Asn	Leu	Leu	Lys	Asn	Leu	Asp	Arg
	210					215					220				
Val	Lys	Pro	Glu	Asn	Val	Arg	Glu	Lys	Ile	Lys	Ala	His	Leu	Glu	Asp
225					230					235					240
Leu	Arg	Leu	Ser	Leu	Glu	Leu	Ser	Arg	Val	Arg	Thr	Asp	Leu	Pro	Leu
				245					250						255
Glu	Val	Asp	Leu	Ala	Gln	Gly	Arg	Glu	Pro	Asp	Arg	Glu	Gly	Leu	Arg
			260					265					270		
Ala	Phe	Leu	Glu	Arg	Leu	Glu	Phe	Gly	Ser	Leu	Leu	His	Glu	Phe	Gly
		275					280					285			
Leu	Leu	Glu	Ala	Pro	Ala	Pro	Leu	Glu	Glu	Ala	Pro	Trp	Pro	Pro	Pro
	290					295					300				
Glu	Gly	Ala	Phe	Val	Gly	Phe	Val	Leu	Ser	Arg	Pro	Glu	Pro	Met	Trp
305					310					315					320
Ala	Glu	Leu	Lys	Ala	Leu	Ala	Ala	Cys	Arg	Asp	Gly	Arg	Val	His	Arg
				325					330					335	
Ala	Ala	Asp	Pro	Leu	Ala	Gly	Leu	Lys	Asp	Leu	Lys	Glu	Val	Arg	Gly
			340					345					350		
Leu	Leu	Ala	Lys	Asp	Leu	Ala	Val	Leu	Ala	Ser	Arg	Glu	Gly	Leu	Asp
		355					360					365			
Leu	Val	Pro	Gly	Asp	Asp	Pro	Met	Leu	Leu	Ala	Tyr	Leu	Leu	Asp	Pro
	370					375					380				
Ser	Asn	Thr	Thr	Pro	Glu	Gly	Val	Ala	Arg	Arg	Tyr	Gly	Gly	Glu	Trp
385					390					395					400
Thr	Glu	Asp	Ala	Ala	His	Arg	Ala	Leu	Leu	Ser	Glu	Arg	Leu	His	Arg
				405					410					415	
Asn	Leu	Leu	Lys	Arg	Leu	Glu	Gly	Glu	Glu	Lys	Leu	Leu	Trp	Leu	Tyr
			420					425					430		
His	Glu	Val	Glu	Lys	Pro	Leu	Ser	Arg	Val	Leu	Ala	His	Met	Glu	Ala
		435					440					445			
Thr	Gly	Val	Arg	Leu	Asp	Val	Ala	Tyr	Leu	Gln	Ala	Leu	Ser	Leu	Glu
	450					455					460				
Leu	Ala	Glu	Glu	Ile	Arg	Arg	Leu	Glu	Glu	Glu	Val	Phe	Arg	Leu	Ala
465					470					475					480
Gly	His	Pro	Phe	Asn	Leu	Asn	Ser	Arg	Asp	Gln	Leu	Glu	Arg	Val	Leu



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1	5	10	15
Val Asp Gly His His Leu Ala Tyr Arg Thr Phe Phe Ala Leu Lys Gly	20	25	30
Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala	35	40	45
Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Tyr Lys Thr Val Phe	50	55	60
Val Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Glu	65	70	80
Ala Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln	85	90	95
Leu Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Phe Thr Arg Leu	100	105	110
Glu Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Thr Leu Ala Lys	115	120	125
Lys Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Arg	130	135	140
Asp Leu Tyr Gln Leu Val Ser Asp Arg Val Ala Val Leu His Pro Glu	145	150	160
Gly His Leu Ile Thr Pro Glu Trp Leu Trp Glu Lys Tyr Gly Leu Thr	165	170	175
Pro Glu Gln Trp Val Asp Phe Arg Ala Leu Val Gly Asp Pro Ser Asp	180	185	190
Asn Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Leu Lys Leu	195	200	205
Leu Lys Glu Trp Gly Ser Leu Glu Asn Leu Leu Lys Asn Leu Asp Arg	210	215	220
Val Lys Pro Glu Asn Val Arg Glu Lys Ile Lys Ala His Leu Glu Asp	225	230	240
Leu Arg Leu Ser Leu Glu Leu Ser Arg Val Arg Thr Asp Leu Pro Leu	245	250	255
Glu Val Asp Leu Ala Gln Gly Arg Glu Pro Asp Arg Glu Gly Leu Arg	260	265	270
Ala Phe Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly	275	280	285
Leu Leu Glu Ala Pro Ala Pro Leu Glu Glu Ala Pro Trp Pro Pro Pro	290	295	300
Glu Gly Ala Phe Val Gly Phe Val Leu Ser Arg Pro Glu Pro Met Trp	305	310	320
Ala Glu Leu Lys Ala Leu Ala Ala Cys Arg Asp Gly Arg Val His Arg	325	330	335
Ala Ala Asp Pro Leu Ala Gly Leu Lys Asp Leu Glu Glu Val Arg Gly	340	345	350
Leu Leu Ala Lys Asp Leu Cys Val Leu Ala Ser Arg Glu Gly Leu Asp	355	360	365
Leu Val Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro	370	375	380
Ser Asn Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp	385	390	400
Thr Glu Asp Ala Ala His Arg Ala Leu Leu Ser Glu Arg Leu His Arg	405	410	415

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Asn Leu Leu Lys Arg Leu Glu Gly Glu Glu Lys Leu Leu Trp Leu Tyr  
                   420                                  425                                  430

His Glu Val Glu Lys Pro Leu Ser Arg Val Leu Ala His Met Glu Ala  
                   435                                  440                                  445

Thr Gly Val Arg Leu Asp Val Ala Tyr Leu Gln Ala Leu Ser Leu Glu  
                   450                                  455                                  460

Leu Ala Glu Glu Ile Arg Arg Leu Glu Glu Glu Val Phe Arg Leu Ala  
 465                                  470                                  475                                  480

Gly His Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu  
                                   485                                  490                                  495

Phe Asp Glu Leu Arg Leu Pro Ala Leu Gly Lys Thr Lys Lys Thr Gly  
                                   500                                  505                                  510

Lys Arg Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His  
                   515                                  520                                  525

Pro Ile Val Glu Lys Ile Leu Gln His Arg Glu Leu Thr Lys Leu Lys  
                   530                                  535                                  540

Asn Thr Tyr Val Asp Pro Leu Pro Ser Leu Val His Pro Arg Thr Gly  
 545                                  550                                  555                                  560

Arg Leu His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu  
                                   565                                  570                                  575

Ser Ser Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu  
                                   580                                  585                                  590

Gly Gln Arg Ile Arg Arg Ala Phe Val Ala Glu Ala Gly Trp Ala Leu  
                   595                                  600                                  605

Val Ala Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu  
                   610                                  615                                  620

Ser Gly Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Lys Asp Ile  
 625                                  630                                  635                                  640

His Thr Gln Thr Ala Ser Trp Met Phe Gly Val Pro Pro Glu Ala Val  
                                   645                                  650                                  655

Asp Pro Leu Met Arg Arg Ala Ala Lys Thr Val Asn Phe Gly Val Leu  
                                   660                                  665                                  670

Tyr Gly Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr  
                   675                                  680                                  685

Glu Glu Ala Val Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys  
                   690                                  695                                  700

Val Arg Ala Trp Met Glu Lys Thr Leu Glu Glu Gly Arg Lys Arg Gly  
 705                                  710                                  715                                  720

Tyr Val Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Asn  
                                   725                                  730                                  735

Ala Arg Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Ile Asn  
                                   740                                  745                                  750

Met Pro Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val  
                   755                                  760                                  765

Lys Leu Phe Pro Arg Leu Arg Glu Met Gly Ala Arg Met Leu Leu Gln  
                   770                                  775                                  780

Val His Asp Glu Leu Leu Leu Glu Ala Pro Gln Ala Arg Ala Glu Glu  
 785                                  790                                  795                                  800

Val Ala Ala Leu Ala Lys Glu Ala Met Glu Lys Ala Tyr Pro Leu Ala  
                                   805                                  810                                  815



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Ile	Ala	Leu	Val	Asn	Glu	His	Gly	Arg	Phe	Phe	Met	Arg	Pro	Glu	Thr
			340					345					350		
Ala	Leu	Ala	Asp	Ser	Gln	Phe	Leu	Ala	Trp	Leu	Ala	Asp	Glu	Thr	Lys
			355				360					365			
Lys	Lys	Ser	Met	Phe	Asp	Ala	Lys	Arg	Ala	Val	Val	Ala	Leu	Lys	Trp
	370					375					380				
Lys	Gly	Ile	Asp	Val	Arg	Gly	Val	Ala	Phe	Asp	Leu	Leu	Leu	Ala	Ala
385					390					395					400
Tyr	Leu	Leu	Asn	Pro	Ala	Gln	Asp	Ala	Gly	Asp	Ile	Ala	Ala	Val	Ala
				405					410					415	
Lys	Met	Lys	Gln	Tyr	Glu	Ala	Val	Arg	Ser	Asp	Glu	Ala	Val	Tyr	Gly
			420					425					430		
Lys	Gly	Val	Lys	Arg	Ser	Leu	Pro	Asp	Glu	Gln	Thr	Leu	Ala	Glu	His
		435					440					445			
Leu	Val	Arg	Lys	Ala	Ala	Ala	Ile	Trp	Ala	Leu	Glu	Gln	Pro	Phe	Met
	450					455					460				
Asp	Asp	Leu	Arg	Asn	Asn	Glu	Gln	Asp	Gln	Leu	Leu	Thr	Lys	Leu	Glu
465					470					475					480
Gln	Pro	Leu	Ala	Ala	Ile	Leu	Ala	Glu	Met	Glu	Phe	Thr	Gly	Val	Asn
				485					490					495	
Val	Asp	Thr	Lys	Arg	Leu	Glu	Gln	Met	Gly	Ser	Glu	Leu	Ala	Glu	Gln
			500					505					510		
Leu	Arg	Ala	Ile	Glu	Gln	Arg	Ile	Tyr	Glu	His	Ala	Gly	Gln	Glu	Phe
		515					520					525			
Asn	Ile	Asn	Ser	Pro	Lys	Gln	Leu	Gly	Val	Ile	Leu	Phe	Glu	Lys	Leu
	530					535					540				
Gln	Leu	Pro	Val	Leu	Lys	Lys	Thr	Lys	Thr	Gly	Tyr	Ser	Thr	Ser	Ala
545					550					555					560
Asp	Val	Leu	Glu	Lys	Leu	Ala	Pro	His	His	Glu	Ile	Val	Glu	Asn	Ile
				565					570					575	
Leu	His	Tyr	Arg	Gln	Leu	Gly	Lys	Leu	Gln	Ser	Thr	Tyr	Ile	Glu	Gly
			580					585					590		
Leu	Leu	Lys	Val	Val	Arg	Pro	Asp	Thr	Gly	Lys	Val	His	Thr	Met	Phe
		595					600					605			
Asn	Gln	Thr	Leu	Thr	Gln	Thr	Gly	Arg	Leu	Ser	Ser	Ala	Glu	Pro	Asn
	610					615					620				
Leu	Gln	Asn	Ile	Pro	Ile	Arg	Leu	Glu	Glu	Gly	Arg	Lys	Ile	Arg	Gln
625					630					635					640
Ala	Phe	Val	Pro	Ser	Glu	Pro	Asp	Trp	Leu	Ile	Phe	Ala	Ala	Asp	Tyr
				645					650					655	
Ser	Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala	His	Ile	Ala	Asp	Asp	Asp	Asn
			660					665					670		
Leu	Ile	Glu	Ala	Phe	Gln	Arg	Asp	Leu	Asp	Ile	His	Thr	Lys	Thr	Ala
		675					680					685			
Met	Asp	Ile	Phe	His	Val	Ser	Glu	Glu	Glu	Val	Thr	Ala	Asn	Met	Arg
	690					695					700				
Arg	Gln	Ala	Lys	Ala	Val	Asn	Phe	Gly	Ile	Val	Tyr	Gly	Ile	Ser	Asp
705					710					715					720
Tyr	Gly	Leu	Ala	Gln	Asn	Leu	Asn	Ile	Thr	Arg	Lys	Glu	Ala	Ala	Glu
				725					730					735	
Phe	Ile	Glu	Arg	Tyr	Phe	Ala	Ser	Phe	Pro	Gly	Val	Arg	Arg	Tyr	Met





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Gly Glu Lys Val Lys Glu Lys Leu Arg Gln His Arg Asp Leu Ala Leu  
 225 230 235 240

Leu Ser Lys Gln Leu Ala Ser Ile Cys Arg Asp Ala Pro Val Glu Leu  
 245 250 255

Ser Leu Asp Ala Leu Val Tyr Glu Gly Gln Asp Arg Glu Lys Val Ile  
 260 265 270

Ala Leu Phe Lys Glu Leu Gly Phe Gln Ser Phe Leu Glu Lys Met Ala  
 275 280 285

Ala Pro Ala Ala Glu Gly Arg Lys Pro Leu Glu Glu Met Glu Phe Ala  
 290 295 300

Ile Val Asp Val Ile Thr Glu Glu Met Leu Ala Asp Lys Ala Ala Leu  
 305 310 315 320

Val Val Glu Val Met Glu Glu Asn Tyr His Asp Ala Pro Ile Val Gly  
 325 330 335

Ile Ala Leu Val Asn Glu His Gly Arg Phe Phe Met Arg Pro Glu Thr  
 340 345 350

Ala Leu Ala Asp Ser Gln Phe Leu Ala Trp Leu Ala Asp Glu Glu Lys  
 355 360 365

Lys Lys Ser Met Phe Asp Ala Lys Arg Cys Val Val Ala Leu Lys Trp  
 370 375 380

Lys Gly Ile Asp Val Arg Gly Val Ala Phe Asp Leu Leu Leu Ala Ala  
 385 390 395 400

Tyr Leu Leu Asn Pro Ala Gln Asp Ala Gly Asp Ile Ala Ala Val Ala  
 405 410 415

Lys Met Lys Gln Tyr Glu Ala Val Arg Ser Asp Glu Ala Val Tyr Gly  
 420 425 430

Lys Gly Val Lys Arg Ser Leu Pro Asp Glu Gln Thr Leu Ala Glu His  
 435 440 445

Leu Val Arg Lys Ala Ala Ala Ile Trp Ala Leu Glu Gln Pro Phe Met  
 450 455 460

Asp Asp Leu Arg Asn Asn Glu Gln Asp Gln Leu Leu Thr Lys Leu Glu  
 465 470 475 480

Gln Pro Leu Ala Ala Ile Leu Ala Glu Met Glu Phe Thr Gly Val Asn  
 485 490 495

Val Asp Thr Lys Arg Leu Glu Gln Met Gly Ser Glu Leu Ala Glu Gln  
 500 505 510

Leu Arg Ala Ile Glu Gln Arg Ile Tyr Glu His Ala Gly Gln Glu Phe  
 515 520 525

Asn Ile Asn Ser Pro Lys Gln Leu Gly Val Ile Leu Phe Glu Lys Leu  
 530 535 540

Gln Leu Pro Val Leu Lys Lys Thr Lys Thr Gly Tyr Ser Thr Ser Ala  
 545 550 555 560

Asp Val Leu Glu Lys Leu Ala Pro His His Glu Ile Val Glu Asn Ile  
 565 570 575

Leu His Tyr Arg Gln Leu Gly Lys Leu Gln Ser Thr Tyr Ile Glu Gly  
 580 585 590

Leu Leu Lys Val Val Arg Pro Asp Thr Gly Lys Val His Thr Met Phe  
 595 600 605

Asn Gln Thr Leu Thr Gln Thr Gly Arg Leu Ser Ser Ala Glu Pro Asn  
 610 615 620

Leu Gln Asn Ile Pro Ile Arg Leu Glu Glu Gly Arg Lys Ile Arg Gln

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625		630		635		640
Ala Phe Val Pro Ser Glu Pro Asp Trp Leu Ile Phe Ala Ala Asp Tyr						
		645		650		655
Ser Gln Ile Glu Leu Arg Val Leu Ala His Ile Ala Asp Asp Asp Asn						
		660		665		670
Leu Ile Glu Ala Phe Gln Arg Asp Leu Asp Ile His Thr Lys Thr Ala						
		675		680		685
Met Asp Ile Phe His Val Ser Glu Glu Glu Val Thr Ala Asn Met Arg						
		690		695		700
Arg Gln Ala Lys Ala Val Asn Phe Gly Ile Val Tyr Gly Ile Ser Asp						
		705		710		715
Tyr Gly Leu Ala Gln Asn Leu Asn Ile Thr Arg Lys Glu Ala Ala Glu						
		725		730		735
Phe Ile Glu Arg Tyr Phe Ala Ser Phe Pro Gly Val Arg Arg Tyr Met						
		740		745		750
Glu Asn Ile Val Gln Glu Ala Lys Gln Lys Gly Tyr Val Thr Thr Leu						
		755		760		765
Leu His Arg Arg Arg Tyr Leu Pro Asp Ile Thr Ser Arg Asn Phe Asn						
		770		775		780
Val Arg Ser Phe Ala Glu Arg Thr Ala Ile Asn Thr Pro Ile Gln Gly						
		785		790		795
Ser Ala Ala Asp Ile Ile Lys Lys Ala Met Ile Asp Leu Ala Ala Arg						
		805		810		815
Leu Lys Glu Glu Gln Leu Gln Ala Arg Leu Leu Leu Gln Val His Asp						
		820		825		830
Glu Leu Ile Leu Glu Ala Pro Lys Glu Glu Ile Glu Arg Leu Cys Glu						
		835		840		845
Leu Val Pro Glu Val Met Glu Gln Ala Val Ser Ser Val Pro Leu Lys						
		850		855		860
Val Asp Tyr His Tyr Gly Pro Thr Trp Tyr Asp Ala Lys						
		865		870		875

<210> SEQ ID NO 45  
 <211> LENGTH: 928  
 <212> TYPE: PRT  
 <213> ORGANISM: Escherichia coli

<400> SEQUENCE: 45

Met Val Gln Ile Pro Gln Asn Pro Leu Ile Leu Val Asp Gly Ser Ser						
1		5		10		15
Tyr Leu Tyr Arg Ala Tyr His Ala Phe Pro Pro Leu Thr Asn Ser Ala						
		20		25		30
Gly Glu Pro Thr Gly Ala Met Tyr Gly Val Leu Asn Met Leu Arg Ser						
		35		40		45
Leu Ile Met Gln Tyr Lys Pro Thr His Ala Ala Val Val Phe Asp Ala						
		50		55		60
Lys Gly Lys Thr Phe Arg Asp Glu Leu Phe Glu His Tyr Lys Ser His						
		65		70		75
Arg Pro Pro Met Pro Asp Asp Leu Arg Ala Gln Ile Glu Pro Leu His						
		85		90		95
Ala Met Val Lys Ala Met Gly Leu Pro Leu Leu Ala Val Ser Gly Val						
		100		105		110

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Glu Ala Asp Asp Val Ile Gly Thr Leu Ala Arg Glu Ala Glu Lys Ala  
 115 120 125  
 Gly Arg Pro Val Leu Ile Ser Thr Gly Asp Lys Asp Met Ala Gln Leu  
 130 135 140  
 Val Thr Pro Asn Ile Thr Leu Ile Asn Thr Met Thr Asn Thr Ile Leu  
 145 150 155 160  
 Gly Pro Glu Glu Val Val Asn Lys Tyr Gly Val Pro Pro Glu Leu Ile  
 165 170 175  
 Ile Asp Phe Leu Ala Leu Met Gly Asp Ser Ser Asp Asn Ile Pro Gly  
 180 185 190  
 Val Pro Gly Val Gly Glu Lys Thr Ala Gln Ala Leu Leu Gln Gly Leu  
 195 200 205  
 Gly Gly Leu Asp Thr Leu Tyr Ala Glu Pro Glu Lys Ile Ala Gly Leu  
 210 215 220  
 Ser Phe Arg Gly Ala Lys Thr Met Ala Ala Lys Leu Glu Gln Asn Lys  
 225 230 235 240  
 Glu Val Ala Tyr Leu Ser Tyr Gln Leu Ala Thr Ile Lys Thr Asp Val  
 245 250 255  
 Glu Leu Glu Leu Thr Cys Glu Gln Leu Glu Val Gln Gln Pro Ala Ala  
 260 265 270  
 Glu Glu Leu Leu Gly Leu Phe Lys Lys Tyr Glu Phe Lys Arg Trp Thr  
 275 280 285  
 Ala Asp Val Glu Ala Gly Lys Trp Leu Gln Ala Lys Gly Ala Lys Pro  
 290 295 300  
 Ala Ala Lys Pro Gln Glu Thr Ser Val Ala Asp Glu Ala Pro Glu Val  
 305 310 315 320  
 Thr Ala Thr Val Ile Ser Tyr Asp Asn Tyr Val Thr Ile Leu Asp Glu  
 325 330 335  
 Glu Thr Leu Lys Ala Trp Ile Ala Lys Leu Glu Lys Ala Pro Val Phe  
 340 345 350  
 Ala Phe Asp Thr Glu Thr Asp Ser Leu Asp Asn Ile Ser Ala Asn Leu  
 355 360 365  
 Val Gly Leu Ser Phe Ala Ile Glu Pro Gly Val Ala Ala Tyr Ile Pro  
 370 375 380  
 Val Ala His Asp Tyr Leu Asp Ala Pro Asp Gln Ile Ser Arg Glu Arg  
 385 390 395 400  
 Ala Leu Glu Leu Leu Lys Pro Leu Leu Glu Asp Glu Lys Ala Leu Lys  
 405 410 415  
 Val Gly Gln Asn Leu Lys Tyr Asp Arg Gly Ile Leu Ala Asn Tyr Gly  
 420 425 430  
 Ile Glu Leu Arg Gly Ile Ala Phe Asp Thr Met Leu Glu Ser Tyr Ile  
 435 440 445  
 Leu Asn Ser Val Ala Gly Arg His Asp Met Asp Ser Leu Ala Glu Arg  
 450 455 460  
 Trp Leu Lys His Lys Thr Ile Thr Phe Glu Glu Ile Ala Gly Lys Gly  
 465 470 475 480  
 Lys Asn Gln Leu Thr Phe Asn Gln Ile Ala Leu Glu Glu Ala Gly Arg  
 485 490 495  
 Tyr Ala Ala Glu Asp Ala Asp Val Thr Leu Gln Leu His Leu Lys Met  
 500 505 510  
 Trp Pro Asp Leu Gln Lys His Lys Gly Pro Leu Asn Val Phe Glu Asn

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515					520					525					
Ile	Glu	Met	Pro	Leu	Val	Pro	Val	Leu	Ser	Arg	Ile	Glu	Arg	Asn	Gly
530						535					540				
Val	Lys	Ile	Asp	Pro	Lys	Val	Leu	His	Asn	His	Ser	Glu	Glu	Leu	Thr
545					550					555					560
Leu	Arg	Leu	Ala	Glu	Leu	Glu	Lys	Lys	Ala	His	Glu	Ile	Ala	Gly	Glu
				565					570					575	
Glu	Phe	Asn	Leu	Ser	Ser	Thr	Lys	Gln	Leu	Gln	Thr	Ile	Leu	Phe	Glu
			580					585					590		
Lys	Gln	Gly	Ile	Lys	Pro	Leu	Lys	Lys	Thr	Pro	Gly	Gly	Ala	Pro	Ser
		595					600					605			
Thr	Ser	Glu	Glu	Val	Leu	Glu	Glu	Leu	Ala	Leu	Asp	Tyr	Pro	Leu	Pro
		610				615					620				
Lys	Val	Ile	Leu	Glu	Tyr	Arg	Gly	Leu	Ala	Lys	Leu	Lys	Ser	Thr	Tyr
625					630					635					640
Thr	Asp	Lys	Leu	Pro	Leu	Met	Ile	Asn	Pro	Lys	Thr	Gly	Arg	Val	His
				645					650					655	
Thr	Ser	Tyr	His	Gln	Ala	Val	Thr	Ala	Thr	Gly	Arg	Leu	Ser	Ser	Thr
			660					665					670		
Asp	Pro	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Asn	Glu	Glu	Gly	Arg	Arg
		675					680					685			
Ile	Arg	Gln	Ala	Phe	Ile	Ala	Pro	Glu	Asp	Tyr	Val	Ile	Val	Ser	Ala
	690					695					700				
Asp	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Ile	Met	Ala	His	Leu	Ser	Arg	Asp
705					710					715					720
Lys	Gly	Leu	Leu	Thr	Ala	Phe	Ala	Glu	Gly	Lys	Asp	Ile	His	Arg	Ala
				725					730					735	
Thr	Ala	Ala	Glu	Val	Phe	Gly	Leu	Pro	Leu	Glu	Thr	Val	Thr	Ser	Glu
			740					745					750		
Gln	Arg	Arg	Ser	Ala	Lys	Ala	Ile	Asn	Phe	Gly	Leu	Ile	Tyr	Gly	Met
		755					760					765			
Ser	Ala	Phe	Gly	Leu	Ala	Arg	Gln	Leu	Asn	Ile	Pro	Arg	Lys	Glu	Ala
		770				775					780				
Gln	Lys	Tyr	Met	Asp	Leu	Tyr	Phe	Glu	Arg	Tyr	Pro	Gly	Val	Leu	Gln
785					790					795					800
Tyr	Met	Glu	Arg	Thr	Arg	Ala	Gln	Ala	Lys	Glu	Gln	Gly	Tyr	Val	Glu
				805					810					815	
Thr	Leu	Asp	Gly	Arg	Arg	Leu	Tyr	Leu	Pro	Asp	Ile	Lys	Ser	Ser	Asn
			820					825					830		
Gly	Ala	Arg	Arg	Ala	Ala	Ala	Glu	Arg	Ala	Ala	Ile	Asn	Ala	Pro	Met
		835					840					845			
Gln	Gly	Thr	Ala	Ala	Asp	Ile	Ile	Lys	Arg	Ala	Met	Ile	Ala	Val	Asp
		850				855					860				
Ala	Trp	Leu	Gln	Ala	Glu	Gln	Pro	Arg	Val	Arg	Met	Ile	Met	Gln	Val
865					870					875					880
His	Asp	Glu	Leu	Val	Phe	Glu	Val	His	Lys	Asp	Asp	Val	Asp	Ala	Val
				885					890					895	
Ala	Lys	Gln	Ile	His	Gln	Leu	Met	Glu	Asn	Cys	Thr	Arg	Leu	Asp	Val
			900					905					910		
Pro	Leu	Leu	Val	Glu	Val	Gly	Ser	Gly	Glu	Asn	Trp	Asp	Gln	Ala	His
			915				920						925		

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<210> SEQ ID NO 46
<211> LENGTH: 928
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli

<400> SEQUENCE: 46

Met Val Gln Ile Pro Gln Asn Pro Leu Ile Leu Val Asp Gly Ser Ser
 1                               5                               10
Tyr Leu Tyr Arg Ala Tyr His Ala Phe Pro Pro Leu Thr Asn Ser Ala
 20                               25                               30
Gly Glu Pro Thr Gly Ala Met Tyr Gly Val Leu Asn Met Leu Arg Ser
 35                               40                               45
Leu Ile Met Gln Tyr Lys Pro Thr Thr Ala Ala Val Val Phe Asp Ala
 50                               55                               60
Lys Gly Lys Thr Phe Arg Asp Glu Leu Phe Glu His Tyr Lys Ser His
 65                               70                               75                               80
Arg Pro Pro Met Pro Asp Asp Leu Arg Ala Gln Ile Glu Pro Leu His
 85                               90                               95
Ala Met Val Lys Ala Met Gly Leu Pro Leu Leu Ala Val Ser Gly Val
 100                              105                              110
Glu Ala Asp Asp Val Ile Gly Thr Leu Ala Arg Glu Ala Glu Lys Ala
 115                              120                              125
Gly Arg Pro Val Leu Ile Ser Thr Gly Asp Lys Asp Met Ala Gln Leu
 130                              135                              140
Val Thr Pro Asn Ile Thr Leu Ile Asn Thr Met Thr Asn Thr Ile Leu
 145                              150                              155                              160
Gly Pro Glu Glu Val Val Asn Lys Tyr Gly Val Pro Pro Glu Leu Ile
 165                              170                              175
Ile Asp Phe Leu Ala Leu Met Gly Asp Ser Ser Asp Asn Ile Pro Gly
 180                              185                              190
Val Pro Gly Val Gly Glu Lys Thr Ala Gln Ala Leu Leu Gln Gly Leu
 195                              200                              205
Gly Gly Leu Asp Thr Leu Tyr Ala Glu Pro Glu Lys Ile Ala Gly Leu
 210                              215                              220
Ser Phe Arg Gly Ala Lys Thr Met Ala Ala Lys Leu Glu Gln Asn Lys
 225                              230                              235                              240
Glu Val Ala Tyr Leu Ser Tyr Gln Leu Ala Thr Ile Lys Thr Asp Val
 245                              250                              255
Glu Leu Glu Leu Thr Cys Glu Gln Leu Glu Val Gln Gln Pro Ala Ala
 260                              265                              270
Glu Glu Leu Leu Gly Leu Phe Lys Lys Tyr Glu Phe Lys Arg Trp Thr
 275                              280                              285
Ala Asp Val Glu Ala Gly Lys Trp Leu Gln Ala Lys Gly Ala Lys Pro
 290                              295                              300
Ala Ala Lys Pro Gln Glu Thr Ser Val Ala Asp Glu Ala Pro Glu Val
 305                              310                              315                              320
Thr Ala Thr Val Ile Ser Tyr Asp Asn Tyr Val Thr Ile Leu Asp Glu
 325                              330                              335
Glu Thr Leu Lys Ala Trp Ile Ala Lys Leu Glu Lys Ala Pro Val Phe
 340                              345                              350
Ala Phe Asp Thr Glu Thr Asp Ser Leu Asp Asn Ile Ser Ala Asn Leu

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355					360					365					
Val	Gly	Leu	Ser	Phe	Ala	Ile	Glu	Pro	Gly	Val	Ala	Ala	Tyr	Ile	Pro
370						375					380				
Val	Ala	His	Asp	Tyr	Leu	Asp	Ala	Pro	Asp	Gln	Ile	Ser	Arg	Glu	Arg
385					390					395					400
Ala	Leu	Glu	Leu	Leu	Lys	Pro	Leu	Leu	Glu	Asp	Glu	Lys	Cys	Leu	Lys
				405					410						415
Val	Gly	Gln	Asn	Leu	Lys	Tyr	Asp	Arg	Gly	Ile	Leu	Ala	Asn	Tyr	Gly
			420					425						430	
Ile	Glu	Leu	Arg	Gly	Ile	Ala	Phe	Asp	Thr	Met	Leu	Glu	Ser	Tyr	Ile
	435						440					445			
Leu	Asn	Ser	Val	Ala	Gly	Arg	His	Asp	Met	Asp	Ser	Leu	Ala	Glu	Arg
450						455					460				
Trp	Leu	Lys	His	Lys	Thr	Ile	Thr	Phe	Glu	Glu	Ile	Ala	Gly	Lys	Gly
465					470					475					480
Lys	Asn	Gln	Leu	Thr	Phe	Asn	Gln	Ile	Ala	Leu	Glu	Glu	Ala	Gly	Arg
				485					490						495
Tyr	Ala	Ala	Glu	Asp	Ala	Asp	Val	Thr	Leu	Gln	Leu	His	Leu	Lys	Met
			500					505							510
Trp	Pro	Asp	Leu	Gln	Lys	His	Lys	Gly	Pro	Leu	Asn	Val	Phe	Glu	Asn
		515					520						525		
Ile	Glu	Met	Pro	Leu	Val	Pro	Val	Leu	Ser	Arg	Ile	Glu	Arg	Asn	Gly
530						535						540			
Val	Lys	Ile	Asp	Pro	Lys	Val	Leu	His	Asn	His	Ser	Glu	Glu	Leu	Thr
545					550						555				560
Leu	Arg	Leu	Ala	Glu	Leu	Glu	Lys	Lys	Ala	His	Glu	Ile	Ala	Gly	Glu
				565					570						575
Glu	Phe	Asn	Leu	Ser	Ser	Thr	Lys	Gln	Leu	Gln	Thr	Ile	Leu	Phe	Glu
			580					585						590	
Lys	Gln	Gly	Ile	Lys	Pro	Leu	Lys	Lys	Thr	Lys	Gly	Gly	Ala	Pro	Ser
		595					600						605		
Thr	Ser	Glu	Glu	Val	Leu	Glu	Glu	Leu	Ala	Leu	Asp	Tyr	Pro	Leu	Pro
610						615						620			
Lys	Val	Ile	Leu	Glu	Tyr	Arg	Gly	Leu	Ala	Lys	Leu	Lys	Ser	Thr	Tyr
625					630					635					640
Thr	Asp	Lys	Leu	Pro	Leu	Met	Ile	Asn	Pro	Lys	Thr	Gly	Arg	Val	His
				645					650						655
Thr	Ser	Tyr	His	Gln	Ala	Val	Thr	Ala	Thr	Gly	Arg	Leu	Ser	Ser	Thr
			660					665							670
Asp	Pro	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Asn	Glu	Glu	Gly	Arg	Arg
		675					680						685		
Ile	Arg	Gln	Ala	Phe	Ile	Ala	Pro	Glu	Asp	Tyr	Val	Ile	Val	Ser	Ala
690						695									700
Asp	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Ile	Met	Ala	His	Leu	Ser	Arg	Asp
705					710					715					720
Lys	Gly	Leu	Leu	Thr	Ala	Phe	Ala	Glu	Gly	Lys	Asp	Ile	His	Arg	Ala
				725						730					735
Thr	Ala	Ala	Glu	Val	Phe	Gly	Leu	Pro	Leu	Glu	Thr	Val	Thr	Ser	Glu
			740						745						750
Gln	Arg	Arg	Ser	Ala	Lys	Ala	Ile	Asn	Phe	Gly	Leu	Ile	Tyr	Gly	Met
			755					760							765

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Ser Ala Phe Gly Leu Ala Arg Gln Leu Asn Ile Pro Arg Lys Glu Ala  
770 775 780

Gln Lys Tyr Met Asp Leu Tyr Phe Glu Arg Tyr Pro Gly Val Leu Gln  
785 790 795 800

Tyr Met Glu Arg Thr Arg Ala Gln Ala Lys Glu Gln Gly Tyr Val Glu  
805 810 815

Thr Leu Asp Gly Arg Arg Leu Tyr Leu Pro Asp Ile Lys Ser Ser Asn  
820 825 830

Gly Ala Arg Arg Ala Ala Ala Glu Arg Ala Ala Ile Asn Ala Pro Met  
835 840 845

Gln Gly Thr Ala Ala Asp Ile Ile Lys Arg Ala Met Ile Ala Val Asp  
850 855 860

Ala Trp Leu Gln Ala Glu Gln Pro Arg Val Arg Met Ile Met Gln Val  
865 870 875 880

His Asp Glu Leu Val Phe Glu Val His Lys Asp Asp Val Asp Ala Val  
885 890 895

Ala Lys Gln Ile His Gln Leu Met Glu Asn Cys Thr Arg Leu Asp Val  
900 905 910

Pro Leu Leu Val Glu Val Gly Ser Gly Glu Asn Trp Asp Gln Ala His  
915 920 925

<210> SEQ ID NO 47  
<211> LENGTH: 832  
<212> TYPE: PRT  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Clone 15 mutated bacterial DNA polymerase

<400> SEQUENCE: 47

Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu  
1 5 10 15

Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly  
20 25 30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala  
35 40 45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Thr Val Ile Val  
50 55 60

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly  
65 70 75 80

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu  
85 90 95

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu  
100 105 110

Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys  
115 120 125

Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp  
130 135 140

Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly  
145 150 155 160

Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro  
165 170 175

Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn  
180 185 190

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Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu  
 195 200 205  
 Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu  
 210 215 220  
 Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys  
 225 230 235 240  
 Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val  
 245 250 255  
 Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe  
 260 265 270  
 Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu  
 275 280 285  
 Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly  
 290 295 300  
 Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp  
 305 310 315 320  
 Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro  
 325 330 335  
 Glu Pro Tyr Lys Ala Leu Arg Asp Leu Glu Glu Ala Arg Gly Leu Leu  
 340 345 350  
 Ala Lys Asp Leu Cys Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro  
 355 360 365  
 Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn  
 370 375 380  
 Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu  
 385 390 395 400  
 Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu  
 405 410 415  
 Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu  
 420 425 430  
 Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly  
 435 440 445  
 Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala  
 450 455 460  
 Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His  
 465 470 475 480  
 Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp  
 485 490 495  
 Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Lys Lys Thr Gly Lys Arg  
 500 505 510  
 Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile  
 515 520 525  
 Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr  
 530 535 540  
 Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu  
 545 550 555 560  
 His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser  
 565 570 575  
 Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln  
 580 585 590



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Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala  
 595 600 605

Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly  
 610 615 620

Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr  
 625 630 635 640

Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro  
 645 650 655

Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly  
 660 665 670

Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu  
 675 680 685

Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg  
 690 695 700

Ala Trp Met Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val  
 705 710 715 720

Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg  
 725 730 735

Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Ile Asn Met Pro  
 740 745 750

Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu  
 755 760 765

Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His  
 770 775 780

Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala  
 785 790 795 800

Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro  
 805 810 815

Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu  
 820 825 830

<210> SEQ ID NO 48  
 <211> LENGTH: 832  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Clone 15 mutated bacterial DNA polymerase

<400> SEQUENCE: 48

Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu  
 1 5 10 15

Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly  
 20 25 30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala  
 35 40 45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Thr Val Ile Val  
 50 55 60

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly  
 65 70 75 80

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu  
 85 90 95

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu  
 100 105 110

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Val	Pro	Gly	Tyr	Glu	Ala	Asp	Asp	Val	Leu	Ala	Ser	Leu	Ala	Lys	Lys	115	120	125	
Ala	Glu	Lys	Glu	Gly	Tyr	Glu	Val	Arg	Ile	Leu	Thr	Ala	Asp	Lys	Asp	130	135	140	
Leu	Tyr	Gln	Leu	Leu	Ser	Asp	Arg	Ile	His	Val	Leu	His	Pro	Glu	Gly	145	150	155	160
Tyr	Leu	Ile	Thr	Pro	Ala	Trp	Leu	Trp	Glu	Lys	Tyr	Gly	Leu	Arg	Pro	165	170	175	
Asp	Gln	Trp	Ala	Asp	Tyr	Arg	Ala	Leu	Thr	Gly	Asp	Glu	Ser	Asp	Asn	180	185	190	
Leu	Pro	Gly	Val	Lys	Gly	Ile	Gly	Glu	Lys	Thr	Ala	Arg	Lys	Leu	Leu	195	200	205	
Glu	Glu	Trp	Gly	Ser	Leu	Glu	Ala	Leu	Leu	Lys	Asn	Leu	Asp	Arg	Leu	210	215	220	
Lys	Pro	Ala	Ile	Arg	Glu	Lys	Ile	Leu	Ala	His	Met	Asp	Asp	Leu	Lys	225	230	235	240
Leu	Ser	Trp	Asp	Leu	Ala	Lys	Val	Arg	Thr	Asp	Leu	Pro	Leu	Glu	Val	245	250	255	
Asp	Phe	Ala	Lys	Arg	Arg	Glu	Pro	Asp	Arg	Glu	Arg	Leu	Arg	Ala	Phe	260	265	270	
Leu	Glu	Arg	Leu	Glu	Phe	Gly	Ser	Leu	Leu	His	Glu	Phe	Gly	Leu	Leu	275	280	285	
Glu	Ser	Pro	Lys	Ala	Leu	Glu	Glu	Ala	Pro	Trp	Pro	Pro	Pro	Glu	Gly	290	295	300	
Ala	Phe	Val	Gly	Phe	Val	Leu	Ser	Arg	Lys	Glu	Pro	Met	Trp	Ala	Asp	305	310	315	320
Leu	Leu	Ala	Leu	Ala	Ala	Ala	Arg	Gly	Gly	Arg	Val	His	Arg	Ala	Pro	325	330	335	
Glu	Pro	Tyr	Lys	Ala	Leu	Arg	Asp	Leu	Glu	Glu	Ala	Arg	Gly	Leu	Leu	340	345	350	
Ala	Lys	Asp	Leu	Cys	Val	Leu	Ala	Leu	Arg	Glu	Gly	Leu	Gly	Leu	Pro	355	360	365	
Pro	Gly	Asp	Asp	Pro	Met	Leu	Leu	Ala	Tyr	Leu	Leu	Asp	Pro	Ser	Asn	370	375	380	
Thr	Thr	Pro	Glu	Gly	Val	Ala	Arg	Arg	Tyr	Gly	Gly	Glu	Trp	Thr	Glu	385	390	395	400
Glu	Ala	Gly	Glu	Arg	Ala	Ala	Leu	Ser	Glu	Arg	Leu	Phe	Ala	Asn	Leu	405	410	415	
Trp	Gly	Arg	Leu	Glu	Gly	Glu	Glu	Arg	Leu	Leu	Trp	Leu	Tyr	Arg	Glu	420	425	430	
Val	Glu	Arg	Pro	Leu	Ser	Ala	Val	Leu	Ala	His	Met	Glu	Ala	Thr	Gly	435	440	445	
Val	Arg	Leu	Asp	Val	Ala	Tyr	Leu	Arg	Ala	Leu	Ser	Leu	Glu	Val	Ala	450	455	460	
Glu	Glu	Ile	Ala	Arg	Leu	Glu	Ala	Glu	Val	Phe	Arg	Leu	Ala	Gly	His	465	470	475	480
Pro	Phe	Asn	Leu	Asn	Ser	Arg	Asp	Gln	Leu	Glu	Arg	Val	Leu	Phe	Asp	485	490	495	
Glu	Leu	Gly	Leu	Pro	Ala	Ile	Gly	Lys	Thr	Lys	Lys	Thr	Gly	Lys	Arg	500	505	510	
Ser	Thr	Ser	Ala	Ala	Val	Leu	Glu	Ala	Leu	Arg	Glu	Ala	His	Pro	Ile				

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515					520					525					
Val	Glu	Lys	Ile	Leu	Gln	Tyr	Arg	Glu	Leu	Thr	Lys	Leu	Lys	Ser	Thr
530						535					540				
Tyr	Ile	Asp	Pro	Leu	Pro	Asp	Leu	Ile	His	Pro	Arg	Thr	Gly	Arg	Leu
545					550					555					560
His	Thr	Arg	Phe	Asn	Gln	Thr	Ala	Thr	Ala	Thr	Gly	Arg	Leu	Ser	Ser
				565					570					575	
Ser	Asp	Pro	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Thr	Pro	Leu	Gly	Gln
			580					585					590		
Arg	Ile	Arg	Arg	Ala	Phe	Ile	Ala	Glu	Glu	Gly	Trp	Leu	Leu	Val	Ala
				595			600					605			
Leu	Asp	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala	His	Leu	Ser	Gly
610					615					620					
Asp	Glu	Asn	Leu	Ile	Arg	Val	Phe	Gln	Glu	Gly	Arg	Asp	Ile	His	Thr
625					630					635					640
Glu	Thr	Ala	Ser	Trp	Met	Phe	Gly	Val	Pro	Arg	Glu	Ala	Val	Asp	Pro
				645					650					655	
Leu	Met	Arg	Arg	Ala	Ala	Lys	Thr	Ile	Asn	Phe	Gly	Val	Leu	Tyr	Gly
				660					665					670	
Met	Ser	Ala	His	Arg	Leu	Ser	Gln	Glu	Leu	Ala	Ile	Pro	Tyr	Glu	Glu
				675					680					685	
Ala	Gln	Ala	Phe	Ile	Glu	Arg	Tyr	Phe	Gln	Ser	Phe	Pro	Lys	Val	Arg
690					695					700					
Ala	Trp	Met	Glu	Lys	Thr	Leu	Glu	Glu	Gly	Arg	Arg	Arg	Gly	Tyr	Val
705					710					715					720
Glu	Thr	Leu	Phe	Gly	Arg	Arg	Arg	Tyr	Val	Pro	Asp	Leu	Glu	Ala	Arg
				725					730					735	
Val	Lys	Ser	Val	Arg	Glu	Ala	Ala	Glu	Arg	Met	Ala	Leu	Asn	Met	Pro
				740					745					750	
Val	Gln	Gly	Thr	Ala	Ala	Asp	Leu	Met	Lys	Leu	Ala	Met	Val	Lys	Leu
				755					760					765	
Phe	Pro	Arg	Leu	Glu	Glu	Met	Gly	Ala	Arg	Met	Leu	Leu	Gln	Val	His
770					775					780					
Asp	Glu	Leu	Val	Leu	Glu	Ala	Pro	Lys	Glu	Arg	Ala	Glu	Ala	Val	Ala
785					790					795					800
Arg	Leu	Ala	Lys	Glu	Val	Met	Glu	Gly	Val	Tyr	Pro	Leu	Ala	Val	Pro
				805					810					815	
Leu	Glu	Val	Glu	Val	Gly	Ile	Gly	Glu	Asp	Trp	Leu	Ser	Ala	Lys	Glu
				820					825					830	

&lt;210&gt; SEQ ID NO 49

&lt;211&gt; LENGTH: 832

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Thermus aquaticus

&lt;400&gt; SEQUENCE: 49

Met	Arg	Gly	Met	Leu	Pro	Leu	Phe	Glu	Pro	Lys	Gly	Arg	Val	Leu	Leu
1				5					10					15	

Val	Asp	Gly	His	His	Leu	Ala	Tyr	Arg	Thr	Phe	His	Ala	Leu	Lys	Gly
			20						25					30	

Leu	Thr	Thr	Ser	Arg	Gly	Glu	Pro	Val	Gln	Ala	Val	Tyr	Gly	Phe	Ala
				35					40					45	

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Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val  
 50 55 60  
 Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly  
 65 70 75 80  
 Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu  
 85 90 95  
 Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu  
 100 105 110  
 Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys  
 115 120 125  
 Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp  
 130 135 140  
 Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly  
 145 150 155 160  
 Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro  
 165 170 175  
 Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn  
 180 185 190  
 Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu  
 195 200 205  
 Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu  
 210 215 220  
 Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys  
 225 230 235 240  
 Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val  
 245 250 255  
 Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe  
 260 265 270  
 Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu  
 275 280 285  
 Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly  
 290 295 300  
 Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp  
 305 310 315 320  
 Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro  
 325 330 335  
 Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu  
 340 345 350  
 Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro  
 355 360 365  
 Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn  
 370 375 380  
 Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu  
 385 390 395 400  
 Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu  
 405 410 415  
 Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu  
 420 425 430  
 Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly  
 435 440 445  
 Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala

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450					455					460					
Glu	Glu	Ile	Ala	Arg	Leu	Glu	Ala	Glu	Val	Phe	Arg	Leu	Ala	Gly	His
465					470					475					480
Pro	Phe	Asn	Leu	Asn	Ser	Arg	Asp	Gln	Leu	Glu	Arg	Val	Leu	Phe	Asp
				485					490					495	
Glu	Leu	Gly	Leu	Pro	Ala	Ile	Gly	Lys	Thr	Glu	Lys	Thr	Gly	Lys	Arg
			500					505					510		
Ser	Thr	Ser	Ala	Ala	Val	Leu	Glu	Ala	Leu	Arg	Glu	Ala	His	Pro	Ile
		515					520					525			
Val	Glu	Lys	Ile	Leu	Gln	Tyr	Arg	Glu	Leu	Thr	Lys	Leu	Lys	Ser	Thr
	530					535					540				
Tyr	Ile	Asp	Pro	Leu	Pro	Asp	Leu	Ile	His	Pro	Arg	Thr	Gly	Arg	Leu
545				550						555					560
His	Thr	Arg	Phe	Asn	Gln	Thr	Ala	Thr	Ala	Thr	Gly	Arg	Leu	Ser	Ser
				565					570					575	
Ser	Asp	Pro	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Thr	Pro	Leu	Gly	Gln
			580					585					590		
Arg	Ile	Arg	Arg	Ala	Phe	Ile	Ala	Glu	Glu	Gly	Trp	Leu	Leu	Val	Ala
		595					600					605			
Leu	Asp	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala	His	Leu	Ser	Gly
610					615					620					
Asp	Glu	Asn	Leu	Ile	Arg	Val	Phe	Gln	Glu	Gly	Arg	Asp	Ile	His	Thr
625				630						635					640
Glu	Thr	Ala	Ser	Trp	Met	Phe	Gly	Val	Pro	Arg	Glu	Ala	Val	Asp	Pro
				645					650					655	
Leu	Met	Arg	Arg	Ala	Ala	Lys	Thr	Ile	Asn	Phe	Gly	Val	Leu	Tyr	Gly
		660					665						670		
Met	Ser	Ala	His	Arg	Leu	Ser	Gln	Glu	Leu	Ala	Ile	Pro	Tyr	Glu	Glu
		675					680					685			
Ala	Gln	Ala	Phe	Ile	Glu	Arg	Tyr	Phe	Gln	Ser	Phe	Pro	Lys	Val	Arg
690					695					700					
Ala	Trp	Ile	Glu	Lys	Thr	Leu	Glu	Glu	Gly	Arg	Arg	Arg	Gly	Tyr	Val
705					710					715					720
Glu	Thr	Leu	Phe	Gly	Arg	Arg	Arg	Tyr	Val	Pro	Asp	Leu	Glu	Ala	Arg
			725						730					735	
Val	Lys	Ser	Val	Arg	Glu	Ala	Ala	Glu	Arg	Met	Ala	Phe	Asn	Met	Pro
			740					745					750		
Val	Gln	Gly	Thr	Ala	Ala	Asp	Leu	Met	Lys	Leu	Ala	Met	Val	Lys	Leu
		755				760						765			
Phe	Pro	Arg	Leu	Glu	Glu	Met	Gly	Ala	Arg	Met	Leu	Leu	Gln	Val	His
770					775					780					
Asp	Glu	Leu	Val	Leu	Glu	Ala	Pro	Lys	Glu	Arg	Ala	Glu	Ala	Val	Ala
785				790						795					800
Arg	Leu	Ala	Lys	Glu	Val	Met	Glu	Gly	Val	Tyr	Pro	Leu	Ala	Val	Pro
			805						810					815	
Leu	Glu	Val	Glu	Val	Gly	Ile	Gly	Glu	Asp	Trp	Leu	Ser	Ala	Lys	Glu
			820					825						830	

&lt;210&gt; SEQ ID NO 50

&lt;211&gt; LENGTH: 834

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Thermus thermophilus

-continued

&lt;400&gt; SEQUENCE: 50

Met Glu Ala Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu  
 1 5 10 15  
 Val Asp Gly His His Leu Ala Tyr Arg Thr Phe Phe Ala Leu Lys Gly  
 20 25 30  
 Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala  
 35 40 45  
 Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Tyr Lys Ala Val Phe  
 50 55 60  
 Val Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Glu  
 65 70 75 80  
 Ala Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln  
 85 90 95  
 Leu Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Phe Thr Arg Leu  
 100 105 110  
 Glu Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Thr Leu Ala Lys  
 115 120 125  
 Lys Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Arg  
 130 135 140  
 Asp Leu Tyr Gln Leu Val Ser Asp Arg Val Ala Val Leu His Pro Glu  
 145 150 155 160  
 Gly His Leu Ile Thr Pro Glu Trp Leu Trp Glu Lys Tyr Gly Leu Arg  
 165 170 175  
 Pro Glu Gln Trp Val Asp Phe Arg Ala Leu Val Gly Asp Pro Ser Asp  
 180 185 190  
 Asn Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Leu Lys Leu  
 195 200 205  
 Leu Lys Glu Trp Gly Ser Leu Glu Asn Leu Leu Lys Asn Leu Asp Arg  
 210 215 220  
 Val Lys Pro Glu Asn Val Arg Glu Lys Ile Lys Ala His Leu Glu Asp  
 225 230 235 240  
 Leu Arg Leu Ser Leu Glu Leu Ser Arg Val Arg Thr Asp Leu Pro Leu  
 245 250 255  
 Glu Val Asp Leu Ala Gln Gly Arg Glu Pro Asp Arg Glu Gly Leu Arg  
 260 265 270  
 Ala Phe Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly  
 275 280 285  
 Leu Leu Glu Ala Pro Ala Pro Leu Glu Glu Ala Pro Trp Pro Pro Pro  
 290 295 300  
 Glu Gly Ala Phe Val Gly Phe Val Leu Ser Arg Pro Glu Pro Met Trp  
 305 310 315 320  
 Ala Glu Leu Lys Ala Leu Ala Ala Cys Arg Asp Gly Arg Val His Arg  
 325 330 335  
 Ala Ala Asp Pro Leu Ala Gly Leu Lys Asp Leu Lys Glu Val Arg Gly  
 340 345 350  
 Leu Leu Ala Lys Asp Leu Ala Val Leu Ala Ser Arg Glu Gly Leu Asp  
 355 360 365  
 Leu Val Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro  
 370 375 380  
 Ser Asn Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp

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385		390		395		400									
Thr	Glu	Asp	Ala	Ala	His	Arg	Ala	Leu	Leu	Ser	Glu	Arg	Leu	His	Arg
			405						410					415	
Asn	Leu	Leu	Lys	Arg	Leu	Glu	Gly	Glu	Glu	Lys	Leu	Leu	Trp	Leu	Tyr
			420					425					430		
His	Glu	Val	Glu	Lys	Pro	Leu	Ser	Arg	Val	Leu	Ala	His	Met	Glu	Ala
		435						440					445		
Thr	Gly	Val	Arg	Leu	Asp	Val	Ala	Tyr	Leu	Gln	Ala	Leu	Ser	Leu	Glu
	450				455						460				
Leu	Ala	Glu	Glu	Ile	Arg	Arg	Leu	Glu	Glu	Glu	Val	Phe	Arg	Leu	Ala
465					470					475					480
Gly	His	Pro	Phe	Asn	Leu	Asn	Ser	Arg	Asp	Gln	Leu	Glu	Arg	Val	Leu
				485					490						495
Phe	Asp	Glu	Leu	Arg	Leu	Pro	Ala	Leu	Gly	Lys	Thr	Gln	Lys	Thr	Gly
		500							505					510	
Lys	Arg	Ser	Thr	Ser	Ala	Ala	Val	Leu	Glu	Ala	Leu	Arg	Glu	Ala	His
		515					520						525		
Pro	Ile	Val	Glu	Lys	Ile	Leu	Gln	His	Arg	Glu	Leu	Thr	Lys	Leu	Lys
	530					535							540		
Asn	Thr	Tyr	Val	Asp	Pro	Leu	Pro	Ser	Leu	Val	His	Pro	Arg	Thr	Gly
545					550					555					560
Arg	Leu	His	Thr	Arg	Phe	Asn	Gln	Thr	Ala	Thr	Ala	Thr	Gly	Arg	Leu
				565					570						575
Ser	Ser	Ser	Asp	Pro	Asn	Leu	Gln	Asn	Ile	Pro	Val	Arg	Thr	Pro	Leu
			580					585						590	
Gly	Gln	Arg	Ile	Arg	Arg	Ala	Phe	Val	Ala	Glu	Ala	Gly	Trp	Ala	Leu
		595					600						605		
Val	Ala	Leu	Asp	Tyr	Ser	Gln	Ile	Glu	Leu	Arg	Val	Leu	Ala	His	Leu
	610					615					620				
Ser	Gly	Asp	Glu	Asn	Leu	Ile	Arg	Val	Phe	Gln	Glu	Gly	Lys	Asp	Ile
625				630						635					640
His	Thr	Gln	Thr	Ala	Ser	Trp	Met	Phe	Gly	Val	Pro	Pro	Glu	Ala	Val
				645					650						655
Asp	Pro	Leu	Met	Arg	Arg	Ala	Ala	Lys	Thr	Val	Asn	Phe	Gly	Val	Leu
			660					665						670	
Tyr	Gly	Met	Ser	Ala	His	Arg	Leu	Ser	Gln	Glu	Leu	Ala	Ile	Pro	Tyr
	675						680						685		
Glu	Glu	Ala	Val	Ala	Phe	Ile	Glu	Arg	Tyr	Phe	Gln	Ser	Phe	Pro	Lys
	690					695					700				
Val	Arg	Ala	Trp	Ile	Glu	Lys	Thr	Leu	Glu	Glu	Gly	Arg	Lys	Arg	Gly
705					710					715					720
Tyr	Val	Glu	Thr	Leu	Phe	Gly	Arg	Arg	Arg	Tyr	Val	Pro	Asp	Leu	Asn
				725					730						735
Ala	Arg	Val	Lys	Ser	Val	Arg	Glu	Ala	Ala	Glu	Arg	Met	Ala	Phe	Asn
			740					745						750	
Met	Pro	Val	Gln	Gly	Thr	Ala	Ala	Asp	Leu	Met	Lys	Leu	Ala	Met	Val
		755					760						765		
Lys	Leu	Phe	Pro	Arg	Leu	Arg	Glu	Met	Gly	Ala	Arg	Met	Leu	Leu	Gln
	770					775						780			
Val	His	Asp	Glu	Leu	Leu	Leu	Glu	Ala	Pro	Gln	Ala	Arg	Ala	Glu	Glu
785					790					795					800

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Val Ala Ala Leu Ala Lys Glu Ala Met Glu Lys Ala Tyr Pro Leu Ala  
805 810 815

Val Pro Leu Glu Val Glu Val Gly Met Gly Glu Asp Trp Leu Ser Ala  
820 825 830

Lys Gly

<210> SEQ ID NO 51  
<211> LENGTH: 832  
<212> TYPE: PRT  
<213> ORGANISM: Thermus aquaticus

<400> SEQUENCE: 51

Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu  
1 5 10 15

Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly  
20 25 30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala  
35 40 45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val  
50 55 60

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly  
65 70 75 80

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu  
85 90 95

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu  
100 105 110

Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys  
115 120 125

Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp  
130 135 140

Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly  
145 150 155 160

Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro  
165 170 175

Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn  
180 185 190

Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu  
195 200 205

Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu  
210 215 220

Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys  
225 230 235 240

Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val  
245 250 255

Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe  
260 265 270

Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu  
275 280 285

Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly  
290 295 300

Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp  
305 310 315 320







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Ser Leu Asp Ala Leu Val Tyr Glu Gly Gln Asp Arg Glu Lys Val Ile  
                   260                                  265                                  270

Ala Leu Phe Lys Glu Leu Gly Phe Gln Ser Phe Leu Glu Lys Met Ala  
                   275                                  280                                  285

Ala Pro Ala Ala Glu Gly Arg Lys Pro Leu Glu Glu Met Glu Phe Ala  
                   290                                  295                                  300

Ile Val Asp Val Ile Thr Glu Glu Met Leu Ala Asp Lys Ala Ala Leu  
 305                                  310                                  315                                  320

Val Val Glu Val Met Glu Glu Asn Tyr His Asp Ala Pro Ile Val Gly  
                                   325                                  330                                  335

Ile Ala Leu Val Asn Glu His Gly Arg Phe Phe Met Arg Pro Glu Thr  
                                   340                                  345                                  350

Ala Leu Ala Asp Ser Gln Phe Leu Ala Trp Leu Ala Asp Glu Thr Lys  
                   355                                  360                                  365

Lys Lys Ser Met Phe Asp Ala Lys Arg Ala Val Val Ala Leu Lys Trp  
                   370                                  375                                  380

Lys Gly Ile Asp Val Arg Gly Val Ala Phe Asp Leu Leu Leu Ala Ala  
 385                                  390                                  395                                  400

Tyr Leu Leu Asn Pro Ala Gln Asp Ala Gly Asp Ile Ala Ala Val Ala  
                                   405                                  410                                  415

Lys Met Lys Gln Tyr Glu Ala Val Arg Ser Asp Glu Ala Val Tyr Gly  
                   420                                  425                                  430

Lys Gly Val Lys Arg Ser Leu Pro Asp Glu Gln Thr Leu Ala Glu His  
                   435                                  440                                  445

Leu Val Arg Lys Ala Ala Ala Ile Trp Ala Leu Glu Gln Pro Phe Met  
                   450                                  455                                  460

Asp Asp Leu Arg Asn Asn Glu Gln Asp Gln Leu Leu Thr Lys Leu Glu  
 465                                  470                                  475                                  480

Gln Pro Leu Ala Ala Ile Leu Ala Glu Met Glu Phe Thr Gly Val Asn  
                                   485                                  490                                  495

Val Asp Thr Lys Arg Leu Glu Gln Met Gly Ser Glu Leu Ala Glu Gln  
                   500                                  505                                  510

Leu Arg Ala Ile Glu Gln Arg Ile Tyr Glu His Ala Gly Gln Glu Phe  
                   515                                  520                                  525

Asn Ile Asn Ser Pro Lys Gln Leu Gly Val Ile Leu Phe Glu Lys Leu  
                   530                                  535                                  540

Gln Leu Pro Val Leu Lys Lys Thr Lys Thr Gly Tyr Ser Thr Ser Ala  
 545                                  550                                  555                                  560

Asp Val Leu Glu Lys Leu Ala Pro His His Glu Ile Val Glu Asn Ile  
                                   565                                  570                                  575

Leu His Tyr Arg Gln Leu Gly Lys Leu Gln Ser Thr Tyr Ile Glu Gly  
                   580                                  585                                  590

Leu Leu Lys Val Val Arg Pro Asp Thr Gly Lys Val His Thr Met Phe  
                   595                                  600                                  605

Asn Gln Thr Leu Thr Gln Thr Gly Arg Leu Ser Ser Ala Glu Pro Asn  
                   610                                  615                                  620

Leu Gln Asn Ile Pro Ile Arg Leu Glu Glu Gly Arg Lys Ile Arg Gln  
 625                                  630                                  635                                  640

Ala Phe Val Pro Ser Glu Pro Asp Trp Leu Ile Phe Ala Ala Asp Tyr  
                   645                                  650                                  655

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Ser Gln Ile Glu Leu Arg Val Leu Ala His Ile Ala Asp Asp Asp Asn  
660 665 670

Leu Ile Glu Ala Phe Gln Arg Asp Leu Asp Ile His Thr Lys Thr Ala  
675 680 685

Met Asp Ile Phe His Val Ser Glu Glu Glu Val Thr Ala Asn Met Arg  
690 695 700

Arg Gln Ala Lys Ala Val Asn Phe Gly Ile Val Tyr Gly Ile Ser Asp  
705 710 715 720

Tyr Gly Leu Ala Gln Asn Leu Asn Ile Thr Arg Lys Glu Ala Ala Glu  
725 730 735

Phe Ile Glu Arg Tyr Phe Ala Ser Phe Pro Gly Val Arg Arg Tyr Met  
740 745 750

Glu Asn Ile Val Gln Glu Ala Lys Gln Lys Gly Tyr Val Thr Thr Leu  
755 760 765

Leu His Arg Arg Arg Tyr Leu Pro Asp Ile Thr Ser Arg Asn Phe Asn  
770 775 780

Val Arg Ser Phe Ala Glu Arg Thr Ala Met Asn Thr Pro Ile Gln Gly  
785 790 795 800

Ser Ala Ala Asp Ile Ile Lys Lys Ala Met Ile Asp Leu Ala Ala Arg  
805 810 815

Leu Lys Glu Glu Gln Leu Gln Ala Arg Leu Leu Leu Gln Val His Asp  
820 825 830

Glu Leu Ile Leu Glu Ala Pro Lys Glu Glu Ile Glu Arg Leu Cys Glu  
835 840 845

Leu Val Pro Glu Val Met Glu Gln Ala Val Ser Ser Val Pro Leu Lys  
850 855 860

Val Asp Tyr His Tyr Gly Pro Thr Trp Tyr Asp Ala Lys  
865 870 875

<210> SEQ ID NO 53  
<211> LENGTH: 832  
<212> TYPE: PRT  
<213> ORGANISM: Thermus aquaticus

<400> SEQUENCE: 53

Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu  
1 5 10 15

Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly  
20 25 30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala  
35 40 45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val  
50 55 60

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly  
65 70 75 80

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu  
85 90 95

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu  
100 105 110

Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys  
115 120 125

Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp  
130 135 140

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Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly  
 145 150 155 160  
 Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro  
 165 170 175  
 Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn  
 180 185 190  
 Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu  
 195 200 205  
 Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu  
 210 215 220  
 Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys  
 225 230 235 240  
 Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val  
 245 250 255  
 Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe  
 260 265 270  
 Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu  
 275 280 285  
 Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly  
 290 295 300  
 Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp  
 305 310 315 320  
 Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro  
 325 330 335  
 Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu  
 340 345 350  
 Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro  
 355 360 365  
 Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn  
 370 375 380  
 Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu  
 385 390 395 400  
 Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu  
 405 410 415  
 Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu  
 420 425 430  
 Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly  
 435 440 445  
 Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala  
 450 455 460  
 Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His  
 465 470 475 480  
 Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp  
 485 490 495  
 Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg  
 500 505 510  
 Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile  
 515 520 525  
 Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr  
 530 535 540

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Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu
545                    550                    555                    560

His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser
                    565                    570                    575

Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln
                    580                    585                    590

Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala
                    595                    600                    605

Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly
610                    615                    620

Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr
625                    630                    635                    640

Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro
                    645                    650                    655

Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly
660                    665                    670

Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu
675                    680                    685

Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg
690                    695                    700

Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val
705                    710                    715                    720

Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg
725                    730                    735

Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro
740                    745                    750

Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu
755                    760                    765

Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His
770                    775                    780

Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala
785                    790                    795                    800

Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro
805                    810                    815

Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu
820                    825                    830

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&lt;210&gt; SEQ ID NO 54

&lt;211&gt; LENGTH: 928

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Escherichia coli

&lt;400&gt; SEQUENCE: 54

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Met Val Gln Ile Pro Gln Asn Pro Leu Ile Leu Val Asp Gly Ser Ser
1                    5                    10                    15

Tyr Leu Tyr Arg Ala Tyr His Ala Phe Pro Pro Leu Thr Asn Ser Ala
20                    25                    30

Gly Glu Pro Thr Gly Ala Met Tyr Gly Val Leu Asn Met Leu Arg Ser
35                    40                    45

Leu Ile Met Gln Tyr Lys Pro Thr His Ala Ala Val Val Phe Asp Ala
50                    55                    60

Lys Gly Lys Thr Phe Arg Asp Glu Leu Phe Glu His Tyr Lys Ser His
65                    70                    75                    80

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	885		890		895										
Ala	Lys	Gln	Ile	His	Gln	Leu	Met	Glu	Asn	Cys	Thr	Arg	Leu	Asp	Val
		900						905					910		
Pro	Leu	Leu	Val	Glu	Val	Gly	Ser	Gly	Glu	Asn	Trp	Asp	Gln	Ala	His
		915					920					925			

We claim:

**1.-51.** (canceled)

**52.** A polynucleotide encoding a modified Taq DNA polymerase whose amino acid sequence shares 95% identity with that of SEQ ID NO: 38, but differs from a reference Taq DNA polymerase of SEQ ID NO: 38 at a position corresponding to F749 of SEQ ID NO:38 and furthermore differs from SEQ ID NO:38 by at least two additional amino acids at positions corresponding to at least two of A61, K346, S357, E507 or I707 of SEQ ID NO:38.

**53.** The polynucleotide of claim **52** encoding an amino acid at a position corresponding to F749 of SEQ ID NO: 38 which amino acid is selected from the group consisting of L, I, V, T, Y, and M.

**54.** The polynucleotide of claim **52** encoding an amino acid at a position corresponding to 749 of SEQ ID NO: 38 is L, and the at least two additional amino acids correspond to amino acids in SEQ ID NO: 38, but are substitutions relative to SEQ ID NO: 38 and are selected from the group consisting of: A61T, K346E, S357C, E507K, I707M and combinations thereof.

**55.** The polynucleotide of claim **54** encoding a modified Taq DNA polymerase wherein the modified Taq DNA polymerase has increased resistance to a plant-derived PCR inhibitor as compared to that of SEQ ID NO: 38.

**56.** The polynucleotide of claim **52** encoding a modified Taq DNA polymerase whose amino acid sequence shares 96% identity with that of SEQ ID NO: 38.

**57.** The polynucleotide of claim **52** encoding a modified Taq DNA polymerase whose amino acid sequence shares 97% identity with that of SEQ ID NO: 38.

**58.** The polynucleotide of claim **52** encoding a modified Taq DNA polymerase whose amino acid sequence shares 98% identity with that of SEQ ID NO: 38.

**59.** The polynucleotide of claim **52** encoding a modified Taq DNA polymerase that has increased resistance to salt relative to that of SEQ ID NO: 38.

**60.** A vector comprising the polynucleotide of claim **52**.

**61.** A cell comprising the polynucleotide of claim **52**.

**62.** A cell comprising the vector of claim **52**.

**63.** A kit comprising:

(i) a package unit with a container comprising a modified Taq DNA polymerase encoded by the polynucleotide of claim **52**; and

(ii) instructions.

**64.** A polynucleotide encoding a modified Taq DNA polymerase whose amino acid sequence shares at least 95% identity with that of SEQ ID NO: 48, including in that it has L at a position corresponding to F749 of a reference Taq

DNA polymerase of SEQ ID NO. 38, and in that it has at least two other amino acid substitutions at positions relative SEQ ID NO: 38, which substitutions are selected from the group consisting of T at 61, E at 346, C at 357, K at 507, M at 707 and combinations thereof.

**65.** The polynucleotide of claim **64** encoding a modified Taq DNA polymerase wherein the modified Taq DNA polymerase has increased resistance to a plant-derived PCR inhibitor as compared to that of SEQ ID NO: 38.

**66.** The polynucleotide of claim **64** encoding a modified Taq DNA polymerase whose amino acid sequence shares 96% identity with that of SEQ ID NO: 48.

**67.** The polynucleotide of claim **64** encoding a modified Taq DNA polymerase whose amino acid sequence shares 97% identity with that of SEQ ID NO: 48.

**68.** The polynucleotide of claim **64** encoding a modified Taq DNA polymerase whose amino acid sequence shares 98% identity with that of SEQ ID NO: 48.

**69.** A vector comprising the polynucleotide of claim **64**.

**70.** A cell comprising the polynucleotide of claim **64**.

**71.** A cell comprising the vector of claim **69**.

**72.** A kit comprising:

(i) a package unit with a container comprising a modified Taq DNA polymerase encoded by the polynucleotide of claim **64**; and

(ii) instructions.

**73.** A polynucleotide encoding a modified Taq DNA polymerase having the amino acid sequence of SEQ ID NO: 47 or SEQ ID NO: 48.

**74.** The polynucleotide of claim **73** encoding a modified Taq DNA polymerase, wherein the modified Taq DNA polymerase has increased resistance to a plant-derived PCR inhibitor as compared to that of SEQ ID NO: 38.

**75.** A vector comprising the polynucleotide of claim **73**.

**76.** A cell comprising the polynucleotide of claim **73**.

**77.** A cell comprising the vector of claim **75**.

**78.** A kit comprising:

(i) a package unit with a container comprising a modified Taq DNA polymerase encoded by the polynucleotide of claim **73**; and

(ii) instructions.

**79.** The polynucleotide of claim **73** encoding a modified Taq DNA polymerase having the amino acid sequence of SEQ ID NO: 47.

**80.** The polynucleotide of claim **73** encoding a modified Taq DNA polymerase having the amino acid sequence of SEQ ID NO: 48.

\* \* \* \* \*