Title: ISOLATION AND IDENTIFICATION OF NOVEL POLYMERASES

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ISOLATION AND IDENTIFICATION OF NOVEL POLYMERASES

Field of the Invention

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production and isolation of such polynucleotides and polypeptides. More particularly, the polypeptides of the present invention have been identified as polymerases

Background of the Invention

Thermophilic bacteria have received considerable attention as sources of highly active and thermostable enzymes. Recently, the most extremely thermophilic organotrophic eubacteria presently known have been isolated and characterized. These bacteria, which belong to the genus *thermotoga*, are fermentative microorganisms metabolizing a variety of carbohydrates (Huber, R. and Stetter, K.O., in Ballows, et al., (Ed.), The Procaryotes. 2nd Ed., Springer-Verlag, New York, pgs. 3809-3819 (1992)).

In Huber et al., 1986, Arch. Microbiol. 144:324-333, the isolation of the bacterium *Thermotoga maritima* is described. *T. maritima* is a eubacterium that is strictly anaerobic, rod-shaped, fermentative, hyperthermophilic, and grows between 55°C and 90°C, with an optimum growth temperature of about 80°C. This eubacterium has been isolated from geothermally heated sea floors in Italy and the Azores. *T. maritima* cells have a sheath-like structure and monotrichous flagellation. *T. maritima* is classified in the eubacterium kingdom by virtue of having murein and fatty acid-containing lipids, diphtheria-toxin-resistant elongation factor 2, an RNA polymerase subunit pattern, and sensitivity to antibiotics.

Since, to date, most organisms identified from the archaenal domain are thermophiles or hyperthermophiles, archaea are also considered a fertile source of thermophilic enzymes.
Summary of the Invention

The present invention provides polynucleotides and polypeptides encoded thereby which have been identified as polymerase enzymes. In accordance with one aspect of the present invention, there is provided novel enzymes, as well as active fragments, analogs and derivatives thereof.

In accordance with another aspect of the present invention, there are provided isolated nucleic acid molecules encoding enzymes of the present invention including mRNAs, DNAs, cDNAs, genomic DNAs as well as active analogs and fragments of such enzymes.

In accordance with another aspect of the present invention there are provided isolated nucleic acid molecules encoding mature polypeptides expressed by the DNA in SEQ ID Nos: 1, 3, 5, 7, 9, 11.

In accordance with yet a further aspect of the present invention, there is provided a process for producing such polypeptide by recombinant techniques comprising culturing recombinant prokaryotic and/or eukaryotic host cells, containing a nucleic acid sequence encoding an enzyme of the present invention, under conditions promoting expression of said enzyme and subsequent recovery of said enzyme.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such enzymes, or polynucleotide encoding such enzymes for polymerizing DNA.

In accordance with yet a further aspect of the present invention, there is also provided nucleic acid probes comprising nucleic acid molecules of sufficient length to specifically hybridize to a nucleic acid sequence of the present invention.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such enzymes, or polynucleotides encoding such enzymes, for in vitro purposes related to scientific research, for example, to generate probes for identifying similar sequences which might encode similar enzymes from other organisms.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.
Brief Description of the Drawings

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims. Sequencing was performed using a 378 automated DNA sequencer (Applied Biosystems, Inc.).

FIGURE 1 shows the nucleotide and deduced amino acid sequence of DNA polymerase (3py1) from Ammonifex degensii.

FIGURE 2 shows the nucleotide and deduced amino acid sequence of DNA polymerase (1PY2) from Pyrolobus fumarius.

FIGURE 3 shows the nucleotide and deduced amino acid sequence of DNA polymerase (5PY1) from Archaeoglobus lithotrophicus.

FIGURE 4 shows the nucleotide and deduced amino acid sequence of DNA polymerase (23PY1) from Metallosphaera prunae.

FIGURE 5 shows the nucleotide and deduced amino acid sequence of DNA polymerase (29PY1) from Desulfurococcus.

FIGURE 6 shows the nucleotide and deduced amino acid sequence of DNA polymerase (34PY1) from Aquifex VF-5.
Description of the Preferred Embodiments

The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

A coding sequence is "operably linked to" another coding sequence when RNA polymerase will transcribe the two coding sequences into a single mRNA, which is then translated into a single polypeptide having amino acids derived from both coding sequences. The coding sequences need not be contiguous to one another so long as the expressed sequences are ultimately processed to produce the desired protein.

"Recombinant" enzymes refer to enzymes produced by recombinant DNA techniques; i.e., produced from cells transformed by an exogenous DNA construct encoding the desired enzyme. "Synthetic" enzymes are those prepared by chemical synthesis.

A DNA "coding sequence of" or a "nucleotide sequence encoding" a particular enzyme, is a DNA sequence which is transcribed and translated into an enzyme when placed under the control of appropriate regulatory sequences. A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. The promoter is part of the DNA sequence. This sequence region has a start codon at its 3' terminus. The promoter sequence does include the minimum number of bases where elements necessary to initiate transcription at levels detectable above background. However, after the RNA polymerase binds the sequence and transcription is initiated at the start codon (3' terminus with a promoter), transcription proceeds downstream in the 3' direction. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1) as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

The present invention provides purified thermostable enzymes that catalyze DNA synthesis by addition of deoxynucleotides to the 3' end of a polynucleotide chain, using a complementary polynucleotide strand as a template. An exemplary purified enzyme is a polymerase derived from an organism referred herein as "Ammonifex degensii KC4" is a gram negative, chemolithoautotrophic eubacteria and has a very high temperature optimum. Ammonifex degensii KC4 was discovered in a deep sea isolate from the Middle Atlantic Ridge. Ammonifex degensii
KC4 grows optimally at 70°C and pH 7.0 in a low salt medium. This exemplary enzyme is shown in Figure 1.

The polynucleotide encoding SEQ ID NO:1 was originally recovered from a genomic gene library derived from Ammonifex degensii KC4 as described below. It contains an open reading frame encoding a protein of 867 amino acid residues.

In one embodiment, the representative polymerase of SEQ ID NO:1 of the present invention has a molecular weight of about 95.6 kilodaltons as measured by SDS-PAGE gel electrophoresis and an inferred molecular weight from the nucleotide sequence of the gene. This purified enzyme may be used to polymerize DNA where desired. The polymerase enzyme of the present invention has a very high thermostability and has the closest homology to polymerase from Bacillus steaerothermophilus with 56% identity and 75% similarity at the amino acid level.

In accordance with an aspect of the present invention, there are provided isolated nucleic acid molecules (polynucleotides) which encode for the mature enzymes having the deduced amino acid sequence of Figures 1-6 and SEQ ID NOs:1, 3, 5, 7, 9, 11.

This invention, in addition to the isolated nucleic acid molecule encoding an polymerase enzyme disclosed in Figures 1-6 (SEQ ID NOs:1, 3, 5, 7, 9, 11), also provides substantially similar sequences. Isolated nucleic acid sequences are substantially similar if: (i) they are capable of hybridizing under stringent conditions, hereinafter described, to SEQ ID NO:1; or (ii) they encode DNA sequences which are degenerate to SEQ ID NO:1. Degenerate DNA sequences encode the amino acid sequence of SEQ ID NO:2, but have variations in the nucleotide coding sequences. As used herein, "substantially similar" refers to the sequences having similar identity to the sequences of the instant invention. The nucleotide sequences that are substantially similar can be identified by hybridization or by sequence comparison. Enzyme sequences that are substantially similar can be identified by one or more of the following: proteolytic digestion, gel electrophoresis and/or microsequencing. One means for isolating a nucleic acid molecule encoding a polymerase enzyme is to probe a genomic gene library with a natural or artificially designed probe using art recognized procedures (see, for example: Current Protocols in Molecular Biology, Ausubel F.M. et al. (EDS.) Green Publishing Company Assoc. and John Wiley Interscience, New York. 1989. 1992). It is appreciated to one skilled in the art that SEQ ID NO:1, or fragments thereof (comprising at least 10 contiguous nucleotides and at least 70%
complementary to a target sequence), is a particularly useful probe. Other particular useful probes for this purpose are hybridizable fragments to the sequences of SEQ ID NO:1 (i.e., comprising at least 10 contiguous nucleotides and at least 70% complementary to a target sequence).

With respect to nucleic acid sequences which hybridize to specific nucleic acid sequences disclosed herein, hybridization may be carried out under conditions of reduced stringency, medium stringency or even stringent conditions. As an example of oligonucleotide hybridization, a polymer membrane containing immobilized denatured nucleic acid is first prehybridized for 30 minutes at 45°C in a solution consisting of 0.9 M NaCl, 50 mM NaH₂PO₄, pH 7.0. 5.0 mM Na₂EDTA, 0.5% SDS, 10X Denhardt’s, and 0.5 mg/mL polyriboadenylic acid. Approximately 2 X 10⁷ cpm (specific activity 4-9 X 10⁶ cpm/μg) of ³²P end-labeled oligonucleotide probe are then added to the solution. After 12-16 hours of incubation, the membrane is washed for 30 minutes at room temperature in 1X SET (150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8. 1 mM Na₂EDTA) containing 0.5% SDS, followed by a 30 minute wash in fresh 1X SET at Tm-10°C for the oligo-nucleotide probe. The membrane is then exposed to auto-radiographic film for detection of hybridization signals.

In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (e.g., GC v. AT content), and nucleic acid type (e.g., RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

An example of progressively higher stringency conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.2 x SSC/0.1% SDS at about room temperature (low stringency conditions); 0.2 x SSC/0.1% SDS at about 42°C (moderate stringency conditions); and 0.1 x SSC at about 68°C (high stringency conditions). Washing can be carried out using only one of these conditions, e.g., high stringency conditions, or each of the conditions can be used, e.g., for 10-15 minutes each, in the order listed above, repeating any or all of the steps listed. However, as mentioned above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically.
"Identity" as the term is used herein, refers to a polynucleotide sequence which comprises a percentage of the same bases as a reference polynucleotide (SEQ ID NO:1). For example, a polynucleotide which is at least 90% identical to a reference polynucleotide, has polynucleotide bases which are identical in 90% of the bases which make up the reference polynucleotide and may have different bases in 10% of the bases which comprise that polynucleotide sequence.

The present invention also relates to polynucleotides which differ from the reference polynucleotide such that the changes are silent changes, for example the changes do not alter the amino acid sequence encoded by the polynucleotide. The present invention also relates to nucleotide changes which result in amino acid substitutions, additions, deletions, fusions and truncations in the enzyme encoded by the reference polynucleotide (SEQ ID NO:1). In a preferred aspect of the invention these enzymes retain the same biological action as the enzyme encoded by the reference polynucleotide.

It is also appreciated that such probes can be and are preferably labeled with an analytically detectable reagent to facilitate identification of the probe. Useful reagents include but are not limited to radioactivity, fluorescent dyes or enzymes capable of catalyzing the formation of a detectable product. The probes are thus useful to isolate complementary copies of DNA from other animal sources or to screen such sources for related sequences.

The present invention provides substantially pure polymerase enzymes. The term "substantially pure" is used herein to describe a molecule, such as a polypeptide (e.g., a polymerase polypeptide, or a fragment thereof) that is substantially free of other proteins, lipids, carbohydrates, nucleic acids, and other biological materials with which it is naturally associated. For example, a substantially pure molecule, such as a polypeptide, can be at least 60%, by dry weight, the molecule of interest. The purity of the polypeptides can be determined using standard methods including, e.g., polyacrylamide gel electrophoresis (e.g., SDS-PAGE), column chromatography (e.g., high performance liquid chromatography (HPLC)), and amino-terminal amino acid sequence analysis.

Polymerase polypeptides included in the invention can have one of the amino acid sequences of polymerases shown in Figures 1 through 6 (SEQ ID Nos:2, 4, 6, 8, 10, 12), for example, the amino acid sequence of Ammonifex degensii KC4 (SEQ ID NO:2). Polymerase
polypeptides, such as those isolated from Ammonifex degensii KC4, can be characterized by polymerizing DNA.

Also included in the invention are polypeptides having sequences that are "substantially identical" to the sequence of a polymerase polypeptide, such as one of SEQ ID NO:2. e.g., SEQ ID NO:4. A "substantially identical" amino acid sequence is a sequence that differs from a reference sequence only by conservative amino acid substitutions, for example, substitutions of one amino acid for another of the same class (e.g., substitution of one hydrophobic amino acid, such as isoleucine, valine, leucine, or methionine, for another, or substitution of one polar amino acid for another, such as substitution of arginine for lysine, glutamic acid for aspartic acid, or glutamine for asparagine), or by one or more non-conservative substitutions, deletions, or insertions, provided that the polypeptide retains at least one polymerase-specific activity or a polymerase-specific epitope. For example, one or more amino acids can be deleted from a polymerase polypeptide, resulting in modification of the structure of the polypeptide, without significantly altering its biological activity. For example, amino- or carboxyl-terminal amino acids that are not required for polymerase biological activity, can be removed. Such modifications can result in the development of smaller active polymerase polypeptides.

Other polymerase polypeptides included in the invention are polypeptides having amino acid sequences that are at least 50% identical to the amino acid sequence of a polymerase polypeptide, such as any of polymerases in SEQ ID Nos:2. 4, 6, 8, 10, 12, e.g., SEQ ID NO:12. The length of comparison in determining amino acid sequence homology can be, for example, at least 15 amino acids, for example, at least 20, 25, or 35 amino acids. Homology can be measured using standard sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705; also see Ausubel, et al.. supra). Such procedures and algorithms include, for example, a BLAST program (Basic Local Alignment Search Tool at the National Center for Biological Information), ALIGN, AMAS (Analysis of Multiply Aligned Sequences), AMPS (Protein Multiple Sequence Alignment), ASSET (Aligned Segment Statistical Evaluation Tool), BANDS, BESTCOR, BIOSCAN (Biological Sequence Comparative Analysis Node), BLIMPS (BBlocks IMProved Searcher), FASTA, Intervals & Points, BMB, CLUSTAL V, CLUSTAL W, CONSENSUS, LCONSENSUS, WCONSENSUS, Smith-Waterman algorithm.
DARWIN, Las Vegas algorithm, FNAT (Forced Nucleotide Alignment Tool), Framealign, Framesearch, DYNAMIC, FILTER, FSAP (Frisiensi Sequence Analysis Package), GAP (Global Alignment Program), GENAL, GIBBS, GenQuest, ISSC (Sensitive Sequence Comparison), LALIGN (Local Sequence Alignment), LCP (Local Content Program), MACAW (Multiple Alignment Construction & Analysis Workbench), MAP (Multiple Alignment Program), MBLKP, MBLKN, PIMA (Pattern-Induced Multi-sequence Alignment), SAGA (Sequence Alignment by Genetic Algorithm) and WHAT-IF.

The invention also includes fragments of polymerase polypeptides that retain at least one polymerase-specific activity or epitope. Polymerase activity can be assayed by examining the polymerizing of DNA. For example, a polymerase polypeptide fragment containing, e.g., at least 8-10 amino acids can be used as an immunogen in the production of polymerase-specific antibodies. The fragment can contain, for example, an amino acid sequence that is conserved in polymerases, and this amino acid sequence can contain amino acids that are conserved in polymerases. Such fragments can easily be identified by comparing the sequences of polymerases found in Figures 1-X. In addition to their use as peptide immunogens, the above-described polymerase fragments can be used in immunoassays, such as ELISAs, to detect the presence of polymerase-specific antibodies in samples.

The polymerase polypeptides of the invention can be obtained using any of several standard methods. For example, polymerase polypeptides can be produced in a standard recombinant expression systems (see below), chemically synthesized (this approach may be limited to small polymerase peptide fragments), or purified from organisms in which they are naturally expressed.

The invention also provides isolated nucleic acid molecules that encode the polymerase polypeptides described above, as well as fragments thereof. For example, nucleic acids that encode any of SEQ ID NOs:1, 3, 5, 7, 9, 11 are included in the invention. These nucleic acids can contain naturally occurring nucleotide sequences, or sequences that differ from those of the naturally occurring nucleic acids that encode polymerases, but encode the same amino acids, due to the degeneracy of the genetic code. The nucleic acids of the invention can contain DNA or RNA nucleotides, or combinations or modifications thereof. Exemplary nucleic acids of the invention are shown in SEQ ID NO:1.
By "isolated nucleic acid" is meant a nucleic acid, e.g., a DNA or RNA molecule, that is not immediately contiguous with the 5' and 3' flanking sequences with which it normally is immediately contiguous when present in the naturally occurring genome of the organism from which it is derived. The term thus describes, for example, a nucleic acid that is incorporated into a vector, such as a plasmid or viral vector: a nucleic acid that is incorporated into the genome of a heterologous cell (or the genome of a homologous cell, but at a site different from that at which it naturally occurs); and a nucleic acid that exists as a separate molecule, e.g., a DNA fragment produced by PCR amplification or restriction enzyme digestion, or an RNA molecule produced by in vitro transcription. The term also describes a recombinant nucleic acid that forms part of a hybrid gene encoding additional polypeptide sequences that can be used, for example, in the production of a fusion protein.

The nucleic acid molecules of the invention can be used as templates in standard methods for production of polymerase gene products (e.g., polymerase RNAs and polymerase polypeptides). In addition, the nucleic acid molecules that encode polymerase polypeptides (and fragments thereof) and related nucleic acids, such as (1) nucleic acids containing sequences that are complementary to, or that hybridize to, nucleic acids encoding polymerase polypeptides, or fragments thereof (e.g., fragments containing at least 10, 12, 15, 20, or 25 nucleotides); and (2) nucleic acids containing sequences that hybridize to sequences that are complementary to nucleic acids encoding polymerase polypeptides, or fragments thereof (e.g., fragments containing at least 10, 12, 15, 20, or 25 nucleotides): can be used in methods focused on their hybridization properties. For example, as is described in further detail below, such nucleic acid molecules can be used in the following methods: PCR methods for synthesizing polymerase nucleic acids, methods for detecting the presence of an polymerase nucleic acid in a sample, screening methods for identifying nucleic acids encoding new polymerase family members. Oligonucleotide probes useful for screening methods are from 10 to about 150 nucleotides in length. Further, such probes are preferably 10 to about 100 nucleotides in length and more preferably from 10 to about 50 nucleotides in length.

The invention also includes methods for identifying nucleic acid molecules that encode members of the polymerase polypeptide family in addition to SEQ ID NOs: 1, 3, 5, 7, 9, 11. In these methods, a sample, e.g., a nucleic acid library, such as a cDNA library, that contains a
nucleic acid encoding a polymerase polypeptide is screened with a polymerase-specific probe. e.g., a polymerase-specific nucleic acid probe. Polymerase-specific nucleic acid probes are nucleic acid molecules (e.g., molecules containing DNA or RNA nucleotides, or combinations or modifications thereof) that specifically hybridize to nucleic acids encoding polymerase polypeptides, or to complementary sequences thereof. The term "polymerase-specific probe", in the context of this method of invention, refers to probes that bind to nucleic acids encoding polymerase polypeptides, or to complementary sequences thereof, to a detectably greater extent than to nucleic acids encoding other enzymes, or to complementary sequences thereof.

The invention facilitates production of polymerase-specific nucleic acid probes. Methods for obtaining such probes can be designed based on the amino acid sequences shown in Figure 1. The probes, which can contain at least 10, e.g., 15, 25, 35, 50, 100, or 150 nucleotides, can be produced using any of several standard methods (see, e.g., Ausubel et al., supra). For example, preferably, the probes are generated using PCR amplification methods. In these methods, primers are designed that correspond to polymerase-conserved sequences (see Figure 1), which can include polymerase-specific amino acids, and the resulting PCR product is used as a probe to screen a nucleic acid library, such as a cDNA library.

The coding sequences for the polymerase enzymes of the present invention were identified by preparing an Ammonifex degensii KC4 genomic DNA library, for example, and screening the library for the clones having polymerase activity. Such methods for constructing a genomic gene library are well-known in the art. One means, for example, comprises shearing DNA isolated from Ammonifex degensii KC4 by physical disruption. A small amount of the sheared DNA is checked on an agarose gel to verify that the majority of the DNA is in the desired size range (approximately 3-6 kb). The DNA is then blunt ended using Mung Bean Nuclease, incubated at 37°C and phenol/chloroform extracted. The DNA is then methylated using Eco RI Methylase. Eco RI linkers are then ligated to the blunt ends through the use of T4 DNA ligase and incubation at 4°C. The ligation reaction is then terminated and the DNA is cut-back with Eco RI restriction enzyme. The DNA is then size fractionated on a sucrose gradient following procedures known in the art, for example, Maniatis, T., et al., Molecular Cloning, Cold Spring Harbor Press, New York. 1982.
A plate assay is then performed to get an approximate concentration of the DNA. Ligation reactions are then performed and 1 μl of the ligation reaction is packaged to construct a library. Packaging, for example, may occur through the use of purified (λgt11 phage arms cut with EcoRI and DNA cut with EcoRI after attaching EcoRI linkers. The DNA and (λgt11 arms are ligated with DNA ligase. The ligated DNA is then packaged into infectious phage particles. The packaged phages are used to infect E. coli cultures and the infected cells are spread on agar plates to yield plates carrying thousands of individual phage plaques. The library is then amplified.

Fragments of the full length gene of the present invention may be used as a hybridization probe for a cDNA or a genomic library to isolate the full length DNA and to isolate other DNAs which have a high sequence similarity to the gene or similar biological activity. Probes of this type have at least 10, preferably at least 15, and even more preferably at least 30 bases and may contain, for example, at least 50 or more bases. The probe may also be used to identify a DNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete gene including regulatory and promotor regions, exons, and introns.

The isolated nucleic acid sequences and other enzymes may then be measured for retention of biological activity characteristic to the enzyme of the present invention, for example, in an assay for detecting enzymatic polymerase activity. Such enzymes include truncated forms of polymerase. and variants such as deletion and insertion variants.

The polynucleotide of the present invention may be in the form of DNA which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature enzyme may be identical to the coding sequences shown in Figures 1-6, or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature enzyme as the DNA of Figures 1-6 (e.g., SEQ ID NO:1).

The polynucleotide which encodes the mature enzyme of Figure 1 (e.g., SEQ ID NO:1) may include, but is not limited to: only the coding sequence for the mature enzyme; the coding sequence for the mature enzyme and additional coding sequence such as a leader sequence or a
proprotein sequence: the coding sequence for the mature enzyme (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature enzyme.

Thus, the term "polynucleotide encoding an enzyme (protein)" encompasses a polynucleotide which includes only coding sequence for the enzyme as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the enzyme having the deduced amino acid sequence of Figure 1 (e.g., SEQ ID NO:2). The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same mature enzyme as shown in Figure 1 as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the enzyme of Figure 1. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotide may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in Figure 1. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded enzyme.

The present invention also includes polynucleotides wherein the coding sequence for the mature enzyme may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of an enzyme from a host cell, for example, a leader sequence which functions to control transport of an enzyme from the cell. The enzyme having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the mature form of the enzyme. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.
Thus, for example, the polynucleotide of the present invention may encode for a mature enzyme, or for an enzyme having a prosequence or for an enzyme having both a prosequence and a presequencing (leader sequence).

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode enzymes which either retain substantially the same biological function or activity as the mature enzyme encoded by the DNA of Figure 1.

Alternatively, the polynucleotide may have at least 15 bases, preferably at least 30 bases, and more preferably at least 50 bases which hybridize to a polynucleotide of the present invention and which has an identity thereto, as hereinabove described, and which may or may not retain activity. For example, such polynucleotides may be employed as probes for the polynucleotide of SEQ ID NO:1, for example, for recovery of the polynucleotide or as a PCR primer.

Thus, the present invention is directed to polynucleotides having at least a 70% identity, preferably at least 90% identity and more preferably at least a 95% identity to a polynucleotide which encodes the enzyme of SEQ ID NO:1 as well as fragments thereof. which fragments have at least 30 bases and preferably at least 50 bases to enzymes encoded by such polynucleotides.

The present invention further relates to an enzyme which has the deduced amino acid sequence of Figures 1-6, as well as fragments, analogs and derivatives of such enzyme.

The terms "fragment," "derivative" and "analog" when referring to the enzyme of Figure 1 means a enzyme which retains essentially the same biological function or activity as such enzyme. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature enzyme.

The enzyme of the present invention may be a recombinant enzyme, a natural enzyme or a synthetic enzyme, preferably a recombinant enzyme.
The fragment, derivative or analog of the enzyme of Figure 1 may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature enzyme is fused with another compound, such as a compound to increase the half-life of the enzyme (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature enzyme. Such as a leader or secretory sequence or a sequence which is employed for purification of the mature enzyme or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The enzymes and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or enzyme present in a living animal is not isolated, but the same polynucleotide or enzyme, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or enzymes could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The enzymes of the present invention include an enzyme of Figures 1-6 (in particular the mature enzyme) as well as enzymes which have at least 70% similarity (preferably at least 70% identity) to an enzyme of Figures 1-6 and more preferably at least 90% similarity (more preferably at least 90% identity) to an enzyme of Figures 1-6 and still more preferably at least 95% similarity (still more preferably at least 95% identity) to an enzyme of Figures 1-6 and also include portions of such enzymes with such portion of the enzyme generally containing at least 30 amino acids and more preferably at least 50 amino acids.

As known in the art "similarity" between two enzymes is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one enzyme to the sequence of a second enzyme. Similarity in nucleic acid and amino acid sequences may be determined by procedures and algorithms which are well-known in the art. Such procedures and algorithms
include, for example, a BLAST program (Basic Local Alignment Search Tool at the National Center for Biological Information), AMPS (Protein Multiple Sequence Alignment), ASSET (Aligned Segment Statistical Evaluation Tool), BANDS, BESTSCOR, BIOSCAN (Biological Sequence Comparative Analysis Node), BLIMPS (BLocks IMProved Searcher), FASTA, Intervals & Points, BMB, CLUSTAL V, CLUSTAL W, CONSENSUS, LCONSENSUS, WCONSENSUS, Smith-Waterman algorithm, DARWIN, Las Vegas algorithm, FNAT (Forced Nucleotide Alignment Tool), Framealign, Framesearch, DYNAMIC, FILTER, FSAP (Fristensky Sequence Analysis Package), GAP (Global Alignment Program), GENAL, GIBBS, GenQuest, ISSC (Sensitive Sequence Comparison), LALIGN (Local Sequence Alignment), LCP (Local Content Program), MACAW (Multiple Alignment Construction & Analysis Workbench), MAP (Multiple Alignment Program), MBLKP, MBLKN, PIMA (Pattern-Induced Multi-sequence Alignment), SAGA (Sequence Alignment by Genetic ALgorithm) and WHAT-IF.

A variant, i.e. a "fragment", "analog" or "derivative" enzyme, and reference enzyme may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions and truncations, which may be present in any combination.

Among preferred variants are those that vary from a reference by conservative amino acid substitutions. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu and Ile: interchange of the hydroxyl residues Ser and Thr, exchange of the acidic residues Asp and Glu, substitution between the amide residues Asn and Gln, exchange of the basic residues Lys and Arg and replacements among the aromatic residues Phe, Tyr.

Most highly preferred are variants which retain the same biological function and activity as the reference polypeptide from which it varies.

Fragments or portions of the enzymes of the present invention may be employed for producing the corresponding full-length enzyme by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length enzymes. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.
The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of enzymes of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors containing the polynucleotides of this invention. Such vectors may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes of the present invention. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing enzymes by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing an enzyme. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids: phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA. viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the E. coli lac or trp, the phage lambda P L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.
In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as *E. coli*. Streptomyces, *Bacillus subtilis*; fungal cells, such as yeast; insect cells such as *Drosophila S2* and *Spodoptera Sf9*; animal cells such as CHO, COS or Bowes melanoma: adenoviruses; plant cells, *etc*. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example: Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pBluescript II (Stratagene); pTRC99a, pKK223-3, pDR540, pRT2T (Pharmacia); Eukaryotic: pXT1, pSG5 (Stratagene) pSVK3, pBPV, pMSG, pSVLSV40 (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_r, P_l and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.
In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the enzymes of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989).

Transcription of the DNA encoding the enzymes of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples include the SV40 enhancer on the late side of the replication origin bp 100 to 270. a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated enzyme. Optionally, the
heterologous sequence can encode a fusion enzyme including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli, Bacillus subtilis, Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysis agents, such methods are well known to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, *Cell.*, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and
also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The enzyme can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The enzymes of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the enzymes of the present invention may be glycosylated or may be non-glycosylated. Enzymes of the invention may or may not also include an initial methionine amino acid residue.

The enzyme of this invention may be employed for any purpose in which such enzyme activity is necessary or desired. In a preferred embodiment the enzyme is employed for catalyzing DNA synthesis by addition of deoxynucleotides to the 3' end of a polynucleotide chain, using a complementary polynucleotide strand as a template.

In a preferred embodiment, the enzyme of the present invention is a thermostable enzyme which is stable to heat and is heat resistant and polymerizes DNA, i.e., the enzyme is able to renature and regain activity after a brief (i.e., 5 to 30 seconds), or longer period, for example, minutes or hours, exposure to temperatures of up to 70°C and has a temperature optimum above 60°C.

The enzymes, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab
expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the enzymes corresponding to a sequence of the present invention can be obtained by direct injection of the enzymes into an animal or by administering the enzymes to an animal, preferably a nonhuman. The antibody so obtained will then bind the enzymes itself. In this manner, even a sequence encoding only a fragment of the enzymes can be used to generate antibodies binding the whole native enzymes. Such antibodies can then be used to isolate the enzyme from cells expressing that enzyme.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein. 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic enzyme products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic enzyme products of this invention.

Antibodies generated against the enzyme of the present invention may be used in screening for similar enzymes from other organisms and samples. Such screening techniques are known in the art, for example, one such screening assay is described in "Methods for Measuring Cellulase Activities". Methods in Enzymology, Vol 160, pp. 87-116.

Antibodies may also be employed as a probe to screen gene libraries generated from this or other organisms to identify this or cross reactive activities.

Isolation and purification of polypeptides produced in the systems described above can be carried out using conventional methods, appropriate for the particular system. For example, preparative chromatography and immunological separations employing antibodies such as monoclonal or polyclonal antibodies can be used.

The term "antibody," as used herein, refers to intact immunoglobulin molecules, as well as fragments of immunoglobulin molecules, such as Fab, Fab', (Fab')2, Fv, and SCA fragments.
that are capable of binding to an epitope of a polymerase polypeptide. These antibody fragments, which retain some ability to selectively bind to the antigen (e.g., a polymerase antigen) of the antibody from which they are derived, can be made using well known methods in the art (see, e.g., Harlow and Lane. *supra*), and are described further, as follows.

1. A Fab fragment consists of a monovalent antigen-binding fragment of an antibody molecule, and can be produced by digestion of a whole antibody molecule with the enzyme papain, to yield a fragment consisting of an intact light chain and a portion of a heavy chain.

2. A Fab' fragment of an antibody molecule can be obtained by treating a whole antibody molecule with pepsin, followed by reduction, to yield a molecule consisting of an intact light chain and a portion of a heavy chain. Two Fab' fragments are obtained per antibody molecule treated in this manner.

3. A (Fab')₂ fragment of an antibody can be obtained by treating a whole antibody molecule with the enzyme pepsin, without subsequent reduction. A (Fab')₂ fragment is a dimer of two Fab' fragments, held together by two disulfide bonds.

4. An Fv fragment is defined as a genetically engineered fragment containing the variable region of a light chain and the variable region of a heavy chain expressed as two chains.

5. A single chain antibody ("SCA") is a genetically engineered single chain molecule containing the variable region of a light chain and the variable region of a heavy chain, linked by a suitable, flexible polypeptide linker.

As used in this invention, the term "epitope" refers to an antigenic determinant on an antigen, such as a polymerase polypeptide, to which the paratope of an antibody, such as a polymerase-specific antibody, binds. Antigenic determinants usually consist of chemically active surface groupings of molecules, such as amino acids or sugar side chains, and can have specific three-dimensional structural characteristics, as well as specific charge characteristics.

As is mentioned above, antigens that can be used in producing polymerase-specific antibodies include polymerase polypeptides, e.g., any of the polymerases shown in Figures 1-X polypeptide fragments. The polypeptide or peptide used to immunize an animal can be obtained by standard recombinant, chemical synthetic, or purification methods. As is well known in the
art, in order to increase immunogenicity. An antigen can be conjugated to a carrier protein. Commonly used carriers include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit). In addition to such carriers, well known adjuvants can be administered with the antigen to facilitate induction of a strong immune response.

Polymerase-specific polyclonal and monoclonal antibodies can be purified, for example, by binding to, and elution from, a matrix containing a polymerase polypeptide, e.g., the polymerase polypeptide (or fragment thereof) to which the antibodies were raised. Additional methods for antibody purification and concentration are well known in the art and can be practiced with the polymerase-specific antibodies of the invention (see, for example, Coligan, et al., Unit 9, Current Protocols in Immunology, Wiley Interscience. 1994).

Anti-idiotype antibodies corresponding to polymerase-specific antigens are also included in the invention, and can be produced using standard methods. These antibodies are raised to polymerase-specific antibodies, and thus mimic polymerase-specific epitopes.

The members of a pair of molecules (e.g., an antibody-antigen pair or a nucleic acid pair) are said to "specifically bind" to each other if they bind to each other with greater affinity than to other, non-specific molecules. For example, an antibody raised against an antigen to which it binds more efficiently than to a non-specific protein can be described as specifically binding to the antigen. (Similarly, a nucleic acid probe can be described as specifically binding to a nucleic acid target if it forms a specific duplex with the target by base pairing interactions (see above).)

The present invention is further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In one aspect of the invention, a method for producing a polymerase enzyme, such as those shown in Figures 1-6, is provided. The method includes growing a host cell which contains a polynucleotide encoding the enzyme (e.g., SEQ ID Nos:2, 4, 6, 8, 10, 12), under conditions which allow the expression of the nucleic acid, and isolating the enzyme encoded by the nucleic acid. Methods of culturing the host cell are described in the Examples and are known by those of skill in the art.
In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution.

For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier’s instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is generally performed using 8 percent polyacrylamide gel described by Goeddel, D. et al., Nucleic Acids Res., 8:4057 (1980), for example.

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides may or may not have a 5’ phosphate. Those that do not will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units of T4 DNA
ligase ("ligase") per 0.5 µg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Sambrook, Fritsch and Maniatis, 1989. The following examples are intended to illustrate, but not to limit, the invention. While the procedures described in the examples are typical of those that can be used to carry out certain aspects of the invention, other procedures known to those skilled in the art can also be used. The following materials and methods were used in carrying out the experiments described in the examples.
Example 1

DNA Isolation and Library Construction

The following outlines the procedures used to generate a gene library from a sample.

5

Isolate DNA.

IsoQuick Procedure as per manufacturer's instructions (Orca, Research Inc., Bothell, WA).

Shear DNA

10

Vigorously push and pull DNA through a 25G double-hub needle and 1-cc syringes about 500 times.

Check a small amount (0.5 μg) on a 0.8% agarose gel to make sure the majority of the DNA is in the desired size range (about 3-6 kb).

15

Blunt DNA

Add:

H₂O to a final volume of 405 μl
45 μl 10X Mung Bean Buffer
2.0 μl Mung Bean Nuclease (150 u/μl)

20

Incubate 37°C, 15 minutes.

Phenol/chloroform extract once.

Chloroform extract once.

Add 1 ml ice cold ethanol to precipitate.

Place on ice for 10 minutes.

25

Spin in microfuge, high speed, 30 minutes.

Wash with 1 ml 70% ethanol.

Spin in microfuge, high speed, 10 minutes and dry.
Methylate DNA

Gently resuspend DNA in 26 µl TE.

Add:

5 µl 10X EcoRI Methylase Buffer
0.5 µl SAM (32 mM)
5.0 µl EcoRI Methylase (40 u/µl)

Incubate 37°, 1 hour.

Insure Blunt Ends

Add to the methylation reaction:

5.0 µl 100 mM MgCl₂
8.0 µl dNTP mix (2.5 mM of each dGTP, dATP, dTTP, dCTP)
4.0 µl Klenow (5 u/µl)

Incubate 12°C, 30 minutes.

Add 450 µl 1X STE.

Phenol/chloroform extract once.

Chloroform extract once.

Add 1 ml ice cold ethanol to precipitate and place on ice for 10 minutes.

Spin in microfuge, high speed, 30 minutes.

Wash with 1 ml 70% ethanol.

Spin in microfuge, high speed, 10 minutes and dry.

Adaptor Ligation

Gently resuspend DNA in 8 µl EcoRI adaptors (from Stratagene’s cDNA Synthesis Kit).

Add:

1.0 µl 10X Ligation Buffer
1.0 µl 10 mM rATP
1.0 µl T4 DNA Ligase (4 u/µl)

Incubate 4°C, 2 days.
Phosphorylate Adaptors

Heat kill ligation reaction 70°C, 30 minutes.

Add:

<table>
<thead>
<tr>
<th>Volume</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 µl</td>
<td>10X Ligation Buffer</td>
</tr>
<tr>
<td>2.0 µl</td>
<td>10mM rATP</td>
</tr>
<tr>
<td>6.0 µl</td>
<td>H₂O</td>
</tr>
<tr>
<td>1.0 µl</td>
<td>Polynucleotide kinase (PNK)</td>
</tr>
</tbody>
</table>

Incubate 37°C, 30 minutes.

Add 31 µl H₂O and 5 µl 10X STE.

Size fractionate on a Sephacryl S-500 spin column (pool fractions 1-3).

Phenol/chloroform extract once.

Chloroform extract once.

Add ice cold ethanol to precipitate.

Place on ice, 10 minutes.

Spin in microfuge, high speed. 30 minutes.

Wash with 1 ml 70% ethanol.

Spin in microfuge, high speed. 10 minutes and dry.

Resuspend in 10.5 µl TE buffer.

Do not plate assay. Instead, ligate directly to arms as above except use 2.5 µl of DNA and no water.

Sucrose Gradient (2.2 ml) Size Fractionation

Heat sample to 65°C, 10 minutes.

Gently load on 2.2 ml sucrose gradient.

Spin in mini-ultracentrifuge, 45K, 20°C, 4 hours (no brake).

Collect fractions by puncturing the bottom of the gradient tube with a 20G needle and allowing the sucrose to flow through the needle. Collect the first 20 drops in a Falcon™ 2059 tube then collect 10 1-drop fractions (labelled 1-10). Each drop is about 60 µl in volume.

Run 5 µl of each fraction on a 0.8% agarose gel to check the size.
Pool fractions 1-4 (about 10-1.5 kb) and, in a separate tube, pool fractions 5-7 (about 5-0.5 kb).
Add 1 ml ice cold ethanol to precipitate and place on ice for 10 minutes.
Spin in microfuge, high speed, 30 minutes.
Wash with 1 ml 70% ethanol.
Spin in microfuge, high speed, 10 minutes and dry.
Resuspend each in 10 µl TE buffer.

Test Ligation to Lambda Arms

Plate assay to get an approximate concentration. Spot 0.5 µl of the sample on agarose containing ethidium bromide along with standards (DNA samples of known concentration). View in UV light and estimate concentration compared to the standards. Fraction 1-4 = >1.0 µg/µl. Fraction 5-7 = 500 ng/µl.
Prepare the following ligation reactions (5 µl reactions) and incubate 4°C. overnight:

<table>
<thead>
<tr>
<th>Sample</th>
<th>H₂O</th>
<th>10X Ligase Buffer</th>
<th>10mM rATP</th>
<th>Lambda arms (ZAP)</th>
<th>Insert DNA</th>
<th>T4 DNA Ligase (4 u/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction 1-4</td>
<td>0.5µl</td>
<td>0.5µl</td>
<td>0.5µl</td>
<td>1.0µl</td>
<td>2.0µl</td>
<td>0.5µl</td>
</tr>
<tr>
<td>Fraction 5-7</td>
<td>0.5µl</td>
<td>0.5µl</td>
<td>0.5µl</td>
<td>1.0µl</td>
<td>2.0µl</td>
<td>0.5µl</td>
</tr>
</tbody>
</table>

Test Package and Plate

Package the ligation reactions following manufacturer’s protocol.
Stop packaging reactions with 500 µl SM buffer and pool packaging that came from the same ligation.
Titer 1.0 µl of each on appropriate host (OD₆₀₀ = 1.0) [XLI-Blue MRF]
Add 200 µl host (in mM MgSO₄) to Falcon 2059 tubes
Inoculate with 1 µl packaged phage
Incubate 37°C. 15 minutes
Add about 3 ml 48°C top agar
[50ml stock containing 150 µl IPTG (0.5M) and 300 µl X-GAL (350 mg/ml)]
Plate on 100mm plates and incubate 37°C, overnight.
Amplification of Libraries (5.0 x 10^4 recombinants from each library)

Add 3.0 ml host cells (OD_{600}=1.0) to two 50 ml conical tube.
Inoculate with 2.5 X 10^5 pfu per conical tube.
Incubate 37°C. 20 minutes.
Add top agar to each tube to a final volume of 45 ml.
Plate the tube across five 150 mm plates.
Incubate 37°C, 6-8 hours or until plaques are about pin-head in size.
Overlay with 8-10 ml SM Buffer and place at 4°C overnight (with gentle rocking if possible).

Harvest Phage

Recover phage suspension by pouring the SM buffer off each plate into a 50 ml conical tube.
Add 3 ml chloroform, shake vigorously and incubate at room temperature, 15 minutes.
Centrifuge at 2K rpm, 10 minutes to remove cell debris.
Pour supernatant into a sterile flask, add 500 µl chloroform.
Store at 4°C.

Titer Amplified Library

Make serial dilutions:

$10^{-3} = 1 \, \mu l$ amplified phage in 1 ml SM Buffer
$10^{-6} = 1 \, \mu l$ of the $10^3$ dilution in 1 ml SM Buffer
Add 200 µl host (in 10 mM MgSO_4) to two tubes.
Inoculate one with 10 µl $10^{-6}$ dilution ($10^3$).
Inoculate the other with 1 µl $10^{-6}$ dilution ($10^4$).
Incubate 37°C, 15 minutes.
Add about 3 ml 48°C top agar.
[50ml stock containing 150 µl IPTG (0.5M) and 375 µl X-GAL (350 mg/ml)]
Plate on 100 mm plates and incubate 37°C, overnight.
Excise the ZAP II library to create the pBluescript library according to manufacturers protocols (Stratagene).

Example 2
Activated Calf Thymus DNA Polymerase Assay

Streak out the clone to isolation:

1. Inoculate 5ml LB/Amp/Meth/Kan culture with isolated clone
2. Grow to turbidity
3. Inoculate a 50ml culture of LB/Amp/Meth/Kan
   Grow to OD600 of 0.7 to 0.9; induce culture with IPTG at a final concentration of 1mM for 3 hours
   Centrifuge at 4500 RPM for 20 minutes and discard supernate
   Resuspend pellet in 3mLs of 20mM Tris pH 8.0 and sonicate twice for 1 minute each
   Microcentrifuge 1ml of sonicate for 30 minutes at 4°C
   Remove 1uL of the sonicate supernatant and add to 10uL of the following Activated Calf Thymus Reaction Cocktail in a 0.5ml eppendorf:
   5 units/ml activated calf thymus DNA (Pharmacia 27-4575-01)
   1mMDTT
   40mg/ml BSA
   50uM dATP, 50uM dCTP, 50uM dGTP, 5uM dTTP
   50mM Tris pH 7.6
   5mM MgCl2
   50μCi/ml H²-dTTP
   bring to volume with H2O
   Incubate at 70°C for 10-30 minutes
   Stop reaction by cooling the tube
   Spot sample onto Whatman DE-81 filter paper (catalog#3658-323)
   Dry completely
   Wash filters in 2X SSC five times for 2 minutes each
   Final wash in 100% ethanol to remove most of remaining water
Allow the filters to dry to completion
Count incorporation of H\textsuperscript{3}-dTTP using a scintillation counter


**Example 3**

**PCR Screening**

Polymerase sequences from *Thermococcus litoralis*, *Pyrococcus* GB-D (Deep Vent), and *Pyrococcus furiosus* were scanned to determine conserved regions. The following nucleic acid sequences were identified and corresponding amino acid sequences were utilized to derive degenerate oligonucleotide primers to be used in downstream screening:

- *Thermococcus litoralis*: 37-45, 1045-1051
- *Pyrococcus* GB-D (Deep Vent): 37-45, 1042-1049
- *Pyrococcus furiosus*: 37-45, 505-512

The following corresponding amino acid sequences were used to produce degenerate oligonucleotide primers:

- YIYALL\textsuperscript{5}/RDD
- WY\textsuperscript{C}/SKECAE

The primers have been labeled Poldgen1 forward and Poldgen2 reverse:

Poldgen1 forward (26mer): \textasciitilde5'-TA\textsuperscript{C}/TAT\textsuperscript{A}/TTA\textsuperscript{C}/TGCTCT\textsuperscript{C}/TCTCA\textsuperscript{A}/GAGATGA-3'\textasciitilde

Poldgen2 reverse (23mer): \textasciitilde5'-TC\textsuperscript{A}/TGCA\textsuperscript{A}/GCA\textsuperscript{C}/TTC\textsuperscript{C}/TTTACA\textsuperscript{A}/GTACCA-3'\textasciitilde

These primers were used to amplify potential polymerase genes directly from genomic DNA (Template DNA).
100 µl PCR conditions:
1 µl Poldgen1 forward (500 ng/µl)
1 µl Poldgen2 reverse (500 ng/µl)
1 µl 25 mM dNTP mix
1 µl Template DNA (~100 ng/µl)
1 µl TaqPlus Polymerase (Stratagene)
10 µl 10X low salt reaction buffer (Stratagene)
85 µl H₂O

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PCR products (1.4kb bands from both organisms) were phenol chloroform extracted (ref. Maniatis) and cloned using the TA cloning system into the pGemT PCR Cloning Vector (Promega) using the following ligation reaction:

0.5 µl pGemT Cloning Vector (50 ng/µl)
2 µl PCR Product (~1000 ng/µl)
2 µl rATP (10 mM)
2 µl 10X T4 Ligase Buffer
1 µl T4 Ligase
12.5 µl H₂O

Incubate 4°C overnight.
2.5μl of the above reaction was transformed into XL1-Blue MRF' competent cells (Stratagene).

1.4kb PCR products were also restriction analyzed using the appropriate restriction enzymes:

Potential clones were verified by restriction analysis and sequenced.

BLASTX and BLASTN database comparisons of the sequences indicated whether the sequences were homologous to the nucleic acid sequence of a known polymerase from another organism. Amplification primers were then generated to both ends of the known polymerase gene, and were used in an amplification reaction on the genomic DNA in an attempt to pull out a full length polymerase gene from this organism. These primers include restriction sites and a new Ribosome Binding Site for downstream processing of the gene:

**PCR Conditions:**

1μl forward primer (250ng/μl)
1μl reverse primer (250ng/μl)
1μl 25mM dNTP
1μl template DNA (100ng/μl)
1μl Taq polymerase
10μl 10X Taq Buffer
85μl H₂O
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**GENE LIBRARY SCREENING**

PCR products generated in the above reactions (using degenerate primers) were used to make long "run off" single stranded DNA probes using (P³² as a label).

The genomic library was screened using the single stranded (P³² labeled probe. Hybridization conditions for these screenings were as per Maniatis (maximum stringency for aqueous solutions; 68°C in rolling hybridization chamber).

All positive clones were then excised into pBluescript SK and sequenced.

**Example 4**

**Expression**

Positive clones were identified and isolated from the genomic library by the above methods. DNA from the clones were then used as templates in a 100 ul PCR reaction. DNA encoding the enzymes of the present invention were initially amplified from a pBluescript vector containing the DNA by the PCR technique. The amplified sequences were then inserted into a PQE vector and the enzyme was expressed according to the protocols set forth herein.

The pQE vector (Qiagen, Inc. Chatsworth, CA) encodes antibiotic resistance (Amp'), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites.

The pQE vector was digested with the appropriate restriction enzymes. The amplified sequences were ligated into the respective pQE vector and inserted in frame with the sequence
encoding for the RBS. The ligation mixture was then used to transform the E. coli strain M15/pREP4 (Qiagen, Inc.) by electroporation. M15/pREP4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan'). Transformants were identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies were selected. Plasmid DNA was isolated and confirmed by restriction analysis. Clones containing the desired constructs were grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 µg/ml) and Kan (25 µg/ml). The O/N culture was used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells were grown to an optical density 600 (O.D.600) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalactopyranoside") was then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells were grown an extra 3 to 4 hours. Cells were then harvested by centrifugation.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described. It is to be understood that, while the invention has been described with reference to the above detailed description, the foregoing description is intended to illustrate, but not to limit, the scope of the invention. Other aspects, advantages, and modifications of the invention are within the scope of the following claims.
SEQUENCE LISTING

Diversa Corporation

ISOLATION AND IDENTIFICATION OF NOVEL POLYMERASES

581-179

2,310,854

1998-08-06

US 08/907,166

1997-08-06

12

FastSEQ™ for Windows™ Version 3.0

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2607

DNA

Ammonifex degensii

CDS

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Val Arg Lys Ile Arg Ser Lys Phe Asn Thr Ala Trp Glu Ala Lys Ile
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Lys Tyr His Ala Asn Tyr Ile Tyr Asp Asn Arg Leu Ile Pro Gly Met
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Arg Tyr Val Thr Asp Phe Ser Asn Gly Ala Glu Lys Leu Val Met Val
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Lys Pro Glu Ile Pro Gln Ser Leu Val Glu Lys Val Arg Glu Leu Phe
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Ile Pro Asn Lys Glu Asp Ile Leu Arg His Val Arg Gly Thr Gly Thr
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Lys Ala Ile Glu Gly Lys Lys Tyr Ala Gly Ala Leu Val Val Glu
370 375 380

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Pro Pro Lys Gly Ala Phe Phe Asn Val Val Leu Asp Ile Ala Ser
385 390 395 400

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Leu Tyr Pro Ser Ile Ile Lys Tyr Asn Leu Ser Tyr Glu Thr Val
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Asp Met Lys Trp Cys Ser Lys Thr Ile Asp Ile Val Asp Glu Thr Gly
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Arg Arg Leu His Glu Val Cys Val Asp Lys Pro Gly Leu Thr Ala Gln
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Leu Thr Gly Ile Leu Arg Asp Tyr Arg Val Gly Ile Tyr Lys Lys Arg
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Ser Lys Asp Lys Ser Leu Pro Pro Glu Thr Leu Ala Trp Tyr Glu Val
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1872

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Val Lys Thr Ile His Asn Ala Arg Ile Arg Lys Glu Ile Thr Leu Asp
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1968

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2016

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Lys Pro Glu Ile Pro Gln Ser Leu Val Glu Lys Val Arg Glu Leu Phe
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Lys Arg Leu Glu Asn Ser Pro Tyr Leu Tyr Leu Asp Thr Glu Thr Thr
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1722

taa

1725

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THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. Substantially pure polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10 and 12.

2. An isolated polynucleotide sequence encoding a polypeptide of claim 1.

3. An isolated polynucleotide selected from the group consisting of:
   a) SEQ ID NO:1, 3, 5, 7, 9, 11;
   b) SEQ ID NO:1, 3, 5, 7, 9, 11 wherein T can be replaced by U;
   c) nucleic acid sequences complementary to a) and b); and
   d) fragments of a), b), or c) that are at least 15 bases in length and that will hybridize to DNA which encodes the amino acid sequences of SEQ ID NO:2, 4, 6, 8, 10 or 12 under moderate to highly stringent conditions.

4. The polynucleotide of claim 2, wherein the polynucleotide is isolated from a prokaryote.

5. An expression vector including the polynucleotide of claim 2.

6. The vector of claim 5, wherein the vector is a plasmid.

7. The vector of claim 5, wherein the vector is derived from a virus.

8. A host cell stably transformed with the vector of claim 5.

9. The host cell of claim 8, wherein the cell is prokaryotic.

10. The host cell of claim 8, wherein the cell is eukaryotic.
11. Antibodies that specifically bind to the polypeptide of claim 1 or to a fragment thereof, wherein said fragment is at least 8 amino acids in length.

12. The antibodies according to claim 11, wherein said fragment is at least 10 amino acids in length.

13. The antibodies according to claim 11, wherein said fragment is at least 30 amino acids in length.

14. The antibodies according to claim 11, wherein said fragment is at least 50 amino acids in length.

15. The antibodies of any one of claims 11 to 14, wherein the antibodies are polyclonal.

16. An enzyme selected from the group consisting of:
   a) an enzyme comprising an amino acid sequence which is at least 70% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10, and 12; and
   b) an enzyme which comprises at least 30 consecutive amino acid residues having at least 70% identity to the amino acid sequence as set forth in any one of SEQ ID NOs: 2, 5, 6, 8, 10 and 12, wherein the enzyme is a polymerase.

17. A method for producing a polypeptide comprising:
   a) culturing the host cell of claim 8;
   b) expressing from the host cell of claim 8 a polypeptide encoded by said DNA; and
   c) isolating the polypeptide.
18. A process for producing a transformed cell comprising transforming or transfeciting the cell with the vector of claim 5 such that the cell expresses the polypeptide encoded by the DNA contained in the vector.

19. An isolated polynucleotide selected from the group consisting of:
a) a polynucleotide having at least 70% identity to a nucleic acid encoding an enzyme comprising an amino acid sequence selected from the group of amino acid sequences set forth in SEQ ID NOs: 2, 4, 6, 8, 10, and 12, wherein the polynucleotide encodes a polymerase;
b) a polynucleotide which is complementary to the polynucleotide of a); and
c) a polynucleotide comprising at least 15 bases of the polynucleotide of a) or b).

20. An oligonucleotide probe that hybridizes to a nucleic acid target region corresponding to a region selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9 and 11, under hybridization conditions comprising hybridization in a solution comprising 0.9M NaCl, 50 mM NaH₂PO₄, pH 7.0, 5.0 mM Na₂EDTA, 0.5% SDS, 10X Denhardt’s, and 0.5 mg/mL polyriboadenylic acid at 45°C.

21. The oligonucleotide probe of claim 20, wherein the hybridization conditions further comprise: a wash for 30 minutes at room temperature in a buffer comprising 150 mM NaCl, 20 mM Tris hydrochloride, pH 7.8, 1 mM Na₂EDTA, 0.5% SDS, followed by a 30 minute wash in fresh buffer at Tm-10°C.

22. The oligonucleotide probe of claim 20, wherein the probe is from 10 to about 150 nucleotides in length.

23. The oligonucleotide probe of claim 20, wherein the probe is from 10 to about 100 nucleotides in length.

24. The oligonucleotide probe of claim 20, wherein the probe is from 10 to about 50 nucleotides in length.
25. The oligonucleotide probe of claim 20, wherein the probe is from 10 to about 30 nucleotides in length.

26. The oligonucleotide probe of claim 20, wherein the probe is from 10 to about 15 nucleotides in length.

27. The oligonucleotide probe of claim 20, wherein the probe comprises a segment of 10 contiguous bases which is at least 70% complementary to a target sequence of 10 contiguous nucleotides present in the target region.

28. The oligonucleotide probe of claim 20, wherein the probe is detectably labeled.

29. The oligonucleotide probe of claim 28, wherein the detectable label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemiluminescent compound, a metal chelator and an enzyme.

30. An antibody that specifically binds to a polypeptide as set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10 and 12.

31. The antibody of claim 30, wherein the antibody is polyclonal.

32. The antibody of claim 30, wherein the antibody is detectably labeled.
FIGURE 1

*Ammonifex degensii* DNA Polymerase (3py1)

1

(SEQ ID NO: 1) GTG AAG GGA ALA ACC TTG CTC CTG TCG GAC CGC ATG ATA GCC TAC CGG

(SEQ ID NO: 2) Val Lys Gly Lys Thr Leu Leu Leu Leu Asp Gly Ser Ser Ser Ile Ala Tyr Arg

GCC TTT TCC GCC CTT CCC TCC CTC CGC ACC CGT ACC GGC CTG CCC ACC GGT
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GCC GTG TAG GCC TTT ACC GCC ATG CTC TCC AAA GTG CTG GAA GAA AGG CGT
Ala Val Tyr Gly Phe Thr Ser Met Leu Phe Lys Val Leu Glu Arg Arg

CCC ACG GCC ATA GTG GCG GCT TCC GTT GAT AAA AGC AAG ACC ACC TTC CGG CAC
Pro Thr Ala Ile Val Ala Ala Phe Asp Lys Ser Lys Thr Thr Phe Arg His

GCC CTG GCG GAG ACC TAC AAG GCC ACC CGC CCC GCC ACT CCG GAT GAA CTG
 Ala Leu Ala Glu Thr Tyr Lys Ala His Arg Pro Ala Thr Pro Asp Glu Leu

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GTA GTG GAA AAG GAG GGT TTT GAG GCC GAC GTC ATC GGC ACT CTG GTA
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GAC CGG GCG GAA AAA GAG GTG TGT CAG TTC ATC GTC ACC GCC GAC CTG
Asp Arg Ala Glu Lys Gly Gly Trp Gln Cys Leu Ile Val Thr Gly Asp Leu

GAC GCC CTG CAG CTG GTT TCC CCC CTC ACC ACC GTC GTC GTC ATG GCC AAG
Asp Ala Leu Gln Leu Val Ser Pro Leu Thr Thr Val Val Leu Met Arg Lys

GGG ATA AGC GAA ATA GCG GTC TTT AAC GAG GCG GAG GTG AAA CGC CGC TTC
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GCC GTC ACA CCC CGC CAA CTC CCC GAC TTC AAA GCC TTG GCC GGA GAT GCC
Gly Val Thr Pro Arg Gln Leu Pro Asp Phe Lys Ala Leu Ala Gly Asp Ala

TCG GAC AAC ATC CCC GGG CTG CGC GAC ATA GGG CCC AAA ACT GCC TCC CTG
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CTG CTA CAG TCC CAC CAG AGC CTG GAG AAA TTG CTG GAG AGC AAG GAA TTT
Leu Leu Gln Ser His Gln Ser Leu Glu Leu Leu Glu Ser Lys Glu Phe

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Phe Pro Ala Lys Leu Arg Glu Thr Leu Glu Arg His Lys Glu Glu Ala Val

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Ser Arg Leu Glu Phe Arg Thr Leu Ala Lys Arg Phe Leu Glu Leu Phe Pro

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Lys Val Glu Arg Pro Glu Glu Leu Glu Arg Leu Gly Glu Glu Leu Gly Arg

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Gln Glu Phe Ala Ala Leu Ala Tyr Pro Pro Val Leu Arg Arg Lys Ala Thr

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Fig. 1 CONTINUED

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Arg Glu Leu Lys Gly Gin Gly Phe Lys Cys Arg Met Ile Leu Gin Val His

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2602

GAA TGA Glu End
**Pyrolobus fumarius** DNA Polymerase (1PY2)

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GTC CTC ATT GAC AGG TCT TTT CGC CCA TAC TCC TAT GCG CTG CTT GCA CCG
Val Leu Ile Asp Arg Ser Phe Arg Pro Tyr Phe Tyr Ala Leu Leu Ala Pro
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Val Asp Lys Val Tyr Leu Val Lys Ser Arg Pro Glu Pro Leu Tyr Gly Glu
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GCT CTC GCA CCA ACC AAG CTT CCC GAT CTT AGG ATA CTC GGC TTC GAT ATT
Ala Leu Ala Pro Thr Lys Leu Pro Asp Leu Arg Ile Leu Ala Phe Asp Ile

GAA GTT TAT AGC AAG CAA GGG TCG CGT CCA GAC GGC GAT CCT GTA ATA
Glu Val Tyr Ser Lys Gln Gly Ser Pro Arg Pro Glu Arg Asp Pro Val Ile

GTG ATA GCT GTG AAG ACT GAC GAT GGC GAT GAG GTG CTA TTC ATT GCA GAG
Val Ile Ala Val Lys Thr Asp Asp Glu Val Leu Phe Ile Ala Glu

GCC AAA GAC GAT CGA AAA CCG ATA CGC GAG TTT GTA GAG TAC GTG AAG AGG
Gly Lys Asp Arg Lys Pro Ile Arg Glu Phe Val Glu Tyr Val Lys Arg

TAT GAC CCC GAC ATA ATA GTC GGT TAT AAC AAT CAT TTC GAT TGG CCT
Tyr Asp Pro Asp Ile Ile Val Gly Tyr Asn Asn His Phe Asp Trp Pro

TAT CTT TTG AGG CGC GCC CGC ATC CTA GGC ATA AAG CTT GAT GTG ACT AGA
Tyr Leu Leu Arg Arg Ala Arg Ile Leu Gly Ile Lys Leu Asp Val Thr Arg

AGA GTT GCC GCC GAG CCC ACC ACT AGC GTA CAT GGG CAC GTC TCT GTC CCT
Arg Val Gly Ala Glu Pro Thr Thr Ser Val His Gly His Val Ser Val Pro

GCC AGG CTT AAC GTA GAT CTG TAC GAC TAT GCC GAA GAG ATG CCA GAG ATC
Gly Arg Leu Asn Val Asp Leu Tyr Asp Tyr Ala Glu Glu Met Pro Glu Ile

AAG ATA AAG AGT CTC GAG GAG GTC GCA GAG TAT CTA GGC GTG ATG AAG AGG
Lys Ile Lys Ser Leu Glu Glu Val Ala Glu Tyr Leu Gly Val Met Lys Lys

AGT GAA CGC GTC ATC ATC AAT TGG TGG GAG ATT CCA GAC TAT TGG GAC GAC
Ser Glu Arg Val Ile Ile Asn Trp Trp Glu Ile Pro Asp Tyr Trp Asp Asp

CCG AAG AAG AGA CCA CTA TTA CTG CAA TAC GCG CCG GAC GAT GTC CGC GCT
Pro Lys Lys Arg Pro Leu Leu Leu Gln Tyr Ala Arg Asp Asp Val Arg Ala

ACT TAC GGC TTA GCC GAG AAG ATA TTG CCG TTT GCT ATC CAG TTG TCG TAC
Thr Tyr Gly Leu Ala Glu Lys Ile Leu Pro Phe Ala Ile Gln Leu Ser Tyr

GTA ACA GGT CTC CCA CTA GAC CAG GTA GGT GCC ATG AGT GTT GCC TTT CGA
Val Thr Gly Leu Pro Leu Asp Glu Val Gly Ala Met Ser Val Gly Phe Arg

CTT GAA TGG TAC CTG ATA CCG GCC GCG TTT AAG ATG AAA GAG CTT GTG CCG
Leu Glu Trp Tyr Leu Ile Arg Ala Ala Phe Lys Met Lys Glu Leu Val Pro

AAC CGC GTT GAG CGC CCA GAA GAG ACT TAC CGT GCC GCT ATA GTT CTT GAG
Asn Arg Val Glu Arg Pro Glu Glu Thr Tyr Arg Gly Ala Ile Val Leu Glu

CCG TTG AGA GGC GTG CAC GAG AAT ATA GCC GTA CTC GAC TTT AGC TCG ATG
Pro Leu Arg Gly Val His Glu Asn Ile Ala Val Leu Asp Phe Ser Ser Met

TAC CCA AAC ATC ATG ATA AAG TAC AAT GTT GGT CCT GAC ACG CTT GTG AGG
Tyr Pro Asn Ile Met Ile Lys Tyr Asn Val Gly Pro Asp Thr Leu Val Arg

CCT GGT GAA AAG TGT GCC GAG TGT GGT TGC TGG GAG GCC CCG GAG GTC AAG
Pro Gly Glu Lys Cys Gly Glu Cys Gly Cys Trp Glu Ala Pro Glu Val Lys

CAC AGG TTC CGT AGG TGT CCG CCC GCC TTC TTC AAG ACA GTT CTT GAG AGG
His Arg Phe Arg Arg Cys Pro Pro Gly Phe Phe Lys Thr Val Leu Glu Arg

CTG TTA GAG CTT CGT AAG CGT GTG CTT GCT GAA ATG AAG AAG TAT CCT CCG
Leu Leu Glu Leu Arg Lys Arg Val Arg Ala Glu Met Lys Tyr Pro Pro

GAT AGC CCA GAA TAT CGA CTG TTG GAT GAA AGG CAG AAG GCC TTG AAG GTT
Asp Ser Pro Glu Tyr Arg Leu Leu Asp Glu Arg Gln Lys Ala Leu Lys Val

CTT GCA AAC GCT AGT TAC GCC TAC ATG GGT TGG AGC GCC GCT AGG TGG TAT
Leu Ala Asn Ala Ser Tyr Gly Tyr Met Gly Trp Ser Gly Ala Arg Trp Tyr

TGC AGG GAG TGC GCA AAG GCT GTC ACG GCT TGG GGT AGG CAC CTC ATA CGC
Cys Arg Glu Cys Ala Lys Ala Val Thr Ala Trp Gly Arg His Leu Ile Arg
ACC GCC ATC AAC ATA GCT CGT AAA CTA GGC CTC AAG GTG ATC TAC GGT GAC
Thr Ala Ile Asn Ile Ala Arg Lys Leu Gly Leu Lys Val Ile Tyr Gly Asp

ACA GAT TCG CTC TTC GTG ACC TAT GAT CCG GAG AAG GTG GAA AAT TTC ATC
Thr Asp Ser Leu Phe Val Thr Tyr Asp Pro Glu Val Glu Asn Phe Ile

AAA ATT ATA AAG GAG GAG CTG GGG TTC GAA ATC GTA AAG GTG TAC
Lys Ile Ile Lys Glu Glu Leu Gly Phe Glu Ile Lys Leu Glu Lys Val Tyr

AAA CGC TTA TTC TTT ACA GAG GCT AAG AAG TAC GCT GCC CTT CTC GAG
Lys Arg Leu Phe Phe Thr Glu Ala Lys Tyr Ala Gly Leu Leu Glu

GAC GGA CGT ATA GAT ATT GTC GGT TTC GAG CTT GCG CCT CTC GAG
Asp Gly Arg Ile Asp Ile Val Gly Phe Glu Ala Val Arg Gly Asp Trp Cys

GAA CTC GCC AAG GAG GTT CAG ACT AAG GTT GTC GAA ATA GTA TTG AAG ACG
Glu Leu Ala Lys Glu Val Gln Thr Lys Val Val Glu Ile Val Leu Lys Thr

AGT GAG GTG AAC AAG CTC GAT GAG TAC GTG AGG AAG ATT GTG AAA GAG TTG
Ser Glu Val Asn Lys Ala Val Glu Tyr Val Arg Lys Ile Val Lys Glu Leu

GAG GAG GCC AAG GTT CCC ATA GAG AAG CTT GTA ATC TGG AAG ACC CTT AGT
Glu Glu Gly Lys Val Pro Ile Glu Lys Leu Val Ile Trp Lys Thr Leu Ser

AAG CGT CTT GAG GAG TAC ACA ACG GAG GCA CCA CAC GTC GTT GCA CGG AAG
Lys Arg Leu Glu Tyr Thr Thr Glu Ala Pro His Val Val Ala Ala Lys

AGG ATG CTG TCA GCA GGC TAC CGG GTA AGC CCA GGC GAC AAG ATA GGG TAT
Arg Met Leu Ser Ala Gly Tyr Arg Val Ser Pro Gly Asp Lys Ile Gly Tyr

GTA ATA GTG AAG GGT GTG GCC CTC ATG AGT CAA AGA GCA TGG CCA TAC TTC
Val Ile Val Lys Gly Gly Gly Arg Ile Ser Gln Arg Ala Trp Pro Tyr Phe
ATG GTC AAG GAT CCT AGC CAG ATA GAC GTG ACC TAC TAT GTT GAC CAC CAA
Met Val Lys Asp Pro Ser Gln Ile Asp Val Thr Tyr Tyr Val Asp His Gln

ATC ATC CCG GCT GCA TTG AGA ATA CTG GCC TAC TTG GCC GCC ATC ACC GAG AAG
Ile Ile Pro Ala Ala Leu Arg Ile Leu Gly Tyr Phe Gly Ile Thr Glu Lys

AAG CTG AAA GCA AGT GCA ACT GGG CAG AAG ACT CTC TTC GAC TTT CTA GCC
Lys Leu Lys Ala Ser Ala Thr Gly Gln Lys Thr Leu Phe Asp Phe Leu Ala

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AAG AAG AGC AAG TAA
Lys Lys Ser Lys End
**FIGURE 3**

*Archaeoglobus lithotrophicus* DNA Polymerase (5PY1)

1

(SEQ ID NO:5) ATG ATA AAG GTC AAG GGC TGG CTG CTC GAT GCA GAT TAT ATC ACC GAA AAC

(SEQ ID NO:6) Met Ile Lys Val Lys Gly Trp Leu Leu Asp Ala Asp Tyr Ile Thr Glu Asn

GAT CGA GCC GTT ATA AGG CTA TGG TGT AAG GAT GAG GAA GGA ATA TTT ATC Asp Arg Ala Val Ile Arg Leu Trp Cys Lys Asp Glu Glu Gly Ile Phe Ile

GCA TAC GAT CAC TCA TTC CAG CCC TAC TTT TAC GCA CTC AAA GAA GAG GGT Ala Tyr Asp His Ser Phe Gln Pro Tyr Phe Tyr Ala Leu Lys Glu Glu Gly

ATC ACT GCC GAA GAT ATA GTG AAA ATA AAG GTT CAA ACG AAA AAA GAA GTA Ile Thr Ala Glu Asp Ile Val Lys Ile Lys Val Gln Thr Lys Lys Glu Val

ATT ACG CCG TTA AAA GTT GAG GAA ACC ACA GCC AAA AAT CTT GTT AGG GAG Ile Thr Pro Leu Lys Val Glu Glu Thr Thr Ala Asn Leu Gly Arg Glu

GTT GAA GTT TTC AAG ATA TAT GCA AGA CAC CCT CAG CAC GTC CCC AAA CTT Val Glu Val Phe Lys Ile Tyr Ala Arg His Pro Gln His Val Pro Lys Leu

CGT GAG GTT GTT TCG TAT GAG ATT AGG GAG GCA GAC ATA CCT TTT Arg Glu Val Val Ser Gln Tyr Leu Glu Ile Arg Glu Ala Asp Ile Pro Phe

GCC TAT CGA TAC CTC ATA GAT AAA AAT CTT GCG TGT ATG GAT GGA GTT GTA Ala Tyr Arg Tyr Leu Ile Asp Lys Asn Leu Ala Cys Met Asp Gly Val Val

ATT GAA GCC GTT GAA AGA CGT GAG AAG GGG TTG AGA TGT TAC GAA ATC AAG Ile Glu Gly Val Glu Arg Arg Glu Lys Gly Leu Arg Cys Tyr Glu Ile Lys

AGA ATA GAA AGA GAT TCC AGA CAG GTT TTT CCC GAA CTC AAG GTT ATG GCG Arg Ile Glu Arg Asp Ser Arg Gln Asp Phe Pro Glu Leu Lys Val Met Ala
Fig. 3

TTT  GAT  TGC  GAA  ATG  TGG  GTT  GGT  ATG  CCC  GAT  CCA  GAG  AAA  GAT
Phe  Asp  Cys  Glu  Met  Leu  Ser  Glu  Val  Gly  Met  Pro  Asp  Pro  Glu  Lys  Asp

CCT  ATC  ATA  GTC  ATA  TCA  ATT  AAA  TGG  GAT  ATG  CAG  GAA  ATC  CTC  AAC
Pro  Ile  Ile  Val  Leu  Ser  Ile  Lys  Ser  Gly  Glu  Tyr  Glu  Glu  Ile  Leu  Asn

GGT  GAT  AAC  GAG  AGA  GAA  TTG  CTT  ACC  AGA  TTT  GTC  GAG  ATA  ATT  CGT  GAT
Gly  Asp  Asn  Glu  Arg  Glu  Leu  Leu  Thr  Arg  Phe  Val  Lys  Ile  Ile  Arg  Asp

ATT  GAT  CCC  GAC  ATT  ATA  GTT  GGA  TAC  AAT  CAG  GAC  AGC  TTT  GAC  TCG  CCC
Ile  Asp  Pro  Asp  Ile  Val  Gly  Tyr  Asn  Gln  Asp  Ser  Phe  Asp  Trp  Pro

TAT  ATC  AAG  AGA  GCT  GAG  AAA  CTG  AGG  GTT  AAG  CTT  GAC  ATC  GGA  AGA
Tyr  Ile  Lys  Lys  Arg  Ala  Glu  Lys  Leu  Arg  Val  Lys  Leu  Asp  Ile  Gly  Arg

GAT  AGA  AGC  GAA  CTG  GCT  ATC  AGG  GGA  GGA  AGA  CCA  AAG  ATT  GCT  GGC  AGG
Asp  Arg  Ser  Glu  Leu  Ala  Ile  Arg  Gly  Gly  Arg  Pro  Lys  Ile  Ala  Gly  Arg

TTG  AAC  GTG  GAT  CTC  TAT  GAT  ATT  GCA  ATG  AGG  AGT  CTC  GAT  GTA  AAG  GTG
Leu  Asn  Val  Asp  Leu  Tyr  Asp  Ile  Ala  Met  Arg  Ser  Leu  Asp  Val  Lys  Val

AAG  AAG  CTC  GAA  AAC  GCT  GGA  GAG  CGG  TCT  CGT  GGT  AAG  AAA  ATA  GAG  CTT  GCA
Lys  Lys  Leu  Glu  Ala  Glu  Val  Ala  Glu  Phe  Leu  Gly  Lys  Lys  Ile  Glu  Leu  Ala

GAT  ATT  GAA  GCC  AGG  GAT  ATC  TAC  AAG  CAC  TGG  ACA  TCG  GCC  GAC  AGG  GAA
Asp  Ile  Glu  Ala  Lys  Asp  Ile  Tyr  Lys  His  Trp  Thr  Ser  Gly  Asp  Arg  Glu

AGC  GTA  ATC  AAA  TAC  TCC  CGG  CAG  GAC  ATC  CTG  CAC  ACG  TAC  TTC  ATA  GCT
Ser  Val  Ile  Lys  Tyr  Ser  Arg  Gln  Asp  Ile  Leu  His  Thr  Tyr  Phe  Ile  Ala

GAA  GAA  TTG  CTA  GAG  CAA  CTT  TCC  AGA  ATG  ATA  CGC  ATA  CCT
Glu  Glu  Leu  Leu  Pro  Met  His  Tyr  Glu  Leu  Ser  Arg  Met  Ile  Arg  Ile  Pro
Fig. 3

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CTC GAT GAT GTG ACA AGG AGC GGG AGA GGT AAG CAG GTT GAG TGG CTG CTG
Leu Asp Asp Val Thr Arg Ser Gly Arg Gly Lys Gln Val Glu Trp Leu Leu

TTA AGC GAA GCA CAC AAA CTT GGC GAA CTT GCA CCC AAC CCC AGA GAG ATG
Leu Ser Glu Ala His Lys Leu Gly Glu Leu Ala Pro Asn Pro Arg Glu Met

GCC GAC AGC TAT GAA GGA GCA TTC GTG CTC GAG CCC GCA AGA GGA TTG CAT
Ala Asp Ser Tyr Glu Gly Ala Phe Val Leu Glu Pro Ala Arg Gly Leu His

GAG AAC GTA ATC TGC CTG GAC TTT GCG TCC ATG TAT CCC TCA ATA ATG ATT
Glu Asn Val Ile Cys Leu Asp Phe Ala Ser Met Tyr Pro Ser Ile Met Ile

TCA TAC AAC ATC AGC CCC GAC ACG CTT GTA ATA GGC TAC AAA TGC GAC GAT TGC
Ser Tyr Asn Ile Ser Pro Asp Thr Leu Val Ile Gly Lys Cys Asp Asp Cys

AAT GTA GCG CCG GAG GTG GGG CAC AAA TTC AGG AAA CAT CCT GAT GTT TTT
Asn Val Ala Pro Glu Val Gly His Lys Phe Arg Lys His Pro Asp Gly Phe

TTC AAA AGA ATA CTC AAA ATG CTG ATT GAG AAA AGA AGA GAA ATA AAG AAG
Phe Lys Arg Ile Leu Lys Met Leu Ile Glu Lys Arg Glu Ile Lys Lys

GTT ATG AAA ACA CTT GAC TAC AAC TCG CCA GAA TAC AAG CTG CTC GAT ATA
Val Met Lys Thr Leu Asp Tyr Asn Ser Pro Glu Tyr Lys Leu Leu Asp Ile

AAG CAG GCA ACG CTG AAA GTT CTT ACA AAC TCG TTT TAC GTG TAT ACT GGG
Lys Gln Ala Thr Leu Lys Val Leu Thr Asp Ser Phe Tyr Gly Tyr Thr Gly

TGG AGT CTT GCG AGA TGG TAC TGC AAG GAG TGC GCT GAA GCT ACA ACG GCA
Trp Ser Leu Ala Arg Trp Tyr Cys Lys Glu Cys Ala Glu Ala Thr Thr Ala

TGG GGC AGA CAC TTT ATC AAA ACA TCT GCA AGA ATT GCG AAA GAG CTT GGA
Trp Gly Arg His Phe Ile Lys Thr Ser Ala Arg Ile Ala Lys Glu Leu Gly

TTT GAA GTG CTA TAT GGG GAT ACA GAT AGC ATC TTT GTT AAA AAA GAT GGA
Phe Glu Val Leu Tyr Gly Asp Thr Asp Ser Ile Phe Val Lys Lys Asp Gly

TTG AGC CTG GAA GAG CTC AAA AAA GAA GTT AAA AAG CTC ATA GGT AAA CTG
Leu Ser Leu Glu Glu Leu Lys Lys Glu Val Lys Leu Ile Gly Lys Leu

TCG GAA GAG ATG CCA ATA ATA GAG ATG GAA TAC TAC GAG ACA ATA
Ser Glu Glu Met Pro Ile Gln Ile Glu Ile Asp Glu Tyr Tyr Glu Thr Ile

TTC TTC GTT GAA AAG AAA AGG TAT GCT GGA TTG ACA CAG GAT GGA AGA ATA
Phe Phe Val Glu Lys Arg Tyr Ala Gly Leu Thr Gln Asp Gly Arg Ile

ATT GTA AAG GGT CTT GAA GTC AGA AGA GGC GAC TGG TGC GAG CTT GCA AAG
Ile Val Lys Gly Leu Glu Val Arg Arg Gly Asp Trp Cys Glu Leu Ala Lys

AAG ATA CAG AAA GGT GTA ATA GAA ATC ATT CTG AAG GAA AAG AAT CCT GAA
Lys Ile Gln Lys Gly Val Ile Glu Ile Leu Lys Gly Lys Asn Pro Glu

AAA GCT GCT GAG TAT GTG AAA GGA GTC ATA GAG GAG GAT AAG GCA GGC AAA
Lys Ala Ala Glu Tyr Val Lys Gly Val Ile Glu Glu Ile Lys Ala Gly Lys

ATT CCG CTT GAA GAT TAT ATC ATC TAC TAC GAA GGA TTG AGA AAA CCA TCA
Ile Pro Leu Glu Asp Tyr Ile Ile Tyr Lys Leu Thr Arg Lys Pro Ser

AAG TAC GAG AGT ATG CAG GCT CAC GTA AAA GCT GCC ATG AAG GCG GCA AAG
Lys Tyr Glu Ser Met Gln Ala His Val Lys Ala Met Lys Ala Ala Lys

AGA GGA ATA GTA TAC ACA ATC GCC TCA AAG GTT GGT TTT GTC GTT ACA AAA
Arg Gly Ile Val Tyr Thr Ile Gly Ser Lys Val Gly Phe Val Val Thr Lys

GGT GTG GGG AAC ATA GGT GAT AGG GCT TTT CCA TCT GAT CTG ATA GAG GAC
Gly Val Gly Asn Ile Gly Asp Arg Ala Phe Pro Ser Asp Leu Ile Glu Asp

TTT GAC GGT GAA GTG ATC ACA GAT CTT GAC GGA AAC AAG TAC AAG ATC GAC
Phe Asp Gly Glu Val Ile Thr Asp Leu Asp Gly Asn Lys Tyr Lys Ile Asp
AAG GAA TAC TAT ATA GAC CAT CAG GTA CTG CCA TCG GTT CTT CGA ATT CTC
Lys Glu Tyr Tyr Ile Asp His Gln Val Leu Pro Ser Val Leu Arg Ile Leu

GAG AGG TTC GGA TAC ACC GAG GCA CAG CTA AAA GTT GCT GCG GAG CAG CAA
Glu Arg Phe Gly Tyr Thr Glu Ala Gln Leu Lys Gly Ala Ala Glu Gln Gln

ACG CTA GAT GCT TTC TGG TAA
Thr Leu Asp Ala Phe Trp End

Fig. 3 continued
FIGURE 4

_Metallophaira prunae_ DNA Polymerase (23PY1)

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1
(SEQ ID NO: 7) ATG AGT ATA ATG GCC AGA CAG CTT ACC CTT GCT GAC TTC
TCT GGG ATC AAG

(SEQ ID NO: 8) Met Ser Ile Met Ala Arg Gln Leu Thr Leu Ala Asp Phe
Ser Gly Ile Lys

AGA GAG GAA CCA GTT AAA CAG GAA GAG AAG ACG CAG GAG GAA GAG AGG CCT
Arg Glu Glu Pro Val Lys Gln Glu Glu Lys Thr Gln Glu Glu Arg Pro

CTG GAA AGG CCA GCG AGG CTA AGA AAG GAC ACA GTT AAA CAG GCG CAG GAG
Leu Glu Arg Pro Ala Arg Leu Arg Lys Asp Thr Val Lys Gln Ala Gln Glu

GAG AGA AAG TAC TTT CTT CTC TCC GTA GAC TAT GAT GGT AAA ATG GGG AAG
Glu Arg Lys Tyr Phe Leu Leu Ser Val Asp Tyr Asp Gly Lys Met Gly Lys

GCT GTC TGC AAG CTT TAT GAT CCT GAA ACG GGT GAG CTA CAC GTC CTT TAC
Ala Val Cys Lys Leu Tyr Asp Pro Glu Thr Gly Glu Leu His Val Leu Tyr

GAC AGC ACG GGT CAC AAG TCA TAC TTC CTT GTG GAT TTA GAG CCA GAT CAG
Asp Ser Thr Gly His Lys Ser Tyr Phe Leu Val Asp Leu Glu Pro Asp Gln

ATC CAA AAA ATT CCA AAG ATT GTT AAG GAT GAG TCC TTT GTT AGG CTT GAG
Ile Gln Lys Ile Pro Lys Ile Val Lys Asp Glu Ser Phe Val Arg Leu Glu

AAG ACC ACT AAA ATA GAC CCC TAC ACT TGG AAA CCT ATT AAC CTA ACC AAG
Lys Thr Thr Lys Ile Asp Pro Tyr Thr Trp Lys Pro Ile Asn Leu Thr Lys

ATT GTG GTG AAT GAC CCC CTC GCT GTG AGA CGC CTA AGA GAA TAT GTC CCA
Ile Val Val Asn Asp Pro Leu Ala Val Arg Leu Arg Glu Tyr Val Pro

AGG GCC TAT GAA GCT CAT ATA AAA TAT TTT AAC AAT TAT ATT TAC GAT TTC
Arg Ala Tyr Glu Ala His Ile Lys Tyr Phe Asn Asn Tyr Ile Tyr Asp Phe
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AGC CTC ATA CCA GGG ATG CCC TAC GTG GTA AAG AAG GGG AAG CTA GTG CCC
Ser Leu Ile Pro Gly Met Pro Tyr Val Val Lys Gly Lys Leu Val Pro

CTT AAG CCG GAG GGT GAC GTC AAA GAG GTA AAG GAA GCG TTC AAG GAT GCT
Leu Lys Pro Glu Val Asp Val Lys Glu Val Lys Ala Phe Lys Asp Ala

GAC CAG ATA GCT CAA GAG ATG GCG CTA GAC TGG GCT CCC CTC TTT GAG TCC
Asp Gln Ile Ala Glu Met Ala Leu Asp Trp Ala Pro Leu Phe Glu Ser

GAG ATT CCG TCG GTG AAG AGG GTC GCA ATA GAT ATA GAg GTT TAT ACT CCC
Glu Ile Pro Ser Val Lys Arg Val Ala Ile Asp Ile Glu Val Tyr Thr Pro

ATG ATG GGT AGG GTA CCG GAT CCA GTA AAG GCC GAG TAC CCC GTG ATA AGC
Met Met Gly Arg Val Pro Asp Pro Val Lys Ala Glu Tyr Pro Val Ile Ser

GTA GCC CTA GCA GGG AGC GAT GGC CTG AAA CTG GTC CTA GTC CTT GAT AGG
Val Ala Leu Ala Gly Ser Asp Gly Leu Lys Leu Val Leu Val Leu Asp Arg

GGA GAT AGT CCG ATT CAA AGT AAG GAT ATC AAG GTT GAG GTC TTC CGC ACA
Gly Asp Ser Pro Ile Gln Ser Lys Asp Ile Lys Val Glu Val Phe Arg Thr

GAG AGG GAG CTT CTC TCC AGG TTG TTT GAC ATT CTT AAG GAA TAT CCC ATG
Glu Arg Glu Leu Leu Ser Arg Leu Phe Asp Ile Leu Lys Glu Tyr Pro Met

GTT CTG ACC TTT AAC GGA GAC GAC TTC GAT ATC CCA TAC CTG ATC TTC AGA
Val Leu Thr Phe Asn Gly Asp Phe Asp Pro Tyr Leu Ile Phe Arg

GGT TTC AAG CTC GGG TTA CTA CAG GAT GAG ATA CCC TTC GAG ATC TCT AGT
Gly Phe Lys Leu Gly Leu Leu Gln Asp Glu Ile Pro Phe Glu Ile Ser Ser

TTT GGC AGG AAA CCT GAC GCG AAG TTC AGA TAT GGA TTT CAC ATA GAT TTG
Phe Gly Arg Lys Pro Asp Ala Lys Phe Arg Tyr Gly Phe His Ile Asp Leu
TAC AGG TTC TTC TTC AAC AAG GCG GTC AGG AAC TAT GCA TTT GAG GGG AAG
Tyr Arg Phe Phe Phe Asn Lys Ala Val Arg Asn Tyr Ala Phe Glu Gly Lys

TAC TCA GAG TAC AAC CTT GAC ACC GTA GCC CAG GCA CTC TTG GGT CTC TCC
Tyr Ser Glu Tyr Asn Leu Asp Thr Val Ala Gln Ala Leu Leu Gly Leu Ser

AAG GTC AAG TTG GAC GAG TCC ATT AGC GAC CTA AAC ATG TCT AAA CTC GTG
Lys Val Lys Leu Asp Glu Ser Ile Ser Asp Leu Asn Met Ser Lys Leu Val

GAG TAC AAC TAC AGG GAC TCG GAG ATC ACG AAG TTG ACC ACG TTC AAC
Glu Tyr Asn Tyr Arg Asp Ser Glu Ile Thr Leu Lys Leu Thr Thr Phe Asn

AAC GAA CTA GTA TGG AAG TTG ATT GTA CTC TTC TCC AGA ATT TCC AAG CTT
Asn Glu Leu Val Trp Lys Leu Ile Val Leu Phe Ser Arg Ile Ser Lys Leu

GGT ATA GAG GAG CTA ACT AGG ACA GAG ATA TCA GCC TGG GTA AAG AAC CTG
Gly Ile Glu Glu Leu Thr Arg Thr Glu Ile Ser Ala Trp Val Lys Asn Leu

TAC TAC TGG GAA CAT AGG AAA AGG AAC TGG TTA ATC CCC CTC AAG GAG GAA
Tyr Tyr Trp Glu His Arg Lys Arg Asn Trp Leu Ile Pro Leu Lys Glu Glu

ATC CTT GAA CGC TCC TCT GGG TTG AAG ACA GCT GCC ATT ATC AAG GGA AAG
Ile Leu Glu Arg Ser Ser Gly Leu Lys Thr Ala Ala Ile Ile Lys Gly Lys

GGA TAC AAG GGC GCA GTG GTC ATA GAC CCA CCT GTG GGG GTT TAC TTT GAC
Gly Tyr Lys Gly Ala Val Val Ile Asp Pro Pro Val Gly Val Tyr Phe Asp

GTA GTT GTT CTG GAC TCC TCA CTG TAT CCC TCC ATC ATC TAC AGG TGG
Val Val Val Leu Asp Phe Ala Ser Leu Tyr Pro Ser Ile Ile Arg Asn Trp

AAC CTC AGT TAT GAA ACC GTT GAT GTG AAG GAA TGT AAC AAG AAA AGG GAT
Asn Leu Ser Tyr Glu Thr Val Asp Val Lys Glu Cys Asn Lys Lys Arg Asp

ATA AGG GAT GAG AGT GGG GCG AAA ATC CAT GAG GTG TGC GTG GAC AGG CCC
Ile Arg Asp Glu Ser Gly Ala Lys Ile His Glu Val Cys Val Asp Arg Pro

GGG ATT ACT GCA GTG GTA ACT GGC TTA CTT AGG GAC TTC AGG GTC AAA ATT
Gly Ile Thr Ala Val Val Thr Gly Leu Leu Arg Asp Phe Arg Val Lys Ile

TAC AAG AAG AAA GGG AAA CAG AGC AAC ATA GAC GAG GAG AGA AAG ATG TTG
Tyr Lys Lys Lys Gly Lys Gln Ser Asn Ile Asp Glu Glu Arg Lys Met Leu

TAC GAC GTG GTA CAG AGG GGC ATG AAG GTG TTC ATT AAT GCG ACC TAT GGC
Tyr Asp Val Val Gln Arg Gly Met Lys Val Phe Ile Asn Ala Thr Tyr Gly

GTC TTC GGT GCG GAG ACC TTC CCC TTG TAC GCC CCA GCA GTT GCA GAG ACC
Val Phe Gly Ala Glu Thr Phe Pro Leu Tyr Ala Pro Ala Val Ala Glu Ser

GTT ACA GCC CTA GGT AGG TAC GTA ATC ACG TCC ACC AAG GAA ATG GCT AAC
Val Thr Ala Leu Gly Arg Tyr Val Ile Thr Ser Thr Lys Glu Met Ala Asn

AAG CTT GGG CTG AAG GTT GTG TAC GGG GAT ACG GAC TCG CTC TAC ATT CAC
Lys Leu Gly Leu Lys Val Val Tyr Gly Asp Thr Asp Ser Leu Phe Ile His

CAG CCT GAT AAG AAG AAG CTG GAG GAA CTG GTG GAG TGG ACC AGG CAG AAC
Gln Pro Asp Lys Lys Leu Glu Glu Leu Val Glu Trp Thr Arg Gln Asn

TTC GGG CTT GAT CTA GAG GTG GAC AAA ACT TAC AGG TTC ATT GCC TCC TCC
Phe Gly Leu Asp Leu Glu Val Asp Lys Thr Tyr Arg Phe Ile Ala Phe Ser

GGT CTT AAG AAG AAC TAC TTC GGT GTG TTC AAG GAT TCC AAG GTT GAC ATA
Gly Leu Lys Asn Tyr Phe Gly Val Phe Lys Asp Ser Lys Val Asp Ile

AAG GCC ATG TTG GCA AAG AAG AGG AAC ACC CCA GAG TTT CTG AAG CAG GCC
Lys Gly Met Leu Ala Lys Lys Arg Asn Thr Pro Glu Phe Leu Lys Gln Ala

TTC AAT GAG GCT AAG GAG AGG CTA GCG AAG GTT CAG AAC CAG GAG GAG CTC
Phe Asn Glu Ala Lys Glu Arg Leu Ala Lys Val Gln Asn Gln Glu Glu Leu
GAA AAG GCA ATT CAA GAC TTA ACG GCC CAG GTT AAG GAG GTG TAC AGG AAG
Glu Lys Ala Ile Gln Asp Leu Thr Ala Gln Val Lys Glu Val Tyr Arg Lys

CTT AAG ATG AAG GAA TAT AAC TTG GAT GAG CTC GCC TGC AGG GTC ATG TTA
Leu Lys Met Lys Glu Tyr Asn Leu Asp Glu Leu Ala Phe Arg Val Met Leu

TCC AGG GAC GTG AAG TCC TAT GAG AAG AAC ACC CCA CAG CAC GTT AAG GCT
Ser Arg Asp Val Lys Ser Tyr Glu Lys Asn Thr Pro Gln His Val Lys Ala

GCG GCA CAG CTG GCG GAG ATG AAC GTA CAA GTG ATG TCA AGG GAT ATA ATT
Ala Ala Gln Leu Ala Glu Met Asn Val Gln Val Met Ser Arg Asp Ile Ile

AGC TTC GTA AAG GTA AAG ACT AAG GAG GGA GTT AAA CCT GTC CAG CTA GCT
Ser Phe Val Lys Val Lys Thr Lys Glu Gly Val Lys Pro Val Gln Leu Ala

AAG CTT TCA GAG ATT GAT GTG GAT AAA TAC TAT GAG AGC GTG AGA AGT ACC
Lys Leu Ser Glu Ile Asp Val Asp Lys Tyr Tyr Glu Ser Val Arg Ser Thr

TTC GAA CAG TTA TTG AAA AGC TTC AAT GTG AGC TGG GAT AGA ATA GAG TCC
Phe Glu Gln Leu Leu Lys Ser Phe Asn Val Ser Trp Asp Arg Ile Glu Ser

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ACG ACA TCA ATC GAC TCG TTC TTC AAG ACT TAG
Thr Thr Ser Ile Asp Ser Phe Phe Lys Thr End
FIGURE 5

*Desulfiurococcus MII TL DNA Polymerase (29PY1)*

1

(SEQ ID NO:9) ATG GAG AGG GTT CGC CTA GTG AAG GTG GTT ACC AAG GAT CCT CTA ATC GTG

(SEQ ID NO:10) Met Glu Arg Val Arg Leu Val Lys Val Val Thr Lys Asp Pro Leu Ile Val

AGG AAG ATT AGG AGC AAG TTT AAC ACT GCG TGG GAG GCT AAG ATA AAG TAT Arg Lys Ile Arg Ser Lys Phe Asn Thr Ala Trp Glu Ala Lys Ile Lys Tyr

CAT GCA AAC TAC ATC TAC GAT AAT AGG CTG ATA CCT GGA ATG AGG TAT GTT His Ala Asn Tyr Ile Tyr Asp Asn Arg Leu Ile Pro Gly Met Arg Tyr Val

ACA GAC TTC TCC AAC AGT GCG CAA AAG CTT GTC ATG GTT AAG CCA GAG ATA Thr Asp Phe Ser Asn Gly Ala Gln Lys Leu Val Met Val Lys Pro Glu Ile

CCC CAA TCC CTT GTT GAG AAA GTA AGG GAG TTG AGG AAT GAG CCT CCT Pro Gln Ser Leu Val Glu Lys Val Arg Glu Leu Phe Arg Asn Glu Pro Pro

GAA ACA GTG AAG CTG GCT GAG GAA CTC CTC TTC TTG GAG GAG TCA CGG Glu Thr Val Lys Leu Ala Glu Leu Leu Leu Leu Phe Glu Glu Ser Pro

CCC AAG GTG AAG CGC GTA GTC GAC ATA GAG GTT TTC ACC CCA TTC AAA Pro Lys Val Lys Arg Val Ala Val Asp Ile Glu Val Phe Thr Pro Phe Lys

GGG CGT ATC CCC AGC CCG AAG CTC GCC GAA TAC CCT GTG ATT AGC ATA GCA Gly Arg Ile Pro Ser Pro Lys Leu Ala Glu Tyr Pro Val Ile Ser Ile Ala

TTG GCC GGT AGC GGC TTG AAG AAA ATC CTC CTG CTG GCC AGG GAA TAC Leu Ala Gly Ser Asp Gly Leu Lys Ile Leu Leu Leu Ala Arg Glu Tyr

AAG CAT GAT TTC GAC TAC ATG GAG GAT TAC CCT GTT GAA GCC GAG GTG Lys His Asp Phe Asp Tyr Met Met Glu Asp Tyr Pro Val Glu Ala Glu Val
Fig. 5 Continued

GAG GTG TTC GAC TCC GAG AAA GAC ATG TTG CTG GAA GCC TTC AGA ATA ATG
Glu Val Phe Asp Ser Glu Lys Asp Met Leu Leu Glu Ala Phe Arg Ile Met

GGG AGC TAT CCC GTC GTC CTC ACT TAC AAC GGT GAT AAT TTC GAC CTT CAA
Gly Ser Tyr Pro Val Val Val Leu Thr Tyr Asn Gly Asp Phe Asp Leu Gln

TAC CTG TAC GTG AGA GCC TTC AAG CTG GGG ATT CTG AGA AGC CAT ATC CCG
Tyr Leu Tyr Val Arg Ala Phe Lys Leu Gly Ile Leu Arg Ser His Ile Pro

TTG AAG ATA GGG GAG GAT ATG ATT AGA ATT GAC ACA AGC ATA CAC CTA GAT
Leu Lys Ile Gly Glu Asp Met Ile Arg Ile Asp Thr Ser Ile His Leu Asp

CTA TAC AAG TTC TCC TCG AAC AGG GCT GTT AAA AAC TAT GCT TTC GGG GGG
Leu Tyr Lys Phe Phe Ser Asn Arg Ala Val Lys Asn Tyr Ala Phe Gly Gly

AAA TAC CAG GAG GAG AAG CTT GAC GCT GTT TCA GGG GCA CTG CTA GGA GTG
Lys Tyr Gln Glu Glu Lys Leu Asp Ala Val Ser Gly Ala Leu Leu Gly Val

TCG AAA ATA GGT TTC GAG GAA ACA ATC GCC GCC GTA TGG GCC TCA CTA TTA
Ser Lys Ile Gly Phe Glu Glu Thr Ile Gly Gly Ile Ser Ala Ser Leu Leu

GCC GCC TAC AAC TAC AGG GAT GCC GAG ATC ACG TTA AAG CTA ACC ATG TTC
Ala Ala Tyr Asn Tyr Arg Asp Ala Glu Ile Thr Leu Asn Leu Thr Met Phe

AGT AAT GAA CTC GTT TGG AAA CTC ATT ATT CTT CTA GCT AGG GTT TCC AAG
Ser Asn Glu Leu Val Trp Lys Leu Ile Leu Leu Ala Arg Val Ser Lys

ACA AGC ATT GAA GAC CTG TGT AGG AGG CAG ATT TCC TAC TGG ATT CAA AAT
Thr Ser Ile Glu Asp Leu Cys Arg Arg Glu Ile Ser Tyr Trp Ile Glu Asn

CTG TTC TTC TGG GAG CGC AGG AAG CTC GCC TAC CTC ATA CCT AAC AAG GAG
Leu Phe Phe Trp Glu Arg Arg Lys Leu Gly Tyr Leu Ile Pro Asn Lys Glu
Trp Ala Pro Thr Gln Glu Gln Ser Trp Ile Leu Glu
AAG CTA GCC CTG GAG ATC GAG ATT GAC AAG TCT TTT ACA TAC GTG GTT TTC
Lys Leu Gly Leu Glu Ile Glu Ile Asp Lys Ser Phe Thr Tyr Val Val Phe
ACA GGG CTT AAG AAG AAC TAC CTG GCC AGA ACG GTT GAC GGC GGC ATA GAG
Thr Gly Leu Lys Asn Tyr Leu Gly Arg Thr Val Asp Gly Gly Ile Glu
ATC AAG GGG CTT GTC SCG AAG AAG AAT ACT CCG GAG TTC CTG AAA GAC
Ile Lys Gly Leu Val XXX Lys Lys Arg Asn Thr Pro Glu Phe Leu Lys Asp
TTG TTC GAG AAT GTT ATC GAA AAG CTT AAA AGC GTT GAA AAC CCC GCG GTT
Leu Phe Glu Asn Val Ile Glu Lys Leu Lys Ser Val Glu Asn Pro Ala Gly
TTC ATA GAG TTC GTC AAG TGG TTG GAG CAT CAG GTG AAG ACA ATA CAT AAC
Phe Ile Glu Phe Val Lys Trp Leu Glu His Gln Val Val Thr Ile His Asn
GAT ATT AGG AGG AAG GAG ATA AGC CTC GAC CGG CTC GCC ATA AGG GTG GCC
Asp Ile Arg Arg Lys Glu Ile Thr Leu Asp Arg Leu Ala Ile Arg Val Ala
TTA ACC AAG ACG CCA TCC CTC TAC ACT AAG ACT AAG CCG CCG CAT GTT AAG
Leu Thr Lys Thr Pro Ser Leu Tyr Thr Lys Thr Lys Pro Pro His Val Lys
GCA GCC CTC CAA TTA ATG AAC TAC GGG TAC AGC GTG GAG GAG GGG GAT ATT
Ala Ala Leu Gln Leu Met Asn Tyr Gly Tyr Ser Val Glu Gly Glu Asp Ile
ATA ACG TTT GTC AAG GTG AAG AGC AAG GAG GGC TAT AAG GCT ATA CAG TTA
Ile Thr Phe Val Lys Val Lys Ser Lys Glu Gly Tyr Lys Ala Ile Gln Leu
ACG AGG CTT CAC GAA GTA GAC CCT GAT AAG TAC ATT GAG CTT GTT AAA AGC
Thr Arg Leu His Glu Val Asp Pro Asp Lys Tyr Ile Glu Leu Val Lys Ser
GGT CTT GAA CAA TTC TCT TCA GCC TTC GGA ATA AGG TGG GAG GAT ATC ATA
Gly Leu Glu Gln Phe Leu Ser Ala Phe Gly Ile Arg Trp Glu Asp Ile Ile
Fig. 5 Continued

GCC TCC GCC GGG TTA ACC GAG CTT TTG AGA AAC AAT AGG GCG TAG

Gly Ser Gly Gly Leu Thr Glu Leu Leu Arg Asn Asn Arg Ala End
FIGURE 6

Aquifex VF-5 DNA Polymerase (34PY)

(SEQ ID NO:11) ATG GAT TTT GAA TAC GTA ACG GGA GAA GAG GGA TTA AAA AAG
GCA ATA AAA

(SEQ ID NO:12) Met Asp Phe Glu Tyr Val Thr Gly Glu Glu Gly Leu Lys
Lys Ala Ile Lys

AGG CTC GAA AAT TCT CCA TAC CTT TAC CTG GAT ACG GAA ACC ACA GGA GAC
Arg Leu Glu Asn Ser Pro Tyr Leu Tyr Leu Asp Thr Glu Thr Thr Gly Asp

AGG ATA AGG CTC GTA CAA ATC GGA GAC GAA GAA AAC ACC TAC GTT ATT GAC
Arg Ile Arg Leu Val Gln Ile Gly Asp Glu Glu Asn Thr Tyr Val Ile Asp

CTC TAC GAA ATT CAG GAT ATA GAA CCT CTG AGG AAA TTA ATA AAC GAA AGG
Leu Tyr Glu Ile Gln Asp Ile Glu Pro Leu Arg Lys Leu Ile Asn Glu Arg

GGG ATA GTA GGG CAC AAC CTT AAG TTC GAT CTT AAG TAC CTC TAC AGG TAC
Gly Ile Val Gly His Asn Leu Lys Phe Asp Leu Lys Tyr Leu Tyr Arg Tyr

GGG ATA TTT CCC TCG GCA ACG TTT GAC ACT ATG ATA GCG AGC TAC CTC CTC
Gly Ile Phe Pro Ser Ala Thr Phe Asp Thr Met Ile Ala Ser Tyr Leu Leu

GGA TAC GAG AGA CAC TCC CTC AAT CAC ATA GTT TCA AAC CTA CTC GGA TAT
Gly Tyr Glu Arg His Ser Leu Asn His Ile Val Ser Asn Leu Leu Gly Tyr

TCC ATG GAC AAG AGT TAT CAG ACT TCC GAC TGG GGA GCG AGC GTT CTG AGC
Ser Met Asp Lys Ser Tyr Glu Thr Ser Asp Trp Gly Ala Ser Val Leu Ser

GAC GCT CAG CTC AAG TAC GCT GCA AAC GAC GTT ATA GTC AGA GAA CTC
Asp Ala Gln Leu Lys Tyr Ala Ala Asn Asp Val Ile Val Leu Arg Glu Leu

TTC CCT AAG ATG AGG GAC ATG TTA AAC GAG CTA GAC GCT GAG AGG GGA GAG
Phe Pro Lys Met Arg Asp Met Leu Asn Glu Leu Asp Ala Glu Arg Gly Glu
Fig. 6 Continued...

GAA CTG CTC AAG ACT AGA ACG GCA AAG ATT TTC GAT CTG AAG AGT CCC GTA
Glu Leu Leu Lys Thr Arg Thr Ala Lys Ile Phe Asp Leu Lys Ser Pro Val

GCA ATA GTG GAA ATG GCT TTC GTA AGG GTA AAG GTG GCA AAA CTC GAG ATA AAC
Ala Ile Val Glu Met Ala Phe Val Arg Glu Val Ala Lys Leu Glu Ile Asn

GGA TTT CCC GTG GAC GTA GAA GAG CTA ACC AAC AAG TTA AAA GCT GTG GAA
Gly Phe Pro Val Asp Val Glu Glu Leu Thr Asn Lys Leu Lys Ala Val Glu

AGG GAA ACC CAG AAG AGG ATA CAG GAG TTT TAC ATA AAG TAC AGA GTT GAC
Arg Glu Thr Gln Lys Arg Ile Gln Glu Phe Tyr Ile Lys Tyr Arg Val Asp

CCT CTC TCT CGG AAA CAG CTC GCC TCA CTC CTG ACG AAG AAG TTT AAA CTG
Pro Leu Ser Pro Lys Gln Leu Ala Ser Leu Leu Thr Lys Phe Lys Leu

AAC CTT CCC AAG ACT CCT AAA GGG AAC GTA TCT ACA GAC GAC AAG GCT CTT
Asn Leu Pro Lys Thr Pro Lys Gly Asn Val Ser Thr Asp Asp Lys Ala Leu

ACT TCC TAT CAG GAC GTA GAA CCC GTA AAA CTC GTT CTG GAA ATA AGA AAG
Thr Ser Tyr Gln Asp Val Glu Pro Val Lys Leu Val Leu Glu Ile Arg Lys

CTT AAG AAG ATC GCG GAC AAG TTA AAG GAG TTA AAA GAA CAC TTG AAG AAC
Leu Lys Lys Ile Ala Asp Lys Leu Lys Glu Leu Lys Glu His Leu Lys Asn

GGG AGA GTT TAC CGG GAG TTC AAGCAA ATA GGA GCT GTA ACG GGA AGG ATG
Gly Arg Val Tyr Pro Glu Phe Lys Gin Ile Gly Ala Val Thr Gly Arg Met

TCC TCA CAC CAA AAT ATC CAG AAC ATA CAC AGG GAT ATG AGA AGA ATT
Ser Ser Ala His Pro Asn Ile Gln Asn Ile Asp Met Arg Gly Ile

TTC AAG GCG GAG GGA AAT ACT TTC GTC ATT TCG GAC TTT TCT CAG ATA
Phe Lys Ala Glu Glu Gly Asn Thr Phe Val Ile Ser Asp Phe Ser Gln Ile
GAG CTC AGG ATT GCG GCC GAA TAC GTA AAG GAC CCG CTT ATG CTG GAC GCC
Glu Leu Arg Ile Ala Ala Glu Tyr Val Lys Asp Pro Leu Met Leu Asp Ala

TTC AAA AAG GGA AAG GAC ATG CAC AGG TAC ACC GCT TCA GTG GTA CTC GGA
Phe Lys Lys Gly Lys Asp Met His Arg Tyr Thr Ala Ser Val Val Leu Gly

AAG AAA GAG GAA GAA ATA ACA AAA GAG GAG AGA CAG CTC GCA AAA GCT ATA
Lys Lys Glu Glu Ile Thr Lys Glu Glu Arg Gln Leu Ala Lys Ala Ile

AAC TTC GGT CTC ATA TAC GCC ATT TCC GCT AAA GGG CTT GCA GAA TAC GCA
Asn Phe Gly Leu Ile Tyr Gly Ile Ser Ala Lys Gly Leu Ala Glu Tyr Ala

AAG CTT GGT TAC GCC GGT AAA ATT TCT TTA GAA GAA GCT CAG GTT TTG AGA
Lys Leu Gly Tyr Gly Val Glu Ile Ser Leu Glu Glu Ala Gln Val Leu Arg

GAG AGG TTT TTC AAG AAC TTC AAA GCT TTT AAA GAG TGG CAC GAC AGA GTT
Glu Arg Phe Phe Lys Asn Phe Lys Ala Phe Lys Glu Trp His Asp Arg Val

AAG AAA GAA CTA AAG GAA AAG GGA GAG GTA AAA GGT CAT ACG CTT CTT GGA
Lys Lys Glu Leu Lys Glu Lys Gly Glu Val Lys Gly His Thr Leu Leu Gly

AGG AGA TTT TCC GCA AAT ACC TTT AAC GAC GCT GTA AAT TAC CCC ATA CAG
Arg Arg Phe Ser Ala Asn Thr Phe Asn Asp Ala Val Asn Tyr Pro Ile Gln

GGA ACG GGT GCG GAC CTA CTA AAA CTG GCA GTT CTA CTT TTT GAC GCA AAC
Gly Thr Gly Ala Asp Leu Leu Lys Leu Ala Val Leu Phe Asp Ala Asn

CTC CAG AAA AAG GGA ATA GAT GCA AAG CTC GTG AAC CTC GTG CAC GAC GAG
Leu Gln Lys Lys Gly Ile Asp Ala Lys Leu Val Leu Val His Asp Glu

ATA GTC GTA GAG TGC GAA AAG GAA AAA GCG GAA GTA AAA GAA ATA CTC
Ile Val Val Glu Cys Glu Lys Glu Lys Ala Glu Glu Val Lys Glu Ile Leu

GAA AAA TCC ATG AAA ACG GCG GGA AAG ATA ATA CTG AAA GAG GTT CCC GTG
Fig. 6  Continued

Glu Lys Ser Met Lys Thr Ala Gly Lys Ile Ile Leu Lys Glu Val Pro Val

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GAA GTA GAA AGC GTT ATA AAC GAA AGG TGG ACG AAA GAT TAA
Glu Val Glu Ser Val Ile Asn Glu Arg Trp Thr Lys Asp End