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(54) Title: SECRETED PROTEINS AND USES THEREOF

(57) Abstract

The invention provides isolated nucleic acid molecules, designated TANGO 228 nucleic acid molecules, which encode secreted proteins with homology to the rat MCA–32 protein, isolated nucleic acid molecules, designated TANGO 240 nucleic acid molecules, which encode secreted proteins with homology to the Mycobacterium tuberculosis hypothetical protein Rv0712, and isolated nucleic acid molecules, designated TANGO 243 nucleic acid molecules, which encode proteins with homology to human PLAP (phospholipase A2–activating protein). The invention also provides antisense nucleic acid molecules, expression vectors containing the nucleic acid molecules of the invention, host cells into which the expression vectors have been introduced, and non–human transgenic animals in which a nucleic acid molecule of the invention has been introduced or disrupted. The invention still further provides isolated polypeptides, fusion polypeptides, antigenic polypeptides and antibodies. Diagnostic, screening and therapeutic methods utilizing compositions of the invention are also provided.

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                                                  M  W  S  H  L  S  R  L  L  F  W      11
CTTCTGACCCCGTCTTGGACTTCAACTGGGAGA ATG TGG AGC CAT TTG AGC AGG CTC CTC TTC TGG      66

 S  I  F  S  S  V  T  C  R  K  A  Y  L  D  C  E  A  M  K  T      31
AGC ATA TTT TCT TCT GTC ACT TGT AGA AAA GCT GTA TTG GAT TGT GAG GCA ATG AAA ACA   126

 N  E  F  P  S  P  C  L  D  S  K  T  K  V  V  M  K  G  Q  N      51
AAT GAA TTC CCT TCT CCA TGT TTG GAC TCA AAG ACT AAG GTG GTT ATG AAG GGT CAA AAT   186

 V  S  M  F  C  S  H  K  N  K  S  L  Q  I  T  Y  S  L  F  R      71
GTA TCT ATG TTT TGT TCC CAT AAG AAC AAA TCA CTG CAG ATC ACC TAT TCA TTG TTT CGA   246

 R  K  T  H  L  G  T  Q  D  G  K  G  E  P  A  I  F  N  L  S      91
CGT AAG ACA CAC CTG GGA ACC CAG GAT GGA AAA GGT GAA CCT GCG ATT TTT AAC CTA AGC   306

 I  T  E  A  H  E  S  G  P  Y  K  C  K  A  Q  V  T  S  C  S     111
ATC ACA GAA GCC CAT GAA TCA GGC CCC TAC AAA TGC AAA GCC CAA GTT ACC AGC TGT TCA   366

 K  Y  S  R  D  F  S  F  T  I  V  D  P  V  T  S  P  V  L  N     131
AAA TAC AGT CGT GAC TTC AGC TTC ACG ATT GTC GAC CCG GTG ACT TCC CCA GTG CTG AAC   426

 I  M  V  I  Q  T  E  D  R  H  I  T  L  H  C  L  S  V  N     151
ATT ATG GTC ATT CAA ACA GAA ACA GAC CGA CAT ATA ACA TTA CAT TGC CTC TCA GTC AAT   486

 G  S  L  P  I  N  Y  T  F  F  E  N  H  V  A  I  S  P  A  I     171
GGC TCG CTG CCC ATC AAT TAC ACT TTC TTT GAA AAC CAT GTT GCC ATA TCA CCA GCT ATT   546

 S  K  Y  D  R  E  P  A  E  F  N  L  T  K  K  N  P  G  E  E     191
TCC AAG TAT GAC AGG GAG CCT GCT GAA TTT AAC TTA ACC AAG AAG AAT CCT GGA GAA GAG   606

 E  E  Y  R  C  E  A  K  N  R  L  P  N  Y  A  T  Y  S  H  P     211
GAA GAG TAT AGG TGT GAA GCT AAA AAC AGA TTG CCT AAC TAT GCA ACA TAC AGT CAC CCT   666

 V  T  M  P  S  T  G  G  D  S  C  P  F  C  L  K  L  L  L  P     231
GTC ACC ATG CCC TCA ACA GGC GGA GAC AGC TGT CCT TTC TGT CTG AAG CTA CTA CTT CCA   726

 G  L  L  L  L  L  V  V  I  I  L  I  L  A  F  W  V  L  P  K     251
GGG TTA TTA CTG TTG CTG GTG GTG ATA ATC CTA ATT CTG GCT TTT TGG GTA CTG CCC AAA   786

 Y  K  T  R  K  A  M  R  N  N  V  P  R  D  R  G  D  T  A  M     271
TAC AAA ACA AGA AAA GCT ATG AGA AAT AAT GTG CCC AGG GAC CGT GGA GAC ACA GCC ATG   846

 E  V  G  I  Y  A  N  I  L  E  K  Q  A  K  E  E  S  V  P  E     291
GAA GTT GGA ATC TAT GCA AAT ATC CTT GAA AAA CAA GCA AAG GAG GAA TCT GTG CCA GAA   906

 V  G  S  R  P  C  V  S  T  A  Q  D  E  A  K  H  S  Q  E  L     311
GTG GGA TCC AGG CCG TGT GTT TCC ACA GCC CAA GAT GAG GCC AAA CAC TCC CAG GAG CTA   966

 Q  Y  A  T  P  V  F  Q  E  V  A  P  R  E  Q  E  A  C  D  S     331
CAG TAT GCC ACC CCC GTG TTC CAG GAG GTG GCA CCA AGA GAG CAA GAA GCC TGT GAT TCT  1026
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SECRETED PROTEINS AND USES THEREOF

Background of the Invention

Many secreted proteins, for example, cytokines and cytokine receptors, play a vital role in the regulation of cell growth, cell differentiation, and a variety of specific cellular responses. A number of medically useful proteins, including erythropoietin, granulocyte-macrophage colony stimulating factor, human growth hormone, and various interleukins, are secreted proteins. Thus, an important goal in the design and development of new therapies is the identification and characterization of secreted and transmembrane proteins and the genes which encode them.

Many secreted proteins are receptors which bind a ligand and transduce an intracellular signal, leading to a variety of cellular responses. The identification and characterization of such a receptor enables one to identify both the ligands which bind to the receptor and the intracellular molecules and signal transduction pathways associated with the receptor, permitting one to identify or design modulators of receptor activity, e.g., receptor agonists or antagonists and modulators of signal transduction.

Summary of the Invention

The present invention is based, at least in part, on the discovery of cDNA molecules which encode the TANGO 228, 240, and 243 proteins, all of which are either wholly secreted or transmembrane proteins.

The TANGO 228 proteins are homologous to rat surface protein MCA-32 (mast cell Ag-32), a component of the immunologic pathway.

The TANGO 240 proteins are homologous to the Mycobacterium tuberculosis conserved hypothetical protein Rv0712.

The TANGO 243 proteins share significant homology to human PLAP (phospholipase A2-activating protein), a modulator of arthropathic disorders.

The TANGO 228, TANGO 240, and TANGO 243 proteins, fragments, derivatives, and variants thereof are collectively referred to herein as "polypeptides of the invention" or "proteins of the invention." Nucleic acid molecules encoding the polypeptides or proteins of the invention are collectively referred to as "nucleic acids of the invention."

The nucleic acids and polypeptides of the present invention are useful as modulating agents in regulating a variety of cellular processes. Accordingly, in one aspect, this invention provides isolated nucleic acid molecules encoding a polypeptide of the invention or a biologically active portion thereof. The present invention also provides nucleic acid molecules which are suitable for use as primers or hybridization probes for the detection of nucleic acids encoding a polypeptide of the invention.

The invention features nucleic acid molecules which are at least 30% (or 35%, 40%, 45%, 50%, 55%, 65%, 75%, 85%, 95%, or 98%) identical to the nucleotide sequence of SEQ ID NO:1, the nucleotide sequence of the cDNA insert of a clone deposited with ATCC as Accession Number 207116, or a complement thereof.

The invention features nucleic acid molecules which are at least 48% (or 50%, 55%, 60%, 65%, 75%, 85%, 95%, or 98%) identical to the nucleotide sequence of SEQ ID NO:2, the nucleotide sequence of the cDNA insert of a clone deposited with ATCC as Accession Number 207116, or a complement thereof.

The invention features nucleic acid molecules which are at least 30% (or 40%, 45%, 50%, 55%, 65%, 75%, 85%, 95%, or 98%) identical to the nucleotide sequence of SEQ ID NO:13 or 14, the nucleotide sequence of the cDNA insert of a clone deposited with ATCC as Accession Number 207116, or a complement thereof.

The invention features nucleic acid molecules which are at least 80% (or 82%, 85%, 87%, 90%, 92%, 95%, or 98%) identical to the nucleotide sequence of SEQ ID NO:19, the nucleotide sequence of the cDNA insert of a clone deposited with ATCC as Accession Number 207116, or a complement thereof.

The invention features nucleic acid molecules which are at least 93% (or 94%, 95%, 96%, 97%, or 98%) identical to the nucleotide sequence of SEQ ID NO:20, the nucleotide sequence of the cDNA insert of a clone deposited with ATCC as Accession Number 207116, or a complement thereof.

The invention features nucleic acid molecules which include a fragment of at least 310 (400, 500, 600, 800, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 2750, 3000, 3250, 3500, 3750, 4000, or 4020) nucleotides of the nucleotide sequence of SEQ ID NO:1 the nucleotide sequence of the cDNA of ATCC Accession Number 207116, or a complement thereof.

The invention features nucleic acid molecules which are at least 30% (or 40%, 45%, 50%, 55%, 65%, 75%, 85%, 95%, or 98%) identical to the nucleotide sequence of SEQ ID NO:31 or 32, or a complement thereof.

The invention features nucleic acid molecules which are at least 30% (or 40%, 45%, 50%, 55%, 65%, 75%, 85%, 95%, or 98%) identical to the nucleotide sequence of SEQ ID NO:39 or 40, or a complement thereof.

The invention features nucleic acid molecules which include a fragment of at least 515 (530, 550, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, or 2150) nucleotides of the nucleotide sequence of SEQ ID NO:13, the nucleotide sequence of the cDNA of ATCC Accession Number 207116, or a complement thereof.

The invention features nucleic acid molecules which include a fragment of at least 2220 (2260, 2300, 2340, 2380, 2420, 2460, 2500, 2540, 2580, 2620, 2660, 2700, 2740, 2780, or 2800) nucleotides of the nucleotide sequence of SEQ ID NO:19, the nucleotide sequence of the cDNA of ATCC Accession Number 207116, or a complement thereof.

The invention features nucleic acid molecules which include a fragment of at least 50 (100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, or 900) nucleotides of the nucleotide sequence of SEQ ID NO:31 or 32, or a complement thereof.

The invention features nucleic acid molecules which include a fragment of at least 50 (100, 150, 200, 250, 300, 350, 400, 450 500, 550, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, or 220) nucleotides of the nucleotide sequence of SEQ ID NO:39 or 40, or a complement thereof.

The invention also features nucleic acid molecules which include a nucleotide sequence encoding a protein having an amino acid sequence that is at least 30% (or 35%, 40%, 45%, 50%, 55%, 60%, 65%, 75%, 85%, 95%, or 98%) identical to the amino acid sequence of SEQ ID NO:3, the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116, or a complement thereof.

The invention also features nucleic acid molecules which include a nucleotide sequence encoding a protein having an amino acid sequence that is at least 35% (or 40%, 45%, 50%, 55%, 65%, 75%, 85%, 95%, or 98%) identical to the amino acid sequence of SEQ ID NO:15, the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116, or a complement thereof.

The invention also features nucleic acid molecules which include a nucleotide sequence encoding a protein having an amino acid sequence that is at least 93% (or 94%, 95%, 96%, 97% or 98%) identical to the amino acid sequence of SEQ ID NO:21, the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116, or a complement thereof.

The invention also features nucleic acid molecules which include a nucleotide sequence encoding a protein having an amino acid sequence that is at least 35% (or 40%, 45%, 50%, 55%, 65%, 75%, 85%, 95%, or 98%) identical to the amino acid sequence of SEQ ID NO:33, the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116, or a complement thereof.

The invention also features nucleic acid molecules which include a nucleotide sequence encoding a protein having an amino acid sequence that is at least 35% (or 40%, 45%, 50%, 55%, 65%, 75%, 85%, 95%, or 98%) identical to the amino acid sequence of SEQ ID NO:41, the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116, or a complement thereof.

The invention also features nucleic acid molecules which include a nucleotide sequence encoding a protein having an amino acid sequence that is at least 35% (or 40%, 45%, 50%, 55%, 65%, 75%, 85%, 95%, or 98%) identical to the amino acid sequence of SEQ ID NO:45, the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116, or a complement thereof.

In preferred embodiments, the nucleic acid molecules have the nucleotide sequence of SEQ ID NO:1, 2, 13, 14, 19, 20, 31, 32, 39, 40, 44 or the nucleotide sequence of the cDNA of ATCC Accession Number 207116.

Also within the invention are nucleic acid molecules which encode a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:3, or a fragment including at least 15 (25, 30, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, or 340) contiguous amino acids of SEQ ID NO:3, or the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116.

Also within the invention are nucleic acid molecules which encode a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:15, or a fragment including at least 15 (25, 30, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 360, or 370) contiguous amino acids of SEQ ID NO:15, or the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116.

Also within the invention are nucleic acid molecules which encode a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:21, or a fragment including at least 740 (745, 750, 755, 760, 765, 770, 775, 780, 785, or 790) contiguous amino acids of SEQ ID NO:21, or the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116.

Also within the invention are nucleic acid molecules which encode a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:33, or a fragment including at least 15 (25, 30, 50, 75, 100, 125, 150, 175, 200, 225, or 240) contiguous amino acids of SEQ ID NO:33.

Also within the invention are nucleic acid molecules which encode a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:41, or a fragment including at least 15 (25, 30, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350 or 370) contiguous amino acids of SEQ ID NO:41.

Also within the invention are nucleic acid molecules which encode a fragment of a polypeptide having the amino acid sequence of SEQ ID NO:21, or a fragment including at least 740 (745, 750, 755, 760, 765, 770, 775, 780, 785, 790, 800, 825, 850, or 880) contiguous amino acids of SEQ ID NO:21, or the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116.

The invention includes nucleic acid molecules which encode a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:3, 15, 21, 33, 41, or 45, or the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule consisting of a nucleic acid sequence encoding SEQ ID NO:3, 15, 21, 33, 41, or 45, the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116, or a complement thereof under stringent conditions.

Also within the invention are isolated polypeptides or proteins having an amino acid sequence that is at least about 30%, preferably 35%, 45%, 55%, 65%, 75%, 85%, 95%, or 98% identical to the amino acid sequence of SEQ ID NO:3, or the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116.

Also within the invention are isolated polypeptides or proteins having an amino acid sequence that is at least about 35%, preferably 40%, 45%, 50%, 55%, 65%, 75%, 85%, 95%, or 98% identical to the amino acid sequence of SEQ ID NO:15, or the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116.

Also within the invention are isolated polypeptides or proteins having an amino acid sequence that is at least about 93%, preferably 94%, 95%, 96%, 97% or 98% identical to the amino acid sequence of SEQ ID NO:21, or the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116.

Also within the invention are isolated polypeptides or proteins having an amino acid sequence that is at least about 35%, preferably 40%, 45%, 50%, 55%, 65%, 75%, 85%, 95%, or 98% identical to the amino acid sequence of SEQ ID NO:33.

Also within the invention are isolated polypeptides or proteins having an amino acid sequence that is at least about 35%, preferably 40%, 45%, 50%, 55%, 65%, 75%, 85%, 95%, or 98% identical to the amino acid sequence of SEQ ID NO:41

Also within the invention are isolated polypeptides or proteins having an amino acid sequence that is at least about 35%, preferably 40%, 45%, 50%, 55%, 65%, 75%, 85%, 95%, or 98% identical to the amino acid sequence of SEQ ID NO:45.

Also within the invention are isolated polypeptides or proteins which are encoded by a nucleic acid molecule having a nucleotide sequence that is at least about 48%, preferably 50%, 55%, 60%, 65%, 75%, 85%, or 95% identical to the nucleic acid sequence encoding SEQ ID NO:3, and isolated polypeptides or proteins which are encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 or 2, a complement thereof, or the non-coding strand of the cDNA of ATCC Accession Number 207116.

Also within the invention are isolated polypeptides or proteins which are encoded by a nucleic acid molecule having a nucleotide sequence that is at least about 30%, preferably 35%, 40%, 45%, 50%, 55%, 60%, 65%, 75%, 85%, or 95% identical to the nucleic acid sequence encoding SEQ ID NO:15, and isolated polypeptides or proteins which are encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:13 or 14, a complement thereof, or the non-coding strand of the cDNA of ATCC Accession Number 207116.

Also within the invention are isolated polypeptides or proteins which are encoded by a nucleic acid molecule having a nucleotide sequence that is at least about 93%, preferably 94%, 95%, 96%, 97%, or 98% identical to the nucleic acid sequence encoding SEQ ID NO:21, and isolated polypeptides or proteins which are encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:19, 20, or 44, a complement thereof, or the non-coding strand of the cDNA of ATCC Accession Number 207116.

Also within the invention are isolated polypeptides or proteins which are encoded by a nucleic acid molecule having a nucleotide sequence that is at least about 30%, preferably 35%, 40%, 45%, 50%, 55%, 60%, 65%, 75%, 85%, or 95% identical to the nucleic acid sequence encoding SEQ ID NO:33, and isolated polypeptides or proteins which are encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:31 or 32, a complement thereof.

Also within the invention are isolated polypeptides or proteins which are encoded by a nucleic acid molecule having a nucleotide sequence that is at least about 30%, preferably 35%, 40%, 45%, 50%, 55%, 60%, 65%, 75%, 85%, or 95% identical to the nucleic acid sequence encoding SEQ ID NO:41, and isolated polypeptides or proteins which are encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:39 or 40.

Also within the invention are isolated polypeptides or proteins which are encoded by a nucleic acid molecule having a nucleotide sequence that is at least about 30%, preferably 35%, 40%, 45%, 50%, 55%, 60%, 65%, 75%, 85%, or 95% identical to the nucleic acid sequence encoding SEQ ID NO:45, and isolated polypeptides or proteins which are encoded by a nucleic acid molecule having a nucleotide sequence which hybridizes under stringent hybridization conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:19 or 44.

Also within the invention are polypeptides which are naturally occurring allelic variants of a polypeptide that includes the amino acid sequence of SEQ ID NO:3, 15, 21, 33, 41, or 45, or the amino acid sequence encoded by the cDNA of ATCC Accession Number 207116, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule having the sequence of SEQ ID NO:1, 2, 3, 13, 14, 19, 20, 31, 32, 39, 40, or 44 or a complement thereof under stringent conditions.

The invention also features nucleic acid molecules that hybridize under stringent conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1 or 2, the cDNA of ATCC Accession Number 207116, or a complement thereof. In other embodiments, the nucleic acid molecules are at least 310 (400, 500, 600, 800, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 2750, 3000, 3250, 3500, 3750, 4000, or 4020) nucleotides in length and hybridize under stringent conditions to a nucleic acid molecule comprising

the nucleotide sequence of SEQ ID NO:1 or 2, the cDNA of ATCC Accession Number 207116, or a complement thereof.

The invention also features nucleic acid molecules that hybridize under stringent conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:13 or 14, the cDNA of ATCC Accession Number 207116, or a complement thereof. In other embodiments, the nucleic acid molecules are at least 515 (530, 550, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, or 2150) nucleotides in length and hybridize under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:13 or 14, the cDNA of ATCC Accession Number 207116, or a complement thereof.

The invention also features nucleic acid molecules that hybridize under stringent conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:19 or 20, or the cDNA of ATCC Accession Number 207116, or a complement thereof. In other embodiments, the nucleic acid molecules are at least 2220 (2260, 2300, 2340, 2380, 2420, 2460, 2500, 2540, 2580, 2620, 2660, 2700, 2740, 2780, or 2800) nucleotides in length and hybridize under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:19 or 20, the cDNA of ATCC Accession Number 207116, or a complement thereof.

The invention also features nucleic acid molecules that hybridize under stringent conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:31 or 32, or a complement thereof. In other embodiments, the nucleic acid molecules are at least 100 (150, 200, 250, 300, 350, 400, 500, 600, or 700) nucleotides in length and hybridize under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:31 or 32, or a complement thereof.

The invention also features nucleic acid molecules that hybridize under stringent conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:39 or 40, or a complement thereof. In other embodiments, the nucleic acid molecules are at least 515 (530, 550, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, or 2150) nucleotides in length and hybridize under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:39 or 40, or a complement thereof.

The invention also features nucleic acid molecules that hybridize under stringent conditions to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:19 or 44, the cDNA of ATCC Accession Number 207116, or a complement thereof. In other

embodiments, the nucleic acid molecules are at least 515 (530, 550, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, or 2150) nucleotides in length and hybridize under stringent conditions to a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:19 or 44, the cDNA of ATCC Accession Number 207116, or a complement thereof.

In one embodiment, the invention provides an isolated nucleic acid molecule which is antisense to the coding strand of a nucleic acid of the invention.

Another aspect of the invention provides vectors, e.g., recombinant expression vectors, comprising a nucleic acid molecule of the invention. In another embodiment, the invention provides host cells containing such a vector or a nucleic acid molecule of the invention. The invention also provides methods for producing a polypeptide of the invention by culturing, in a suitable medium, a host cell of the invention containing a recombinant expression vector such that a polypeptide is produced.

Another aspect of this invention features isolated or recombinant proteins and polypeptides of the invention. Preferred proteins and polypeptides possess at least one biological activity possessed by the corresponding naturally-occurring human polypeptide. An activity, a biological activity, or a functional activity of a polypeptide or nucleic acid of the invention refers to an activity exerted by a protein, polypeptide or nucleic acid molecule of the invention on a responsive cell as determined in vivo, or in vitro, according to standard techniques. Such activities can be a direct activity, such as an association with or an enzymatic activity on a second protein, or an indirect activity, such as a cellular signaling activity mediated by interaction of the protein with a second protein.

For TANGO 228, biological activities include, e.g., (1) the ability to modulate, e.g., stabilize, protein-protein interactions (e.g., homophilic and/or heterophilic), and protein-ligand interactions, e.g., in receptor-ligand recognition; (2) ability to interact with (e.g., noncovalently bind to) antigens, e.g., the antigens that elicited their formation; (3) the ability to initiate the immune response; and (4) the ability to modulate the activity (e.g., the development and/or activation) of connective tissue cells (e.g., mast cells and/or monocytes).

Other activities of TANGO 228 include: (1) the ability to modulate intracellular signaling cascades (e.g., signal transduction cascade), e.g., by interacting with target peptides (e.g., phosphotyrosine-containing target peptides); and (2) the ability to mediate

(e.g., initiate) the allergic response, e.g., by serving as a receptor to antigens and/or a signaling molecule to other immune response mediators (e.g., histamines).

Still other activities of TANGO 228 include: (1) the ability to modulate (e.g., inhibit) lipid associated processes (e.g., exocytosis and/or lipid mediator generation) by, e.g., interacting with (e.g., binding to) a cell surface protein (e.g., a receptor) on a cell type involved in the immune response (e.g., mast cell); and (2) the ability to perform one or more of the functions of rat surface protein MCA-32 described, for example, in Pirozzi et al. (1995) Journal of Immunology. 155:5811-5818, the contents of which are incorporated herein by reference.

For TANGO 240, biological activities include, e.g., (1) the ability to modulate the tuberculosis pathology pathway in the same fashion as Mycobacterium tuberculosis conserved hypothetical protein Rv0712; and (2) the ability to modulate the function, migration, proliferation (e.g., suppress cell growth), and/or differentiation of cells, e.g., cells in tissues in which it is expressed (see description of expression data below).

For TANGO 243, biological activities include, e.g., (1) the ability to modulate (e.g., activate) the activity of enzymes (e.g., phospholipases) that hydrolyze lipids (e.g., phospholipids); (2) the ability to modulate (e.g., activate) the activity of enzymes that release precursors (e.g., arachidonic acid) of regulatory molecules (e.g., prostaglandins and/or eicosanoids) associated with the arthropathy pathway; (3) the ability to mediate (e.g., activate) the arthropathy pathway, e.g., through biological activity (1); and (4) the ability to modulate (e.g., induce) the activity (e.g., proliferation) of cell types associated with the arthropathic pathway (e.g., leukocytes, e.g., polymorphonuclear leukocytes, and/or mononuclear inflammatory cells).

Other activities of TANGO 243 include: (1) the ability to modulate, e.g., by causing cells to alter (e.g., increase) the synthesis of, and/or by causing cells (e.g., macrophages) to release, cell mediating molecules, e.g., cytokines (e.g., IL-1 and/or TNF); (2) the ability to modulate (e.g., initiate) an immune and/or inflammatory response, e.g., through biological activity (1); and (3) the ability to modulate (e.g., perpetuate) an immune and/or inflammatory response, e.g., through biological activity (1), whereby TANGO 243 itself is stimulated by the cell mediating molecule that it modulates (e.g., cytokine (e.g., IL-1 and/or TNF)).

Additional activities of TANGO 243 include: (1) the ability to modulate (e.g., initiate) the activity of enzymes (e.g., phospholipases (e.g., phospholipase A2), e.g., by modulating their signal transduction; (2) the ability to modulate (e.g., promote) cell-cell

interaction (e.g., chemotaxis) by modulating (e.g., initiating) phospolipase A2 activation and/or signal transduction; (3) the ability to modulate (e.g., increase) cell permeability (e.g., endothelial cell permeability), e.g., via biological activity (2); and (4) the ability to modulate (e.g., promote) cell-cell adhesion (e.g., membrane fusion), e.g., via biological activity (2).

Still other activities of TANGO 243 include: (1) the ability to modulate (e.g., inhibit) cellular functions, e.g., cell division, cell-fate determination, gene transcription, transmembrane signaling, mRNA modification, and/or vesicle fusion; (2) the ability to modulate signal transduction, e.g., that of transmembrane receptors; and (3) the ability to perform one or more of the functions of human PLAP described, for example, in Bomalaski et al. (1990) J Lab Clin Med. 16 (6):814-825, the contents of which are incorporated herein by reference.

In one embodiment, a polypeptide of the invention has an amino acid sequence sufficiently identical to an identified domain of a polypeptide of the invention. As used herein, the term "sufficiently identical" refers to a first amino acid or nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., with a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have a common structural domain and/or common functional activity. For example, amino acid or nucleotide sequences which contain a common structural domain having about 60% identity, preferably 65% identity, more preferably 75%, 85%, 95%, 98% or more identity are defined herein as sufficiently identical.

In one embodiment, a TANGO 228 protein includes at least one or more of the following domains: a signal sequence, an extracellular domain, an Ig domain, a transmembrane domain, and an intracellular domain. In another embodiment, a TANGO 228 protein includes an extracellular domain, two Ig domains, and is wholly secreted. In yet another, a TANGO 228 protein includes an extracellular domain, two Ig domains, a transmembrane domain, and a cytoplasmic domain, and is a transmembrane protein.

In one embodiment, a TANGO 240 protein includes a signal peptide.

In one embodiment, a TANGO 243 protein includes a G-beta domain. In another embodiment, a TANGO 243 protein includes at least about two G-beta domains. In still another embodiment, a TANGO 243 protein includes at least three, four, five, six, or seven G-beta domains.

The polypeptides of the present invention, or biologically active portions thereof, can be operably linked to a heterologous amino acid sequence to form fusion proteins. The invention further features antibodies that specifically bind a polypeptide of the invention such as monoclonal or polyclonal antibodies. In addition, the polypeptides of the invention or biologically active portions thereof can be incorporated into pharmaceutical compositions, which optionally include pharmaceutically acceptable carriers.

In another aspect, the present invention provides methods for detecting the presence of the activity or expression of a polypeptide of the invention in a biological sample by contacting the biological sample with an agent capable of detecting an indicator of activity such that the presence of activity is detected in the biological sample.

In another aspect, the invention provides methods for modulating activity of a polypeptide of the invention comprising contacting a cell with an agent that modulates (inhibits or stimulates) the activity or expression of a polypeptide of the invention such that activity or expression in the cell is modulated. In one embodiment, the agent is an antibody that specifically binds to a polypeptide of the invention.

In another embodiment, the agent modulates expression of a polypeptide of the invention by modulating transcription, splicing, or translation of an mRNA encoding a polypeptide of the invention. In yet another embodiment, the agent is a nucleic acid molecule having a nucleotide sequence that is antisense to the coding strand of an mRNA encoding a polypeptide of the invention.

The present invention also provides methods to treat a subject having a disorder characterized by aberrant activity of a polypeptide of the invention or aberrant expression of a nucleic acid of the invention by administering an agent which is a modulator of the activity of a polypeptide of the invention or a modulator of the expression of a nucleic acid of the invention to the subject. In one embodiment, the modulator is a protein of the invention. In another embodiment, the modulator is a nucleic acid of the invention. In other embodiments, the modulator is a peptide, peptidomimetic, or other small molecule.

The present invention also provides diagnostic assays for identifying the presence or absence of a genetic lesion or mutation characterized by at least one of: (i) aberrant modification or mutation of a gene encoding a polypeptide of the invention, (ii) mis-regulation of a gene encoding a polypeptide of the invention, and (iii) aberrant post-translational modification of the invention wherein a wild-type form of the gene encodes a protein having the activity of the polypeptide of the invention.

In another aspect, the invention provides a method for identifying a compound that binds to or modulates the activity of a polypeptide of the invention. In general, such methods entail measuring a biological activity of the polypeptide in the presence and absence of a test compound and identifying those compounds which alter the activity of the polypeptide.

The invention also features methods for identifying a compound which modulates the expression of a polypeptide or nucleic acid of the invention by measuring the expression of the polypeptide or nucleic acid in the presence and absence of the compound.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

Brief Description of the Drawings

Figures 1A-1C depict the cDNA sequence of human TANGO 228 (SEQ ID NO:1) and the predicted amino acid sequence of TANGO 228 (SEQ ID NO:3). The open reading frame of SEQ ID NO:1 extends from nucleotide 34 to nucleotide 1062 of SEQ ID NO:1 (SEQ ID NO:2).

Figure 2 depicts a hydropathy plot of human TANGO 228. Relatively hydrophobic regions of the protein are above the dashed horizontal line, and relatively hydrophilic regions of the protein are below the dashed horizontal line. The cysteine residues (cys) and potential N-glycosylation sites (Ngly) are indicated by short vertical lines just below the hydropathy trace. The dashed vertical line separates the signal sequence (amino acids 1 to 19 of SEQ ID NO:3; SEQ ID NO:5) on the left from the mature protein (amino acids 20 to 343 of SEQ ID NO:3; SEQ ID NO:4) on the right. Thicker gray horizontal bars below the dashed horizontal line indicate extracellular ("out"), transmembrane ("TM"), and intracellular ("in") regions of the molecule. Below the hydropathy plot, the amino acid sequence of TANGO 228 is depicted.

Figures 3A-3H depict an alignment of the nucleotide sequence of rat surface protein MCA-32 (SEQ ID NO:11; GenBank Accession Number U39546) and the nucleotide sequence of human TANGO 228 (SEQ ID NO:1). The nucleotide sequences of rat MCA-32 and human TANGO 228 are 28.3% identical. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4.

Figures 4A-4C depict an alignment of the nucleotide sequence of the open reading frames of rat MCA-32 (nucleotides 8 to 826 of SEQ ID NO:11) and human TANGO 228 (SEQ ID NO:2). The nucleotide sequences of the open reading frames of rat MCA-32 and human TANGO 228 (SEQ ID NO:2) are 45.4% identical. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4.

Figure 5 depicts an alignment of the amino acid sequence of rat MCA-32 (SEQ ID NO:12) and the amino acid sequence of human TANGO 228 (SEQ ID NO:3). The amino acid sequences of rat MCA-32 and human TANGO 228 are 26.8% identical. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4.

Figures 6A-6B depict the cDNA sequence of human TANGO 240 (SEQ ID NO:13) and the predicted amino acid sequence of TANGO 240 (SEQ ID NO:15). The open reading frame of SEQ ID NO:13 extends from nucleotide 2 to nucleotide 1123 of SEQ ID NO:13 (SEQ ID NO:14).

Figure 7 depicts a hydropathy plot of human TANGO 240. Relatively hydrophobic regions of the protein are shown above the horizontal line, and relatively hydrophilic regions of the protein are below the horizontal line. The cysteine residues (cys) and potential N-glycosylation sites (Ngly) are indicated by short vertical lines just below the hydropathy trace. The dashed vertical line separates the signal sequence (amino acids 1 to 34 of SEQ ID NO:15; SEQ ID NO:17) on the left from the mature protein (amino acids 35 to 374 of SEQ ID NO:15; SEQ ID NO:16) on the right. Below the hydropathy plot, the amino acid sequence of TANGO 240 is depicted.

Figure 8 depicts an alignment of the amino acid sequence of the Mycobacterium tuberculosis conserved hypothetical protein Rv0712 (SEQ ID NO:18; GenBank Accession Number Z84395) and the amino acid sequence of human TANGO 240 (SEQ ID NO:15). The amino acid sequences of Rv0712 and human TANGO 240 are 31.2% identical. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4.

Figures 9A-9C depict the cDNA sequence of human TANGO 243 (SEQ ID NO:19) and the predicted amino acid sequence of TANGO 243 (SEQ ID NO:21). The open reading frame of SEQ ID NO:19 extends from nucleotide 183 to nucleotide 2567 of SEQ ID NO:19 (SEQ ID NO:20).

Figure 10 depicts a hydropathy plot of human TANGO 243. Relatively hydrophobic regions of the protein are above the dashed horizontal line, and relatively hydrophilic regions of the protein are below the dashed horizontal line. The cysteine residues (cys) and potential N-glycosylation sites (Ngly) are indicated by short vertical lines just below the hydropathy trace. Below the hydropathy plot, the amino acid sequence of TANGO 243 is depicted.

Figures 11A-11F depict an alignment of the nucleotide sequence of human PLAP (SEQ ID NO:29; GenBank Accession Number AF083395) and the nucleotide sequence of human TANGO 243 (SEQ ID NO:19). The nucleotide sequences of human PLAP and human TANGO 243 are 78.8% identical. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4.

Figures 12A-12E depict an alignment of the nucleotide sequence of the open reading frames of human PLAP (nucleotides 1 to 2217 of SEQ ID NO:29) and human TANGO 243 (SEQ ID NO:20). The nucleotide sequences of the open reading frames of human PLAP and human TANGO 243 (SEQ ID NO:20) are 92.7% identical. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4.

Figures 13A-13B depict an alignment of the amino acid sequence of human PLAP (SEQ ID NO:30) and the amino acid sequence of human TANGO 243 (SEQ ID NO:21). The amino acid sequences of human PLAP and human TANGO 243 are 92.8% identical. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4.

Figure 14 depicts the cDNA sequence of murine TANGO 228 (SEQ ID NO:31) and the predicted amino acid sequence of murine TANGO 228 (SEQ ID NO:33). The open reading frame of SEQ ID NO:31 extends from nucleotide 27 to nucleotide 743 of SEQ ID NO:31 (SEQ ID NO:33).

Figures 15A-15B depict an alignment of the nucleotide sequence of murine TANGO 228 (SEQ ID NO:31; upper line of each pair) and the nucleotide sequence of human TANGO 228 (SEQ ID NO:1; lower line of each pair). The nucleotide sequences of murine TANGO 228 and human TANGO 228 are 58.2% identical, over the length of the murien cDNA. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4.

Figures 16A-16B depict an alignment of the nucleotide sequence of the open reading frames of murine TANGO 228 (SEQ ID NO:33; upper line of each pair) and human TANGO 228 (SEQ ID NO:3; lower line of each pair). The nucleotide sequences of the open reading frames of murine TANGO 228 human TANGO 228 are 58.2% identical, over the length of the murine ORF. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4.

Figure 17 depicts an alignment of the amino acid sequence of murine TANGO 228 (SEQ ID NO:32; upper line of each pair) and human TANGO 228 (SEQ ID NO:3; lower line of each pair). The amino acid sequences of murine TANGO 228 and human TANGO 228 are 30.6% identical, over the length of the murine protein. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4.

Figures 18A-18B depict the cDNA sequence of murine TANGO 240 (SEQ ID NO:39) and the predicted amino acid sequence of murine TANGO 240 (SEQ ID NO:41). The open reading frame of SEQ ID NO:41 extends from nucleotide 2 to nucleotide 1117 of SEQ ID NO:41 (SEQ ID NO:40).

Figures 19A-19D depict an alignment of the nucleotide sequence of murine TANGO 240 (SEQ ID NO:39; upper line of each pair) and the nucleotide sequence of human TANGO 228 (SEQ ID NO:13; lower line of each pair). In this alignment the sequences are 78.4% identical, over the length of the human cDNA. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4.

Figures 20A-20B depict an alignment of the nucleotide sequence of the open reading frames of murine TANGO 240 (SEQ ID NO:40; upper line of each pair) and human TANGO 240 (SEQ ID NO:14; lower line of each pair). The nucleotide sequences of the open reading frames of murine TANGO 240 human TANGO 240 are 84.4% identical. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4.

Figure 21 depicts an alignment of the amino acid sequence of murine TANGO 240 (SEQ ID NO:41; upper line of each pair) and human TANGO 240 (SEQ ID NO:17; lower line of each pair). The amino acid sequences of murine TANGO 240 and human TANGO 240 are 86% identical. This alignment was performed using the ALIGN

alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4.

Figure 22A-22C depict the cDNA sequence of human TANGO 243 (SEQ ID NO:19) and the predicted amino acid sequence of a longer form of TANGO 243 (SEQ ID NO:45) arising from an alternative translation of the human TANGO 243 cDNA. In this translation the open reading frame of SEQ ID NO:19 extends from nucleotide 3 to nucleotide 2567 of SEQ ID NO:19 (SEQ ID NO:44).

Figure 23 depicts a hydropathy plot of the longer form of human TANGO 243 arising from the alternative translation of the human TANGO 243 cDNA. Relatively hydrophobic regions of the protein are above the dashed horizontal line, and relatively hydrophilic regions of the protein are below the dashed horizontal line. The cysteine residues (cys) and potential N-glycosylation sites (Ngly) are indicated by short vertical lines just below the hydropathy trace. Below the hydropathy plot, the amino acid sequence of TANGO 243 is depicted.

Detailed Description of the Invention

The TANGO 228, TANGO 240, and TANGO 243 proteins and nucleic acid molecules comprise a family of molecules having certain conserved structural and functional features. As used herein, the term "family" is intended to mean two or more proteins or nucleic acid molecules having a common structural domain and having sufficient amino acid or nucleotide sequence identity as defined herein. Family members can be from either the same or different species. For example, a family can comprises two or more proteins of human origin, or can comprise one or more proteins of human origin and one or more of non-human origin. Members of the same family may also have common structural domains.

For example, TANGO 228 proteins and TANGO 240 proteins of the invention have signal sequences. As used herein, a "signal sequence" includes a peptide of at least about 15 or 20 amino acid residues in length which occurs at the N-terminus of secretory and membrane-bound proteins and which contains at least about 70% hydrophobic amino acid residues such as alanine, leucine, isoleucine, phenylalanine, proline, tyrosine, tryptophan, or valine. In a preferred embodiment, a signal sequence contains at least about 10 to 40 amino acid residues, preferably about 19-34 amino acid residues, and has at least about 60-80%, more preferably 65-75%, and more preferably at least about 70% hydrophobic residues. A signal sequence serves to direct a protein containing such a

sequence to a lipid bilayer. Thus, in one embodiment, a TANGO 228 protein contains a signal sequence at about amino acids 1 to 19 of SEQ ID NO:3 (SEQ ID NO:5). In another embodiment, a TANGO 240 protein contains a signal sequence at about amino acids 1 to 34 of SEQ ID NO:15 (SEQ ID NO:17). The signal sequence is cleaved during processing of the mature protein.

In another example, a TANGO 228 family member also includes one or more of the following domains: (1) an extracellular domain; (2) a transmembrane domain; and (3) a cytoplasmic domain. Thus, in one embodiment, a TANGO 228 protein contains an extracellular domain at about amino acids 1 to 227 of SEQ ID NO:3 (SEQ ID NO:6). In another embodiment, a TANGO 228 protein contains a transmembrane domain at about amino acids 228 to 249 of SEQ ID NO:3 (SEQ ID NO:7). In another embodiment, a TANGO 228 protein contains a cytoplasmic domain at about amino acids 250 to 343 of SEQ ID NO:3 (SEQ ID NO:10).

In one embodiment, the extracellular domain of TANGO 228 can also include an Ig domain. In another embodiment, the extracellular domain of TANGO 228 includes about 1 to 10, preferably about 3-8, more preferably about 6, N-glycosylation sites, about 1 to 30, preferably about 10 to 20, more preferably about 15 conserved serine residues (not including residues within the Ig domain), and about 1 to 20, preferably about 5 to 15, more preferably about 11 conserved threonine residues (not including residues within the Ig domain).

In a preferred embodiment, a TANGO 228 family member has the amino acid sequence of SEQ ID NO:3 wherein the extracellular domain is located at about amino acids 1 to 227 (SEQ ID NO:6), the N-glycosylation sites are located at about amino acid residue positions 51 to 54, 60 to 63, 89 to 92, 151 to 154, 157 to 160, and 182 to 185, the conserved serine residues are located at about amino acid positions 3, 6, 12, 15, 16, 36, 41, 109, 111, 114, 118, 127, 209, 216, and 221, and the conserved threonine residues are located at about amino acid positions 18, 31, 43, 108, 120, 126, 137, 139, 207, 213, and 217.

TANGO 228 family members can also include an Ig domain contained within the extracellular domain. As used herein, the term "Ig domain" refers to a protein domain bearing homology to immunoglobulin superfamily members. An Ig domain includes about 30-90 amino acid residues, preferably about 40-80 amino acid residues, more preferably about 50-70 amino acid residues, still more preferably about 55-65 amino acid residues, and most preferably about 57 to 59 amino acid residues. Typically, an Ig

domain contains a conserved cysteine residue within about 5 to 15 amino acid residues, preferably about 7 to 12 amino acid residues, and most preferably about 8 amino acid residues from its N-terminal end, and another conserved cysteine residue within about 1 to 5 amino acid residues, preferably about 2 to 4 amino acid residues, and most preferably about 3 amino acid residues from its C-terminal end.

An Ig domain typically has the following consensus sequence, beginning about 1 to 15 amino acid residues, more preferably about 3 to 10 amino acid residues, and most preferably about 5 amino acid residues from the domain C terminal end of a protein: (FY)-Xaa-C-Xaa-(VA)-COO-, wherein (FY) is either a phenylalanine or a tyrosine residue (preferably tyrosine), Xaa is any amino acid, C is a cysteine residue, (VA) is either a valine or an alanine residue (preferably alanine), and COO- is the protein C terminus.

In one embodiment, a TANGO 228 family member includes one or more Ig domains having an amino acid sequence that is at least about 55%, preferably at least about 65%, more preferably at least about 75%, yet more preferably at least about 85%, and most preferably at least about 95% identical to amino acids 49 to 105 and/or amino acids 140 to 198 of SEQ ID NO:3, which are the Ig domains of TANGO 228 (these Ig domains are also represented as SEQ ID NO:8 and 9, respectively). In another embodiment, a TANGO 228 family member includes one or more Ig domains having an amino acid sequence that is at least about 55%, preferably at least about 65%, more preferably at least about 75%, yet more preferably at least about 85%, and most preferably at least about 95% identical to amino acids 49 to 105 and/or amino acids 140 to 198 of SEQ ID NO:3 (SEQ ID NO:8 and 9, respectively), includes a conserved cysteine residue about 8 residues downstream from the N-terminus of the Ig domain, and has one or more Ig domain consensus sequences described herein. In another embodiment, a TANGO 228 family member includes one or more Ig domains having an amino acid sequence that is at least 55%, preferably at least about 65%, more preferably at least about 75%, yet more preferably at least about 85%, and most preferably at least about 95% identical to amino acids 49 to 105 and/or amino acids 140 to 198 of SEQ ID NO:3 (SEQ ID NO:8 and 9, respectively), includes a conserved cysteine residue 8 residues downstream from the N-terminus of the Ig domain, has one or more Ig domain consensus sequences described herein, and has a conserved cysteine within the consensus sequence that forms a disulfide both with said first conserved cysteine. In yet another embodiment, a TANGO 228 family member includes one or more Ig domains having an

amino acid sequence that is at least 55%, preferably at least about 65%, more preferably at least about 75%, yet more preferably at least about 85%, and most preferably at least about 95% identical to amino acids 49 to 105 and/or amino acids 140 to 198 of SEQ ID NO:3 (SEQ ID NO:8 and 9, respectively), includes a conserved cysteine residue 8 residues downstream from the N-terminus of the Ig domain, has one or more Ig domain consensus sequences described herein, has a conserved cysteine within the consensus sequence that forms a disulfide both with said first conserved cysteine, and has at least one TANGO 228 biological activity as described herein.

In a preferred embodiment, a TANGO 228 family member has the amino acid sequence of SEQ ID NO:3 wherein the aforementioned Ig domain conserved residues are located as follows: the N-terminal conserved cysteine residue is located at about amino acid residue position 56 (within the first Ig domain (SEQ ID NO:8)) and at about amino acid residue position 147 (within the second Ig domain (SEQ ID NO:9)), and the C-terminal conserved cysteine residue is located at about amino acid position 103 (within the first Ig domain (SEQ ID NO:8)) and at about amino acid residue position 196 (within the second Ig domain (SEQ ID NO:9)).

A TANGO 228 family member can also include a cytoplasmic domain. The cytoplasmic domain can include hydrophobic amino acid residues, and can contain several SH2 domain recognition sites, which contain typically begin with a tyrosine residue. This cytoplasmic domain can contain about 1 to 5, more preferably about 2 to 4, and still more preferably about 3 SH2 domain recognition sites.

One type of SH2 domain recognition site typically has the following consensus sequence: Y-Xaa1-Xaa1-(IP), wherein Y is a tyrosine residue, Xaa1 is any hydrophobic amino acid, and (IP) is either an isoleucine or proline residue. Another type of SH2 domain recognition site typically has the following consensus sequence: Y-Xaa1-Xaa-Xaa1, wherein Y is a tyrosine residue, Xaa1 is any hydrophobic amino acid, and Xaa is any amino acid.

In a preferred embodiment, a TANGO 228 family member has the amino acid sequence of SEQ ID NO:3 wherein the cytoplasmic domain is located at about amino acids 250 to 343 (SEQ ID NO:10) and the conserved SH2 domain recognition sites are located at about amino acid positions 276 to 279, 313 to 317, and 338 to 341.

A TANGO 240 family member can include a signal sequence. In a preferred embodiment, a TANGO 240 family member has the amino acid sequence of SEQ ID NO:15, and the signal sequence is located at about amino acids 1 to 34.

A TANGO 243 family member can include one or more G-beta domains. As used herein, a "G-beta domain" refers to a domain which includes about 25 to 55 amino acid residues, preferably about 30 to 50 amino acid residues, more preferably about 35-55 amino acid residues, and most preferably about 38 to 44 amino acid residues. In addition, a G-beta domain contains a conserved glycine residue adjacent to a conserved histidine residue, a conserved aspartic acid residue, and one or more conserved valine or isoleucine residues.

G-beta domains are typically found within the WD-repeat (tryptophan-aspartate repeat) family of proteins. The WD-repeat family of proteins are found in all eukaryotes but not in prokaryotes, and are functionally diverse, in some cases regulating cellular functions, transmembrane signaling, and/or vesicle fusion, among other things.

A G-beta domain typically has the following consensus sequence: G-H-Xaa(n1)-V-Xaa(n2)-[V or L]-Xaa(n3)-D-Xaa(n4)-W, wherein G is glycine, H is histidine, V is valine, D is aspartic acid, L is leucine, W is tryptophan, Xaa is any amino acid, n1 is about 1-8 amino acid residues, more preferably about 2-5 amino acid residues, and more preferably about 3 amino acid residues, n2 is about 1-8 amino acid residues, more preferably about 1-4 amino acid residues, and more preferably about 2 amino acid residues, n3 is about 5-30 amino acid residues, more preferably about 10-25 amino acid residues, and more preferably about 14-19 amino acid residues, and n4 is about 1-8 amino acid residues, more preferably about 3-6 amino acid residues, and more preferably about 5 amino acid residues.

A TANGO 243 family member can also include a G-beta-like domain. As used herein, a "G-beta-like domain" refers to an stretch of amino acid residues, about 25 to 55 residues, preferably about 30 to 50 residues, more preferably about 35-55 amino acid residues, and most preferably about 38 to 44 residues, in length, that is similar in content to a G-beta domain. A G-beta-like domain typically has the following consensus sequence: [V or I]-Xaa(2)-[V or C]-Xaa(n1)-[P or D]-[D or N]-G-Xaa(n2)-G-Xaa(2)-D, wherein V is valine, I is isoleucine, C is cysteine, P is proline, D is aspartic acid, G is glycine, Xaa is any amino acid, n1 is about 1 to 8 amino acid residues, preferably about 2 to 5 amino acid residues, more preferably about 3 amino acid residues, and n2 is about 1 to 8 amino acid residues, preferably about 2 to 5 amino acid residues, more preferably about 4 amino acid residues.

In one embodiment, a TANGO 243 family member includes one or more G-beta domains having an amino acid sequence that is at least about 55%, preferably at least

about 65%, more preferably at least about 75%, yet more preferably at least about 85%, and most preferably at least about 95% identical to amino acids 8 to 47, or amino acids 55 to 98, or amino acids 102 to 139, or amino acids 141 to 179, or amino acids 181 to 218, or amino acids 221 to 259 of SEQ ID NO:21, which are the G-beta domains of TANGO 243 (these G-beta domains are also represented as SEQ ID NO:22, 23, 24, 25, 26, and 27, respectively). In another embodiment, a TANGO 243 family member includes one or more G-beta domains having an amino acid sequence that is at least about 55%, preferably at least about 65%, more preferably at least about 75%, yet more preferably at least about 85%, and most preferably at least about 95% identical to amino acids 8 to 47, or amino acids 55 to 98, or amino acids 102 to 139, or amino acids 141 to 179, or amino acids 181 to 218, or amino acids 221 to 259 of SEQ ID NO:21 (SEQ ID NO:22, 23, 24, 25, 26, and 27, respectively), and has a G-beta domain consensus sequence as described herein. In yet another embodiment, a TANGO 243 family member includes one or more G-beta domains having an amino acid sequence that is at least about 55%, preferably at least about 65%, more preferably at least about 75%, yet more preferably at least about 85%, and most preferably at least about 95% identical to amino acids 8 to 47, or amino acids 55 to 98, or amino acids 102 to 139, or amino acids 141 to 179, or amino acids 181 to 218, or amino acids 221 to 259 of SEQ ID NO:21 (SEQ ID NO:22, 23, 24, 25, 26, and 27, respectively), has a G-beta domain consensus sequence as described herein, and has at least one TANGO 243 biological activity as described herein.

In another embodiment, a TANGO 243 family member includes one or more G-beta domains having an amino acid sequence that is at least about 55%, preferably at least about 65%, more preferably at least about 75%, yet more preferably at least about 85%, and most preferably at least about 95% identical to amino acids 8 to 47, or amino acids 55 to 98, or amino acids 102 to 139, or amino acids 141 to 179, or amino acids 181 to 218, or amino acids 221 to 259 of SEQ ID NO:21, amino acids 68 to 107, or amino acids 115 to 158, or amino acids 162 to 199, or amino acids 201 to 139, or amino acids 141 to 278, or amino acids 281 to 219 of SEQ ID NO:45 (SEQ ID NO:22, 23, 24, 25, 26, and 27, respectively), G-beta-like domain having an amino acid sequence that is at least about 55%, preferably at least about 65%, more preferably at least about 75%, yet more preferably at least about 85%, and most preferably at least about 95% identical to amino acids 261 to 298 of SEQ ID NO:21 (SEQ ID NO:28), has a G-beta domain consensus sequence and a G-beta-like domain consensus sequence as described herein, and has at least one TANGO 243 biological activity as described herein.

A TANGO 243 family member can include a PLAP-like domain. A PLAP-like domain 1 can include at least 20, 25, 30, or 40 contiguous amino acids of SEQ ID NO:47. A PLAP-like domain 2 can include at least 20, 25, 30, 40, or 45 contiguous amino acids of SEQ ID NO:48. A PLAP-like domain 3 can include 20, 25, 30, 40, 45, 50, 60, 70, or 80 contiguous amino acids of SEQ ID NO:49. A PLAP-like domain 1 can include at least 20, 25, 30, or 35 contiguous amino acids of SEQ ID NO:50.

In a preferred embodiment, a TANGO 243 family member has the amino acid sequence of SEQ ID NO:21 wherein the G-beta consensus sequences are located from amino acid 16 to 46 (the first G-beta domain (SEQ ID NO:22)), 63 to 97 (the second G-beta domain (SEQ ID NO:23)), 110 to 138 (the third G-beta domain (SEQ ID NO:24)), 149 to 178 (the fourth G-beta domain (SEQ ID NO:25)), 189 to 217 (the fifth G-beta domain (SEQ ID NO:26)), and 229 to 258 (the sixth G-beta domain (SEQ ID NO:27)), and the G-beta-like consensus sequence is located from amino acid 261 to 298 (SEQ ID NO:28).

Various features of human TANGO 228, murine TANGO 228, human TANGO 240, and human TANGO 243 are summarized below.

Human TANGO 228

A cDNA encoding human TANGO 228 was identified by analyzing the sequences of clones present in a fetal spleen cDNA library. This analysis led to the identification of a clone, jthsa055f08, encoding full-length human TANGO 228. The human TANGO 228 cDNA of this clone is 4043 nucleotides long (Figures 1A-1C; SEQ ID NO:1). The open reading frame of this cDNA, nucleotides 34 to 1062 of SEQ ID NO:1 (SEQ ID NO:2), encodes a 343 amino acid transmembrane protein (Figures 1A-1C; SEQ ID NO:3).

The signal peptide prediction program SIGNALP (Nielsen et al. (1997) Protein Engineering 10:1-6) predicted that human TANGO 228 includes a 19 amino acid signal peptide (amino acid 1 to about amino acid 19 of SEQ ID NO:3)(SEQ ID NO:5) preceding the mature TANGO 228 protein (corresponding to about amino acid 20 to amino acid 343 of SEQ ID NO:3)(SEQ ID NO:4). The TANGO 228 protein molecular weight is 38.7 kDa prior to the cleavage of the signal peptide, 36.4 kDa after cleavage of the signal peptide.

TANGO 228 includes a extracellular domain (about amino acids 1 to 227 of SEQ ID NO:3; SEQ ID NO:6), a transmembrane domain (about amino acids 228 to 249 of SEQ ID NO:3; SEQ ID NO:7), two Ig domains (about amino acids 49 to 105, and about

amino acids140 to 198, of SEQ ID NO:3; SEQ ID NO:8 and 9), and a cytoplasmic domain (about amino acids 250 to 343 of SEQ ID NO:3; SEQ ID NO:10).

An N-glycosylation site having the sequence NVSM is found from amino acids 51 to 54 of SEQ ID NO:3. A second N-glycosylation site having the sequence NKSL is found from amino acids 60 to 63. A third N-glycosylation site having the sequence NLSI is found from amino acids 89 to 92. A fourth N-glycosylation site having the sequence NGSL is found from amino acids 151 to 154. A fifth N-glycosylation site having the sequence NYTF is found from amino acids 157 to 160. A sixth N-glycosylation site having the sequence NLTK is found from amino acids 182 to 185. A cAMP and cGMP-dependent protein kinase phosphorylation site having the sequence RRKT is found from amino acids 71 to 74. A protein kinase C phosphorylation site having the sequence TCR is found from amino acids 18 to 20. A second protein kinase C phosphorylation site having the sequence SHK is found from amino acids 57 to 59. A third protein kinase C phosphorylation site having the sequence TDR is found from amino acids 139 to 141. A fourth protein kinase C phosphorylation site having the sequence TKK is found from amino acids 184 to 186. A fifth protein kinase C phosphorylation site having the sequence TRK is found from amino acids 254 to 256. A sixth protein kinase C phosphorylation site having the sequence SYK is found from amino acids 331 to 333. A casein kinase II phosphorylation site having the sequence SITE is found from amino acids 91 to 94. A second casein kinase II phosphorylation site having the sequence TIVD is found from amino acids 120 to 123. A third casein kinase II phosphorylation site having the sequence TETD is found from amino acids 137 to 140. A fourth casein kinase II phosphorylation site having the sequence TFFE is found from amino acids 159 to 162. A fifth casein kinase II phosphorylation site having the sequence SKYD is found from amino acids 172 to 175. A sixth casein kinase II phosphorylation site having the sequence TGGD is found from amino acids 217 to 220. A seventh casein kinase II phosphorylation site having the sequence TAME is found from amino acids 269 to 272. An eighth casein kinase II phosphorylation site having the sequence SVPE is found from amino acids 288 to 291. A ninth casein kinase II phosphorylation site having the sequence TAQD is found from amino acids 300 to 303. A tyrosine kinase phosphorylation site having the sequence KNPGEEEEY is found from amino acids 186 to 194. A second tyrosine kinase phosphorylation site having the sequence KHSQELQY is found from amino acids 306 to 313. An N-myristoylation site having the sequence GQNVSM is found from amino acids 49 to 54. A second N-myristoylation site having

the sequence GTQDGK is found from amino acids 77 to 82. A third N-myristoylation site having the sequence GIYANI is found from amino acids 274 to 279. A fourth N-myristoylation site having the sequence GSRPCV is found from amino acids 293 to 298. A cell attachment sequence having the sequence RGD is found from amino acids 266 to 268. A leucine zipper pattern having the sequence LLLPGLLLLLVVIILILAFWVL is found from amino acids 228 to 249.

TANGO 228 is homologous to rat MCA-32, which is known to exist in two forms, one of which has a transmembrane domain, and the other which does not. Thus TANGO 228, like rat MCA-32, is likely to exist in two forms, one having domains as follows and is wholly secreted: an extracellular domain (SEQ ID NO:6) containing one or more Ig domains (SEQ ID NO:8 and 9), and the other having the following domains and is a transmembrane, e.g., a cell surface, protein: an extracellular domain (SEQ ID NO:6) containing one or more Ig domains (SEQ ID NO:8 and 9), a transmembrane domain (SEQ ID NO:7), and cytoplasmic domain (SEQ ID NO:10).

Figures 3A-3H show an alignment of the human TANGO 228 full length nucleic acid sequence (SEQ ID NO:1) with the rat MCA-32 full length nucleic acid sequence (SEQ ID NO:11). Figures 4A-4C show an alignment of the human TANGO 228 coding region (SEQ ID NO:2) with the rat MCA-32 coding region. Figure 5 shows an alignment of the human TANGO 228 protein sequence (SEQ ID NO:3) with the rat MCA-32 protein sequence (SEQ ID NO:12). As shown in Figure 5, the human TANGO 228 signal sequence is represented by amino acids 1-19 (and encoded by nucleotides 34 to 90 of SEQ ID NO:1). Though the rat MCA-32 gene structure suggests it can function as a secreted protein or cell surface protein, depending on the splice variant, it does not exhibit the characteristic hydrophobic signal sequence generally found in secretory and transmembrane proteins. The human TANGO 228 extracellular domain sequence (SEQ ID NO:6) is represented by amino acids 1 to 227 (and encoded by nucleotides 34 to 714 of SEQ ID NO:1), and the rat MCA-32 extracellular domain sequence is represented by amino acids 1 to 177 (and encoded by nucleotides 8 to 523 of SEQ ID NO:11). The human TANGO 228 Ig domains (SEQ ID NO:8 and 9) are represented by amino acids 49 to 105, and 140 to 198 (and encoded by nucleotides 178 to 348, and 451 to 627 of SEQ ID NO:1), and the rat MCA-32 Ig domain is represented by amino acids 99 to 153 (and encoded by nucleotides 287 to 451 of SEQ ID NO:11). The human TANGO 228 transmembrane domain (SEQ ID NO:7) is represented by amino acids 228 to 249 (and encoded by nucleotides 715 to 780 of SEQ ID NO:1), and the rat MCA-32

transmembrane domain is represented by amino acids 178 to 199 (and encoded by nucleotides 524 to 589 of SEQ ID NO:11). The human TANGO 228 cytoplasmic domain (SEQ ID NO:10) is represented by amino acids 250 to 343 (and encoded by nucleotides 781 to 1065 of SEQ ID NO:1), and the rat MCA-32 cytoplasmic domain is represented by amino acids 200 to 278 (and encoded by nucleotides 590 to 829 of SEQ ID NO:11).

Figures 3A-3H and Figures 4A-4C show that there is an overall 28.3% identity between the full length human TANGO 228 nucleic acid molecule and the full length rat MCA-32 nucleic acid molecule, and an overall 45.5% identity between the open reading frame of human TANGO 228 nucleic acid molecule and the open reading frame of the rat MCA-32 nucleic acid molecule, respectively. The amino acid alignment in Figure 5 shows a 26.8% overall amino acid sequence identity between human TANGO 228 and rat MCA-32.

Clone EpT228, which encodes human TANGO 228, was deposited with the American Type Culture Collection (10801 University Boulevard, Manassas, VA 20110-2209) on February 18, 1999 and assigned Accession Number 207116. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

Figure 2 depicts a hydropathy plot of human TANGO 228. Relatively hydrophobic regions of the protein are shown above the horizontal line, and relatively hydrophilic regions of the protein are below the horizontal line. The cysteine residues (cys) and N-glycosylation sites are indicated by short vertical lines just below the hydropathy trace. The dashed vertical line separates the signal sequence (amino acids 1 to 19 of SEQ ID NO:3; SEQ ID NO:5) on the left from the mature protein (amino acids 20 to 343 of SEQ ID NO:3; SEQ ID NO:4) on the right. The TANGO 228 transmembrane domain is indicated by the section of the plot under which the number 7.1 can be seen, which represents a score assigned to the predicted transmembrane domain. The extracellular domain (SEQ ID NO:6) and cytoplasmic domain (SEQ ID NO:10) are similarly indicated by gray horizontal bars, labeled as "out" and "in", respectively.

Human TANGO 228 was mapped to h17q23. The nearby flanking markers include: WI-4118 and WI-5110. The known nearby loci include: JPD (periodontitis, juvenile) and DGI1 (dentinogenesis imperfecta). Nearby known human genes include: COIL (coilin P80), TNFAIP1 (tumor necrosis factor), GH1 (growth hormone1, 2),

ICAM2 (intercellular adhesion molecule), APOH (apolipoprotein H), PEPE(peptidase E), MPO (myeloperoxidase), and CDK3(cyclin dependent kinase3). Thus, human TANGO 228 nucleic acid molecules can be used to map these loci and genes.

The syntenic mouse chromosomal location is mo11. Nearby mouse loci include: rimy (rimy), al (alopecia), nog (noggin), and bda (baldarthritic). Near known mouse genes include: rimy (rimy), al (alopecia), hlf (hepatic leukemia factor), chad (ahondroadherin), re (rex), mpo (myeloperoxidase), nog (noggin), bda (bald arthritic), gip(gastric inhibitory polypeptide), and ngfr (nerve growth factor receptor).

Murine TANGO 228

A cDNA encoding murine TANGO 228 was identified by analyzing the sequences of clones present in a bone marrow stromal cell cDNA library. This analysis led to the identification of a clone, jtmMa107f05, encoding full-length murine TANGO 228. The murine TANGO 228 cDNA of this clone is 911 nucleotides long (Figure 14; SEQ ID NO:31). The open reading frame of this cDNA, nucleotides 27 to 743 of SEQ ID NO:31 (SEQ ID NO:32), encodes a 239 amino acid transmembrane protein (Figure 14; SEQ ID NO:33).

The signal peptide prediction program SIGNALP (Nielsen et al. (1997) Protein Engineering 10:1-6) predicted that murine TANGO 228 includes a 34 amino acid signal peptide (amino acid 1 to about amino acid 34 of SEQ ID NO:33; SEQ ID NO:35) preceding the mature murine TANGO 228 protein (corresponding to about amino acid 35 to amino acid 239 of SEQ ID NO:33; SEQ ID NO:34). The murine TANGO 228 protein molecular weight is 26.7 kDa prior to the cleavage of the signal peptide, 22.9 kDa after cleavage of the signal peptide.

Mature murine TANGO 228 includes an extracellular domain (about amino acids 35 to 141 of SEQ ID NO:33; SEQ ID NO:36), a transmembrane domain (about amino acids 142 to 164 of SEQ ID NO:33; SEQ ID NO:37), and a cytoplasmic domain (about amino acids 165 to 239 of SEQ ID NO:33; SEQ ID NO:8). Murine TANGO 228 also includes an Ig domain (about amino acids 59 to 115 of SEQ ID NO:33; SEQ ID NO:46).

Figures 15A-15B show an alignment of the murine TANGO 228 full length nucleic acid sequence (upper line of each pair; SEQ ID NO:31) with the human TANGO 228 full length nucleic acid sequence (lower line of each pair; SEQ ID NO:1). Figures 16A-16B show an alignment of the murine TANGO 228 coding region (upper line of each pair; SEQ ID NO:32) with the human TANGO 228 coding region (lower line of

each pair; SEQ ID NO:2). Figure 17 shows an alignment of the murine TANGO 228 protein sequence (upper line of each pair; SEQ ID NO:33) with the human TANGO 228 protein sequence (lower line of each pair; SEQ ID NO:3).

Figures 15A-15B and Figures 16A-4B show that there is an overall 58.2% identity between the full length murine TANGO 228 nucleic acid molecule and the full length human TANGO 228 nucleic acid molecule over the length of the murine molecule and an overall 58.2% identity between the open reading frame of murine TANGO 228 nucleic acid molecule and the open reading frame of the human TANGO 228 nucleic acid molecule over the length of the murine molecule, respectively. The amino acid alignment in Figure 17 shows a 30.6% overall amino acid sequence identity between murine TANGO 228 and human TANGO 228 over the length of the murine moleceule

Uses of TANGO 228 Nucleic acids, Polypeptides, and Modulators Thereof

As TANGO 228 was originally found in a fetal spleen library, TANGO 228 nucleic acids, proteins, and modulators thereof can be used to modulate the proliferation, differentiation, and/or function of cells that form the spleen, e.g., cells of the splenic connective tissue, e.g., splenic smooth muscle cells and/or endothelial cells of the splenic blood vessels. TANGO 228 nucleic acids, proteins, and modulators thereof can also be used to modulate the proliferation, differentiation, and/or function of cells that are processed, e.g., regenerated or phagocytized within the spleen, e.g., erythrocytes and/or B and T lymphocytes and macrophages. Thus TANGO 228 nucleic acids, proteins, and modulators thereof can be used to treat spleen, e.g., the fetal spleen, associated diseases and disorders. Examples of splenic diseases and disorders include e.g., splenic lymphoma and/or splenomegaly, and/or phagocytotic disorders, e.g., those inhibiting macrophage engulfment of bacteria and viruses in the bloodstream.

Due to TANGO 228's homology to rat MCA-32, which is a cell surface antigen gene up-regulated in activated mast cells, TANGO 228 nucleic acids, proteins, and modulators thereof can be used to modulate mast cell function and thus to treat immunologic diseases and disorders. Examples of immunologic diseases and disorders include allergic disorders, e.g., anaphylaxis and allergic asthma, and inflammatory disorders, e.g., atopic dermatitis.

Because TANGO 228 is homologous to rat MCA-32, which is expressed in monocyte and macrophage cell lines, TANGO 228 nucleic acids, proteins, and modulators thereof can be used to protect the body from antigenic invaders, e.g., by

modulating the activity of macrophages, and can be used to treat allergies, e.g., anaphylactic shock and/or allergic rhinitis.

In addition, as TANGO 228 includes one or more Ig domains, TANGO 228 nucleic acids, proteins, and modulators thereof can be used to modulate immunologic function, e.g., by the modulation of immunoglobulins and the formation of antibodies. For the same reason, TANGO 228 nucleic acids, proteins, and modulators thereof can be used to modulate Type I immunologic disorders, e.g., anaphylaxis and/or rhinitis, by modulating, e.g., stabilizing, the interaction between antigens and mast cell receptors, e.g., high affinity IgE receptors.

Due to its mapping in the same region as TNFAIP1, a tumor necrosis factor, TANGO 228 nucleic acids, proteins, and modulators thereof can be sued to treat TNF related disorders (e.g., acute myocarditis, myocardial infarction, congestive heart failure, T cell disorders (e.g., dermatitis, fibrosis)), differentiative and apoptotic disorders, and disorders related to angiogenesis (e.g., tumor formation and/or metastasis, cancer). Modulators of TANGO 228 expression and/or activity can be used to treat such disorders.

Human TANGO 240

A cDNA encoding human TANGO 240 was identified by analyzing the sequences of clones present in an osteoblast cDNA library. This analysis led to the identification of a clone, jthoc087d01, encoding full-length human TANGO 240. The human TANGO 240 cDNA of this clone is 2165 nucleotides long (Figure 6; SEQ ID NO:13). The open reading frame of this cDNA, nucleotides 2 to 1126 of SEQ ID NO:13 (SEQ ID NO:14), encodes a 374 amino acid secreted protein (Figure 6; SEQ ID NO:15).

The signal peptide prediction program SIGNALP (Nielsen, et al. (1997) Protein Engineering 10:1-6) predicted that human TANGO 240 includes an 34 amino acid signal peptide (amino acid 1 to about amino acid 34 of SEQ ID NO:15)(SEQ ID NO:17) preceding the mature TANGO 240 protein (corresponding to about amino acid 35 to amino acid 374 of SEQ ID NO:15; SEQ ID NO:16). The TANGO 240 protein molecular weight is 40.6 kDa prior to the cleavage of the signal peptide, 37.2 kDa after cleavage of the signal peptide.

An N-glycosylation site having the sequence NSTG is found from amino acids 141 to 144 of SEQ ID NO:15. A cAMP and cGMP-dependent protein kinase phosphorylation site having the sequence RRVT is found from amino acids 117 to 120. A protein kinase C phosphorylation site having the sequence SCR is found from amino

acids 234 to 236. A second protein kinase C phosphorylation site having the sequence SGK is found from amino acids 323 to 325. A casein kinase II phosphorylation site having the sequence SNTE is found from amino acids 132 to 135. A second casein kinase II phosphorylation site having the sequence TEAE is found from amino acids 147 to 150. A third casein kinase II phosphorylation site having the sequence SWND is found from amino acids 210 to 213. A fourth casein kinase II phosphorylation site having the sequence TEAE is found from amino acids 227 to 230. A fifth casein kinase II phosphorylation site having the sequence TGED is found from amino acids 270 to 273. A sixth casein kinase II phosphorylation site having the sequence SVEE is found from amino acids 311 to 314. A seventh casein kinase II phosphorylation site having the sequence SGKD is found from amino acids 323 to 326. A tyrosine kinase phosphorylation site having the sequence RVTIDAFY is found from amino acids 118 to 125. An N-myristoylation site having the sequence GLVCGR is found from amino acids 7 to 12. A second N-myristoylation site having the sequence GAAGSQ is found from amino acids 30 to 35. A third N-myristoylation site having the sequence GTGAGA is found from amino acids 38 to 43. A fourth N-myristoylation site having the sequence GSLAGS is found from amino acids 44 to 49. A fifth N-myristoylation site having the sequence GAHGNS is found from amino acids 59 to 64. A sixth N-myristoylation site having the sequence GGLHNR is found from amino acids 237 to 242. A seventh N-myristoylation site having the sequence GQHYAN is found from amino acids 254 to 259. An eighth N-myristoylation site having the sequence GSYMCH is found from amino acids 332 to 337. An amidation site having the sequence AGKR is found from amino acids 221 to 224. A prokaryotic membrane lipoprotein lipid attachment site having the sequence GAGAGSLAGSC is found from amino acids 40 to 50.

Clone EpT240, which encodes human TANGO 240, was deposited with the American Type Culture Collection (10801 University Boulevard, Manassas, VA 20110-2209) on February 18, 1999 and assigned Accession Number 207116. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

Figure 7 depicts a hydropathy plot of human TANGO 240. Relatively hydrophobic regions of the protein are shown above the horizontal line, and relatively hydrophilic regions of the protein are below the horizontal line. The cysteine residues

(cys) and potential N-glycosylation sites (Ngly) are indicated by short vertical lines just below the hydropathy trace. The dashed vertical line separates the signal sequence (amino acids 1 to 34 of SEQ ID NO:15; SEQ ID NO:17) on the left from the mature protein (amino acids 35 to 374 of SEQ ID NO:15; SEQ ID NO:16) on the right.

Northern analysis of TANGO 240 expression in human tissues showed that an approximately 2.2 kB transcript is weakly expressed in skeletal muscle, brain, colon, thymus, spleen, and small intestine, moderately expressed in heart, liver, lung, and peripheral blood leukocytes, and strongly expressed in kidney and placenta.

Secretion assays indicate that the polypeptide encoded by human TANGO 240 is a secreted protein, as the presence of a 48kD and a 42 kD protein was detected by performing the assays. The secretion assays were performed essentially as follows: $8x10^5$ 293T cells were plated per well in a 6-well plate and the cells were incubated in growth medium (DMEM, 10% fetal bovine serum, penicillin/streptomycin) at 37°C, 5% CO_2 overnight. 293T cells were transfected with 2 g of full-length TANGO 240 inserted in the pMET7 vector/well and 10 g LipofectAMINE (GIBCO/BRL Cat. #18324-012)/well according to the protocol for GIBCO/BRL LipofectAMINE. The transfectant was removed 5 hours later and fresh growth medium was added to allow the cells to recover overnight. The medium was removed and each well was gently washed twice with DMEM without methionine and cysteine (ICN Cat. #16-424-54). Next, 1 ml DMEM without methionine and cysteine with 50 Ci Trans-^{35}S (ICN Cat. #51006) was added to each well and the cells were incubated at 37°C, 5% CO_2 for the appropriate time period. A 150 1 aliquot of conditioned medium was obtained and 150 1 of 2X SDS sample buffer was added to the aliquot. The sample was heat-activated and loaded on a 4-20% SDS-PAGE gel. The gel was fixed and the presence of secreted protein was detected by autoradiography.

Murine TANGO 240

A cDNA encoding murine TANGO 240 was identified by analyzing the sequences of clones present in a mouse bone marrow stromal cell cDNA library. This analysis led to the identification of a clone, jtmMa100b11, encoding full-length murine TANGO 240. The murine TANGO 240 cDNA of this clone is 2426 nucleotides long (Figures 18A-18B; SEQ ID NO:39). The open reading frame of this cDNA, nucleotides 2 to 1117 of SEQ ID NO:39 (SEQ ID NO:40), encodes a 372 amino acid secreted protein (Figures 18A-18B; SEQ ID NO:41).

The signal peptide prediction program SIGNALP (Nielsen, et al. (1997) Protein Engineering 10:1-6) predicted that murine TANGO 240 includes a 31 amino acid signal peptide (amino acid 1 to about amino acid 31 of SEQ ID NO:41; SEQ ID NO:43) preceding the mature murine TANGO 240 protein (corresponding to about amino acid 32 to amino acid 372 of SEQ ID NO:11; SEQ ID NO:42). The murine TANGO 240 protein molecular weight is 40.7 kDa prior to the cleavage of the signal peptide, 37.3 kDa after cleavage of the signal peptide.

Figures 19A-19D show an alignment of the murine TANGO 240 full length nucleic acid sequence (upper line of each pair; SEQ ID NO:39) with the human TANGO 240 full length nucleic acid sequence (lower line of each pair; SEQ ID NO:13). Figures 20A-20B show an alignment of the murine TANGO 240 coding region (upper line of each pair; SEQ ID NO:40) with the human TANGO 240 coding region (lower line of each pair; SEQ ID NO:14). Figure 21 shows an alignment of the murine TANGO 240 protein sequence (upper line of each pair; SEQ ID NO:41) with the human TANGO 240 protein sequence (lower line of each pair; SEQ ID NO:15).

Figures 19A-19D and Figures 20A-20B show that there is an overall 78.4% identity between the full length murine TANGO 240 nucleic acid molecule and the full length human TANGO 240 nucleic acid molecule, over the length of the human molecule, and an overall 84.4% identity between the open reading frame of murine TANGO 240 nucleic acid molecule and the open reading frame of the human TANGO 240 nucleic acid molecule, respectively. The amino acid alignment in Figure 21 shows an 86% overall amino acid sequence identity between murine TANGO 240 and human TANGO 240.

Northern analysis of TANGO 240 expression in mouse tissues showed that an approximately 2.2 kB transcript is weakly expressed in spleen and skeletal muscle, moderately expressed in testis, and strongly expressed in heart, brain, lung, liver, and kidney.

Uses of TANGO 240 Nucleic acids, Polypeptides, and Modulators Thereof

As TANGO 240 was originally found in an osteoblast library, TANGO 240 nucleic acids, proteins, and modulators thereof can be used to modulate the proliferation, differentiation, and/or function of bone and cartilage cells, e.g., chondrocytes and osteoblasts, and to treat bone and/or cartilage associated diseases or disorders. Examples of bone and/or cartilage diseases and disorders include bone and/or cartilage injury due to

for example, trauma (e.g., bone breakage, cartilage tearing), degeneration (e.g., osteoporosis), degeneration of joints, e.g., arthritis, e.g., osteoarthritis, and bone wearing.

As TANGO 240 is homologous to Mycobacterium tuberculosis conserved hypothetical protein Rv0712, TANGO 240 nucleic acids, proteins, and modulators thereof can be used to treat diseases associated with bacterial infection, e.g., tuberculosis, e.g., pulmonary tuberculosis. In addition, TANGO 240 can be used to modulate, e.g., trigger, the immune response and can be used to treat immunologic disease and disorders, e.g., those associated with the respiratory system, e.g., asthma.

TANGO 240 nucleic acids, proteins, and modulators thereof can also be used to treat disorders of the cells and tissues in which it is expressed. As TANGO 240 is expressed in heart, brain, spleen, lung, liver, skeletal muscle, kidney, testis, colon, thymus, peripheral blood leukocytes, small intestine, and placenta, TANGO 240 nucleic acids, proteins, and modulators thereof can be used to treat disorders of these cells, tissues, or organs, e.g., ischemic heart disease or atherosclerosis, head trauma, brain cancer, splenic lymphoma, splenomegaly, lung cancer, cystic fibrosis, rheumatoid lung disease, liver cirrhosis, hepatitis, muscular dystrophy, stroke, muscular atrophy, glomerulonephritis, end stage renal disease, uremia, testicular cancer, colon cancer and colonic volvulus, DiGeorge syndrome, thymoma, autoimmune disorders, atresia, Crohns disease, and various placental disorders.

Human TANGO 243

A cDNA encoding human TANGO 243 was identified by analyzing the sequences of clones present in a fetal spleen cDNA library. This analysis led to the identification of a clone, jthsa049g04, encoding potentially full-length human TANGO 243. The human TANGO 243 cDNA of this clone is 2811 nucleotides long (Figure 9; SEQ ID NO:19). The open reading frame of this cDNA, nucleotides 183 to 2567 of SEQ ID NO:19 (SEQ ID NO:20), encodes a 795 amino acid protein (Figure 9; SEQ ID NO:21). The TANGO 243 protein molecular weight is 87.1 kDa.

Like TANGO 243, proteins with a WD domain contain a number of conserved G-beta repeats and are intracellular. The functions associated with WD domains and G-beta repeats, e.g., signal transduction, cell division control, transcriptional regulation, vesicular trafficking, are performed within the cell.

TANGO 243 includes 6 G-beta domains and one G-beta-like domain. G-beta domain 1 is located at about amino acids 8 to 47 of SEQ ID NO:21 (SEQ ID NO:22). G-

beta domain 2 is located at about amino acids 55 to 98 of SEQ ID NO:21 (SEQ ID NO:23). G-beta domain 3 is located at about amino acids 102 to 139 of SEQ ID NO:21 (SEQ ID NO:24). G-beta domain 4 is located at about amino acids 141 to 179 of SEQ ID NO:21 (SEQ ID NO:25). G-beta domain 5 is located at about amino acids 181 to 218 of SEQ ID NO:21 (SEQ ID NO:26). G-beta domain 6 is located at about amino acids 221 to 259 of SEQ ID NO:21 (SEQ ID NO:27). The G-beta-like domain is located at about amino acids 261 to 298 of SEQ ID NO:21 (SEQ ID NO:28).

An N-glycosylation site having the sequence NRSF is found from amino acids 52 to 55 of SEQ ID NO:21. A second N-glycosylation site having the sequence NTSD is found from amino acids 421 to 424. A third N-glycosylation site having the sequence NGTA is found from amino acids 559 to 562. A fourth N-glycosylation site having the sequence NSSS is found from amino acids 585 to 588. A fifth N-glycosylation site having the sequence NYSV is found from amino acids 708 to 711. A cAMP and cGMP-dependent protein kinase phosphorylation site having the sequence KKLT is found from amino acids 566 to 569. A second cAMP and cGMP-dependent protein kinase phosphorylation site having the sequence KKYS is found from amino acids 773 to 776. A protein kinase C phosphorylation site having the sequence SLR is found from amino acids 13 to 15. A second protein kinase C phosphorylation site having the sequence TTR is found from amino acids 42 to 44. A third protein kinase C phosphorylation site having the sequence SGK is found from amino acids 120 to 122. A fourth protein kinase C phosphorylation site having the sequence TAK is found from amino acids 134 to 136. A fifth protein kinase C phosphorylation site having the sequence TVK is found from amino acids 174 to 176. A sixth protein kinase C phosphorylation site having the sequence SIR is found from amino acids 213 to 215. A seventh protein kinase C phosphorylation site having the sequence SLR is found from amino acids 254 to 256. An eighth protein kinase C phosphorylation site having the sequence TIR is found from amino acids 266 to 268. A ninth protein kinase C phosphorylation site having the sequence SGK is found from amino acids 391 to 393. A tenth protein kinase C phosphorylation site having the sequence SYK is found from amino acids 415 to 417. An eleventh protein kinase C phosphorylation site having the sequence SEK is found from amino acids 588 to 590. A twelfth protein kinase C phosphorylation site having the sequence SIK is found from amino acids 620 to 622. A thirteenth protein kinase C phosphorylation site having the sequence SQR is found from amino acids 678 to 680. A fourteenth protein kinase C phosphorylation site having the sequence SNK is found from amino acids 694 to 696. A

fifteenth protein kinase C phosphorylation site having the sequence TFR is found from amino acids 742 to 744. A casein kinase II phosphorylation site having the sequence SFTE is found from amino acids 54 to 57. A second casein kinase II phosphorylation site having the sequence SGHE is found from amino acids 188 to 191. A third casein kinase II phosphorylation site having the sequence SETE is found from amino acids 201 to 204. A fourth casein kinase II phosphorylation site having the sequence TTAE is found from amino acids 248 to 251. A fifth casein kinase II phosphorylation site having the sequence TESE is found from amino acids 298 to 301. A sixth casein kinase II phosphorylation site having the sequence SAEE is found from amino acids 306 to 309. A seventh casein kinase II phosphorylation site having the sequence SVSE is found from amino acids 368 to 371. An eighth casein kinase II phosphorylation site having the sequence TSDD is found from amino acids 422 to 425. A ninth casein kinase II phosphorylation site having the sequence SFSD is found from amino acids 466 to 469. A tenth casein kinase II phosphorylation site having the sequence TAPE is found from amino acids 561 to 564. An eleventh casein kinase II phosphorylation site having the sequence TEDD is found from amino acids 569 to 572. A twelfth casein kinase II phosphorylation site having the sequence SSSE is found from amino acids 586 to 589. A thirteenth casein kinase II phosphorylation site having the sequence SVNE is found from amino acids 625 to 628. A fourteenth casein kinase II phosphorylation site having the sequence SQRE is found from amino acids 678 to 681. A fifteenth casein kinase II phosphorylation site having the sequence TILE is found from amino acids 731 to 734. A sixteenth casein kinase II phosphorylation site having the sequence SVSE is found from amino acids 777 to 780. An N-myristoylation site having the sequence GLVCCA is found from amino acids 23 to 28. A second N-myristoylation site having the sequence GAFVSV is found from amino acids 32 to 37. A third N-myristoylation site having the sequence GLIATG is found from amino acids 83 to 88. A fourth N-myristoylation site having the sequence GNDHNI is found from amino acids 89 to 94. A fifth N-myristoylation site having the sequence GTLLSG is found from amino acids 124 to 129. A sixth N-myristoylation site having the sequence GLMLTG is found from amino acids 164 to 169. A seventh N-myristoylation site having the sequence GASDGI is found from amino acids 288 to 293. An eighth N-myristoylation site having the sequence GTREGQ is found from amino acids 347 to 352. A ninth N-myristoylation site having the sequence GSSGAN is found from amino acids 382 to 387. A tenth N-myristoylation site having the sequence GQMLGL is found from amino acids 457 to 462. An eleventh N-myristoylation site having the sequence GSSGSS

is found from amino acids 480 to 485. A twelfth N-myristoylation site having the sequence GTTMAG is found from amino acids 508 to 513. A thirteenth N-myristoylation site having the sequence GAQFSS is found from amino acids 636 to 641. A fourteenth N-myristoylation site having the sequence GSNKNI is found from amino acids 693 to 698. A fifteenth N-myristoylation site having the sequence GTLISD is found from amino acids 750 to 755.

Clone EpT243, which encodes human TANGO 243, was deposited with the American Type Culture Collection (10801 University Boulevard, Manassas, VA 20110-2209) on February 18, 1999 and assigned Accession Number 207116. This deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. This deposit was made merely as a convenience for those of skill in the art and is not an admission that a deposit is required under 35 U.S.C. §112.

Figure 10 depicts a hydropathy plot of human TANGO 243. Relatively hydrophobic regions of the protein are shown above the horizontal line, and relatively hydrophilic regions of the protein are below the horizontal line. The cysteine residues (cys) and potential N-glycosylation sites (Ngly) are indicated by short vertical lines just below the hydropathy trace.

TANGO 243 bears some similarity to human PLAP (GenBank Accession Number AF083395). An alignment of the nucleotide sequence of human PLAP (SEQ ID NO:29; GenBank Accession Number AF083395) and the nucleotide sequence of human TANGO 243 (SEQ ID NO:19) is depicted in Figures 11A-11F. In this alignment, the nucleotide sequences of human PLAP and human TANGO 243 are 78.8% identical. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4. Figures 12A-12E depict an alignment of the nucleotide sequence of the open reading frames of human PLAP (nucleotides 1 to 2217 of SEQ ID NO:29) and human TANGO 243 (SEQ ID NO:20). The nucleotide sequences of the open reading frames of human PLAP and human TANGO 243 (SEQ ID NO:20) are 92.7% identical. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4. An alignment of the amino acid sequence of human PLAP (SEQ ID NO:30) and the amino acid sequence of human TANGO 243 (SEQ ID NO:21) is depicted in Figures 13A-13B. The amino acid sequences of human PLAP and human TANGO 243 are 92.8%

identical. This alignment was performed using the ALIGN alignment program with a PAM120 scoring matrix, a gap length penalty of 12, and a gap penalty of 4.

It is possible that the human TANGO 243 clone described above, jthsa049g04, is a less than full-length clone and encodes a portion of human TANGO 243. If so, the start site of translation is located upstream of the 5' end of this clone. Figure 22 depicts an alternative translation of the 2811 nucleotide long human TANGO 243 cDNA clone described above. In this translation, the open reading frame of the cDNA, nucleotides 3 to 2567 of SEQ ID NO:19 (SEQ ID NO:44), encodes a 885 amino acid protein (Figure 21; SEQ ID NO:45). The molecular weight of this TANGO 243 protein is 93.7 kDa.

In this alternative translation of the human TANGO 243, G-beta domain 1 is located at about amino acids 68 to 107 of SEQ ID NO:45 (SEQ ID NO:22), G-beta domain 2 is located at about amino acids 115 to 158 of SEQ ID NO:45 (SEQ ID NO:23), G-beta domain 3 is located at about amino acids 162 to 199 of SEQ ID NO:45 (SEQ ID NO:24), G-beta domain 4 is located at about amino acids 201 to 239 of SEQ ID NO:45 (SEQ ID NO:25), G-beta domain 5 is located at about amino acids 241 to 278 of SEQ ID NO:45 (SEQ ID NO:26), G-beta domain 6 is located at about amino acids 281 to 319 of SEQ ID NO:45 (SEQ ID NO:27), and the G-beta-like domain is located at about amino acids 321 to 358 of SEQ ID NO:45 (SEQ ID NO:28).

Within this longer form of TANGO 243, there are regions with considerable similarity to human PLAP. For example, a region of human TANGO 243 from amino acids 358 to 609 of SEQ ID NO:45 (SEQ ID NO:47) constitute PLAP-like domain 1. A region of human TANGO 243 from amino acids 528 to 758 of SEQ ID NO:45 (SEQ ID NO:48) constitute PLAP-like domain 2. A region of human TANGO 243 from amino acids 118 to 205 of SEQ ID NO:45 (SEQ ID NO:49) constitute PLAP-like domain 3. A region of human TANGO 243 from amino acids 239 to 273 of SEQ ID NO:45 (SEQ ID NO:50) constitute PLAP-like domain 4.

In this alternative translation of the TANGO 243 clone the following sites are present. An N-glycosylation site having the sequence NRSF is found from amino acids 112 to 115 of SEQ ID NO:45. A second N-glycosylation site having the sequence NTSD is found from amino acids 481 to 484. A third N-glycosylation site having the sequence NGTA is found from amino acids 619 to 622. A fourth N-glycosylation site having the sequence NSSS is found from amino acids 645 to 648. A fifth N-glycosylation site having the sequence NYSV is found from amino acids 768 to 771. A cAMP and cGMP-dependent protein kinase phosphorylation site having the sequence KKLT is found from

amino acids 626 to 629. A second cAMP and cGMP-dependent protein kinase phosphorylation site having the sequence KKYS is found from amino acids 833 to 836. A protein kinase C phosphorlyation site having the sequence SGR is found from amino acids 22 to 24. A second protein kinase C phosphorylation site having the sequence SLR is found from amino acids 73 to 75. A third protein kinase C phosphorylation site having the sequence TTR is found from amino acids 102 to 104. A fourth protein kinase C phosphorylation site having the sequence SGK is found from amino acids 180 to 182. A fifth protein kinase C phosphorylation site having the sequence TAK is found from amino acids 194 to 196. A sixth protein kinase C phosphorylation site having the sequence TVK is found from amino acids 234 to 236. A seventh protein kinase C phosphorylation site having the sequence SIR is found from amino acids 273 to 275. An eighth protein kinase C phosphorylation site having the sequence SLR is found from amino acids 314 to 316. A ninth protein kinase C phosphorylation site having the sequence TIR is found from amino acids 326 to 328. A tenth protein kinase C phosphorylation site having the sequence SGK is found from amino acids 451 to 453. An eleventh protein kinase C phosphorylation site having the sequence SYK is found from amino acids 475 to 477. A twelfth protein kinase C phosphorylation site having the sequence SEK is found from amino acids 648 to 650. A thirteenth protein kinase C phosphorylation site having the sequence SIK is found from amino acids 680 to 682. A fourteenth protein kinase C phosphorylation site having the sequence SQR is found from amino acids 738 to 740. A fifteenth protein kinase C phosphorylation site having the sequence SNK is found from amino acids 754 to756. A sixteenth protein kinase C phosphorylation site having the sequence TFR is found from amino acids 802 to 804. A casein kinase II phosphorylation site having the sequence SFTE is found from amino acids 114 to 117. A second casein kinase II phosphorylation site having the sequence SGHE is found from amino acids 248 to 252. A third casein kinase II phosphorylation site having the sequence SETE is found from amino acids 261 to 264. A fourth casein kinase II phosphorylation site having the sequence TTAE is found from amino acids 308 to 331. A fifth casein kinase II phosphorylation site having the sequence TESE is found from amino acids 358 to 361. A sixth casein kinase II phosphorylation site having the sequence SAEE is found from amino acids 366 to 369. A seventh casein kinase II phosphorylation site having the sequence SVSE is found from amino acids 428 to 431. An eighth casein kinase II phosphorylation site having the sequence TSDD is found from amino acids 482 to 485. A ninth casein kinase II phosphorylation site having the sequence SFSD is found from

amino acids 526 to 529. A tenth casein kinase II phosphorylation site having the sequence TAPE is found from amino acids 621 to 624. An eleventh casein kinase II phosphorylation site having the sequence TEDD is found from amino acids 629 to 632. A twelfth casein kinase II phosphorylation site having the sequence SSSE is found from amino acids 646 to 649. A thirteenth casein kinase II phosphorylation site having the sequence SVNE is found from amino acids 685 to 688. A fourteenth casein kinase II phosphorylation site having the sequence SQRE is found from amino acids 738 to 741. A fifteenth casein kinase II phosphorylation site having the sequence TILE is found from amino acids 791 to 794. A sixteenth casein kinase II phosphorylation site having the sequence SVSE is found from amino acids 837 to 840. An N-myristoylation site having the sequence GSSPAA is found from amino acids 29 to 34. A second N-myristoylation site having the sequence GARQTL is found from amino acids 54 to 59. A third N-myristoylation site having the sequence GLVCCA is found from amino acids 83 to 88. A fourth N-myristoylation site having the sequence GAFVSV is found from amino acids 92 to 97. A fifth N-myristoylation site having the sequence GLIATG is found from amino acids 83 to 88. A sixth N-myristoylation site having the sequence GNDHNI is found from amino acids 149 to 154. A seventh N-myristoylation site having the sequence GTLLSG is found from amino acids 184 to 189. An eighth N-myristoylation site having the sequence GLMLTG is found from amino acids 224 to 229. A ninth N-myristoylation site having the sequence GASDGI is found from amino acids 348 to 353. An tenth N-myristoylation site having the sequence GTREGQ is found from amino acids 407 to 412. A eleventh N-myristoylation site having the sequence GSSGAN is found from amino acids 442 to 447. A twelfth N-myristoylation site having the sequence GQMLGL is found from amino acids 517 to 522. A thirteenth N-myristoylation site having the sequence GSSGSS is found from amino acids 540 to 545. A fourteenth N-myristoylation site having the sequence GTTMAG is found from amino acids 568 to 573. A fifteenth N-myristoylation site having the sequence GAQFSS is found from amino acids 696 to 701. A sixteenth N-myristoylation site having the sequence GSNKNI is found from amino acids 753 to 758. A seventeenth N-myristoylation site having the sequence GTLISD is found from amino acids 810 to 815.

Figure 23 depicts a hydropathy plot of the 255 amino acid human TANGO 243 protein arising from the alternative translation. Relatively hydrophobic regions of the protein are shown above the horizontal line, and relatively hydrophilic regions of the protein are below the horizontal line. The cysteine residues (cys) and potential N-

glycosylation sites (Ngly) are indicated by short vertical lines just below the hydropathy trace.

Uses of TANGO 243 Nucleic acids, Polypeptides, and Modulators Thereof

As TANGO 243 was originally found in a fetal spleen library, TANGO 243 nucleic acids, proteins, and modulators thereof can be used to modulate the proliferation, differentiation, and/or function of cells that form the spleen, e.g., cells of the splenic connective tissue, e.g., splenic smooth muscle cells and/or endothelial cells of the splenic blood vessels. TANGO 243 nucleic acids, proteins, and modulators thereof can also be used to modulate the proliferation, differentiation, and/or function of cells that are processed, e.g., regenerated or phagocytized within the spleen, e.g., erythrocytes and/or B and T lymphocytes and macrophages. Thus TANGO 243 nucleic acids, proteins, and modulators thereof can be used to treat spleen, e.g., the fetal spleen, associated diseases and disorders. Examples of splenic diseases and disorders include e.g., splenic lymphoma and/or splenomegaly, and/or phagocytotic disorders, e.g., those inhibiting macrophage engulfment of bacteria and viruses in the bloodstream.

Since TANGO 243 is homologous to human PLAP, which is up-regulated in patients with rheumatoid arthritis, and which activates molecules involved in the arthropathy pathway, TANGO 243 nucleic acids, proteins, and modulators thereof can be used to treat inflammatory arthropathy, and in bone and cartilage diseases and disorders, e.g., bone and cartilage degenerative diseases and disorders, e.g., arthritis, e.g., rheumatoid arthritis.

Because both TANGO 243 and PLAP hydrolyze phospholipids into lysophospholipids, which are known to modulate chemotaxis, regulate blood vessel permeability, and promote membrane fusion, and can cause monocytes to secrete IL-1 (interleukin 1), in turn modulating T-cell-mediated immunity, TANGO 243 nucleic acids, proteins, and modulators thereof can be used to modulate chemotaxis, regulate blood vessel permeability, and promote membrane fusion, and can cause monocytes to secrete IL-1 (interleukin 1), in turn modulating T-cell-mediated immunity.

TANGO 243 and PLAP can also be used to modulate IL-1 and TNF (tumor necrosis factor) synthesis and release (e.g., by modulating, e.g., activating PLA2 (phospholipase A2), which releases arachidonic acid as a byproduct of hydrolysis, which regulates IL-1 and TNF transcription). As IL-1 and TNF are involved in immunologic

processes, TANGO 243 nucleic acids, proteins, and modulators thereof can be used to modulate such processes and to treat immunologic disorders.

By activating PLA2, TANGO 243 nucleic acids, proteins, and modulators thereof can modulate levels of arachidonic acid, and thus control levels of eicosanoids and prostaglandins. By controlling the levels of eicosanoids and prostaglandins, TANGO 243 nucleic acids, proteins, and modulators thereof can modulate the immunologic pathway.

The presence of one or more G-beta domain suggests that TANGO 228 functions in a manner similar to other G-beta-containing proteins, such as members of the WD-repeat family. For example, members of WD-repeat family typically have biological functions that include, but are not limited to, signal transduction, cell divisional control, transcription regulation, and vesicular trafficking.

Tables 1 and 2 below provide a summary of the sequence information for human TANGO 228, human TANGO 240, and human TANGO 243. Tables 3 and 4 below provide a summary of the sequence information for murine TANGO 228 and murine TANGO 240.

TABLE 1: Summary of Human TANGO 228, TANGO 240 and TANGO 243 Sequence Information

Gene	cDNA	ORF	Figure	Accession Number
TANGO 228	SEQ ID NO:1	SEQ ID NO:2	Figures 1A-1C	207116
TANGO 240	SEQ ID NO:13	SEQ ID NO:14	Figures 6A-6B	207116
TANGO 243	SEQ ID NO:19	SEQ ID NO:20	Figures 9A-9C	207116
TANGO 243 Alternative translation	SEQ ID NO:19	SEQ ID NO:44	Figures 22A-22C	207116

TABLE 2: Summary of Domains of Human TANGO 228, TANGO 240, and TANGO 243 Proteins

Protein	Signal Sequence	Mature Protein	Extracellular	Ig	Transmembrane	Cytoplasmic	G-beta
TANGO 228	aa 1-19 of SEQ ID NO:3 (SEQ ID NO:5)	aa 20-343 of SEQ ID NO:3 (SEQ ID NO:4)	aa 1-227 of SEQ ID NO:3 (SEQ ID NO:6)	aa 49-105 and 140-198 of SEQ ID NO:3 (SEQ ID NO:8 and 9)	aa 228-249 of SEQ ID NO:3 (SEQ ID NO:7)	aa 250-343 of SEQ ID NO:3 (SEQ ID NO:10)	
TANGO 240	aa 1-34 of SEQ ID NO:13 (SEQ ID NO:17)	aa 35-374 of SEQ ID NO:13 (SEQ ID NO:16)					
TANGO 243							aa 8-47, 55-98, 102-139, 141-179, 181-218, 221-259, and 261-298 of SEQ ID NO:19 (SEQ ID NO:22-28)
TANGO 243 Alternative Translation							aa 68-107, 115-158, 162-199, 201-239, 241-278, 281-319, and 321-358 of SEQ ID NO:45 (SEQ ID NO:22-28)

TABLE 3: Summary of Murine TANGO 228 and Murine TANGO 240 Sequence Information

Gene	cDNA	ORF	Figure	Accession Number
TANGO 228	SEQ ID NO:31	SEQ ID NO:32	Figure 14	
TANGO 240	SEQ ID NO:43	SEQ ID NO:44	Figure 18	

TABLE 4: Summary of Domains of Murine TANGO 228 and TANGO 240 Proteins

Protein	Signal Sequence	Mature Protein	Extracellular	Transmembrane	Cytoplasmic
TANGO 228	aa 1-34 of SEQ ID NO:33 (SEQ ID NO:35)	aa 35-239 of SEQ ID NO:33 (SEQ ID NO:34)	aa 35-141 of SEQ ID NO:33 (SEQ ID NO:36)	aa 142-164 of SEQ ID NO:33 (SEQ ID NO:37)	aa 165-239 of SEQ ID NO:33 (SEQ ID NO:38)
TANGO 240	aa 1-31 of SEQ ID NO:41 (SEQ ID NO:43)	aa 32-372 of SEQ ID NO:41 (SEQ ID NO:42)			

Various aspects of the invention are described in further detail in the following subsections:

I. Isolated Nucleic Acid Molecules

One aspect of the invention pertains to isolated nucleic acid molecules that encode a polypeptide of the invention or a biologically active portion thereof, as well as nucleic acid molecules sufficient for use as hybridization probes to identify nucleic acid molecules encoding a polypeptide of the invention and fragments of such nucleic acid molecules suitable for use as PCR primers for the amplification or mutation of nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

An "isolated" nucleic acid molecule is one which is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid molecule. Preferably, an "isolated" nucleic acid molecule is free of sequences (preferably protein encoding sequences) which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the

nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kB, 4 kB, 3 kB, 2 kB, 1 kB, 0.5 kB or 0.1 kB of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. As used herein, the term "isolated" when referring to a nucleic acid molecule does not include an isolated chromosome.

A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, 2, 3, 13, 14, 19, 20, 31, 32, 39, 40, or 44, or a complement thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of the nucleic acid sequences of SEQ ID NO:1, 2, 3, 13, 14, 19, 20, 31, 32, 39, 40, or 44 as a hybridization probe, nucleic acid molecules of the invention can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook et al., eds., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

A nucleic acid molecule of the invention can be amplified using cDNA, mRNA or genomic DNA as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to all or a portion of a nucleic acid molecule of the invention can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

In another preferred embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule which is a complement of the nucleotide sequence of SEQ ID NO:1, 2, 3, 13, 14, 19, 20, 31, 32, 39, 40, or 44, or a portion thereof. A nucleic acid molecule which is complementary to a given nucleotide sequence is one which is sufficiently complementary to the given nucleotide sequence that it can hybridize to the given nucleotide sequence thereby forming a stable duplex.

Moreover, a nucleic acid molecule of the invention can comprise only a portion of a nucleic acid sequence encoding a full length polypeptide of the invention for example, a fragment which can be used as a probe or primer or a fragment encoding a biologically

active portion of a polypeptide of the invention. The nucleotide sequence determined from the cloning one gene allows for the generation of probes and primers designed for use in identifying and/or cloning homologues in other cell types, e.g., from other tissues, as well as homologues from other mammals. The probe/primer typically comprises substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 50, 75, 100, 125, 150, 175, 200, 250, 300, 350 or 400 consecutive nucleotides of the sense or anti-sense sequence of SEQ ID NO:1, 2, 3, 13, 14, 19, 20, 31, 32, 39, 40, or 44 or of a naturally occurring mutant of SEQ ID NO:1, 2, 3, 13, 14, 19, 20, 31, 32, 39, 40, or 44.

Probes based on the sequence of a nucleic acid molecule of the invention can be used to detect transcripts or genomic sequences encoding the same protein molecule encoded by a selected nucleic acid molecule. The probe comprises a label group attached thereto, e.g., a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as part of a diagnostic test kit for identifying cells or tissues which mis-express the protein, such as by measuring levels of a nucleic acid molecule encoding the protein in a sample of cells from a subject, e.g., detecting mRNA levels or determining whether a gene encoding the protein has been mutated or deleted.

A nucleic acid fragment encoding a "biologically active portion" of a polypeptide of the invention can be prepared by isolating a portion of any of SEQ ID NO:2, 14, or 20, expressing the encoded portion of the polypeptide protein (e.g., by recombinant expression in vitro) and assessing the activity of the encoded portion of the polypeptide.

The invention further encompasses nucleic acid molecules that differ from the nucleotide sequence of SEQ ID NO:1, 2, 3, 13, 14, 19, 20, 31, 32, 39, 40, or 44, due to degeneracy of the genetic code and thus encode the same protein as that encoded by the nucleotide sequence of SEQ ID NO:2, 14, or 20.

In addition to the nucleotide sequences of SEQ ID NO:2, 14, and 20, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequence may exist within a population (e.g., the human population). Such genetic polymorphisms may exist among individuals within a population due to natural allelic variation. An allele is one of a group of genes which occur alternatively at a given genetic locus. As used herein, the phrase "allelic variant" refers to a nucleotide sequence which occurs at a given locus or to a polypeptide encoded by the nucleotide sequence. As used herein, the terms "gene" and "recombinant gene"

refer to nucleic acid molecules comprising an open reading frame encoding a polypeptide of the invention. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of a given gene. Alternative alleles can be identified by sequencing the gene of interest in a number of different individuals. This can be readily carried out by using hybridization probes to identify the same genetic locus in a variety of individuals. Any and all such nucleotide variations and resulting amino acid polymorphisms or variations that are the result of natural allelic variation and that do not alter the functional activity are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding proteins of the invention from other species (homologues), which have a nucleotide sequence which differs from that of the human protein described herein are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of a cDNA of the invention can be isolated based on their identity to the human nucleic acid molecule disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. For example, a cDNA encoding a soluble form of a membrane-bound protein of the invention isolated based on its hybridization to a nucleic acid molecule encoding all or part of the membrane-bound form. Likewise, a cDNA encoding a membrane-bound form can be isolated based on its hybridization to a nucleic acid molecule encoding all or part of the soluble form.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 300 (325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 800, 900, or 1000) nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence, preferably the coding sequence, of SEQ ID NO:1 or 13, or a complement thereof.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 600 (650, 700, 800, 900, 1000, 1200, 1400, 1600, 1800, 2000, or 2200) nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence, preferably the coding sequence, of SEQ ID NO:19, or a complement thereof.

As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% (65%, 70%, preferably 75%) identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be

found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. A preferred, non-limiting example of stringent hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent conditions to the sequence of SEQ ID NO:1, 2, 3, 13, 14, 19, 20, 31, 32, 39, 40, or 44, or a complement thereof, corresponds to a naturally-occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural protein).

In addition to naturally-occurring allelic variants of a nucleic acid molecule of the invention sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation thereby leading to changes in the amino acid sequence of the encoded protein, without altering the biological activity of the protein. For example, one can make nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are not conserved or only semi-conserved among homologues of various species may be non-essential for activity and thus would be likely targets for alteration. Alternatively, amino acid residues that are conserved among the homologues of various species (e.g., murine and human) may be essential for activity and thus would not be likely targets for alteration.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding a polypeptide of the invention that contain changes in amino acid residues that are not essential for activity. Such polypeptides differ in amino acid sequence from SEQ ID NO:3, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule includes a nucleotide sequence encoding a protein that includes an amino acid sequence that is at least about 30% identical, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 75%, 85%, 95%, or 98% identical to the amino acid sequence of SEQ ID NO:3.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding a polypeptide of the invention that contain changes in amino acid residues that are not essential for activity. Such polypeptides differ in amino acid sequence from SEQ ID NO:15, yet retain biological activity. In one embodiment, the isolated nucleic acid

molecule includes a nucleotide sequence encoding a protein that includes an amino acid sequence that is at least about 35% identical, 40%, 45%, 50%, 55%, 65%, 75%, 85%, 95%, or 98% identical to the amino acid sequence of SEQ ID NO:15.

Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding a polypeptide of the invention that contain changes in amino acid residues that are not essential for activity. Such polypeptides differ in amino acid sequence from SEQ ID NO:21, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule includes a nucleotide sequence encoding a protein that includes an amino acid sequence that is at least about 93% identical, 94%, 95%, 96%, 97% or 98% identical to the amino acid sequence of SEQ ID NO:21.

An isolated nucleic acid molecule encoding a variant protein can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of SEQ ID NO:1, 2, 3, 13, 14, 19, 20, 31, 32, 39, 40, or 44 such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed recombinantly and the activity of the protein can be determined.

In a preferred embodiment, a mutant polypeptide that is a variant of a polypeptide of the invention can be assayed for: (1) the ability to form protein:protein interactions with proteins in a signaling pathway of the polypeptide of the invention; (2) the ability to bind a ligand of the polypeptide of the invention; or (3) the ability to bind to an

intracellular target protein of the polypeptide of the invention. In yet another preferred embodiment, the mutant polypeptide can be assayed for the ability to modulate cellular proliferation, cellular migration or chemotaxis, or cellular differentiation.

The present invention encompasses antisense nucleic acid molecules, i.e., molecules which are complementary to a sense nucleic acid encoding a polypeptide of the invention, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire coding strand, or to only a portion thereof, e.g., all or part of the protein coding region (or open reading frame). An antisense nucleic acid molecule can be antisense to all or part of a non-coding region of the coding strand of a nucleotide sequence encoding a polypeptide of the invention. The non-coding regions ("5' and 3' untranslated regions") are the 5' and 3' sequences which flank the coding region and are not translated into amino acids.

An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides or more in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-

amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated in situ such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a selected polypeptide of the invention to thereby inhibit expression, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

An antisense nucleic acid molecule of the invention can be an □-anomeric nucleic acid molecule. An □-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual, β-units, the strands run parallel to each other (Gaultier et al. (1987) Nucleic Acids Res. 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) Nucleic Acids Res. 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) FEBS Lett. 215:327-330).

The invention also encompasses ribozymes. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes (described in Haselhoff and Gerlach (1988)

Nature 334:585-591)) can be used to catalytically cleave mRNA transcripts to thereby inhibit translation of the protein encoded by the mRNA. A ribozyme having specificity for a nucleic acid molecule encoding a polypeptide of the invention can be designed based upon the nucleotide sequence of a cDNA disclosed herein. For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a Cech et al. U.S. Patent No. 4,987,071; and Cech et al. U.S. Patent No. 5,116,742. Alternatively, an mRNA encoding a polypeptide of the invention can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel and Szostak (1993) Science 261:1411-1418.

The invention also encompasses nucleic acid molecules which form triple helical structures. For example, expression of a polypeptide of the invention can be inhibited by targeting nucleotide sequences complementary to the regulatory region of the gene encoding the polypeptide (e.g., the promoter and/or enhancer) to form triple helical structures that prevent transcription of the gene in target cells. See generally Helene (1991) Anticancer Drug Des. 6(6):569-84; Helene (1992) Ann. N.Y. Acad. Sci. 660:27-36; and Maher (1992) Bioassays 14(12):807-15.

In various embodiments, the nucleic acid molecules of the invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup et al. (1996) Bioorganic & Medicinal Chemistry 4(1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup et al. (1996), supra; Perry-O'Keefe et al. (1996) Proc. Natl. Acad. Sci. USA 93: 14670-675.

PNAs can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, e.g., inducing transcription or translation arrest or inhibiting replication. PNAs can also be used, e.g., in the analysis of single base pair mutations in a gene by, e.g., PNA directed PCR clamping; as artificial restriction enzymes when used in

combination with other enzymes, e.g., S1 nucleases (Hyrup (1996), supra; or as probes or primers for DNA sequence and hybridization (Hyrup (1996), supra; Perry-O'Keefe et al. (1996) Proc. Natl. Acad. Sci. USA 93: 14670-675).

In another embodiment, PNAs can be modified, e.g., to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras can be generated which may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, e.g., RNAse H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup (1996), supra). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996), supra, and Finn et al. (1996) Nucleic Acids Res. 24(17):3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs. Compounds such as 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite can be used as a link between the PNA and the 5' end of DNA (Mag et al. (1989) Nucleic Acids Res. 17:5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn et al. (1996) Nucleic Acids Res. 24(17):3357-63). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser et al. (1975) Bioorganic Med. Chem. Lett. 5:1119-11124).

In other embodiments, the oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al. (1989) Proc. Natl. Acad. Sci. USA 86:6553-6556; Lemaitre et al. (1987) Proc. Natl. Acad. Sci. USA 84:648-652; PCT Publication No. W0 88/09810) or the blood-brain barrier (see, e.g., PCT Publication No. W0 89/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (see, e.g., Krol et al. (1988) Bio/Techniques 6:958-976) or intercalating agents (see, e.g., Zon (1988) Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

II. Isolated Proteins and Antibodies

One aspect of the invention pertains to isolated proteins, and biologically active portions thereof, as well as polypeptide fragments suitable for use as immunogens to raise antibodies directed against a polypeptide of the invention. In one embodiment, the native polypeptide can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, polypeptides of the invention are produced by recombinant DNA techniques. Alternative to recombinant expression, a polypeptide of the invention can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the protein is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein (also referred to herein as a "contaminating protein"). When the protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When the protein is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, i.e., it is separated from chemical precursors or other chemicals which are involved in the synthesis of the protein. Accordingly such preparations of the protein have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the polypeptide of interest.

Biologically active portions of a polypeptide of the invention include polypeptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the protein (e.g., the amino acid sequence shown in any of SEQ ID NO:3, 4, 15, 16, 21, 22, 33, 34, 41, 42, or 45), which include fewer amino acids than the full length protein, and exhibit at least one activity of the corresponding full-length protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the corresponding protein. A biologically active portion of a protein of the invention can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino

acids in length. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of the native form of a polypeptide of the invention.

Preferred polypeptides have the amino acid sequence of SEQ ID NO:3, 4, 15, 16, 21, 22, 33, 34, 41, 42, or 45. Other useful proteins are substantially identical (e.g., at least about 45%, preferably 55%, 65%, 75%, 85%, 95%, or 99%) to any of SEQ ID NO: 3, 4, 15, 16, 21, 22, 33, 34, 41, 42, or 45, or substantially identical (e.g., at least about 93%, preferably 94%, 95%, 96%, or 99%) to any of SEQ ID NO:21 or 22, and retain the functional activity of the protein of the corresponding naturally-occurring protein yet differ in amino acid sequence due to natural allelic variation or mutagenesis.

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = # of identical positions/total # of positions (e.g., overlapping positions) x 100). In one embodiment, the two sequences are the same length.

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-2268, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990) J. Mol. Biol. 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al. (1997) Nucleic Acids Res. 25:3389-3402. Alternatively, PSI-Blast can be

used to perform an iterated search which detects distant relationships between molecules (Id.). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, (1988) CABIOS 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, only exact matches are counted.

The invention also provides chimeric or fusion proteins. As used herein, a "chimeric protein" or "fusion protein" comprises all or part (preferably biologically active) of a polypeptide of the invention operably linked to a heterologous polypeptide (i.e., a polypeptide other than the same polypeptide of the invention). Within the fusion protein, the term "operably linked" is intended to indicate that the polypeptide of the invention and the heterologous polypeptide are fused in-frame to each other. The heterologous polypeptide can be fused to the N-terminus or C-terminus of the polypeptide of the invention.

One useful fusion protein is a GST fusion protein in which the polypeptide of the invention is fused to the C-terminus of GST sequences. Such fusion proteins can facilitate the purification of a recombinant polypeptide of the invention.

In another embodiment, the fusion protein contains a heterologous signal sequence at its N-terminus. For example, the native signal sequence of a polypeptide of the invention can be removed and replaced with a signal sequence from another protein. For example, the gp67 secretory sequence of the baculovirus envelope protein can be used as a heterologous signal sequence (Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley & Sons, 1992). Other examples of eukaryotic heterologous signal sequences include the secretory sequences of melittin and human placental alkaline phosphatase (Stratagene; La Jolla, California). In yet another example, useful prokaryotic heterologous signal sequences include the phoA secretory signal (Sambrook et al., supra) and the protein A secretory signal (Pharmacia Biotech; Piscataway, New Jersey).

In yet another embodiment, the fusion protein is an immunoglobulin fusion protein in which all or part of a polypeptide of the invention is fused to sequences derived from a member of the immunoglobulin protein family. The immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a ligand (soluble or membrane-bound) and a protein on the surface of a cell (receptor), to thereby suppress signal transduction in vivo. The immunoglobulin fusion protein can be used to affect the bioavailability of a cognate ligand of a polypeptide of the invention. Inhibition of ligand/receptor interaction may be useful therapeutically, both for treating proliferative and differentiative disorders and for modulating (e.g., promoting or inhibiting) cell survival. Moreover, the immunoglobulin fusion proteins of the invention can be used as immunogens to produce antibodies directed against a polypeptide of the invention in a subject, to purify ligands and in screening assays to identify molecules which inhibit the interaction of receptors with ligands.

Chimeric and fusion proteins of the invention can be produced by standard recombinant DNA techniques. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, e.g., Ausubel et al., supra). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A nucleic acid encoding a polypeptide of the invention can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the polypeptide of the invention.

A signal sequence of a polypeptide of the invention (SEQ ID NO:5 and 19) can be used to facilitate secretion and isolation of the secreted protein or other proteins of interest. Signal sequences are typically characterized by a core of hydrophobic amino acids which are generally cleaved from the mature protein during secretion in one or more cleavage events. Such signal peptides contain processing sites that allow cleavage of the signal sequence from the mature proteins as they pass through the secretory pathway. Thus, the invention pertains to the described polypeptides having a signal sequence, as well as to the signal sequence itself and to the polypeptide in the absence of the signal sequence (i.e., the cleavage products). In one embodiment, a nucleic acid sequence

encoding a signal sequence of the invention can be operably linked in an expression vector to a protein of interest, such as a protein which is ordinarily not secreted or is otherwise difficult to isolate. The signal sequence directs secretion of the protein, such as from a eukaryotic host into which the expression vector is transformed, and the signal sequence is subsequently or concurrently cleaved. The protein can then be readily purified from the extracellular medium by art recognized methods. Alternatively, the signal sequence can be linked to the protein of interest using a sequence which facilitates purification, such as with a GST domain.

In another embodiment, the signal sequences of the present invention can be used to identify regulatory sequences, e.g., promoters, enhancers, repressors. Since signal sequences are the most amino-terminal sequences of a peptide, it is expected that the nucleic acids which flank the signal sequence on its amino-terminal side will be regulatory sequences which affect transcription. Thus, a nucleotide sequence which encodes all or a portion of a signal sequence can be used as a probe to identify and isolate signal sequences and their flanking regions, and these flanking regions can be studied to identify regulatory elements therein.

The present invention also pertains to variants of the polypeptides of the invention. Such variants have an altered amino acid sequence which can function as either agonists (mimetics) or as antagonists. Variants can be generated by mutagenesis, e.g., discrete point mutation or truncation. An agonist can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of the protein. An antagonist of a protein can inhibit one or more of the activities of the naturally occurring form of the protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the protein of interest. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein can have fewer side effects in a subject relative to treatment with the naturally occurring form of the protein.

Variants of a protein of the invention which function as either agonists (mimetics) or as antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of the protein of the invention for agonist or antagonist activity. In one embodiment, a variegated library of variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of variants can be produced by, for example, enzymatically ligating a

mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential protein sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display). There are a variety of methods which can be used to produce libraries of potential variants of the polypeptides of the invention from a degenerate oligonucleotide sequence. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang (1983) Tetrahedron 39:3; Itakura et al. (1984) Annu. Rev. Biochem. 53:323; Itakura et al. (1984) Science 198:1056; Ike et al. (1983) Nucleic Acid Res. 11:477).

In addition, libraries of fragments of the coding sequence of a polypeptide of the invention can be used to generate a variegated population of polypeptides for screening and subsequent selection of variants. For example, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of the coding sequence of interest with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal and internal fragments of various sizes of the protein of interest.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify variants of a protein of the invention (Arkin and Yourvan (1992) Proc. Natl. Acad. Sci. USA 89:7811-7815; Delgrave et al. (1993) Protein Engineering 6(3):327-331).

An isolated polypeptide of the invention, or a fragment thereof, can be used as an immunogen to generate antibodies using standard techniques for polyclonal and monoclonal antibody preparation. The full-length polypeptide or protein can be used or,

alternatively, the invention provides antigenic peptide fragments for use as immunogens. The antigenic peptide of a protein of the invention comprises at least 8 (preferably 10, 15, 20, or 30) amino acid residues of the amino acid sequence of SEQ ID NO:3, 15, 21, or 45, and encompasses an epitope of the protein such that an antibody raised against the peptide forms a specific immune complex with the protein.

Preferred epitopes encompassed by the antigenic peptide are regions that are located on the surface of the protein, e.g., hydrophilic regions. Figures 2, 7, and 10 are hydropathy plots of the proteins of the invention. These plots or similar analyses can be used to identify hydrophilic regions.

An immunogen typically is used to prepare antibodies by immunizing a suitable subject, (e.g., rabbit, goat, mouse or other mammal). An appropriate immunogenic preparation can contain, for example, recombinantly expressed or chemically synthesized polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or similar immunostimulatory agent.

Accordingly, another aspect of the invention pertains to antibodies directed against a polypeptide of the invention. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site which specifically binds an antigen, such as a polypeptide of the invention. A molecule which specifically binds to a given polypeptide of the invention is a molecule which binds the polypeptide, but does not substantially bind other molecules in a sample, e.g., a biological sample, which naturally contains the polypeptide. Examples of immunologically active portions of immunoglobulin molecules include F(ab) and $F(ab')_2$ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal and monoclonal antibodies. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope.

Polyclonal antibodies can be prepared as described above by immunizing a suitable subject with a polypeptide of the invention as an immunogen. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized polypeptide. If desired, the antibody molecules can be isolated from the mammal (e.g., from the blood) and further purified by well-known techniques, such as protein A chromatography to

obtain the IgG fraction. At an appropriate time after immunization, e.g., when the specific antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein (1975) Nature 256:495-497, the human B cell hybridoma technique (Kozbor et al. (1983) Immunol. Today 4:72), the EBV-hybridoma technique (Cole et al. (1985), Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96) or trioma techniques. The technology for producing hybridomas is well known (see generally Current Protocols in Immunology (1994) Coligan et al. (eds.) John Wiley & Sons, Inc., New York, NY). Hybridoma cells producing a monoclonal antibody of the invention are detected by screening the hybridoma culture supernatants for antibodies that bind the polypeptide of interest, e.g., using a standard ELISA assay.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody directed against a polypeptide of the invention can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (e.g., an antibody phage display library) with the polypeptide of interest. Kits for generating and screening phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene SurfZAP™ Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791; PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047; PCT Publication No. WO 92/09690; PCT Publication No. WO 90/02809; Fuchs et al. (1991) Bio/Technology 9:1370-1372; Hay et al. (1992) Hum. Antibod. Hybridomas 3:81-85; Huse et al. (1989) Science 246:1275-1281; Griffiths et al. (1993) EMBO J. 12:725-734.

Additionally, recombinant antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication No. WO 87/02671; European Patent Application 184,187; European Patent

Application 171,496; European Patent Application 173,494; PCT Publication No. WO 86/01533; U.S. Patent No. 4,816,567; European Patent Application 125,023; Better et al. (1988) Science 240:1041-1043; Liu et al. (1987) Proc. Natl. Acad. Sci. USA 84:3439-3443; Liu et al. (1987) J. Immunol. 139:3521-3526; Sun et al. (1987) Proc. Natl. Acad. Sci. USA 84:214-218; Nishimura et al. (1987) Canc. Res. 47:999-1005; Wood et al. (1985) Nature 314:446-449; and Shaw et al. (1988) J. Natl. Cancer Inst. 80:1553-1559); Morrison (1985) Science 229:1202-1207; Oi et al. (1986) Bio/Techniques 4:214; U.S. Patent 5,225,539; Jones et al. (1986) Nature 321:552-525; Verhoeyan et al. (1988) Science 239:1534; and Beidler et al. (1988) J. Immunol. 141:4053-4060.

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Such antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., U.S. Patent 5,625,126; U.S. Patent 5,633,425; U.S. Patent 5,569,825; U.S. Patent 5,661,016; and U.S. Patent 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA), can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a murine antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al. (1994) Bio/technology 12:899-903).

An antibody directed against a polypeptide of the invention (e.g., monoclonal antibody) can be used to isolate the polypeptide by standard techniques, such as affinity

chromatography or immunoprecipitation. Moreover, such an antibody can be used to detect the protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the polypeptide. The antibodies can also be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

III. Recombinant Expression Vectors and Host Cells

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a polypeptide of the invention (or a portion thereof). As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors, expression vectors, are capable of directing the expression of genes to which they are operably linked. In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids (vectors). However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell. This means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many types of host cell and those which direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein.

The recombinant expression vectors of the invention can be designed for expression of a polypeptide of the invention in prokaryotic (e.g., E. coli) or eukaryotic cells (e.g., insect cells (using baculovirus expression vectors), yeast cells or mammalian cells). Suitable host cells are discussed further in Goeddel, supra. Alternatively, the recombinant expression vector can be transcribed and translated in vitro, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in E. coli with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion

moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion E. coli expression vectors include pTrc (Amann et al., (1988) Gene 69:301-315) and pET 11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

One strategy to maximize recombinant protein expression in E. coli is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant protein (Gottesman, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in E. coli (Wada et al. (1992) Nucleic Acids Res. 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the expression vector is a yeast expression vector. Examples of vectors for expression in yeast S. cerivisae include pYepSec1 (Baldari et al. (1987) EMBO J. 6:229-234), pMFa (Kurjan and Herskowitz, (1982) Cell 30:933-943), pJRY88 (Schultz et al. (1987) Gene 54:113-123), pYES2 (Invitrogen Corporation, San Diego, CA), and pPicZ (Invitrogen Corp, San Diego, CA).

Alternatively, the expression vector is a baculovirus expression vector. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith et al. (1983) Mol. Cell Biol. 3:2156-2165) and the pVL series (Lucklow and Summers (1989) Virology 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed (1987) Nature 329:840) and pMT2PC (Kaufman et al. (1987) EMBO J. 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells see chapters 16 and 17 of Sambrook et al., supra.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al. (1987) Genes Dev. 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) Adv. Immunol. 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) EMBO J. 8:729-733) and immunoglobulins (Banerji et al. (1983) Cell 33:729-740; Queen and Baltimore (1983) Cell 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle (1989) Proc. Natl. Acad. Sci. USA 86:5473-5477), pancreas-specific promoters (Edlund et al. (1985) Science 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example the murine hox promoters (Kessel and Gruss (1990) Science 249:374-379) and the beta-fetoprotein promoter (Campes and Tilghman (1989) Genes Dev. 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operably linked to a regulatory sequence in a manner which allows for expression (by transcription of the DNA molecule) of an RNA molecule which is antisense to the mRNA encoding a polypeptide of the invention. Regulatory sequences operably linked to a nucleic acid cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a

recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub et al. (Reviews - Trends in Genetics, Vol. 1(1) 1986).

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic (e.g., E. coli) or eukaryotic cell (e.g., insect cells, yeast or mammalian cells).

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (supra), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce a polypeptide of the invention. Accordingly, the invention further provides methods for producing a polypeptide of the invention using the host cells of the invention. In one embodiment, the method comprises culturing the host

cell of invention (into which a recombinant expression vector encoding a polypeptide of the invention has been introduced) in a suitable medium such that the polypeptide is produced. In another embodiment, the method further comprises isolating the polypeptide from the medium or the host cell.

The host cells of the invention can also be used to produce nonhuman transgenic animals. For example, in one embodiment, a host cell of the invention is a fertilized oocyte or an embryonic stem cell into which a sequence encoding a polypeptide of the invention has been introduced. Such host cells can then be used to create non-human transgenic animals in which exogenous sequences encoding a polypeptide of the invention have been introduced into their genome or homologous recombinant animals in which endogenous encoding a polypeptide of the invention sequences have been altered. Such animals are useful for studying the function and/or activity of the polypeptide and for identifying and/or evaluating modulators of polypeptide activity. As used herein, a "transgenic animal" is a non-human animal, preferably a mammal, more preferably a rodent such as a rat or mouse, in which one or more of the cells of the animal includes a transgene. Other examples of transgenic animals include non-human primates, sheep, dogs, cows, goats, chickens, amphibians, etc. A transgene is exogenous DNA which is integrated into the genome of a cell from which a transgenic animal develops and which remains in the genome of the mature animal, thereby directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic animal. As used herein, an "homologous recombinant animal" is a non-human animal, preferably a mammal, more preferably a mouse, in which an endogenous gene has been altered by homologous recombination between the endogenous gene and an exogenous DNA molecule introduced into a cell of the animal, e.g., an embryonic cell of the animal, prior to development of the animal.

A transgenic animal of the invention can be created by introducing nucleic acid encoding a polypeptide of the invention (or a homologue thereof) into the male pronuclei of a fertilized oocyte, e.g., by microinjection, retroviral infection, and allowing the oocyte to develop in a pseudopregnant female foster animal. Intronic sequences and polyadenylation signals can also be included in the transgene to increase the efficiency of expression of the transgene. A tissue-specific regulatory sequence(s) can be operably linked to the transgene to direct expression of the polypeptide of the invention to particular cells. Methods for generating transgenic animals via embryo manipulation and microinjection, particularly animals such as mice, have become conventional in the art

and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009, U.S. Patent No. 4,873,191 and in Hogan, Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986). Similar methods are used for production of other transgenic animals. A transgenic founder animal can be identified based upon the presence of the transgene in its genome and/or expression of mRNA encoding the transgene in tissues or cells of the animals. A transgenic founder animal can then be used to breed additional animals carrying the transgene. Moreover, transgenic animals carrying the transgene can further be bred to other transgenic animals carrying other transgenes.

To create an homologous recombinant animal, a vector is prepared which contains at least a portion of a gene encoding a polypeptide of the invention into which a deletion, addition or substitution has been introduced to thereby alter, e.g., functionally disrupt, the gene. In a preferred embodiment, the vector is designed such that, upon homologous recombination, the endogenous gene is functionally disrupted (i.e., no longer encodes a functional protein; also referred to as a "knock out" vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous gene is mutated or otherwise altered but still encodes functional protein (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous protein). In the homologous recombination vector, the altered portion of the gene is flanked at its 5' and 3' ends by additional nucleic acid of the gene to allow for homologous recombination to occur between the exogenous gene carried by the vector and an endogenous gene in an embryonic stem cell. The additional flanking nucleic acid sequences are of sufficient length for successful homologous recombination with the endogenous gene. Typically, several kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (see, e.g., Thomas and Capecchi (1987) Cell 51:503 for a description of homologous recombination vectors). The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced gene has homologously recombined with the endogenous gene are selected (see, e.g., Li et al. (1992) Cell 69:915). The selected cells are then injected into a blastocyst of an animal (e.g., a mouse) to form aggregation chimeras (see, e.g., Bradley in Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, Robertson, ed. (IRL, Oxford, 1987) pp. 113-152). A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term. Progeny harboring the homologously recombined DNA in their germ cells can be used to breed animals in which all cells of the animal contain the

homologously recombined DNA by germline transmission of the transgene. Methods for constructing homologous recombination vectors and homologous recombinant animals are described further in Bradley (1991) Current Opinion in Bio/Technology 2:823-829 and in PCT Publication Nos. WO 90/11354, WO 91/01140, WO 92/0968, and WO 93/04169.

In another embodiment, transgenic non-human animals can be produced which contain selected systems which allow for regulated expression of the transgene. One example of such a system is the cre/loxP recombinase system of bacteriophage P1. For a description of the cre/loxP recombinase system, see, e.g., Lakso et al. (1992) Proc. Natl. Acad. Sci. USA 89:6232-6236. Another example of a recombinase system is the FLP recombinase system of Saccharomyces cerevisiae (O'Gorman et al. (1991) Science 251:1351-1355. If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase.

Clones of the non-human transgenic animals described herein can also be produced according to the methods described in Wilmut et al. (1997) Nature 385:810-813 and PCT Publication NOS. WO 97/07668 and WO 97/07669.

IV. Pharmaceutical Compositions

The nucleic acid molecules, polypeptides, and antibodies (also referred to herein as "active compounds") of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

The invention includes methods for preparing pharmaceutical compositions for modulating the expression or activity of a polypeptide or nucleic acid of the invention. Such methods comprise formulating a pharmaceutically acceptable carrier with an agent which modulates expression or activity of a polypeptide or nucleic acid of the invention. Such compositions can further include additional active agents. Thus, the invention further includes methods for preparing a pharmaceutical composition by formulating a pharmaceutically acceptable carrier with an agent which modulates expression or activity of a polypeptide or nucleic acid of the invention and one or more additional active compounds.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF; Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can

be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a polypeptide or antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed.

Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressurized container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

For antibodies, the preferred dosage is 0.1 mg/kg to 100 mg/kg of body weight (generally 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, lower dosages and less frequent administration is often possible. Modifications such as lipidation can be used to stabilize antibodies and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is described by Cruikshank et al. ((1997) J. Acquired Immune Deficiency Syndromes and Human Retrovirology 14:193).

The nucleic acid molecules of the invention can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (U.S. Patent 5,328,470) or by stereotactic injection (see, e.g., Chen et al. (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

V. Uses and Methods of the Invention

The nucleic acid molecules, proteins, protein homologues, and antibodies described herein can be used in one or more of the following methods: a) screening assays; b) detection assays (e.g., chromosomal mapping, tissue typing, forensic biology); c) predictive medicine (e.g., diagnostic assays, prognostic assays, monitoring clinical trials, and pharmacogenomics); and d) methods of treatment (e.g., therapeutic and prophylactic). The isolated nucleic acid molecules of the invention can be used to express proteins (e.g., via a recombinant expression vector in a host cell in gene therapy applications), to detect mRNA (e.g., in a biological sample) or a genetic lesion, and to modulate activity of a polypeptide of the invention. In addition, the polypeptides of the invention can be used to screen drugs or compounds which modulate activity or expression of a polypeptide of the invention as well as to treat disorders characterized by

insufficient or excessive production of a protein of the invention or production of a form of a protein of the invention which has decreased or aberrant activity compared to the wild type protein. In addition, the antibodies of the invention can be used to detect and isolate a protein of the and modulate activity of a protein of the invention.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

A. Screening Assays

The invention provides a method (also referred to herein as a "screening assay") for identifying modulators, i.e., candidate or test compounds or agents (e.g., peptides, peptidomimetics, small molecules or other drugs) which bind to polypeptide of the invention or have a stimulatory or inhibitory effect on, for example, expression or activity of a polypeptide of the invention.

In one embodiment, the invention provides assays for screening candidate or test compounds which bind to or modulate the activity of the membrane-bound form of a polypeptide of the invention or biologically active portion thereof. The test compounds of the present invention can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam (1997) Anticancer Drug Des. 12:145).

Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al. (1993) Proc. Natl. Acad. Sci. USA 90:6909; Erb et al. (1994) Proc. Natl. Acad. Sci. USA 91:11422; Zuckermann et al. (1994). J. Med. Chem. 37:2678; Cho et al. (1993) Science 261:1303; Carrell et al. (1994) Angew. Chem. Int. Ed. Engl. 33:2059; Carell et al. (1994) Angew. Chem. Int. Ed. Engl. 33:2061; and Gallop et al. (1994) J. Med. Chem. 37:1233.

Libraries of compounds may be presented in solution (e.g., Houghten (1992) Bio/Techniques 13:412-421), or on beads (Lam (1991) Nature 354:82-84), chips (Fodor (1993) Nature 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (Patent NOS. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull et al. (1992) Proc. Natl. Acad. Sci.

USA 89:1865-1869) or phage (Scott and Smith (1990) Science 249:386-390; Devlin (1990) Science 249:404-406; Cwirla et al. (1990) Proc. Natl. Acad. Sci. USA 87:6378-6382; and Felici (1991) J. Mol. Biol. 222:301-310).

In one embodiment, an assay is a cell-based assay in which a cell which expresses a membrane-bound form of a polypeptide of the invention, or a biologically active portion thereof, on the cell surface is contacted with a test compound and the ability of the test compound to bind to the polypeptide determined. The cell, for example, can be a yeast cell or a cell of mammalian origin. Determining the ability of the test compound to bind to the polypeptide can be accomplished, for example, by coupling the test compound with a radioisotope or enzymatic label such that binding of the test compound to the polypeptide or biologically active portion thereof can be determined by detecting the labeled compound in a complex. For example, test compounds can be labeled with ^{125}I, ^{35}S, ^{14}C, or ^{3}H, either directly or indirectly, and the radioisotope detected by direct counting of radioemmission or by scintillation counting. Alternatively, test compounds can be enzymatically labeled with, for example, horseradish peroxidase, alkaline phosphatase, or luciferase, and the enzymatic label detected by determination of conversion of an appropriate substrate to product. In a preferred embodiment, the assay comprises contacting a cell which expresses a membrane-bound form of a polypeptide of the invention, or a biologically active portion thereof, on the cell surface with a known compound which binds the polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the polypeptide, wherein determining the ability of the test compound to interact with the polypeptide comprises determining the ability of the test compound to preferentially bind to the polypeptide or a biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-based assay comprising contacting a cell expressing a membrane-bound form of a polypeptide of the invention, or a biologically active portion thereof, on the cell surface with a test compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the polypeptide or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of the polypeptide or a biologically active portion thereof can be accomplished, for example, by determining the ability of the polypeptide protein to bind to or interact with a target molecule.

Determining the ability of a polypeptide of the invention to bind to or interact with a target molecule can be accomplished by one of the methods described above for determining direct binding. As used herein, a "target molecule" is a molecule with which a selected polypeptide (e.g., a polypeptide of the invention) binds or interacts with in nature, for example, a molecule on the surface of a cell which expresses the selected protein, a molecule on the surface of a second cell, a molecule in the extracellular milieu, a molecule associated with the internal surface of a cell membrane or a cytoplasmic molecule. A target molecule can be a polypeptide of the invention or some other polypeptide or protein. For example, a target molecule can be a component of a signal transduction pathway which facilitates transduction of an extracellular signal (e.g., a signal generated by binding of a compound to a polypeptide of the invention) through the cell membrane and into the cell or a second intercellular protein which has catalytic activity or a protein which facilitates the association of downstream signaling molecules with a polypeptide of the invention. Determining the ability of a polypeptide of the invention to bind to or interact with a target molecule can be accomplished by determining the activity of the target molecule. For example, the activity of the target molecule can be determined by detecting induction of a cellular second messenger of the target (e.g., intracellular Ca^{2+}, diacylglycerol, IP3, etc.), detecting catalytic/enzymatic activity of the target on an appropriate substrate, detecting the induction of a reporter gene (e.g., a regulatory element that is responsive to a polypeptide of the invention operably linked to a nucleic acid encoding a detectable marker, e.g., luciferase), or detecting a cellular response, for example, cellular differentiation, or cell proliferation.

In yet another embodiment, an assay of the present invention is a cell-free assay comprising contacting a polypeptide of the invention or biologically active portion thereof with a test compound and determining the ability of the test compound to bind to the polypeptide or biologically active portion thereof. Binding of the test compound to the polypeptide can be determined either directly or indirectly as described above. In a preferred embodiment, the assay includes contacting the polypeptide of the invention or biologically active portion thereof with a known compound which binds the polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the polypeptide, wherein determining the ability of the test compound to interact with the polypeptide comprises determining the ability of the test compound to preferentially bind to the polypeptide or biologically active portion thereof as compared to the known compound.

In another embodiment, an assay is a cell-free assay comprising contacting a polypeptide of the invention or biologically active portion thereof with a test compound and determining the ability of the test compound to modulate (e.g., stimulate or inhibit) the activity of the polypeptide or biologically active portion thereof. Determining the ability of the test compound to modulate the activity of the polypeptide can be accomplished, for example, by determining the ability of the polypeptide to bind to a target molecule by one of the methods described above for determining direct binding. In an alternative embodiment, determining the ability of the test compound to modulate the activity of the polypeptide can be accomplished by determining the ability of the polypeptide of the invention to further modulate the target molecule. For example, the catalytic/enzymatic activity of the target molecule on an appropriate substrate can be determined as previously described.

In yet another embodiment, the cell-free assay comprises contacting a polypeptide of the invention or biologically active portion thereof with a known compound which binds the polypeptide to form an assay mixture, contacting the assay mixture with a test compound, and determining the ability of the test compound to interact with the polypeptide, wherein determining the ability of the test compound to interact with the polypeptide comprises determining the ability of the polypeptide to preferentially bind to or modulate the activity of a target molecule.

The cell-free assays of the present invention are amenable to use of both a soluble form or the membrane-bound form of a polypeptide of the invention. In the case of cell-free assays comprising the membrane-bound form of the polypeptide, it may be desirable to utilize a solubilizing agent such that the membrane-bound form of the polypeptide is maintained in solution. Examples of such solubilizing agents include non-ionic detergents such as n-octylglucoside, n-dodecylglucoside, n-octylmaltoside, octanoyl-N-methylglucamide, decanoyl-N-methylglucamide, Triton X-100, Triton X-114, Thesit, Isotridecypoly(ethylene glycol ether)n, 3-[(3-cholamidopropyl)dimethylamminio]-1-propane sulfonate (CHAPS), 3-[(3-cholamidopropyl)dimethylamminio]-2-hydroxy-1-propane sulfonate (CHAPSO), or N-dodecyl=N,N-dimethyl-3-ammonio-1-propane sulfonate.

In more than one embodiment of the above assay methods of the present invention, it may be desirable to immobilize either the polypeptide of the invention or its target molecule to facilitate separation of complexed from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Binding of a

test compound to the polypeptide, or interaction of the polypeptide with a target molecule in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows one or both of the proteins to be bound to a matrix. For example, glutathione-S-transferase fusion proteins or glutathione-S-transferase fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical; St. Louis, MO) or glutathione derivatized microtitre plates, which are then combined with the test compound or the test compound and either the non-adsorbed target protein or A polypeptide of the invention, and the mixture incubated under conditions conducive to complex formation (e.g., at physiological conditions for salt and pH). Following incubation, the beads or microtitre plate wells are washed to remove any unbound components and complex formation is measured either directly or indirectly, for example, as described above. Alternatively, the complexes can be dissociated from the matrix, and the level of binding or activity of the polypeptide of the invention can be determined using standard techniques.

Other techniques for immobilizing proteins on matrices can also be used in the screening assays of the invention. For example, either the polypeptide of the invention or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated polypeptide of the invention or target molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (e.g., biotinylation kit, Pierce Chemicals; Rockford, IL), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with the polypeptide of the invention or target molecules but which do not interfere with binding of the polypeptide of the invention to its target molecule can be derivatized to the wells of the plate, and unbound target or polypeptide of the invention trapped in the wells by antibody conjugation. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the polypeptide of the invention or target molecule, as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the polypeptide of the invention or target molecule.

In another embodiment, modulators of expression of a polypeptide of the invention are identified in a method in which a cell is contacted with a candidate compound and the expression of the selected mRNA or protein (i.e., the mRNA or protein

corresponding to a polypeptide or nucleic acid of the invention) in the cell is determined. The level of expression of the selected mRNA or protein in the presence of the candidate compound is compared to the level of expression of the selected mRNA or protein in the absence of the candidate compound. The candidate compound can then be identified as a modulator of expression of the polypeptide of the invention based on this comparison. For example, when expression of the selected mRNA or protein is greater (statistically significantly greater) in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of the selected mRNA or protein expression. Alternatively, when expression of the selected mRNA or protein is less (statistically significantly less) in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of the selected mRNA or protein expression. The level of the selected mRNA or protein expression in the cells can be determined by methods described herein.

In yet another aspect of the invention, a polypeptide of the inventions can be used as "bait proteins" in a two-hybrid assay or three hybrid assay (see, e.g., U.S. Patent No. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-12054; Bartel et al. (1993) Bio/Techniques 14:920-924; Iwabuchi et al. (1993) Oncogene 8:1693-1696; and PCT Publication No. WO 94/10300), to identify other proteins, which bind to or interact with the polypeptide of the invention and modulate activity of the polypeptide of the invention. Such binding proteins are also likely to be involved in the propagation of signals by the polypeptide of the inventions as, for example, upstream or downstream elements of a signaling pathway involving the polypeptide of the invention.

This invention further pertains to novel agents identified by the above-described screening assays and uses thereof for treatments as described herein.

B. Detection Assays

Portions or fragments of the cDNA sequences identified herein (and the corresponding complete gene sequences) can be used in numerous ways as polynucleotide reagents. For example, these sequences can be used to: (i) map their respective genes on a chromosome and, thus, locate gene regions associated with genetic disease; (ii) identify an individual from a minute biological sample (tissue typing); and (iii) aid in forensic identification of a biological sample. These applications are described in the subsections below.

1. Chromosome Mapping

Once the sequence (or a portion of the sequence) of a gene has been isolated, this sequence can be used to map the location of the gene on a chromosome. Accordingly, nucleic acid molecules described herein or fragments thereof, can be used to map the location of the corresponding genes on a chromosome. The mapping of the sequences to chromosomes is an important first step in correlating these sequences with genes associated with disease.

Briefly, genes can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp in length) from the sequence of a gene of the invention. Computer analysis of the sequence of a gene of the invention can be used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers can then be used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the gene sequences will yield an amplified fragment. For a review of this technique, see D'Eustachio et al. ((1983) Science 220:919-924).

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular sequence to a particular chromosome. Three or more sequences can be assigned per day using a single thermal cycler. Using the nucleic acid sequences of the invention to design oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes. Other mapping strategies which can similarly be used to map a gene to its chromosome include in situ hybridization (described in Fan et al. (1990) Proc. Natl. Acad. Sci. USA 87:6223-27), pre-screening with labeled flow-sorted chromosomes (CITE), and pre-selection by hybridization to chromosome specific cDNA libraries. Fluorescence in situ hybridization (FISH) of a DNA sequence to a metaphase chromosomal spread can further be used to provide a precise chromosomal location in one step. For a review of this technique, see Verma et al., (Human Chromosomes: A Manual of Basic Techniques (Pergamon Press, New York, 1988)).

Reagents for chromosome mapping can be used individually to mark a single chromosome or a single site on that chromosome, or panels of reagents can be used for marking multiple sites and/or multiple chromosomes. Reagents corresponding to noncoding regions of the genes actually are preferred for mapping purposes. Coding sequences are more likely to be conserved within gene families, thus increasing the chance of cross hybridizations during chromosomal mapping.

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. (Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man, available on-line through Johns Hopkins University Welch Medical Library). The relationship between genes and disease, mapped to the same chromosomal region, can then be identified through linkage analysis (co-inheritance of physically adjacent genes), described in, e.g., Egeland et al. (1987) Nature 325:783-787.

Moreover, differences in the DNA sequences between individuals affected and unaffected with a disease associated with a gene of the invention can be determined. If a mutation is observed in some or all of the affected individuals but not in any unaffected individuals, then the mutation is likely to be the causative agent of the particular disease. Comparison of affected and unaffected individuals generally involves first looking for structural alterations in the chromosomes such as deletions or translocations that are visible from chromosome spreads or detectable using PCR based on that DNA sequence. Ultimately, complete sequencing of genes from several individuals can be performed to confirm the presence of a mutation and to distinguish mutations from polymorphisms.

2. Tissue Typing

The nucleic acid sequences of the present invention can also be used to identify individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identification. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The sequences of the present invention are useful as additional DNA markers for RFLP (described in U.S. Patent 5,272,057).

Furthermore, the sequences of the present invention can be used to provide an alternative technique which determines the actual base-by-base DNA sequence of selected portions of an individual's genome. Thus, the nucleic acid sequences described herein can be used to prepare two PCR primers from the 5' and 3' ends of the sequences. These primers can then be used to amplify an individual's DNA and subsequently sequence it.

Panels of corresponding DNA sequences from individuals, prepared in this manner, can provide unique individual identifications, as each individual will have a unique set of such DNA sequences due to allelic differences. The sequences of the present invention can be used to obtain such identification sequences from individuals and from tissue. The nucleic acid sequences of the invention uniquely represent portions of the human genome. Allelic variation occurs to some degree in the coding regions of these sequences, and to a greater degree in the noncoding regions. It is estimated that allelic variation between individual humans occurs with a frequency at about once per each 500 bases. Each of the sequences described herein can, to some degree, be used as a standard against which DNA from an individual can be compared for identification purposes. Because greater numbers of polymorphisms occur in the noncoding regions, fewer sequences are necessary to differentiate individuals. The noncoding sequences of SEQ ID NO:1, 13, or 19 can comfortably provide positive individual identification with a panel of perhaps 10 to 1,000 primers which each yield a noncoding amplified sequence of 100 bases. If predicted coding sequences, such as those in SEQ ID NO:2, 14, 20, or 44 are used, a more appropriate number of primers for positive individual identification would be 500-2,000.

If a panel of reagents from the nucleic acid sequences described herein is used to generate a unique identification database for an individual, those same reagents can later be used to identify tissue from that individual. Using the unique identification database, positive identification of the individual, living or dead, can be made from extremely small tissue samples.

3. Use of Partial Gene Sequences in Forensic Biology

DNA-based identification techniques can also be used in forensic biology. Forensic biology is a scientific field employing genetic typing of biological evidence found at a crime scene as a means for positively identifying, for example, a perpetrator of a crime. To make such an identification, PCR technology can be used to amplify DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, or semen found at a crime scene. The amplified sequence can then be compared to a standard, thereby allowing identification of the origin of the biological sample.

The sequences of the present invention can be used to provide polynucleotide reagents, e.g., PCR primers, targeted to specific loci in the human genome, which can

enhance the reliability of DNA-based forensic identifications by, for example, providing another "identification marker" (i.e. another DNA sequence that is unique to a particular individual). As mentioned above, actual base sequence information can be used for identification as an accurate alternative to patterns formed by restriction enzyme generated fragments. Sequences targeted to noncoding regions are particularly appropriate for this use as greater numbers of polymorphisms occur in the noncoding regions, making it easier to differentiate individuals using this technique. Examples of polynucleotide reagents include the nucleic acid sequences of the invention or portions thereof, e.g., fragments derived from noncoding regions having a length of at least 20 or 30 bases.

The nucleic acid sequences described herein can further be used to provide polynucleotide reagents, e.g., labeled or labelable probes which can be used in, for example, an in situ hybridization technique, to identify a specific tissue, e.g., brain tissue. This can be very useful in cases where a forensic pathologist is presented with a tissue of unknown origin. Panels of such probes can be used to identify tissue by species and/or by organ type.

C. Predictive Medicine

The present invention also pertains to the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trails are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, one aspect of the present invention relates to diagnostic assays for determining expression of a polypeptide or nucleic acid of the invention and/or activity of a polypeptide of the invention, in the context of a biological sample (e.g., blood, serum, cells, tissue) to thereby determine whether an individual is afflicted with a disease or disorder, or is at risk of developing a disorder, associated with aberrant expression or activity of a polypeptide of the invention. The invention also provides for prognostic (or predictive) assays for determining whether an individual is at risk of developing a disorder associated with aberrant expression or activity of a polypeptide of the invention. For example, mutations in a gene of the invention can be assayed in a biological sample. Such assays can be used for prognostic or predictive purpose to thereby prophylactically treat an individual prior to the onset of a disorder characterized by or associated with aberrant expression or activity of a polypeptide of the invention.

Another aspect of the invention provides methods for expression of a nucleic acid or polypeptide of the invention or activity of a polypeptide of the invention in an individual to thereby select appropriate therapeutic or prophylactic agents for that individual (referred to herein as "pharmacogenomics"). Pharmacogenomics allows for the selection of agents (e.g., drugs) for therapeutic or prophylactic treatment of an individual based on the genotype of the individual (e.g., the genotype of the individual examined to determine the ability of the individual to respond to a particular agent).

Yet another aspect of the invention pertains to monitoring the influence of agents (e.g., drugs or other compounds) on the expression or activity of a polypeptide of the invention in clinical trials. These and other agents are described in further detail in the following sections.

1. Diagnostic Assays

An exemplary method for detecting the presence or absence of a polypeptide or nucleic acid of the invention in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with a compound or an agent capable of detecting a polypeptide or nucleic acid (e.g., mRNA, genomic DNA) of the invention such that the presence of a polypeptide or nucleic acid of the invention is detected in the biological sample. A preferred agent for detecting mRNA or genomic DNA encoding a polypeptide of the invention is a labeled nucleic acid probe capable of hybridizing to mRNA or genomic DNA encoding a polypeptide of the invention. The nucleic acid probe can be, for example, a full-length cDNA, such as the nucleic acid of SEQ ID NO:1, 2, 3, 13, 14, 19, 20, 31, 32, 39, 40, or 44, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to a mRNA or genomic DNA encoding a polypeptide of the invention. Other suitable probes for use in the diagnostic assays of the invention are described herein.

A preferred agent for detecting a polypeptide of the invention is an antibody capable of binding to a polypeptide of the invention, preferably an antibody with a detectable label. Antibodies can be polyclonal, or more preferably, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or $F(ab')_2$) can be used. The term "labeled", with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (i.e., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity

with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin. The term "biological sample" is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. That is, the detection method of the invention can be used to detect mRNA, protein, or genomic DNA in a biological sample in vitro as well as in vivo. For example, in vitro techniques for detection of mRNA include Northern hybridizations and in situ hybridizations. In vitro techniques for detection of a polypeptide of the invention include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations and immunofluorescence. In vitro techniques for detection of genomic DNA include Southern hybridizations. Furthermore, in vivo techniques for detection of a polypeptide of the invention include introducing into a subject a labeled antibody directed against the polypeptide. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

In one embodiment, the biological sample contains protein molecules from the test subject. Alternatively, the biological sample can contain mRNA molecules from the test subject or genomic DNA molecules from the test subject. A preferred biological sample is a peripheral blood leukocyte sample isolated by conventional means from a subject.

In another embodiment, the methods further involve obtaining a control biological sample from a control subject, contacting the control sample with a compound or agent capable of detecting a polypeptide of the invention or mRNA or genomic DNA encoding a polypeptide of the invention, such that the presence of the polypeptide or mRNA or genomic DNA encoding the polypeptide is detected in the biological sample, and comparing the presence of the polypeptide or mRNA or genomic DNA encoding the polypeptide in the control sample with the presence of the polypeptide or mRNA or genomic DNA encoding the polypeptide in the test sample.

The invention also encompasses kits for detecting the presence of a polypeptide or nucleic acid of the invention in a biological sample (a test sample). Such kits can be used to determine if a subject is suffering from or is at increased risk of developing a disorder associated with aberrant expression of a polypeptide of the invention (e.g., a proliferative disorder, e.g., psoriasis or cancer). For example, the kit can comprise a labeled compound or agent capable of detecting the polypeptide or mRNA encoding the

polypeptide in a biological sample and means for determining the amount of the polypeptide or mRNA in the sample (e.g., an antibody which binds the polypeptide or an oligonucleotide probe which binds to DNA or mRNA encoding the polypeptide). Kits can also include instructions for observing that the tested subject is suffering from or is at risk of developing a disorder associated with aberrant expression of the polypeptide if the amount of the polypeptide or mRNA encoding the polypeptide is above or below a normal level.

For antibody-based kits, the kit can comprise, for example: (1) a first antibody (e.g., attached to a solid support) which binds to a polypeptide of the invention; and, optionally, (2) a second, different antibody which binds to either the polypeptide or the first antibody and is conjugated to a detectable agent.

For oligonucleotide-based kits, the kit can comprise, for example: (1) an oligonucleotide, e.g., a detectably labeled oligonucleotide, which hybridizes to a nucleic acid sequence encoding a polypeptide of the invention or (2) a pair of primers useful for amplifying a nucleic acid molecule encoding a polypeptide of the invention. The kit can also comprise, e.g., a buffering agent, a preservative, or a protein stabilizing agent. The kit can also comprise components necessary for detecting the detectable agent (e.g., an enzyme or a substrate). The kit can also contain a control sample or a series of control samples which can be assayed and compared to the test sample contained. Each component of the kit is usually enclosed within an individual container and all of the various containers are within a single package along with instructions for observing whether the tested subject is suffering from or is at risk of developing a disorder associated with aberrant expression of the polypeptide.

2. Prognostic Assays

The methods described herein can furthermore be utilized as diagnostic or prognostic assays to identify subjects having or at risk of developing a disease or disorder associated with aberrant expression or activity of a polypeptide of the invention. For example, the assays described herein, such as the preceding diagnostic assays or the following assays, can be utilized to identify a subject having or at risk of developing a disorder associated with aberrant expression or activity of a polypeptide of the invention, e.g., an immunologic disorder, e.g., asthma, anaphylaxis, or atopic dermatitis. Alternatively, the prognostic assays can be utilized to identify a subject having or at risk for developing such a disease or disorder. Thus, the present invention provides a method

in which a test sample is obtained from a subject and a polypeptide or nucleic acid (e.g., mRNA, genomic DNA) of the invention is detected, wherein the presence of the polypeptide or nucleic acid is diagnostic for a subject having or at risk of developing a disease or disorder associated with aberrant expression or activity of the polypeptide. As used herein, a "test sample" refers to a biological sample obtained from a subject of interest. For example, a test sample can be a biological fluid (e.g., serum), cell sample, or tissue.

Furthermore, the prognostic assays described herein can be used to determine whether a subject can be administered an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) to treat a disease or disorder associated with aberrant expression or activity of a polypeptide of the invention. For example, such methods can be used to determine whether a subject can be effectively treated with a specific agent or class of agents (e.g., agents of a type which decrease activity of the polypeptide). Thus, the present invention provides methods for determining whether a subject can be effectively treated with an agent for a disorder associated with aberrant expression or activity of a polypeptide of the invention in which a test sample is obtained and the polypeptide or nucleic acid encoding the polypeptide is detected (e.g., wherein the presence of the polypeptide or nucleic acid is diagnostic for a subject that can be administered the agent to treat a disorder associated with aberrant expression or activity of the polypeptide).

The methods of the invention can also be used to detect genetic lesions or mutations in a gene of the invention, thereby determining if a subject with the lesioned gene is at risk for a disorder characterized aberrant expression or activity of a polypeptide of the invention. In preferred embodiments, the methods include detecting, in a sample of cells from the subject, the presence or absence of a genetic lesion or mutation characterized by at least one of an alteration affecting the integrity of a gene encoding the polypeptide of the invention, or the mis-expression of the gene encoding the polypeptide of the invention. For example, such genetic lesions or mutations can be detected by ascertaining the existence of at least one of: 1) a deletion of one or more nucleotides from the gene; 2) an addition of one or more nucleotides to the gene; 3) a substitution of one or more nucleotides of the gene; 4) a chromosomal rearrangement of the gene; 5) an alteration in the level of a messenger RNA transcript of the gene; 6) an aberrant modification of the gene, such as of the methylation pattern of the genomic DNA; 7) the presence of a non-wild type splicing pattern of a messenger RNA transcript of the gene;

8) a non-wild type level of a the protein encoded by the gene; 9) an allelic loss of the gene; and 10) an inappropriate post-translational modification of the protein encoded by the gene. As described herein, there are a large number of assay techniques known in the art which can be used for detecting lesions in a gene.

In certain embodiments, detection of the lesion involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g., U.S. Patent Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran et al. (1988) Science 241:1077-1080; and Nakazawa et al. (1994) Proc. Natl. Acad. Sci. USA 91:360-364), the latter of which can be particularly useful for detecting point mutations in a gene (see, e.g., Abravaya et al. (1995) Nucleic Acids Res. 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to the selected gene under conditions such that hybridization and amplification of the gene (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample. It is anticipated that PCR and/or LCR may be desirable to use as a preliminary amplification step in conjunction with any of the techniques used for detecting mutations described herein.

Alternative amplification methods include: self sustained sequence replication (Guatelli et al. (1990) Proc. Natl. Acad. Sci. USA 87:1874-1878), transcriptional amplification system (Kwoh, et al. (1989) Proc. Natl. Acad. Sci. USA 86:1173-1177), Q-Beta Replicase (Lizardi et al. (1988) Bio/Technology 6:1197), or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers.

In an alternative embodiment, mutations in a selected gene from a sample cell can be identified by alterations in restriction enzyme cleavage patterns. For example, sample and control DNA is isolated, amplified (optionally), digested with one or more restriction endonucleases, and fragment length sizes are determined by gel electrophoresis and compared. Differences in fragment length sizes between sample and control DNA indicates mutations in the sample DNA. Moreover, the use of sequence specific

ribozymes (see, e.g., U.S. Patent No. 5,498,531) can be used to score for the presence of specific mutations by development or loss of a ribozyme cleavage site.

In other embodiments, genetic mutations can be identified by hybridizing a sample and control nucleic acids, e.g., DNA or RNA, to high density arrays containing hundreds or thousands of oligonucleotides probes (Cronin et al. (1996) Human Mutation 7:244-255; Kozal et al. (1996) Nature Medicine 2:753-759). For example, genetic mutations can be identified in two-dimensional arrays containing light-generated DNA probes as described in Cronin et al., supra. Briefly, a first hybridization array of probes can be used to scan through long stretches of DNA in a sample and control to identify base changes between the sequences by making linear arrays of sequential overlapping probes. This step allows the identification of point mutations. This step is followed by a second hybridization array that allows the characterization of specific mutations by using smaller, specialized probe arrays complementary to all variants or mutations detected. Each mutation array is composed of parallel probe sets, one complementary to the wild-type gene and the other complementary to the mutant gene.

In yet another embodiment, any of a variety of sequencing reactions known in the art can be used to directly sequence the selected gene and detect mutations by comparing the sequence of the sample nucleic acids with the corresponding wild-type (control) sequence. Examples of sequencing reactions include those based on techniques developed by Maxim and Gilbert ((1977) Proc. Natl. Acad. Sci. USA 74:560) or Sanger ((1977) Proc. Natl. Acad. Sci. USA 74:5463). It is also contemplated that any of a variety of automated sequencing procedures can be utilized when performing the diagnostic assays ((1995) Bio/Techniques 19:448), including sequencing by mass spectrometry (see, e.g., PCT Publication No. WO 94/16101; Cohen et al. (1996) Adv. Chromatogr. 36:127-162; and Griffin et al. (1993) Appl. Biochem. Biotechnol. 38:147-159).

Other methods for detecting mutations in a selected gene include methods in which protection from cleavage agents is used to detect mismatched bases in RNA/RNA or RNA/DNA heteroduplexes (Myers et al. (1985) Science 230:1242). In general, the technique of "mismatch cleavage" entails providing heteroduplexes formed by hybridizing (labeled) RNA or DNA containing the wild-type sequence with potentially mutant RNA or DNA obtained from a tissue sample. The double-stranded duplexes are treated with an agent which cleaves single-stranded regions of the duplex such as which will exist due to basepair mismatches between the control and sample strands.

RNA/DNA duplexes can be treated with RNase to digest mismatched regions, and DNA/DNA hybrids can be treated with S1 nuclease to digest mismatched regions.

In other embodiments, either DNA/DNA or RNA/DNA duplexes can be treated with hydroxylamine or osmium tetroxide and with piperidine in order to digest mismatched regions. After digestion of the mismatched regions, the resulting material is then separated by size on denaturing polyacrylamide gels to determine the site of mutation. See, e.g., Cotton et al. (1988) Proc. Natl. Acad. Sci. USA 85:4397; Saleeba et al. (1992) Methods Enzymol. 217:286-295. In a preferred embodiment, the control DNA or RNA can be labeled for detection.

In still another embodiment, the mismatch cleavage reaction employs one or more proteins that recognize mismatched base pairs in double-stranded DNA (so called "DNA mismatch repair" enzymes) in defined systems for detecting and mapping point mutations in cDNAs obtained from samples of cells. For example, the mutY enzyme of E. coli cleaves A at G/A mismatches and the thymidine DNA glycosylase from HeLa cells cleaves T at G/T mismatches (Hsu et al. (1994) Carcinogenesis 15:1657-1662). According to an exemplary embodiment, a probe based on a selected sequence, e.g., a wild-type sequence, is hybridized to a cDNA or other DNA product from a test cell(s). The duplex is treated with a DNA mismatch repair enzyme, and the cleavage products, if any, can be detected from electrophoresis protocols or the like. See, e.g., U.S. Patent No. 5,459,039.

In other embodiments, alterations in electrophoretic mobility will be used to identify mutations in genes. For example, single strand conformation polymorphism (SSCP) may be used to detect differences in electrophoretic mobility between mutant and wild type nucleic acids (Orita et al. (1989) Proc. Natl. Acad. Sci. USA 86:2766; see also Cotton (1993) Mutat. Res. 285:125-144; Hayashi (1992) Genet. Anal. Tech. Appl. 9:73-79). Single-stranded DNA fragments of sample and control nucleic acids will be denatured and allowed to renature. The secondary structure of single-stranded nucleic acids varies according to sequence, and the resulting alteration in electrophoretic mobility enables the detection of even a single base change. The DNA fragments may be labeled or detected with labeled probes. The sensitivity of the assay may be enhanced by using RNA (rather than DNA), in which the secondary structure is more sensitive to a change in sequence. In a preferred embodiment, the subject method utilizes heteroduplex analysis to separate double stranded heteroduplex molecules on the basis of changes in electrophoretic mobility (Keen et al. (1991) Trends Genet. 7:5).

In yet another embodiment, the movement of mutant or wild-type fragments in polyacrylamide gels containing a gradient of denaturant is assayed using denaturing gradient gel electrophoresis (DGGE) (Myers et al. (1985) Nature 313:495). When DGGE is used as the method of analysis, DNA will be modified to insure that it does not completely denature, for example by adding a `GC clamp of approximately 40 bp of high-melting GC-rich DNA by PCR. In a further embodiment, a temperature gradient is used in place of a denaturing gradient to identify differences in the mobility of control and sample DNA (Rosenbaum and Reissner (1987) Biophys. Chem. 265:12753).

Examples of other techniques for detecting point mutations include, but are not limited to, selective oligonucleotide hybridization, selective amplification, or selective primer extension. For example, oligonucleotide primers may be prepared in which the known mutation is placed centrally and then hybridized to target DNA under conditions which permit hybridization only if a perfect match is found (Saiki et al. (1986) Nature 324:163); Saiki et al. (1989) Proc. Natl. Acad. Sci. USA 86:6230). Such allele specific oligonucleotides are hybridized to PCR amplified target DNA or a number of different mutations when the oligonucleotides are attached to the hybridizing membrane and hybridized with labeled target DNA.

Alternatively, allele specific amplification technology which depends on selective PCR amplification may be used in conjunction with the instant invention. Oligonucleotides used as primers for specific amplification may carry the mutation of interest in the center of the molecule (so that amplification depends on differential hybridization) (Gibbs et al. (1989) Nucleic Acids Res. 17:2437-2448) or at the extreme 3' end of one primer where, under appropriate conditions, mismatch can prevent or reduce polymerase extension (Prossner (1993) Tibtech 11:238). In addition, it may be desirable to introduce a novel restriction site in the region of the mutation to create cleavage-based detection (Gasparini et al. (1992) Mol. Cell Probes 6:1). It is anticipated that in certain embodiments amplification may also be performed using Taq ligase for amplification (Barany (1991) Proc. Natl. Acad. Sci. USA 88:189). In such cases, ligation will occur only if there is a perfect match at the 3' end of the 5' sequence making it possible to detect the presence of a known mutation at a specific site by looking for the presence or absence of amplification.

The methods described herein may be performed, for example, by utilizing pre-packaged diagnostic kits comprising at least one probe nucleic acid or antibody reagent described herein, which may be conveniently used, e.g., in clinical settings to diagnose

patients exhibiting symptoms or family history of a disease or illness involving a gene encoding a polypeptide of the invention. Furthermore, any cell type or tissue, e.g., chondrocytes, in which the polypeptide of the invention is expressed may be utilized in the prognostic assays described herein.

3. Pharmacogenomics

Agents, or modulators which have a stimulatory or inhibitory effect on activity or expression of a polypeptide of the invention as identified by a screening assay described herein can be administered to individuals to treat (prophylactically or therapeutically) disorders associated with aberrant activity of the polypeptide. In conjunction with such treatment, the pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (e.g., drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of a polypeptide of the invention, expression of a nucleic acid of the invention, or mutation content of a gene of the invention in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, e.g., Linder (1997) Clin. Chem. 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the way drugs act on the body are referred to as "altered drug action." Genetic conditions transmitted as single factors altering the way the body acts on drugs are referred to as "altered drug metabolism". These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase deficiency (G6PD) is a common inherited enzymopathy in which the main clinical complication is haemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, a PM will show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of a polypeptide of the invention, expression of a nucleic acid encoding the polypeptide, or mutation content of a gene encoding the polypeptide in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a modulator of activity or expression of the polypeptide, such as a modulator identified by one of the exemplary screening assays described herein.

4. Monitoring of Effects During Clinical Trials

Monitoring the influence of agents (e.g., drugs, compounds) on the expression or activity of a polypeptide of the invention (e.g., the ability to modulate aberrant cell proliferation chemotaxis, and/or differentiation) can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent, as determined by a screening assay as described herein, to increase gene expression, protein

levels or protein activity, can be monitored in clinical trials of subjects exhibiting decreased gene expression, protein levels, or protein activity. Alternatively, the effectiveness of an agent, as determined by a screening assay, to decrease gene expression, protein levels or protein activity, can be monitored in clinical trials of subjects exhibiting increased gene expression, protein levels, or protein activity. In such clinical trials, expression or activity of a polypeptide of the invention and preferably, that of other polypeptide that have been implicated in for example, a cellular proliferation disorder, can be used as a marker of the immune responsiveness of a particular cell.

For example, and not by way of limitation, genes, including those of the invention, that are modulated in cells by treatment with an agent (e.g., compound, drug or small molecule) which modulates activity or expression of a polypeptide of the invention (e.g., as identified in a screening assay described herein) can be identified. Thus, to study the effect of agents on cellular proliferation disorders, for example, in a clinical trial, cells can be isolated and RNA prepared and analyzed for the levels of expression of a gene of the invention and other genes implicated in the disorder. The levels of gene expression (i.e., a gene expression pattern) can be quantified by Northern blot analysis or RT-PCR, as described herein, or alternatively by measuring the amount of protein produced, by one of the methods as described herein, or by measuring the levels of activity of a gene of the invention or other genes. In this way, the gene expression pattern can serve as a marker, indicative of the physiological response of the cells to the agent. Accordingly, this response state may be determined before, and at various points during, treatment of the individual with the agent.

In a preferred embodiment, the present invention provides a method for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate identified by the screening assays described herein) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting the level of the polypeptide or nucleic acid of the invention in the preadministration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting the level the of the polypeptide or nucleic acid of the invention in the post-administration samples; (v) comparing the level of the polypeptide or nucleic acid of the invention in the pre-administration sample with the level of the polypeptide or nucleic acid of the invention in the post-administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly. For example,

increased administration of the agent may be desirable to increase the expression or activity of the polypeptide to higher levels than detected, i.e., to increase the effectiveness of the agent. Alternatively, decreased administration of the agent may be desirable to decrease expression or activity of the polypeptide to lower levels than detected, i.e., to decrease the effectiveness of the agent.

C. Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant expression or activity of a polypeptide of the invention. For example, disorders characterized by aberrant expression or activity of the polypeptides of the invention include immunologic disorders. In addition, the nucleic acids, polypeptides, and modulators thereof of the invention can be used to treat immunologic diseases and disorders, including but not limited to, allergic disorders (e.g., anaphylaxis and allergic asthma) and inflammatory disorders (e.g., atopic dermatitis). Polypeptides of the invention can also treat diseases associated with bacterial infection (e.g., tuberculosis, e.g., pulmonary tuberculosis), inflammatory arthropathy, and bone and cartilage degenerative diseases and disorders (e.g., arthritis, e.g., rheumatoid arthritis), as well as other disorders described herein.

1. Prophylactic Methods

In one aspect, the invention provides a method for preventing in a subject, a disease or condition associated with an aberrant expression or activity of a polypeptide of the invention, by administering to the subject an agent which modulates expression or at least one activity of the polypeptide. Subjects at risk for a disease which is caused or contributed to by aberrant expression or activity of a polypeptide of the invention can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of aberrancy, for example, an agonist or antagonist agent can be used for treating the subject. For example, an antagonist of a TANGO 240 protein may be used to treat an arthropathic disorder, e.g., rheumatoid arthritis. The appropriate agent can be determined based on screening assays described herein.

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating expression or activity of a polypeptide of the invention for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of the polypeptide. An agent that modulates activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of the polypeptide, a peptide, a peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more of the biological activities of the polypeptide. Examples of such stimulatory agents include the active polypeptide of the invention and a nucleic acid molecule encoding the polypeptide of the invention that has been introduced into the cell. In another embodiment, the agent inhibits one or more of the biological activities of the polypeptide of the invention. Examples of such inhibitory agents include antisense nucleic acid molecules and antibodies. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in vivo (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of a polypeptide of the invention. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulates (e.g., upregulates or downregulates) expression or activity. In another embodiment, the method involves administering a polypeptide of the invention or a nucleic acid molecule of the invention as therapy to compensate for reduced or aberrant expression or activity of the polypeptide.

Stimulation of activity is desirable in situations in which activity or expression is abnormally low or downregulated and/or in which increased activity is likely to have a beneficial effect. Conversely, inhibition of activity is desirable in situations in which activity or expression is abnormally high or upregulated and/or in which decreased activity is likely to have a beneficial effect.

This invention is further illustrated by the following examples which should not be construed as limiting. The contents of all references, patents and published patent applications cited throughout this application are hereby incorporated by reference.

Deposit of Clones

Clones containing cDNA molecules encoding TANGO 228, (clone EpT228), TANGO 240 (EpT240) and TANGO 243 (clone EpT243) were deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, VA, 20110-2209, on February 18, 1999 as Accession Number 207116, as part of a composite deposit representing a mixture of three strains, each carrying one recombinant plasmid harboring a particular cDNA clone.

To distinguish the strains and isolate a strain harboring a particular cDNA clone, an aliquot of the mixture can be streaked out to single colonies on nutrient medium (e.g., LB plates) supplemented with 100μg/ml ampicillin, single colonies grown, and then plasmid DNA extracted using a standard minipreparation procedure. Next, a sample of the DNA minipreparation can be digested with a combination of the restriction enzymes Sal I and Not I and the resultant products resolved on a 0.8% agarose gel using standard DNA electrophoresis conditions. The digest liberates fragments as follows:

TANGO 228: 4.1 kb
TANGO 240: 2.2 kb
TANGO 243: 2.8 kb

The identity of the strains can be inferred from the fragments liberated.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. An isolated nucleic acid molecule selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence which is at least 55% identical to the nucleotide sequence of SEQ ID NO:1, 2, 13, 14, 19, 20, the cDNA insert of the plasmid deposited with the ATCC as Accession Number 207116, or a complement thereof;

b) a nucleic acid molecule comprising a fragment of at least 300 nucleotides of the nucleotide sequence of SEQ ID NO:1, 2, 13, 14, 19, 20, the cDNA insert of the plasmid deposited with the ATCC as Accession Number 207116, or a complement thereof;

c) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:3, 15, 21, 45, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 207116;

d) a nucleic acid molecule which encodes a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:3, 15, 21, 45 or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 207116, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:3, 15, 21, 45, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 207116.

e) a nucleic acid molecule which encodes a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:3, 15, 21, 45 or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 207116, wherein the nucleic acid molecule hybridizes to a nucleic acid molecule comprising SEQ ID NO:2, 14, 20, or a complement thereof under stringent conditions.

2. The isolated nucleic acid molecule of claim 1, which is selected from the group consisting of:

a) a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1, 2, 13, 14, 19, 20, the cDNA insert of the plasmid deposited with the ATCC as Accession Number 207116, or a complement thereof; and

b) a nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:3, 15, 21, 45, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 207116.

3. The nucleic acid molecule of claim 1 further comprising vector nucleic acid sequences.

4. The nucleic acid molecule of claim 1 further comprising nucleic acid sequences encoding a heterologous polypeptide.

5. A host cell which contains the nucleic acid molecule of claim 1.

6. The host cell of claim 5 which is a mammalian host cell.

7. A non-human mammalian host cell containing the nucleic acid molecule of claim 1.

8. An isolated polypeptide selected from the group consisting of:

a) a fragment of a polypeptide comprising the amino acid sequence of SEQ ID NO:3, 17, or 25, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:3, 17, or 25;

b) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:3, 15, 21, 45, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 207116, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:2, 14, 20, or a complement thereof under stringent conditions; and

c) a polypeptide which is encoded by a nucleic acid molecule comprising a nucleotide sequence which is at least 55% identical to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:2, 13, or at least 93% to a nucleic acid comprising the nucleotide sequence of SEQ ID NO:19, or a complement thereof.

9. The isolated polypeptide of claim 8 comprising the amino acid sequence of SEQ ID NO:3, 17, or 25.

10. The polypeptide of claim 8 further comprising heterologous amino acid sequences.

11. An antibody which selectively binds to a polypeptide of claim 8.

12. A method for producing a polypeptide selected from the group consisting of:

a) a polypeptide comprising the amino acid sequence of SEQ ID NO:3, 15, or 21, 45, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 207116;

b) a polypeptide comprising a fragment of the amino acid sequence of SEQ ID NO:3, 15, 21, 45, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 207116, wherein the fragment comprises at least 15 contiguous amino acids of SEQ ID NO:3, 15, 21, 45, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 207116; and

c) a naturally occurring allelic variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:3, 15, 21, 45, or the amino acid sequence encoded by the cDNA insert of the plasmid deposited with the ATCC as Accession Number 207116, wherein the polypeptide is encoded by a nucleic acid molecule which hybridizes to a nucleic acid molecule comprising SEQ ID NO:1, 13, or 19, or a complement thereof under stringent conditions;

comprising culturing the host cell of claim 5 under conditions in which the nucleic acid molecule is expressed.

13. A method for detecting the presence of a polypeptide of claim 8 in a sample, comprising:

a) contacting the sample with a compound which selectively binds to a polypeptide of claim 8; and

b) determining whether the compound binds to the polypeptide in the sample.

14. The method of claim 13, wherein the compound which binds to the polypeptide is an antibody.

15. A kit comprising a compound which selectively binds to a polypeptide of claim 8 and instructions for use.

16. A method for detecting the presence of a nucleic acid molecule of claim 1 in a sample, comprising the steps of:

a) contacting the sample with a nucleic acid probe or primer which selectively hybridizes to the nucleic acid molecule; and

b) determining whether the nucleic acid probe or primer binds to a nucleic acid molecule in the sample.

17. The method of claim 16, wherein the sample comprises mRNA molecules and is contacted with a nucleic acid probe.

18. A kit comprising a compound which selectively hybridizes to a nucleic acid molecule of claim 1 and instructions for use.

19. A method for identifying a compound which binds to a polypeptide of claim 8 comprising the steps of:

a) contacting a polypeptide, or a cell expressing a polypeptide of claim 8 with a test compound; and

b) determining whether the polypeptide binds to the test compound.

20. The method of claim 19, wherein the binding of the test compound to the polypeptide is detected by a method selected from the group consisting of:

a) detection of binding by direct detecting of test compound/polypeptide binding;

b) detection of binding using a competition binding assay;

c) detection of binding using an assay for TANGO 228, TANGO 240, or TANGO 243-mediated signal transduction.

21. A method for modulating the activity of a polypeptide of claim 8 comprising contacting a polypeptide or a cell expressing a polypeptide of claim 8 with a compound which binds to the polypeptide in a sufficient concentration to modulate the activity of the polypeptide.

22. A method for identifying a compound which modulates the activity of a polypeptide of claim 8, comprising:

a) contacting a polypeptide of claim 8 with a test compound; and

b) determining the effect of the test compound on the activity of the polypeptide to thereby identify a compound which modulates the activity of the polypeptide.

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 Y   K   S   G   Y   V   Y   S   E   L   N   F   *   344
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ATCTAGTCTTATATCCACGCTCACCACCTCAGCACGCTCAGACCCACGCTGCTGTGGGCTCCTCTGGCTCCTGGAAGAG 1302

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Figure 1B

CTCAGTGTCTCACCCCAGGCACTGCAAACACCCCAGGACAGAAAGTGAACATGTGTATTGTGCAATTGAAGCAAGACAC 3040

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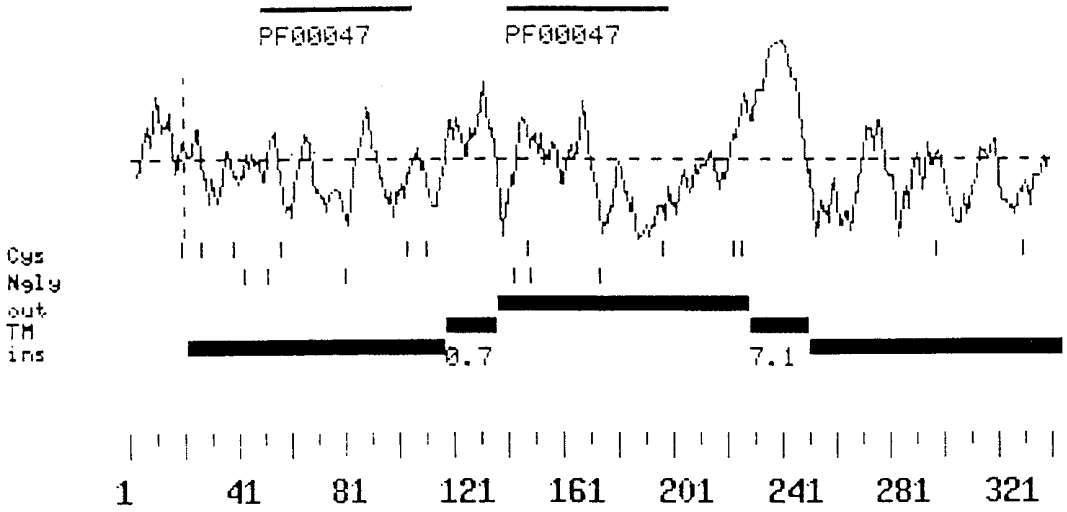
Figure 1C



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HITLHCLSVNGSLPINYTFFENHVAISPAISKYDREPAEFNLTKKNPGE
EEEYRCEAKNRLPNYATYSHPVTMPSTGGDSCPFCLKLLLPGLLLLL
VVIILILAFWVLPKYKTRKAMRNNVPRDRGDTAMEVGIYANILEKQ
AKEESVPEVGSRPCVSTAQDEAKHSQELQYATPVFQEVAPREQEAC
DSYKSGYVYSELNF

Figure 2

```
ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989)
> rat MCA-32                                          1659 aa vs.
> TANGO 228 n.a.                                      4060 aa
scoring matrix: pam120.mat, gap penalties: -12/-4
28.3% identity;          Global alignment score: -4873


MCA-32 ------------------------------------------------------------

T228   GTCGACCCACGCGTCCGCTTCTGACCCCGTCTTGGACTTCAACTGGGAGAATGTGGAGCC
              10         20         30         40         50         60

                      10                    20
MCA-32 -------CTAAATCCTCT-------CAT---TTC--CTG---CATGTGGT---------T
               :... ::::::        :::   :::  :::   :.::::.:         :
       ATTTGAGCAGGCTCCTCTTCTGGAGCATATTTTCTTCTGTCACTTGTAGAAAAGCTGTAT
              70         80         90        100        110        120

      30         40         50                60          70
MCA-32 GGGAAGAGGAATCTTAGAAGACGAG------CCCCACTC--TGTAGCAGCTCAA---CTA
        :::. . ::. :...::::.::.:.       ::: .:::  :::.  ..::::::   :::
       TGGATTGTGAGGCAATGAAAACAAATGAATTCCCTTCTCCATGTTTGGACTCAAAGACTA
              130        140        150        160        170        180

                                                 80
MCA-32 A-------------------------------------CCATGGG-------------
       :                                      ::::..:
       AGGTGGTTATGAAGGGTCAAAATGTATCTATGTTTTGTTCCCATAAGAACAAATCACTGC
              190        200        210        220        230        240

                     90                100
MCA-32 ------------CGATGAT---------GACACCCCTGTGTGCC----------------
                  :.::::.          ::::: :::: :.::
       AGATCACCTATTCATTGTTTCGACGTAAGACACACCTGGGAACCCAGGATGGAAAAGGTG
              250        260        270        280        290        300

                              110       120                      130
MCA-32 ------------------------TCTCTGTCGCC------TCATGC--------AAGGGA
                               ::.:.:. :::       ::: ::         :: : :
       AACCTGCGATTTTTAACCTAAGCATCACAGAAGCCCATGAATCAGGCCCCTACAAATGCA
              310        320        330        340        350        360

                140        150
MCA-32 GTG----AGTTGCTGGCTGGACAAA------------CT---------------------
       ..:     :::::.: .:::: .::::            ::
       AAGCCCAAGTTACCAGCTGTTCAAAATACAGTCGTGACTTCAGCTTCACGATTGTCGACC
              370        380        390        400        410        420

                160                                              170
MCA-32 ------CTTACTCTGGGCT-----------------------------------CTTA---
             ::: : :.: :::                                     :.::
       CGGTGACTTCCCCAGTGCTGAACATTATGGTCATTCAAACAGAAACAGACCGACATATAA
              430        440        450        460        470        480
```

Figure 3A

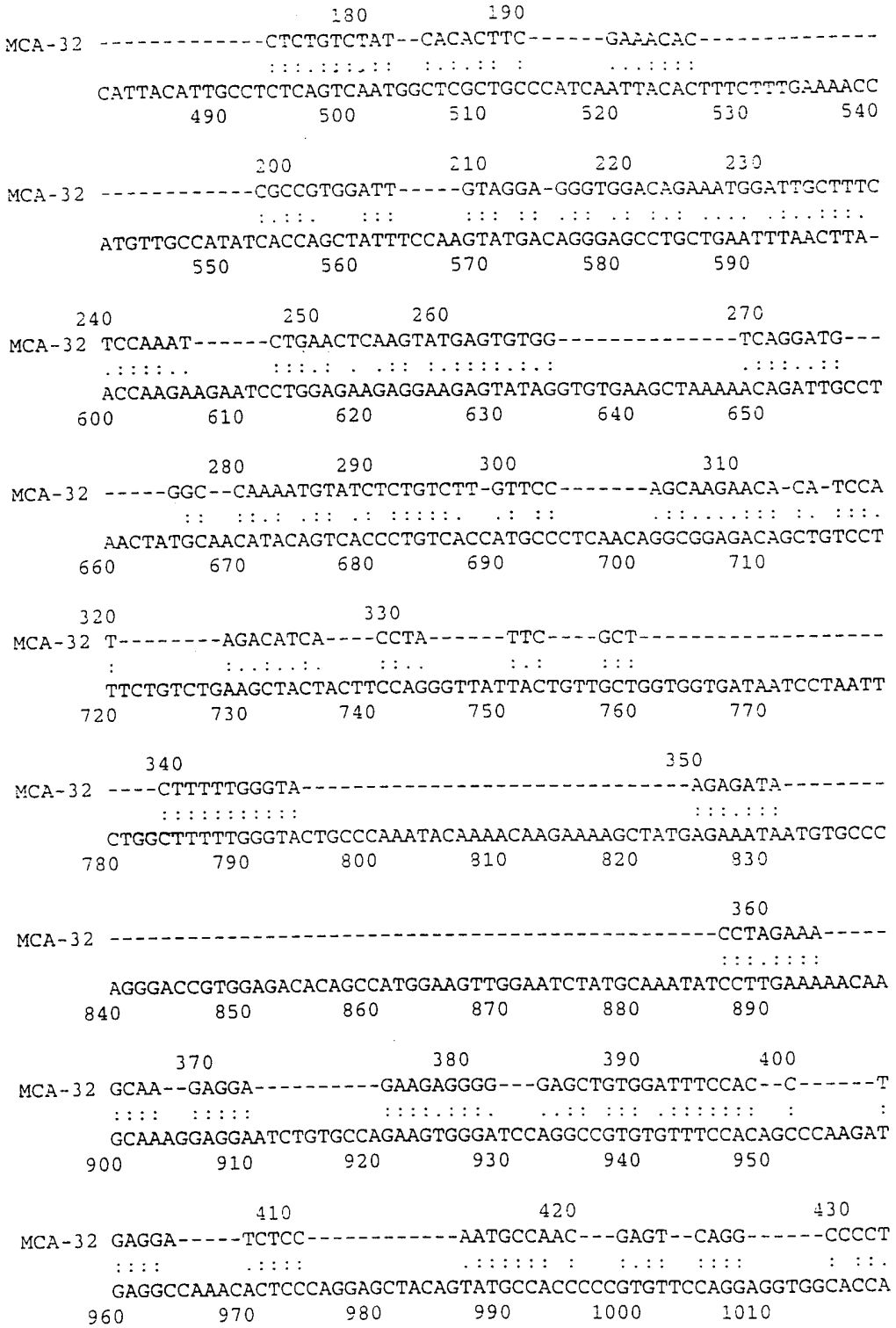


Figure 3B

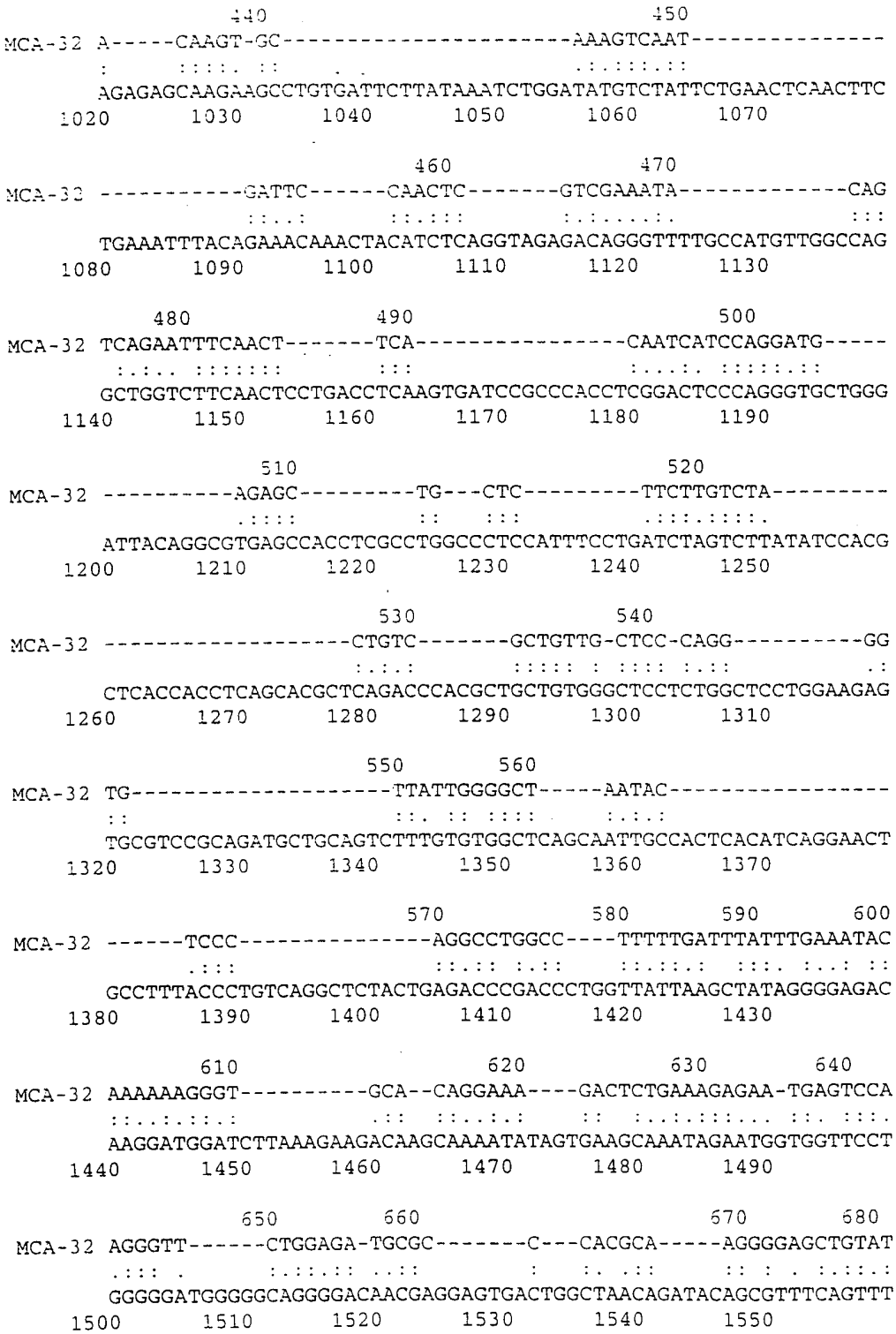

440 450
MCA-32 A-----CAAGT-GC----------AAAGTCAAT----------
AGAGAGCAAGAAGCCTGTGATTCTTATAAATCTGGATATGTCTATTCTGAACTCAACTTC
1020 1030 1040 1050 1060 1070
460 470
MCA-32 ----------GATTC------CAACTC-------GTCGAAATA----------CAG
TGAAATTTACAGAAACAAACTACATCTCAGGTAGAGACAGGGTTTTGCCATGTTGGCCAG
1080 1090 1100 1110 1120 1130
480 490 500
MCA-32 TCAGAATTTCAACT-------TCA----------CAATCATCCAGGATG-----
GCTGGTCTTCAACTCCTGACCTCAAGTGATCCGCCCACCTCGGACTCCCAGGGTGCTGGG
1140 1150 1160 1170 1180 1190
510 520
MCA-32 ----------AGAGC---------TG---CTC---------TTCTTGTCTA---------
ATTACAGGCGTGAGCCACCTCGCCTGGCCCTCCATTTCCTGATCTAGTCTTATATCCACG
1200 1210 1220 1230 1240 1250
530 540
MCA-32 ----------CTGTC-------GCTGTTG-CTCC-CAGG----------GG
CTCACCACCTCAGCACGCTCAGACCCACGCTGCTGTGGGCTCCTCTGGCTCCTGGAAGAG
1260 1270 1280 1290 1300 1310
550 560
MCA-32 TG----------TTATTGGGGCT-----AATAC----------
TGCGTCCGCAGATGCTGCAGTCTTTGTGTGGCTCAGCAATTGCCACTCACATCAGGAACT
1320 1330 1340 1350 1360 1370
570 580 590 600
MCA-32 ------TCCC----------AGGCCTGGCC----TTTTTGATTTATTTGAAATAC
GCCTTTACCCTGTCAGGCTCTACTGAGACCCGACCCTGGTTATTAAGCTATAGGGGAGAC
1380 1390 1400 1410 1420 1430
610 620 630 640
MCA-32 AAAAAAGGGT----------GCA--CAGGAAA----GACTCTGAAAGAGAA-TGAGTCCA
AAGGATGGATCTTAAAGAAGACAAGCAAAATATAGTGAAGCAAATAGAATGGTGGTTCCT
1440 1450 1460 1470 1480 1490
650 660 670 680
MCA-32 AGGGTT------CTGGAGA-TGCGC-------C---CACGCA-----AGGGGAGCTGTAT
GGGGGATGGGGGCAGGGGACAACGAGGAGTGACTGGCTAACAGATACAGCGTTTCAGTTT
1500 1510 1520 1530 1540 1550

Figure 3C

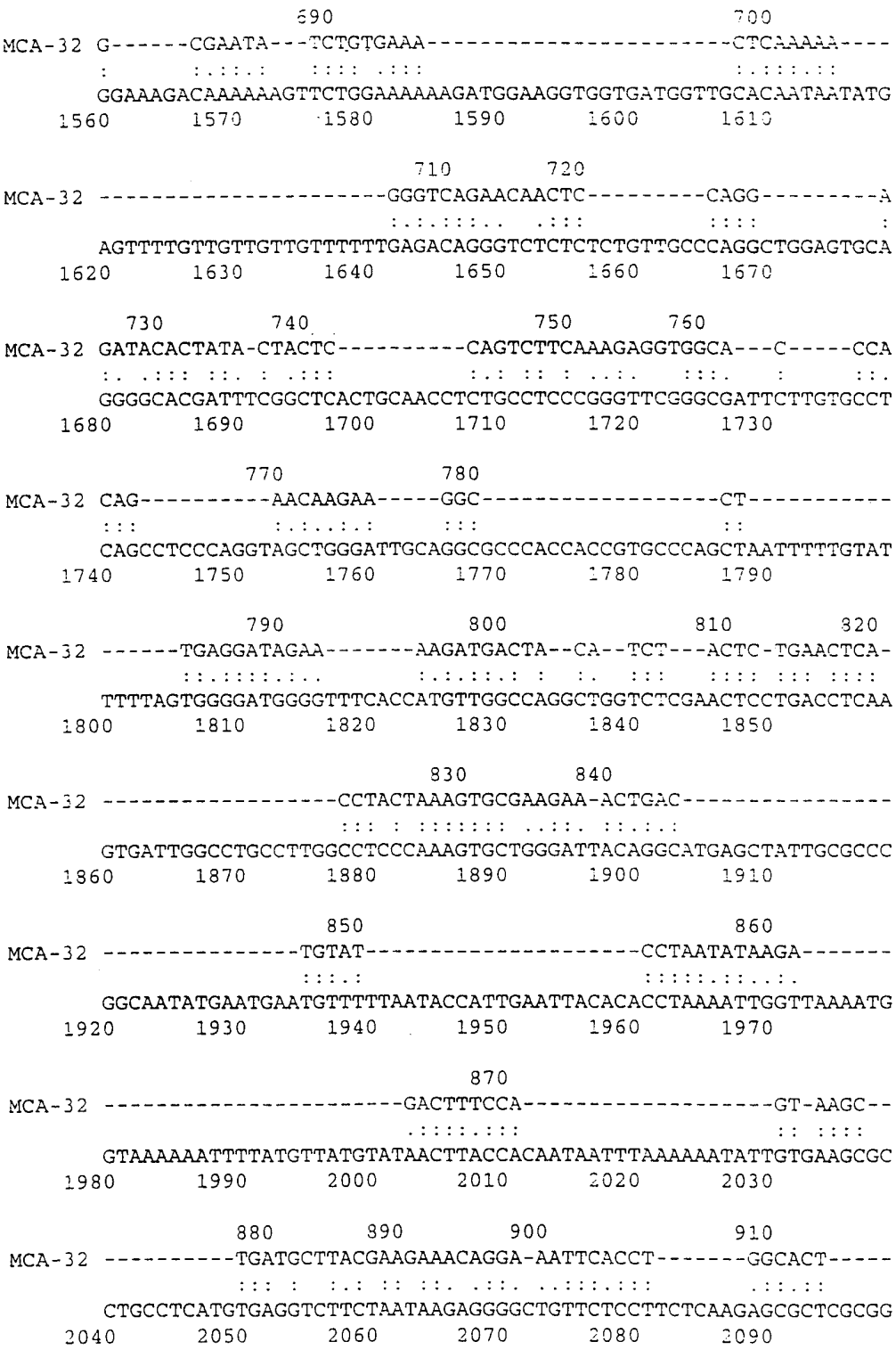

690 700
MCA-32 G------CGAATA---TCTGTGAAA----------CTCAAAAA----
GGAAAGACAAAAAAGTTCTGGAAAAAAGATGGAAGGTGGTGATGGTTGCACAATAATATG
1560 1570 1580 1590 1600 1610
710 720
MCA-32 ----------GGGTCAGAACAACTC---------CAGG---------A
AGTTTTGTTGTTGTTGTTTTTTGAGACAGGGTCTCTCTCTGTTGCCCAGGCTGGAGTGCA
1620 1630 1640 1650 1660 1670
730 740 750 760
MCA-32 GATACACTATA-CTACTC----------CAGTCTTCAAAGAGGTGGCA---C-----CCA
GGGGCACGATTTCGGCTCACTGCAACCTCTGCCTCCCGGGTTCGGGCGATTCTTGTGCCT
1680 1690 1700 1710 1720 1730
770 780
MCA-32 CAG----------AACAAGAA-----GGC----------CT----------
CAGCCTCCCAGGTAGCTGGGATTGCAGGCGCCCACCACCGTGCCCAGCTAATTTTTGTAT
1740 1750 1760 1770 1780 1790
790 800 810 820
MCA-32 ------TGAGGATAGAA-------AAGATGACTA--CA--TCT---ACTC-TGAACTCA-
TTTTAGTGGGGATGGGGTTTCACCATGTTGGCCAGGCTGGTCTCGAACTCCTGACCTCAA
1800 1810 1820 1830 1840 1850
830 840
MCA-32 ----------CCTACTAAAGTGCGAAGAA-ACTGAC----------
GTGATTGGCCTGCCTTGGCCTCCCAAAGTGCTGGGATTACAGGCATGAGCTATTGCGCCC
1860 1870 1880 1890 1900 1910
850 860
MCA-32 ----------TGTAT----------CCTAATATAAGA-------
GGCAATATGAATGAATGTTTTTAATACCATTGAATTACACACCTAAAATTGGTTAAAATG
1920 1930 1940 1950 1960 1970
870
MCA-32 ----------GACTTTCCA----------GT-AAGC--
GTAAAAAATTTTATGTTATGTATAACTTACCACAATAATTTAAAAAATATTGTGAAGCGC
1980 1990 2000 2010 2020 2030
880 890 900 910
MCA-32 ----------TGATGCTTACGAAGAAACAGGA-AATTCACCT-------GGCACT-----
CTGCCTCATGTGAGGTCTTCTAATAAGAGGGGCTGTTCTCCTTCTCAAGAGCGCTCGCGG
2040 2050 2060 2070 2080 2090

Figure 3D

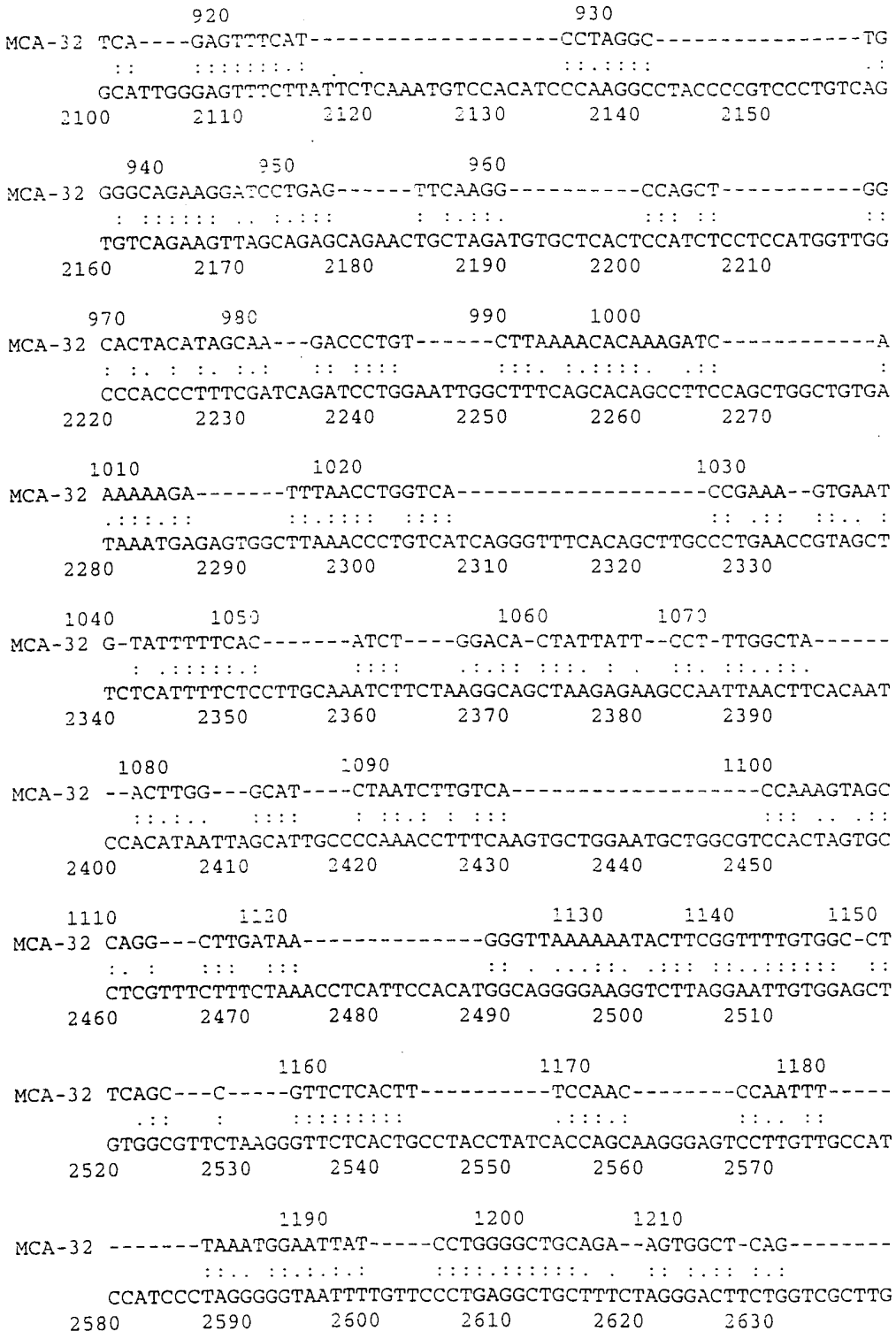

920 930
MCA-32 TCA----GAGTTTCAT----------CCTAGGC----------TG
GCATTGGGAGTTTCTTATTCTCAAATGTCCACATCCCAAGGCCTACCCCGTCCCTGTCAG
2100 2110 2120 2130 2140 2150
940 950 960
MCA-32 GGGCAGAAGGATCCTGAG------TTCAAGG----------CCAGCT----------GG
TGTCAGAAGTTAGCAGAGCAGAACTGCTAGATGTGCTCACTCCATCTCCTCCATGGTTGG
2160 2170 2180 2190 2200 2210
970 980 990 1000
MCA-32 CACTACATAGCAA---GACCCTGT------CTTAAAACACAAAGATC----------A
CCCACCCTTTCGATCAGATCCTGGAATTGGCTTTCAGCACAGCCTTCCAGCTGGCTGTGA
2220 2230 2240 2250 2260 2270
1010 1020 1030
MCA-32 AAAAAGA-------TTTAACCTGGTCA----------CCGAAA--GTGAAT
TAAATGAGAGTGGCTTAAACCCTGTCATCAGGGTTTCACAGCTTGCCCTGAACCGTAGCT
2280 2290 2300 2310 2320 2330
1040 1050 1060 1070
MCA-32 G-TATTTTTCAC-------ATCT----GGACA-CTATTATT--CCT-TTGGCTA------
TCTCATTTTCTCCTTGCAAATCTTCTAAGGCAGCTAAGAGAAGCCAATTAACTTCACAAT
2340 2350 2360 2370 2380 2390
1080 1090 1100
MCA-32 --ACTTGG---GCAT----CTAATCTTGTCA----------CCAAAGTAGC
CCACATAATTAGCATTGCCCCAAACCTTTCAAGTGCTGGAATGCTGGCGTCCACTAGTGC
2400 2410 2420 2430 2440 2450
1110 1120 1130 1140 1150
MCA-32 CAGG---CTTGATAA----------GGGTTAAAAAATACTTCGGTTTTGTGGC-CT
CTCGTTTCTTTCTAAACCTCATTCCACATGGCAGGGGAAGGTCTTAGGAATTGTGGAGCT
2460 2470 2480 2490 2500 2510
1160 1170 1180
MCA-32 TCAGC---C-----GTTCTCACTT----------TCCAAC--------CCAATTT-----
GTGGCGTTCTAAGGGTTCTCACTGCCTACCTATCACCAGCAAGGGAGTCCTTGTTGCCAT
2520 2530 2540 2550 2560 2570
1190 1200 1210
MCA-32 -------TAAATGGAATTAT-----CCTGGGGCTGCAGA--AGTGGCT-CAG--------
CCATCCCTAGGGGGTAATTTTGTTCCCTGAGGCTGCTTTCTAGGGACTTCTGGTCGCTTG
2580 2590 2600 2610 2620 2630

Figure 3E

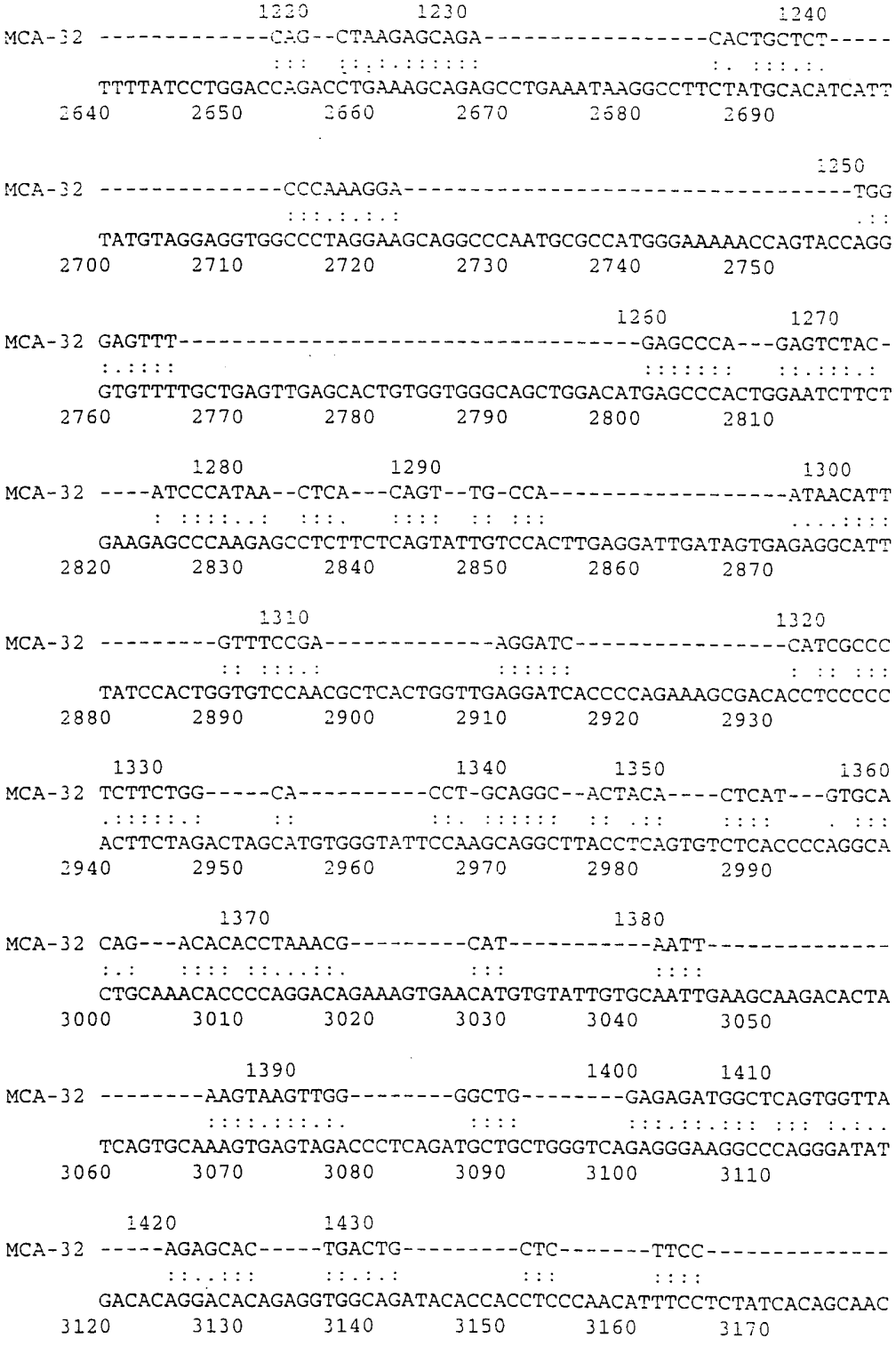

1220 1230 1240
MCA-32 ----------CAG--CTAAGAGCAGA----------CACTGCTCT-----
TTTTATCCTGGACCAGACCTGAAAGCAGAGCCTGAAATAAGGCCTTCTATGCACATCATT
2640 2650 2660 2670 2680 2690
1250
MCA-32 ----------CCCAAAGGA----------TGG
TATGTAGGAGGTGGCCCTAGGAAGCAGGCCCAATGCGCCATGGGAAAAACCAGTACCAGG
2700 2710 2720 2730 2740 2750
1260 1270
MCA-32 GAGTTT----------GAGCCCA---GAGTCTAC-
GTGTTTTGCTGAGTTGAGCACTGTGGTGGGCAGCTGGACATGAGCCCACTGGAATCTTCT
2760 2770 2780 2790 2800 2810
1280 1290 1300
MCA-32 ----ATCCCATAA--CTCA---CAGT--TG-CCA----------ATAACATT
GAAGAGCCCAAGAGCCTCTTCTCAGTATTGTCCACTTGAGGATTGATAGTGAGAGGCATT
2820 2830 2840 2850 2860 2870
1310 1320
MCA-32 ---------GTTTCCGA----------AGGATC----------CATCGCCC
TATCCACTGGTGTCCAACGCTCACTGGTTGAGGATCACCCCAGAAAGCGACACCTCCCCC
2880 2890 2900 2910 2920 2930
1330 1340 1350 1360
MCA-32 TCTTCTGG-----CA----------CCT-GCAGGC--ACTACA----CTCAT---GTGCA
ACTTCTAGACTAGCATGTGGGTATTCCAAGCAGGCTTACCTCAGTGTCTCACCCCAGGCA
2940 2950 2960 2970 2980 2990
1370 1380
MCA-32 CAG---ACACACCTAAACG---------CAT----------AATT----------
CTGCAAACACCCCAGGACAGAAAGTGAACATGTGTATTGTGCAATTGAAGCAAGACACTA
3000 3010 3020 3030 3040 3050
1390 1400 1410
MCA-32 --------AAGTAAGTTGG--------GGCTG--------GAGAGATGGCTCAGTGGTTA
TCAGTGCAAAGTGAGTAGACCCTCAGATGCTGCTGGGTCAGAGGGAAGGCCCAGGGATAT
3060 3070 3080 3090 3100 3110
1420 1430
MCA-32 -----AGAGCAC-----TGACTG---------CTC-------TTCC----------
GACACAGGACACAGAGGTGGCAGATACACCACCTCCCAACATTTCCTCTATCACAGCAAC
3120 3130 3140 3150 3160 3170

Figure 3F

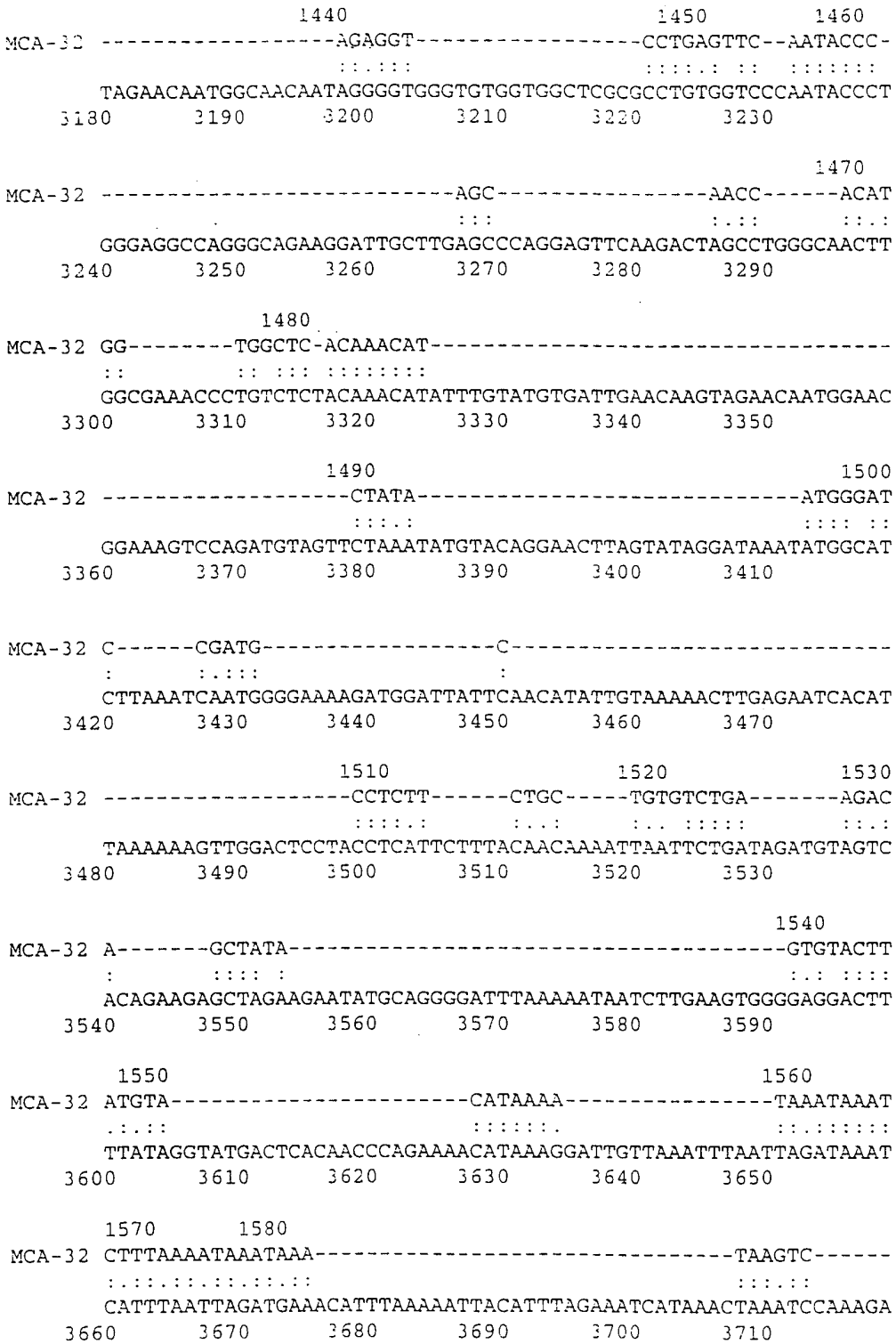

1440 1450 1460
MCA-32 ----------AGAGGT----------CCTGAGTTC--AATACCC-
TAGAACAATGGCAACAATAGGGGTGGGTGTGGTGGCTCGCGCCTGTGGTCCCAATACCCT
3180 3190 3200 3210 3220 3230
1470
MCA-32 ----------AGC----------AACC------ACAT
GGGAGGCCAGGGCAGAAGGATTGCTTGAGCCCAGGAGTTCAAGACTAGCCTGGGCAACTT
3240 3250 3260 3270 3280 3290
1480
MCA-32 GG--------TGGCTC-ACAAACAT----------
GGCGAAACCCTGTCTCTACAAACATATTTGTATGTGATTGAACAAGTAGAACAATGGAAC
3300 3310 3320 3330 3340 3350
1490 1500
MCA-32 ----------CTATA----------ATGGGAT
GGAAAGTCCAGATGTAGTTCTAAATATGTACAGGAACTTAGTATAGGATAAATATGGCAT
3360 3370 3380 3390 3400 3410
MCA-32 C------CGATG----------C----------
CTTAAATCAATGGGGAAAAGATGGATTATTCAACATATTGTAAAAACTTGAGAATCACAT
3420 3430 3440 3450 3460 3470
1510 1520 1530
MCA-32 ----------CCTCTT------CTGC-----TGTGTCTGA-------AGAC
TAAAAAAGTTGGACTCCTACCTCATTCTTTACAACAAAATTAATTCTGATAGATGTAGTC
3480 3490 3500 3510 3520 3530
1540
MCA-32 A-------GCTATA----------GTGTACTT
ACAGAAGAGCTAGAAGAATATGCAGGGGATTTAAAAATAATCTTGAAGTGGGGAGGACTT
3540 3550 3560 3570 3580 3590
1550 1560
MCA-32 ATGTA----------CATAAAA----------TAAATAAAT
TTATAGGTATGACTCACAACCCAGAAAACATAAAGGATTGTTAAATTTAATTAGATAAAT
3600 3610 3620 3630 3640 3650
1570 1580
MCA-32 CTTTAAAATAAATAAA----------TAAGTC------
CATTTAATTAGATGAAACATTTAAAAATTACATTTAGAAATCATAAACTAAATCCAAAGA
3660 3670 3680 3690 3700 3710

Figure 3G

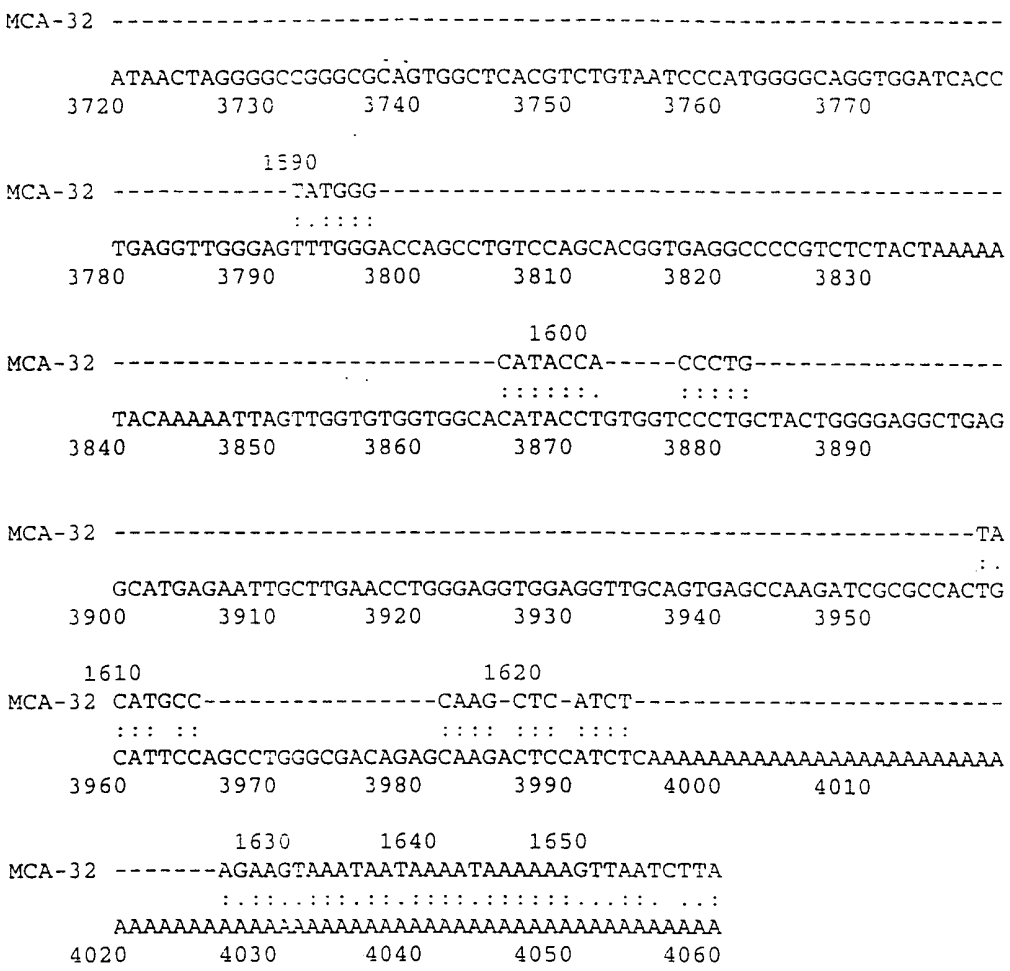

MCA-32 ----------
ATAACTAGGGGCCGGGCGCAGTGGCTCACGTCTGTAATCCCATGGGGCAGGTGGATCACC
3720 3730 3740 3750 3760 3770
1590
MCA-32 ----------TATGGG----------
TGAGGTTGGGAGTTTGGGACCAGCCTGTCCAGCACGGTGAGGCCCCGTCTCTACTAAAAA
3780 3790 3800 3810 3820 3830
1600
MCA-32 ----------CATACCA-----CCCTG----------
TACAAAAATTAGTTGGTGTGGTGGCACATACCTGTGGTCCCTGCTACTGGGGAGGCTGAG
3840 3850 3860 3870 3880 3890
MCA-32 ----------TA
GCATGAGAATTGCTTGAACCTGGGAGGTGGAGGTTGCAGTGAGCCAAGATCGCGCCACTG
3900 3910 3920 3930 3940 3950
1610 1620
MCA-32 CATGCC----------CAAG-CTC-ATCT----------
CATTCCAGCCTGGGCGACAGAGCAAGACTCCATCTCAAAAAAAAAAAAAAAAAAAAAAA
3960 3970 3980 3990 4000 4010
1630 1640 1650
MCA-32 -------AGAAGTAAATAATAAAATAAAAAAGTTAATCTTA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
4020 4030 4040 4050 4060

Figure 3H

```
ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989)
> t228 ORF                                          1032 aa vs.
> rat MCA-32 ORF                                     804 aa
scoring matrix: pam120.mat, gap penalties: -12/-4
45.4% identity;          Global alignment score: 911

                10        20        30        40        50        60
T228    ATGTGGAGCCATTTGAGCAGGCTCCTCTTCTGGAGCATATTTTCTTCTGTCACTTGTAGA
        ::::::      ::::: ::.    :::: ....:...    :  :.  :.:: :::: 
        ATGTGG------TTGGGAAGAGGAATCTTAGAAGACGAG----CCCCA--CTCT-GTAGC
                      10        20        30                40

                70        80        90        100       110
T228    AAAGCTGTATTGGATTGTGAGGCAATGAAAACAAA--TGAATTCCCTTCTCCATGTTTGG
          :::: .: :. :  .:: :::.::::..:::    ::..: ::  :::: :::   :
        --AGCTCAACTA-ACCATG-GGCGATGATGACACCCCTGTGTGCC--TCTC--TGTC--G
           50          60          70        80          90

              120       130       140       150       160       170
T228    ACTCAAAGACTAAGGTGGTTATGAAGGGTCAAAATGTATCTATGTTTTGTTCCCATAAGA
         :::::  .:.:.:::.:  :..:..:   :... :: ..:.:. : ::. ::   :..: 
        CCTCAT--GCAAGGGAG--TGAGTTG---CTGGCTG-GACAAACTCTTACTC---TGGGC
        100          110             120       130       140

              180       190       200       210       220       230
T228    ACAAATCACTGCAGATCACCTATTCATTGTTTCGACGTAAGACACACCTGGGAACCCAGG
        .:..: :.:::   :::::             .: .:::.:: :: :.::  :::.     :
        TCTTA-CTCTGTCTATCAC------------ACTTCGAAACAC-CGCCGTGGAT----TG
         150         160                   170         180

              240       250       260       270       280       290
T228    ATGGAAAAGGTGAACCTGCGATTTTTAACCTAAGCATCACAGAAGCCCATGAATCAGGCC
        ..:::  .:::::.:: .: .::  .:..: :     ::.: .:: :   :::::     :
        TAGGA--GGGTGGAC-AGAAATGGATTGCTT-----TCTCCAAATC---TGAA-----CT
        190          200         210             220          230

              300       310       320       330       340       350
T228    CCTACAAATGCAAAGCCCAAGTTACCAGCTGTTCAAAATACAGTCGTGACTTCAGCTTCA
        : .. :.:.: ...: : ...: . :  :.... ..:. .:.::: ::  :::  :. ::
        CAAGTATGAGTGTGGTCAGGATGGGC--CAAAATGTATCTCTGTCTTG--TTC--CAGCA
             240       250         260       270         280

              360       370       380       390       400       410
T228    CGATTGTCGACCCGGTGACTTCCCCAGTGCTGAACATTATGGTCATTCAAACAGAAACAG
         ::. . :..:: . .:::.:: ::..: : : .:.::.:::  :        :::.:
        AGAACA-CATCC-ATAGACATCACCTATTC-GCTCTTTTTGGGTA-------AGAGA--T
         290         300       310       320               330

              420       430       440       450       460       470
T228    ACCGACATATAACATTACATTGCCTCTCAGTCAATGGCTCGCTGCCCATCAATTACACTT
        ::: :   :.:.::. :    :       ::. .: ::   ::::    : .::::: :
        ACCTA--GAAAGCAAGA---GG------AGAAGAGGGGGAGCTG----TGGATTTC-C--
             340         350             360          370
```

Figure 4A

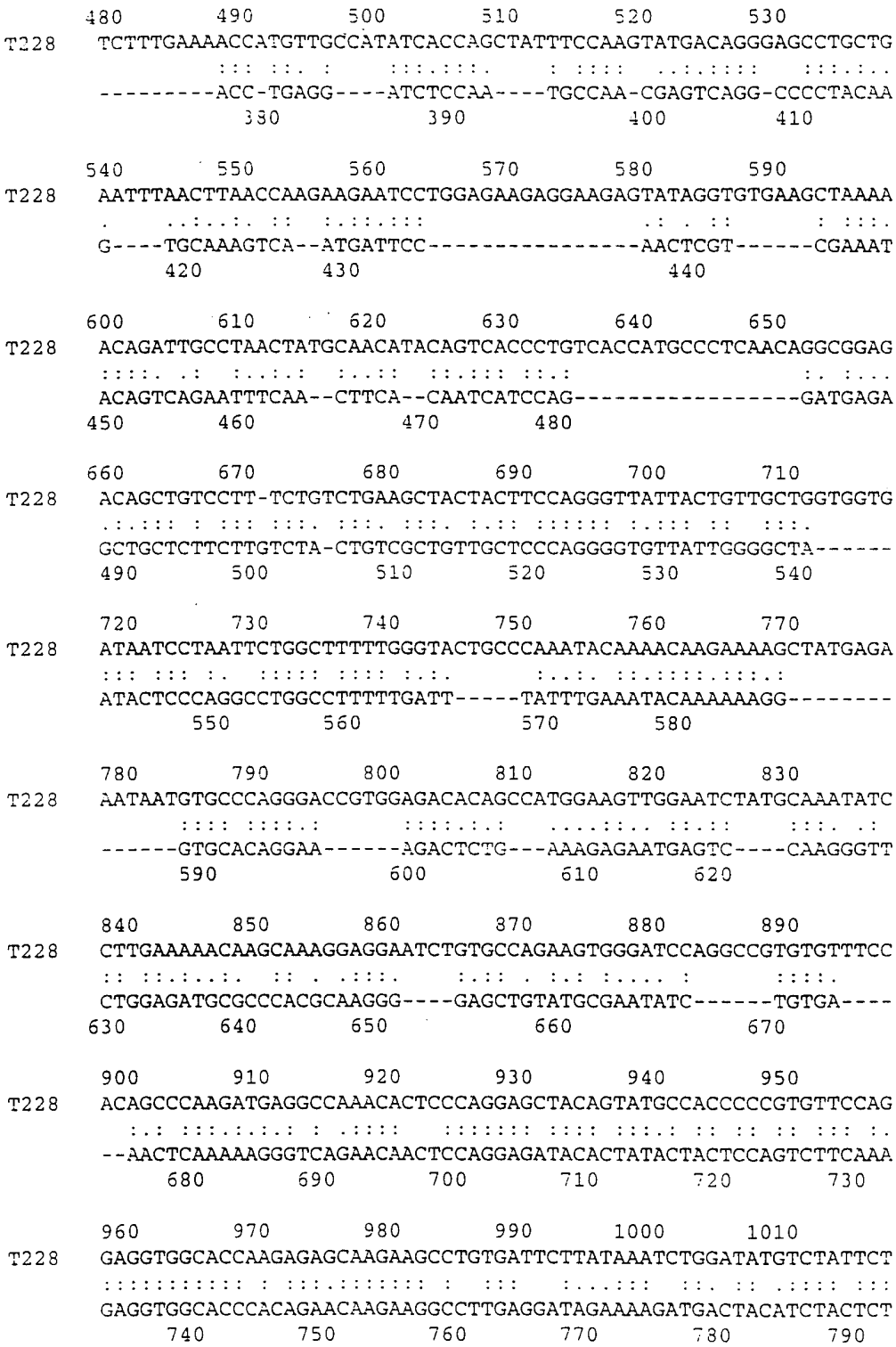

480 490 500 510 520 530
T228 TCTTTGAAAACCATGTTGCCATATCACCAGCTATTTCCAAGTATGACAGGGAGCCTGCTG
---------ACC-TGAGG----ATCTCCAA----TGCCAA-CGAGTCAGG-CCCCTACAA
380 390 400 410
540 550 560 570 580 590
T228 AATTTAACTTAACCAAGAAGAATCCTGGAGAAGAGGAAGAGTATAGGTGTGAAGCTAAAA
G----TGCAAAGTCA--ATGATTCC----------AACTCGT------CGAAAT
420 430 440
600 610 620 630 640 650
T228 ACAGATTGCCTAACTATGCAACATACAGTCACCCTGTCACCATGCCCTCAACAGGCGGAG
ACAGTCAGAATTTCAA--CTTCA--CAATCATCCAG----------GATGAGA
450 460 470 480
660 670 680 690 700 710
T228 ACAGCTGTCCTT-TCTGTCTGAAGCTACTACTTCCAGGGTTATTACTGTTGCTGGTGGTG
GCTGCTCTTCTTGTCTA-CTGTCGCTGTTGCTCCCAGGGGTGTTATTGGGGCTA------
490 500 510 520 530 540
720 730 740 750 760 770
T228 ATAATCCTAATTCTGGCTTTTTGGGTACTGCCCAAATACAAAACAAGAAAAGCTATGAGA
ATACTCCCAGGCCTGGCCTTTTTGATT-----TATTTGAAATACAAAAAAGG--------
550 560 570 580
780 790 800 810 820 830
T228 AATAATGTGCCCAGGGACCGTGGAGACACAGCCATGGAAGTTGGAATCTATGCAAATATC
------GTGCACAGGAA------AGACTCTG---AAAGAGAATGAGTC----CAAGGGTT
590 600 610 620
840 850 860 870 880 890
T228 CTTGAAAAACAAGCAAAGGAGGAATCTGTGCCAGAAGTGGGATCCAGGCCGTGTGTTTCC
CTGGAGATGCGCCCACGCAAGGG----GAGCTGTATGCGAATATC------TGTGA----
630 640 650 660 670
900 910 920 930 940 950
T228 ACAGCCCAAGATGAGGCCAAACACTCCCAGGAGCTACAGTATGCCACCCCCGTGTTCCAG
--AACTCAAAAAGGGTCAGAACAACTCCAGGAGATACACTATACTACTCCAGTCTTCAAA
680 690 700 710 720 730
960 970 980 990 1000 1010
T228 GAGGTGGCACCAAGAGAGCAAGAAGCCTGTGATTCTTATAAATCTGGATATGTCTATTCT
GAGGTGGCACCCACAGAACAAGAAGGCCTTGAGGATAGAAAAGATGACTACATCTACTCT
740 750 760 770 780 790

Figure 4B

```
      1020       1030
T228   GAACTCAACTTCTGA
       ::::::: :::.:
       GAACTCACCTAC---
            800
```

Figure 4C

```
ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989)
> rat MCA-32 a.a.                                    268 aa vs.
> TANGO 228 a.a.                                     343 aa
scoring matrix: pami20.mat, gap penalties: -12/-4
26.8% identity;         Global alignment score: -51

                  10              20          30                40
MCA-32 MW--LGRGILEDEPHSVA---AQL---TMGDDDTPV-CLS-----VASCKGVS--CWL-D
       ::  :.: .. .   ::.   : :   .:  .. :  ::.     :   ..::  :   .
       MWSHLSRLLFWSIFSSVTCRKAVLDCEAMKTNEFPSPCLDSKTKVVMKGQNVSMFCSHKN
               10        20        30        40        50        60

              50                                    60          70
MCA-32 KLLL--WAL---------------------TLSITL---------RNTAVDCRRVDRN---G
       : :    .:                     .::::          .. ...: . .:.
       KSLQITYSLFRRKTHLGTQDGKGEPAIFNLSITEAHESGPYKCKAQVTSCSKYSRDFSFT
               70        80        90        100       110       120

               80            90       100       110          120
MCA-32 LLSPNLNSSMSVVRM----GQNVSLSCSSKNTSIDITYSLFLGKRYLESKRRR--GGAVD
       ...:  .. .... .    .....: : : : :. :.:..: .   . .   .    ...
       IVDPVTSPVLNIMVIQTETDRHITLHCLSVNGSLPINYTFFENHVAISPAISKYDREPAE
               130       140       150       160       170       180

          130       140         150       160       170       180
MCA-32 FHLRISNANESGPYKCKVND--SNSSKYSQNFNFTIIQDESCSSCLLSLLLPGVLLGL--
       :.:   :..:   :.: ...  .: . ::.  .   . ..:::. ::  :::::.:: :
       FNLTKKNPGEEEEYRCEAKNRLPNYATYSHPVTMPSTGGDSCPFCL-KLLLPGLLLLLVV
               190       200       210       220       230

               190       200       210       220
MCA-32 ILPGLAFLIYLKYKKGCTGKTLKENESKGSGDAPTQGELYANI----------------
       :.  ::: .  :::   : :....: ... ::.. .  .::::
       IILILAFWVLPKYK---TRKAMRNNVPRDRGDTAMEVGIYANILEKQAKEESVPEVGSRP
     240       250          260       270       280       290

             230       240       250       260
MCA-32 CET--QKGSEQLQEIHYTTPVFKEVAPTEQEGLEDRKDDYIYSELTY
       : .  :  . . ::..:.:::.:::: :::. .. :..:.:::..
       CVSTAQDEAKHSQELQYATPVFQEVAPREQEACDSYKSGYVYSELNF
        300       310       320       330       340
```

Figure 5

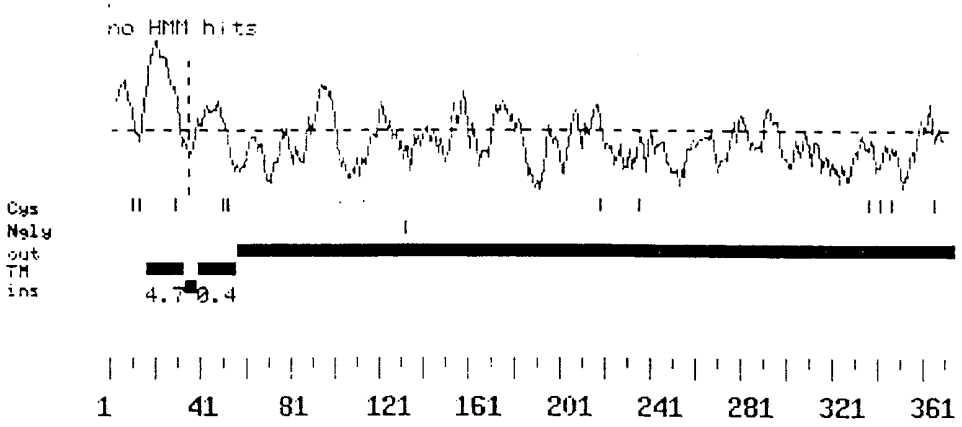
Input file huT240; Output File huT240.pat2
Sequence length 2165

```
   M   A   A   P   A   L   G   L   V   C   G   R   C   P   E   L   G   L   V      19
C ATG GCT GCG CCC GCA CTA GGG CTG GTG TGT GGA CGT TGC CCT GAG CTG GGT CTC GTC      58

 L   L   L   L   L   L   S   L   L   C   G   A   A   G   S   Q   E   A   G   T    39
CTC TTG CTG CTG CTG CTC TCG CTG CTG TGT GGA GCG GCA GGG AGC CAG GAG GCC GGG ACC   118

 G   A   G   A   G   S   L   A   G   S   C   G   C   G   T   P   Q   R   P   G    59
GGT GCG GGC GCG GGG TCC CTT GCG GGT TCT TGC GGC TGC GGC ACG CCC CAG CGG CCT GGC   178

 A   H   G   N   S   A   A   A   H   R   Y   S   R   E   A   N   A   P   G   P    79
GCC CAT GGC AAT TCG GCA GCC GCT CAC CGA TAC TCG CGG GAG GCT AAC GCT CCG GGC CCC   238

 V   P   G   E   R   Q   L   A   H   S   K   M   V   P   I   P   V   G   V   F    99
GTA CCC GGA GAG CGG CAA CTC GCG CAC TCA AAG ATG GTC CCC ATC CCT GTT GGA GTA TTT   298

 T   M   G   T   D   D   P   Q   I   K   Q   D   G   E   A   P   A   R   R   V   119
ACA ATG GGC ACA GAT GAT CCT CAG ATA AAG CAG GAT GGG GAA GCA CCT GCG AGG AGA GTT   358

 T   I   D   A   F   Y   M   D   A   Y   E   V   S   N   T   E   F   E   K   F   139
ACT ATT GAT GCC TTT TAC ATG GAT GCC TAT GAA GTC AGT AAT ACT GAA TTT GAG AAG TTT   418

 V   N   S   T   G   Y   L   T   E   A   E   K   F   G   D   S   F   V   F   E   159
GTG AAC TCA ACT GGC TAT TTG ACA GAG GCT GAG AAG TTT GGC GAC TCC TTT GTC TTT GAA   478

 G   M   L   S   E   Q   V   K   T   N   I   Q   Q   A   V   A   A   A   P   W   179
GGC ATG TTG AGT GAG CAA GTG AAG ACC AAT ATT CAA CAG GCA GTT GCA GCT GCT CCC TGG   538

 W   L   P   V   K   G   A   N   W   R   H   P   E   G   P   D   S   T   I   L   199
TGG TTA CCT GTG AAA GGC GCT AAC TGG AGA CAC CCA GAA GGG CCT GAC TCT ACT ATT CTG   598

 H   R   P   D   H   P   V   L   H   V   S   W   N   D   A   V   A   Y   C   T   219
CAC AGG CCG GAT CAT CCA GTT CTC CAT GTG TCC TGG AAT GAT GCG GTT GCC TAC TGC ACT   658

 W   A   G   K   R   L   P   T   E   A   E   W   E   Y   S   C   R   G   G   L   239
TGG GCA GGG AAG CGG CTG CCC ACG GAA GCT GAG TGG GAA TAC AGC TGT CGA GGA GGC CTG   718

 H   N   R   L   F   P   W   G   N   K   L   Q   P   K   G   Q   H   Y   A   N   259
CAT AAT AGA CTT TTC CCC TGG GGC AAC AAA CTG CAG CCC AAA GGC CAG CAT TAT GCC AAC   778

 I   W   Q   G   E   F   P   V   T   N   T   G   E   D   G   F   Q   G   T   A   279
ATT TGG CAG GGC GAG TTT CCG GTG ACC AAC ACT GGT GAG GAT GGC TTC CAA GGA ACT GCG   838

 P   V   D   A   F   P   P   N   G   Y   G   L   Y   N   I   V   G   N   A   W   299
CCT GTT GAT GCC TTC CCT CCC AAT GGT TAT GGC TTA TAC AAC ATA GTG GGG AAC GCA TGG   898

 E   W   T   S   D   N   W   T   V   H   H   S   V   E   E   T   L   N   P   K   319
GAA TGG ACT TCA GAC TGG TGG ACT GTT CAT CAT TCT GTT GAA GAA ACG CTT AAC CCA AAA   958

 G   P   P   S   G   K   D   R   V   K   K   G   G   S   Y   M   C   H   R   S   339
GGT CCC CCT TCT GGG AAA GAC CGA GTG AAG AAA GGT GGA TCC TAC ATG TGC CAT AGG TCT  1018
```

Figure 6A

```
 Y   C   Y   R   Y   R   C   A   A   R   S   Q   N   T   P   D   S   S   A   S    359
TAT TGT TAC AGG TAT CGC TGT GCT GCT CGG AGC CAG AAC ACA CCT GAT AGC TCT GCT TCG  1078

 N   L   G   F   R   C   A   A   D   R   L   P   T   M   D   *   375
AAT CTG GGA TTC CGC TGT GCA GCC GAC CGC CTG CCC ACT ATG GAC TGA 1126

CAACCAAGGAAAGTCTTCCCCAGTCCAAGGAGCAGTCGTGTCTGACCTACATTGGGCTTTTCTCAGAACTTTGAACGAT 1205

CCCATGCAAAGAATTCCCACCCTGAGGTGGGTTACATACCTGCCCAATGGCCAAAGGAACCGCCTTGTGAGACCAAATT 1284

GCTGACCTGGGTCAGTGCATGTGCTTTATGGTGTGGTGCATCTTTGGAGATCATCGCCATATTTTACTTTTGAGAGTCT 1363

TTAAAGAGGAAGGGGAGTGGAGGGAACCCTGAGCTAGGCTTCAGGAGGCCCGCGTCCTACGCAGGCTCTGCCACAGGGG 1442

TTAGACCCCAGGTCCGACGCTTGACCTTCCTGGGCCTCAAGTGCCCTCCCCTATCAAATGAAGGGATGGACAGCATGAC 1521

CTCTGGGTGTCTCTCCAACTCACCAGTTCTAAAAAGGGTATCAGATTCTATTGTGACTTCATAGTGAGAATTTATGATA 1600

GATTATTTTTTAGCTATTTTTTCCATGTGTGAACCTTGAGTGATACTAATCATGTAAAGTAAGAGTTCTCTTATGTATT 1679

ATTTTCGGAAGAGGGGTGTGGTGACTCCTTTATATTCGTACTGCACTTTGTTTTTCCAAGGAAATCAGTGTCTTTTACG 1758

TTGTTATGATGAATCCCACATGGGGCCGGTGATGGTATGCTGCAGTTCAGCCGTTGAACACATAGGAATGTCTGTGGGG 1837

TGACTCTACTGTGCTTTATCTTTTAACATTAAGTGCCTTTGGTTCAGAGGGGCAGTCATAAGCTCTGTTTCCCCCTCTC 1916

CCCAAAGCCTTCAGCGAACGTGAAATGTGCGCTAAACGGGGAAACCTGTTTAATTCTAGATATAGGGAAAAAGGAACGA 1995

GGACCTTGAATGAGCTATATTCAGGGTATCCGGTATTTTGTAATAGGGAATAGGAAACCTTGTTGGCTGTGGAATATCC 2074

GATGCTTTGAATCATGCACTGTGTTGAATAAACGTATCTGCTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 2153

AAAAAAAAAAAA 2165
```

Figure 6B



MAAPALGLVCGRCPELGLVLLLLLLSLLCGAAGSQEAGTGAGAGSLAGSC
GCGTPQRPGAHGNSAAAHRYSREANAPGPVPGERQLAHSKMVPIPVGVFT
MGTDDPQIKQDGEAPARRVTIDAFYMDAYEVSNTEFEKFVNSTGYLTEAE
KFGDSFVFEGMLSEQVKTNIQQAVAAAPWWLPVKGANWRHPEGPDSTIL
HRPDHPVLHVSWNDAVAYCTWAGKRLPTEAEWEYSCRGGLHNRLFPWG
NKLQPKGQHYANIWQGEFPVTNTGEDGFQGTAPVDAFPPNGYGLYNIVG
NAWEWTSDWWTVHHSVEETLNPKGPPSGKDRVKKGGSYMCHRSYCYRY
RCAARSQNTPDSSASNLGFRCAADRLPTMD

Figure 7

```
ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989)
> TB rv0712                                                299 aa vs.
> TANGO 240a.a.                                            374 aa
scoring matrix: pam120.mat, gap penalties: -12/-4
31.2% identity;         Global alignment score: 101


rv0712 ML-----------------------------------------------------------
       :
       MAAPALGLVCGRCPELGLVLLLLLLSLLCGAAGSQEAGTGAGAGSLAGSCGCGTPQRPGA
                10        20        30        40        50        60

                                          10          20          30
rv0712 ---------------------------------TELVDLPGGSFRMGST--RFYPE-EAPIHTVT
                                        . .: .: : : ::.   .. .. ::: . ::
       HGNSAAAHRYSREANAPGPVPGERQLAHSKMVPIPVGVFTMGTDDPQIKQDGEAPARRVT
                70        80        90        100       110       120

              40        50        60        70        80        90
rv0712 VRAFAVERHPVTNAQFAEFVSATGYVTVAEQPLDPGLYPGVDAADLCPGAMVFCPTAGPV
       . :: ..   :.:..:. ::..::::.: ::.  :. .. :. ....   ..    .:.:
       IDAFYMDAYEVSNTEFEKFVNSTGYLTEAEKFGDSFVFEGMLSEQV-KTNIQQAVAAAP-
              130       140       150       160       170

            100       110       120       130       140       150
rv0712 DLRDWRQWWDWVPGACWRHPFGRDSDIADRAGHPVVQVAYPDAVAYARWAGRRLPTEAEW
             ::  : :: :::: : :: : .:..:::..:.  :::::  :::.:::::::::
       -------WWLPVKGANWRHPEGPDSTILHRPDHPVLHVSWNDAVAYCTWAGKRLPTEAEW
              180       190       200       210       220       230

              160       170       180       190       200       210
rv0712 EYAARGGTTAT-YAWGDQEKPGGMLMANTWQGRFPYRNDGALGWVGTSPVGRFPANGFGL
       ::. :::  .  ..:::. .: :   ::.::: ::  : :. :  ::.::. ::.::.::
       EYSCRGGLHNRLFPWGNKLQPKGQHYANIWQGEFPVTNTGEDGFQGTAPVDAFPPNGYGL
              240       250       260       270       280       290

               220       230       240       250       260       270
rv0712 LDMIGNVWEWTTTEFYPHHRIDPPSTACCAPVKLATAADPTISQTLKGGSHLCAPEYCHR
        ...::.::::.     :: ..   ..          .. .  ... :::: .:   :: :
       YNIVGNAWEWTSDWWTVHHSVEETLNP--------KGPPSGKDRVKKGGSYMCHRSYCYR
              300       310                 320       330       340

               280       290
rv0712 YRPAARSPQSQDTATTHIGFRCVAD--PVSG
       :: ::::.....:.....::::.::  :. .
       YRCAARSQNTPDSSASNLGFRCAADRLPTMD
           350       360       370
```

Figure 8

Input file TANGO 243; Output File TANGO 243.pat2
Sequence length 2811

```
CTCGGCCCGCTCGGCGCGCCCCTTCCCAGCCGCCCTTCCGTACCGGCTCTCGGGCTCTTCCGGTCTCCGGCCGCCCCTT    79

ACCTGCAGGCTCTTCTCCCGCCGCGGCCCGGCGCTCTCCGAGTCGCCCCTGCGGACTGGTCTCGCACAGTGCCTGGGCA   158

                          M  T  S  G  A  T  R  Y  R  L  S  C  S                      13
CCGGGCGCCAGACAGACACTGGCC ATG ACG AGC GGC GCA ACC AGG TAC CGG CTG AGC TGC TCG         221

 L  R  G  H  E  L  D  V  R  G  L  V  C  C  A  Y  P  P  G  A                       33
CTC CGG GGC CAC GAG CTG GAC GTA CGG GGC CTG GTG TGC TGC GCC TAT CCG CCG GGA GCC    281

 F  V  S  V  S  R  D  R  T  T  R  L  W  A  P  D  S  P  N  R                       53
TTT GTG TCC GTG TCC CGA GAC CGC ACC ACC CGC CTC TGG GCC CCA GAC AGT CCA AAC AGG    341

 S  F  T  E  M  H  C  M  S  G  H  S  N  F  V  S  C  V  C  I                       73
AGC TTT ACA GAA ATG CAC TGT ATG AGT GGC CAT TCC AAT TTT GTA TCT TGT GTA TGC ATC    401

 I  P  S  S  D  I  Y  P  H  G  L  I  A  T  G  G  N  D  H  N                       93
ATA CCC TCA AGT GAC ATC TAC CCT CAT GGC CTA ATT GCC ACC GGT GGA AAT GAC CAC AAT    461

 I  C  I  F  S  L  D  S  P  M  P  L  Y  I  L  K  G  H  K  N                      113
ATA TGC ATT TTC TCA CTG GAC AGT CCA ATG CCA CTT TAT ATT CTA AAA GGC CAC AAA AAT    521

 T  V  C  S  L  S  S  G  K  F  G  T  L  L  S  G  S  W  D  T                      133
ACT GTT TGT AGT CTA TCA TCT GGA AAA TTT GGG ACA TTA CTT AGT GGT TCA TGG GAC ACC    581

 T  A  K  V  W  L  N  D  K  C  M  M  T  L  Q  G  H  T  A  A                      153
ACT GCT AAA GTC TGG CTG AAT GAC AAG TGC ATG ATG ACC TTG CAG GGT CAT ACA GCT GCA    641

 V  W  A  V  K  I  L  P  E  Q  G  L  M  L  T  G  S  A  D  K                      173
GTG TGG GCG GTA AAG ATC TTA CCT GAA CAG GGC TTA ATG TTG ACT GGA TCA GCA GAC AAG    701

 T  V  K  L  W  K  A  G  R  C  E  R  T  F  S  G  H  E  D  C                      193
ACT GTT AAA CTG TGG AAG GCT GGA AGA TGT GAG AGG ACT TTT TCA GGG CAT GAA GAC TGT    761

 V  R  G  L  A  I  L  S  E  T  E  F  L  S  C  A  N  D  A  S                      213
GTA AGA GGT TTG GCA ATT TTG AGT GAA ACA GAA TTT CTT TCC TGT GCA AAT GAT GCT AGT    821

 I  R  R  W  Q  I  T  G  E  C  L  E  V  Y  Y  G  H  T  N  Y                      233
ATT AGA AGG TGG CAA ATC ACT GGC GAG TGT CTT GAA GTA TAT TAT GGA CAT ACA AAT TAT    881

 I  Y  S  I  S  V  F  P  N  C  R  D  F  V  T  T  A  E  D  R                      253
ATT TAT AGC ATA TCC GTT TTT CCA AAT TGT AGA GAC TTT GTG ACA ACA GCA GAG GAC AGA    941

 S  L  R  I  W  K  H  G  E  C  A  Q  T  I  R  L  P  A  Q  S                      273
TCT CTG AGA ATC TGG AAA CAT GGG GAA TGT GCT CAA ACT ATC CGA CTT CCA GCT CAG TCT   1001

 I  W  C  C  C  V  L  D  N  G  D  I  V  V  G  A  S  D  G  I                      293
ATA TGG TGC TGC TGT GTG CTC GAC AAT GGT GAC ATT GTG GTT GGT GCG AGT GAT GGC ATT   1061

 I  R  V  F  T  E  S  E  D  R  T  A  S  A  E  E  I  K  A  F                      313
ATT AGA GTG TTT ACA GAA TCA GAA GAT CGA ACA GCA AGT GCT GAA GAA ATC AAG GCT TTT   1121

 E  K  E  L  S  H  A  T  I  D  S  K  T  G  D  L  G  D  I  N                      333
GAA AAA GAA CTG TCT CAC GCA ACC ATT GAT TCT AAA ACT GGC GAT TTA GGG GAC ATC AAT   1181
```

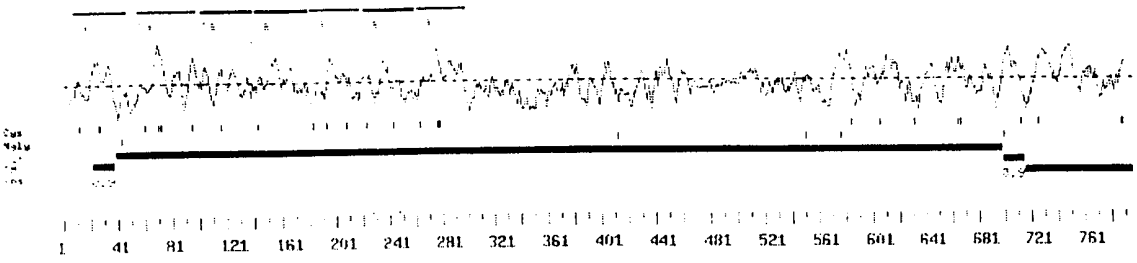
Figure 9A

```
A  E  Q  L  P  G  R  E  H  L  N  E  P  G  T  R  E  G  Q  T   353
GCT GAG CAG CTT CCT GGG AGG GAA CAT CTT AAT GAA CCT GGT ACT AGA GAA GGA CAG ACT  1241

R  L  I  R  D  G  E  K  V  E  A  Y  Q  W  S  V  S  E  G  R   373
CGT CTA ATC AGA GAT GGG GAG AAA GTC GAA GCC TAT CAG TGG AGT GTT AGT GAA GGG AGG  1301

W  I  K  I  G  D  V  V  G  S  S  G  A  N  Q  Q  T  S  G  K   393
TGG ATA AAA ATT GGT GAT GTT GTT GGC TCA TCT GGT GCT AAT CAG CAA ACA TCT GGA AAA  1361

V  L  Y  E  G  K  E  F  D  Y  V  F  S  I  D  V  N  E  G  G   413
GTT TTA TAT GAA GGG AAA GAA TTT GAT TAT GTT TTC TCA ATT GAT GTC AAT GAA GGT GGA  1421

P  S  Y  K  L  P  Y  N  T  S  D  D  P  W  L  T  A  Y  N  F   433
CCA TCA TAT AAA TTG CCA TAT AAT ACC AGT GAT GAC CCT TGG TTA ACT GCA TAC AAC TTC  1481

L  Q  K  N  D  L  N  P  M  F  L  D  Q  V  A  K  F  I  I  D   453
TTA CAG AAG AAT GAT TTG AAT CCT ATG TTT CTG GAT CAA GTA GCT AAA TTT ATT ATT GAT  1541

N  T  K  G  Q  M  L  G  L  G  N  P  S  F  S  D  P  F  T  G   473
AAC ACA AAA GGT CAA ATG TTG GGA CTT GGG AAT CCC AGC TTT TCA GAT CCA TTT ACA GGT  1601

G  G  R  Y  V  P  G  S  S  G  S  S  N  T  L  P  T  A  D  P   493
GGT GGT CGG TAT GTT CCG GGC TCT TCG GGA TCT TCT AAC ACA CTA CCC ACA GCA GAT CCT  1661

F  T  G  A  G  R  Y  V  P  G  S  A  S  M  G  T  T  M  A  G   513
TTT ACA GGT GCT GGT CGT TAT GTA CCA GGT TCT GCA AGT ATG GGA ACT ACC ATG GCC GGA  1721

V  D  P  F  T  G  N  S  A  Y  R  S  A  A  S  K  T  M  N  I   533
GTT GAT CCA TTT ACA GGG AAT AGT GCC TAC CGA TCA GCT GCA TCT AAA ACA ATG AAT ATT  1781

Y  F  P  K  K  E  A  V  T  F  D  Q  A  N  P  T  Q  I  L  G   553
TAT TTC CCT AAA AAA GAG GCT GTC ACA TTT GAC CAA GCA AAC CCT ACA CAA ATA TTA GGT  1841

K  L  K  E  L  N  G  T  A  P  E  E  K  K  L  T  E  D  D  L   573
AAA CTG AAG GAA CTT AAT GGA ACT GCA CCT GAA GAG AAG AAG TTA ACT GAG GAT GAC TTG  1901

I  L  L  E  K  I  L  S  L  I  C  N  S  S  S  E  K  P  T  V   593
ATA CTT CTT GAG AAG ATA CTG TCT CTA ATA TGT AAT AGT TCT TCA GAA AAA CCC ACA GTC  1961

Q  Q  L  Q  I  L  W  K  A  I  N  C  P  E  D  I  V  F  P  A   613
CAG CAA CTT CAG ATT TTG TGG AAA GCT ATT AAC TGT CCT GAA GAT ATT GTC TTT CCT GCA  2021

L  D  I  L  R  L  S  I  K  H  P  S  V  N  E  N  F  C  N  E   633
CTT GAC ATT CTT CGG TTG TCA ATT AAA CAC CCC AGT GTG AAT GAG AAC TTC TGC AAT GAA  2081

K  E  G  A  Q  F  S  S  H  L  I  N  L  L  N  P  K  G  K  P   653
AAG GAA GGG GCT CAG TTC AGC AGT CAT CTT ATC AAT CTT CTG AAC CCT AAA GGA AAG CCA  2141

A  N  Q  L  L  A  L  R  T  F  C  N  C  F  V  G  Q  A  G  Q   673
GCA AAC CAG CTG CTT GCT CTC AGG ACT TTT TGC AAT TGT TTT GTT GGC CAG GCA GGA CAA  2201

K  L  M  M  S  Q  R  E  S  L  M  S  H  A  I  E  L  K  S  G   693
AAA CTC ATG ATG TCC CAG AGG GAA TCA CTG ATG TCC CAT GCA ATA GAA CTG AAA TCA GGG  2261

S  N  K  N  I  H  I  A  L  A  T  L  A  L  N  Y  S  V  C  F   713
AGC AAT AAG AAC ATT CAC ATT GCT CTG GCT ACA TTG GCC CTG AAC TAT TCT GTT TGT TTT  2321
```

Figure 9B

```
 H   K   D   H   N   I   E   G   K   A   Q   C   L   S   L   I   S   T   I   L    733
CAT AAA GAC CAT AAC ATT GAA GGG AAA GCC CAA TGT TTG TCA CTA ATT AGC ACA ATC TTG  2381

 E   V   V   Q   D   L   E   A   T   F   R   L   L   V   A   L   G   T   L   I    753
GAA GTA GTA CAA GAC CTA GAA GCC ACT TTT AGA CTT CTT GTG GCT CTT GGA ACA CTT ATC  2441

 S   D   D   S   N   A   V   Q   L   A   K   S   L   G   V   D   S   Q   I   K    773
AGT GAT GAT TCA AAT GCT GTA CAA TTA GCC AAG TCT TTA GGT GTT GAT TCT CAA ATA AAA  2501

 K   Y   S   S   V   S   E   P   A   K   V   S   E   C   C   R   F   I   L   N    793
AAG TAT TCC TCA GTA TCA GAA CCA GCT AAA GTA AGT GAA TGC TGT AGA TTT ATC CTA AAT  2561

 L   L   *   796
TTG CTG TAG 2570

CAGTGGGGAAGAGGGACGGATATTTTTAATTGATTAGTGTTTTTTTCCTCACATTTGACATGACTGATAACAGATAATT 2649

AAAAAAAGAGAATACGGTGGATTAAGTAAAATTTTACATCTTGTAAAGTGGTGGGGAGGGGAAACAGAAATAAAATTTT 2728

TGCACTGCTGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 2807

AAAA 2811
```

Figure 9C



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PNRSFTEMHCMSGHSNFVSCVCIIPSSDIYPHGLIATGGNDHNICIFSLDSPM
PLYILKGHKNTVCSLSSGKFGTLLSGSWDTTAKVWLNDKCMMTLQGHTA
AVWAVKILPEQGLMLTGSADKTVKLWKAGRCERTFSGHEDCVRGLAILSE
TEFLSCANDASIRRWQITGECLEVYYGHTNYIYSISVFPNCRDFVTTAEDRS
LRIWKHGECAQTIRLPAQSIWCCCVLDNGDIVVGASDGIIRVFTESEDRTAS
AEEIKAFEKELSHATIDSKTGDLGDINAEQLPGREHLNEPGTREGQTRLIRD
GEKVEAYQWSVSEGRWIKIGDVVGSSGANQQTSGKVLYEGKEFDYVFSID
VNEGGPSYKLPYNTSDDPWLTAYNFLQKNDLNPMFLDQVAKFIIDNTKGQ
MLGLGNPSFSDPFTGGGRYVPGSSGSSNTLPTADPFTGAGRYVPGSASMGT
TMAGVDPFTGNSAYRSAASKTMNIYFPKKEAVTFDQANPTQILGKLKELN
GTAPEEKKLTEDDLILLEKILSLICNSSSEKPTVQQLQILWKAINCPEDIVFPA
LDILRLSIKHPSVNENFCNEKEGAQFSSHLINLLNPKGKPANQLLALRTFCN
CFVGQAGQKLMMSQRESLMSHAIELKSGSNKNIHIALATLALNYSVCFHK
DHNIEGKAQCLSLISTILEVVQDLEATFRLLVALGTLISDDSNAVQLAKSLG
VDSQIKKYSSVSEPAKVSECCRFILNLL

Figure 10

```
ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989)
> TANGO 243 n.a.                          2811 aa vs.
> PLAP n.a.                               2217 aa
scoring matrix: pami20.mat, gap penalties: -12/-4
78.8% identity;         Global alignment score: 8334

              10        20        30        40        50        60
T243    CTCGGCCCGCTCGGCGCGCCCCTTCCCAGCCGCCCTTCCGTACCGGCTCTCGGGCTCTTC

        ------------------------------------------------------------


              70        80        90       100       110       120
T243    CGGTCTCCGGCCGCCCCTTACCTGCAGGCTCTTCTCCCGCCGCGGCCCGGCGCTCTCCGA

        ------------------------------------------------------------


             130       140       150       160       170       180
T243    GTCGCCCCTGCGGACTGGTCTCGCACAGTGCCTGGGCACCGGGCGCCAGACAGACACTGG

        ------------------------------------------------------------


             190       200       210       220       230       240
T243    CCATGACGAGCGGCGCAACCAGGTACCGGCTGAGCTGCTCGCTCCGGGGCCACGAGCTGG

        ------------------------------------------------------------


             250       260       270       280       290       300
T243    ACGTACGGGGCCTGGTGTGCTGCGCCTATCCGCCGGGAGCCTTTGTGTCCGTGTCCCGAG

        ------------------------------------------------------------


             310       320       330       340       350       360
T243    ACCGCACCACCCGCCTCTGGGCCCCAGACAGTCCAAACAGGAGCTTTACAGAAATGCACT
                                                           :::::::
        -----------------------------------------------------ATGCACT


             370       380       390       400       410       420
T243    GTATGAGTGGCCATTCCAATTTTGTATCTTGTGTATGCATCATACCCTCAAGTGACATCT
        ::::::: ::::: ::::::::::::::::::::::::::::::::::::::::::::
        GTATGAGCGGCCACTCCAATTTTGTATCTTGTGTATGCATCATACCCTCAAGTGACATCT
         10        20        30        40        50        60

             430       440       450       460       470       480
T243    ACCCTCATGGCCTAATTGCCACCGGTGGAAATGACCACAATATATGCATTTTCTCACTGG
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        ACCCTCATGGCCTAATTGCCACCGGTGGAAATGACCACAATATATGCATTTTCTCACTGG
         70        80        90       100       110       120
```

Figure 11A

```
          490        500        510        520        530        540
T243      ACAGTCCAATGCCACTTTATATTCTAAAAGGCCACAAAAATACTGTTTGTAGTCTATCAT
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          ACAGTCCAATGCCACTTTATATTCTAAAAGGCCACAAAAATACTGTTTGTAGTCTATCAT
          130        140        150        160        170        180

          550        560        570        580        590        600
T243      CTGGAAAATTTGGGACATTACTTAGTGGTTCATGGGACACCACTGCTAAAGTCTGGCTGA
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          CTGGAAAATTTGGGACATTACTTAGTGGTTCATGGGACACCACTGCTAAAGTCTGGCTGA
          190        200        210        220        230        240

          610        620        630        640        650        660
T243      ATGACAAGTGCATGATGACCTTGCAGGGTCATACAGCTGCAGTGTGGGCGGTAAAGATCT
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          ATGACAAGTGCATGATGACCTTGCAGGGTCATACAGCTGCAGTGTGGGCGGTAAAGATCT
          250        260        270        280        290        300

          670        680        690        700        710        720
T243      TACCTGAACAGGGCTTAATGTTGACTGGATCAGCAGACAAGACTGTTAAACTGTGGAAGG
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          TACCTGAACAGGGCTTAATGTTGACTGGATCAGCAGACAAGACTGTTAAACTGTGGAAGG
          310        320        330        340        350        360

          730        740        750        760        770        780
T243      CTGGAAGATGTGAGAGGACTTTTTCAGGGCATGAAGACTGTGTAAGAGGTTTGGCAATTT
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          CTGGAAGATGTGAGAGGACTTTTTCAGGGCATGAAGACTGTGTAAGAGGTTTGGCAATTT
          370        380        390        400        410        420

          790        800        810        820        830        840
T243      TGAGTGAAACAGAATTTCTTTCCTGTGCAAATGATGCTAGTATTAGAAGGTGGCAAATCA
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          TGAGTGAAACAGAATTTCTTTCCTGTGCAAATGATGCTAGTATTAGAAGGTGGCAAATCA
          430        440        450        460        470        480

          850        860        870        880        890        900
T243      CTGGCGAGTGTCTTGAAGTATATTATGGACATACAAATTATATTTATAGCATATCCGTTT
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          CTGGCGAGTGTCTTGAAGTATATTATGGACATACAAATTATATTTATAGCATATCCGTTT
          490        500        510        520        530        540

          910        920        930        940        950        960
T243      TTCCAAATTGTAGAGACTTTGTGACAACAGCAGAGGACAGATCTCTGAGAATCTGGAAAC
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          TTCCAAATTGTAGAGACTTTGTGACAACAGCAGAGGACAGATCTCTGAGAATCTGGAAAC
          550        560        570        580        590        600

          970        980        990       1000       1010       1020
T243      ATGGGGAATGTGCTCAAACTATCCGACTTCCAGCTCAGTCTATATGGTGCTGCTGTGTGC
          ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
          ATGGGGAATGTGCTCAAACTATCCGACTTCCAGCTCAGTCTATATGGTGCTGCTGTGTGC
          610        620        630        640        650        660
```

Figure 11B

```
          1030       1040       1050       1060       1070       1080
T243  TCGACAATGGTGACATTGTGGTTGGTGCGAGTGATGGCATTATTAGAGTGTTTACAGAAT
      :::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      TCGACAATGGTGACATTGTGGTTGGTGCGAGTGATGGCATTATTAGAGTGTTTACAGAGT
      670        680        690        700        710        720

          1090       1100       1110       1120       1130       1140
T243  CAGAAGATCGAACAGCAAGTGCTGAAGAAATCAAGGCTTTTGAAAAAGAACTGTCTCACG
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      CAGAAGATCGAACAGCAAGTGCTGAAGAAATCAAGGCTTTTGAAAAAGAACTGTCTCACG
      730        740        750        760        770        780

          1150       1160       1170       1180       1190       1200
T243  CAACCATTGATTCTAAAACTGGCGATTTAGGGGACATCAATGCTGAGCAGCTTCCTGGGA
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      CAACCATTGATTCTAAAACTGGCGATTTAGGGGACATCAATGCTGAGCAGCTTCCTGGGA
      790        800        810        820        830        840

          1210       1220       1230       1240       1250       1260
T243  GGGAACATCTTAATGAACCTGGTACTAGAGAAGGACAGACTCGTCTAATCAGAGATGGGG
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      GGGAACATCTTAATGAACCTGGTACTAGAGAAGGACAGACTCGTCTAATCAGAGATGGGG
      850        860        870        880        890        900

          1270       1280       1290       1300       1310       1320
T243  AGAAAGTCGAAGCCTATCAGTGGAGTGTTAGTGAAGGGAGGTGGATAAAAATTGGTGATG
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      AGAAAGTCGAAGCCTATCAGTGGAGTGTTAGTGAAGGGAGGTGGATAAAAATTGGTGATG
      910        920        930        940        950        960

          1330       1340       1350       1360       1370       1380
T243  TTGTTGGCTCATCTGGTGCTAATCAGCAAACATCTGGAAAAGTTTTATATGAAGGGAAAG
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      TTGTTGGCTCATCTGGTGCTAATCAGCAAACATCTGGAAAAGTTTTATATGAAGGGAAAG
      970        980        990       1000       1010       1020

          1390       1400       1410       1420       1430       1440
T243  AATTTGATTATGTTTTCTCAATTGATGTCAATGAAGGTGGACCATCATATAAATTGCCAT
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      AATTTGATTATGTTTTCTCAATTGATGTCAATGAAGGTGGACCATCATATAAATTGCCAT
     1030       1040       1050       1060       1070       1080

          1450       1460       1470       1480       1490       1500
T243  ATAATACCAGTGATGACCCTTGGTTAACTGCATACAACTTCTTACAGAAGAATGATTTGA
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      ATAATACCAGTGATGACCCTTGGTTAACTGCATACAACTTCTTACAGAAGAATGATTTGA
     1090       1100       1110       1120       1130       1140

          1510       1520       1530       1540       1550       1560
T243  ATCCTATGTTTCTGGATCAAGTAGCTAAATTTATTATTGATAACACAAAAGGTCAAATGT
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      ATCCTATGTTTCTGGATCAAGTAGCTAAATTTATTATTGATAACACAAAAGGTCAAATGT
     1150       1160       1170       1180       1190       1200
```

Figure 11C

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          1570       1580       1590       1600       1610       1620
T243   TGGGACTTGGGAATCCCAGCTTTTCAGATCCATTTACAGGTGGTGGTCGGTATGTTCCGG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TGGGACTTGGGAATCCCAGCTTTTCAGATCCATTTACAGGTGGTGGTCGGTATGTTCCGG
      1210       1220       1230       1240       1250       1260

          1630       1640       1650       1660       1670       1680
T243   GCTCTTCGGGATCTTCTAACACACTACCCACAGCAGATCCTTTTACAGGTGCTGGTCGTT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GCTCTTCGGGATCTTCTAACACACTACCCACAGCAGATCCTTTTACAGGTGCTGGTCGTT
      1270       1280       1290       1300       1310       1320

          1690       1700       1710       1720       1730       1740
T243   ATGTACCAGGTTCTGCAAGTATGGGAACTACCATGGCCGGAGTTGATCCATTTACAGGGA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       ATGTACCAGGTTCTGCAAGTATGGGAACTACCATGGCCGGAGTTGATCCATTTACAGGGA
      1330       1340       1350       1360       1370       1380

          1750       1760       1770       1780       1790       1800
T243   ATAGTGCCTACCGATCAGCTGCATCTAAAACAATGAATATTTATTTCCCTAAAAAAGAGG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       ATAGTGCCTACCGATCAGCTGCATCTAAAACAATGAATATTTATTTCCCTAAAAAAGAGG
      1390       1400       1410       1420       1430       1440

          1810       1820       1830       1840       1850       1860
T243   CTGTCACATTTGACCAAGCAAACCCTACACAAATATTAGGTAAACTGAAGGAACTTAATG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       CTGTCACATTTGACCAAGCAAACCCTACACAAATATTAGGTAAACTGAAGGAACTTAATG
      1450       1460       1470       1480       1490       1500

          1870       1880       1890       1900       1910       1920
T243   GAACTGCACCTGAAGAGAAGAAGTTAACTGAGGATGACTTGATACTTCTTGAGAAGATAC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GAACTGCACCTGAAGAGAAGAAGTTAACTGAGGATGACTTGATACTTCTTGAGAAGATAC
      1510       1520       1530       1540       1550       1560

          1930       1940       1950       1960       1970       1980
T243   TGTCTCTAATATGTAATAGTTCTTCAGAAAAACCCACAGTCCAGCAACTTCAGATTTTGT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TGTCTCTAATATGTAATAGTTCTTCAGAAAAACCCACAGTCCAGCAACTTCAGATTTTGT
      1570       1580       1590       1600       1610       1620

          1990       2000       2010       2020       2030       2040
T243   GGAAAGCTATTAACTGTCCTGAAGATATTGTCTTTCCTGCACTTGACATTCTTCGGTTGT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GGAAAGCTATTAACTGTCCTGAAGATATTGTCTTTCCTGCACTTGACATTCTTCGGTTGT
      1630       1640       1650       1660       1670       1680

          2050       2060       2070       2080       2090       2100
T243   CAATTAAACACCCCAGTGTGAATGAGAACTTCTGCAATGAAAAGGAAGGGGCTCAGTTCA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       CAATTAAACACCCCAGTGTGAATGAGAACTTCTGCAATGAAAAGGAAGGGGCTCAGTTCA
      1690       1700       1710       1720       1730       1740
```

Figure 11D

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          2110       2120       2130       2140       2150       2160
T243   GCAGTCATCTTATCAATCTTCTGAACCCTAAAGGAAAGCCAGCAAACCAGCTGCTTGCTC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GCAGTCATCTTATCAATCTTCTGAACCCTAAAGGAAAGCCAGCAAACCAGCTGCTTGCTC
      1750       1760       1770       1780       1790       1800

          2170       2180       2190       2200       2210       2220
T243   TCAGGACTTTTTGCAATTGTTTTGTTGGCCAGGCAGGACAAAAACTCATGATGTCCCAGA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TCAGGACTTTTTGCAATTGTTTTGTTGGCCAGGCAGGACAAAAACTCATGATGTCCCAGA
      1810       1820       1830       1840       1850       1860

          2230       2240       2250       2260       2270       2280
T243   GGGAATCACTGATGTCCCATGCAATAGAACTGAAATCAGGGAGCAATAAGAACATTCACA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GGGAATCACTGATGTCCCATGCAATAGAACTGAAATCAGGGAGCAATAAGAACATTCACA
      1870       1880       1890       1900       1910       1920

          2290       2300       2310       2320       2330       2340
T243   TTGCTCTGGCTACATTGGCCCTGAACTATTCTGTTTGTTTTCATAAAGACCATAACATTG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TTGCTCTGGCTACATTGGCCCTGAACTATTCTGTTTGTTTTCATAAAGACCATAACATTG
      1930       1940       1950       1960       1970       1980

          2350       2360       2370       2380       2390       2400
T243   AAGGGAAAGCCCAATGTTTGTCACTAATTAGCACAATCTTGGAAGTAGTACAAGACCTAG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       AAGGGAAAGCCCAATGTTTGTCACTAATTAGCACAATCTTGGAAGTAGTACAAGACCTAG
      1990       2000       2010       2020       2030       2040

          2410       2420       2430       2440       2450       2460
T243   AAGCCACTTTTAGACTTCTTGTGGCTCTTGGAACACTTATCAGTGATGATTCAAATGCTG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       AAGCCACTTTTAGACTTCTTGTGGCTCTTGGAACACTTATCAGTGATGATTCAAATGCTG
      2050       2060       2070       2080       2090       2100

          2470       2480       2490       2500       2510       2520
T243   TACAATTAGCCAAGTCTTTAGGTGTTGATTCTCAAATAAAAAAAGTATTCCTCAGTATCAG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       TACAATTAGCCAAGTCTTTAGGTGTTGATTCTCAAATAAAAAAAGTATTCCTCAGTATCAG
      2110       2120       2130       2140       2150       2160

          2530       2540       2550       2560       2570       2580
T243   AACCAGCTAAAGTAAGTGAATGCTGTAGATTTATCCTAAATTTGCTGTAGCAGTGGGGAA
       ::::::::::::::::::::::::::::::::::::::::::::::::
       AACCAGCTAAAGTAAGTGAATGCTGTAGATTTATCCTAAATTTGCTGTAG----------
      2170       2180       2190       2200       2210

          2590       2600       2610       2620       2630       2640
T243   GAGGGACGGATATTTTTAATTGATTAGTGTTTTTTTCCTCACATTTGACATGACTGATAA

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```

Figure 11E

```
          2650       2660       2670       2680       2690       2700
T243  CAGATAATTAAAAAAAGAGAATACGGTGGATTAAGTAAAATTTTACATCTTGTAAAGTGG

      ------------------------------------------------------------

          2710       2720       2730       2740       2750       2760
T243  TGGGGAGGGGAAACAGAAATAAAATTTTTGCACTGCTGAAAAAAAAAAAAAAAAAAAAAA

      ------------------------------------------------------------

          2770       2780       2790       2800       2810
T243  AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

      ----------------------------------------------------
```

Figure 11F

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ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989)
> PLAP n.a.                                  2217 aa vs.
> TANGO 243 ORF                              2388 aa
scoring matrix: pam120.mat, gap penalties: -12/-4
92.7% identity;          Global alignment score: 10034


PLAP    ATG-------------------------------------------------------
        :::
        ATGACGAGCGGCGCAACCAGGTACCGGCTGAGCTGCTCGCTCCGGGGCCACGAGCTGGAC
                10        20        30        40        50        60


PLAP    ------------------------------------------------------------

        GTACGGGGCCTGGTGTGCTGCGCCTATCCGCCGGGAGCCTTTGTGTCCGTGTCCCGAGAC
                70        80        90       100       110       120


PLAP    -----------------------------------------------------CACTGT
                                                              ::::::
        CGCACCACCCGCCTCTGGGCCCCAGACAGTCCAAACAGGAGCTTTACAGAAATGCACTGT
               130       140       150       160       170       180

      10        20        30        40        50        60
PLAP  ATGAGCGGCCACTCCAATTTTGTATCTTGTGTATGCATCATACCCTCAAGTGACATCTAC
      ::::: ::::: ::::::::::::::::::::::::::::::::::::::::::::::::
      ATGAGTGGCCATTCCAATTTTGTATCTTGTGTATGCATCATACCCTCAAGTGACATCTAC
               190       200       210       220       230       240

      70        80        90       100       110       120
PLAP  CCTCATGGCCTAATTGCCACCGGTGGAAATGACCACAATATATGCATTTTCTCACTGGAC
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      CCTCATGGCCTAATTGCCACCGGTGGAAATGACCACAATATATGCATTTTCTCACTGGAC
               250       260       270       280       290       300

     130       140       150       160       170       180
PLAP  AGTCCAATGCCACTTTATATTCTAAAAGGCCACAAAAATACTGTTTGTAGTCTATCATCT
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      AGTCCAATGCCACTTTATATTCTAAAAGGCCACAAAAATACTGTTTGTAGTCTATCATCT
               310       320       330       340       350       360

     190       200       210       220       230       240
PLAP  GGAAAATTTGGGACATTACTTAGTGGTTCATGGGACACCACTGCTAAAGTCTGGCTGAAT
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      GGAAAATTTGGGACATTACTTAGTGGTTCATGGGACACCACTGCTAAAGTCTGGCTGAAT
               370       380       390       400       410       420

     250       260       270       280       290       300
PLAP  GACAAGTGCATGATGACCTTGCAGGGTCATACAGCTGCAGTGTGGGCGGTAAAGATCTTA
      ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
      GACAAGTGCATGATGACCTTGCAGGGTCATACAGCTGCAGTGTGGGCGGTAAAGATCTTA
               430       440       450       460       470       480
```

Figure 12A

```
     310        320        330        340        350        360
PLAP   CCTGAACAGGGCTTAATGTTGACTGGATCAGCAGACAAGACTGTTAAACTGTGGAAGGCT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       CCTGAACAGGGCTTAATGTTGACTGGATCAGCAGACAAGACTGTTAAACTGTGGAAGGCT
              490        500        510        520        530        540

     370        380        390        400        410        420
PLAP   GGAAGATGTGAGAGGACTTTTTCAGGGCATGAAGACTGTGTAAGAGGTTTGGCAATTTTG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GGAAGATGTGAGAGGACTTTTTCAGGGCATGAAGACTGTGTAAGAGGTTTGGCAATTTTG
              550        560        570        580        590        600

     430        440        450        460        470        480
PLAP   AGTGAAACAGAATTTCTTTCCTGTGCAAATGATGCTAGTATTAGAAGGTGGCAAATCACT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       AGTGAAACAGAATTTCTTTCCTGTGCAAATGATGCTAGTATTAGAAGGTGGCAAATCACT
              610        620        630        640        650        660

     490        500        510        520        530        540
PLAP   GGCGAGTGTCTTGAAGTATATTATGGACATACAAATTATATTTATAGCATATCCGTTTTT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GGCGAGTGTCTTGAAGTATATTATGGACATACAAATTATATTTATAGCATATCCGTTTTT
              670        680        690        700        710        720

     550        560        570        580        590        600
PLAP   CCAAATTGTAGAGACTTTGTGACAACAGCAGAGGACAGATCTCTGAGAATCTGGAAACAT
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       CCAAATTGTAGAGACTTTGTGACAACAGCAGAGGACAGATCTCTGAGAATCTGGAAACAT
              730        740        750        760        770        780

     610        620        630        640        650        660
PLAP   GGGGAATGTGCTCAAACTATCCGACTTCCAGCTCAGTCTATATGGTGCTGCTGTGTGCTC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GGGGAATGTGCTCAAACTATCCGACTTCCAGCTCAGTCTATATGGTGCTGCTGTGTGCTC
              790        800        810        820        830        840

     670        680        690        700        710        720
PLAP   GACAATGGTGACATTGTGGTTGGTGCGAGTGATGGCATTATTAGAGTGTTTACAGAGTCA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::.:::
       GACAATGGTGACATTGTGGTTGGTGCGAGTGATGGCATTATTAGAGTGTTTACAGAATCA
              850        860        870        880        890        900

     730        740        750        760        770        780
PLAP   GAAGATCGAACAGCAAGTGCTGAAGAAATCAAGGCTTTTGAAAAAGAACTGTCTCACGCA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GAAGATCGAACAGCAAGTGCTGAAGAAATCAAGGCTTTTGAAAAAGAACTGTCTCACGCA
              910        920        930        940        950        960

     790        800        810        820        830        840
PLAP   ACCATTGATTCTAAAACTGGCGATTTAGGGGACATCAATGCTGAGCAGCTTCCTGGGAGG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       ACCATTGATTCTAAAACTGGCGATTTAGGGGACATCAATGCTGAGCAGCTTCCTGGGAGG
              970        980        990       1000       1010       1020
```

Figure 12B

```
     850        860        870        880        890        900
PLAP GAACATCTTAATGAACCTGGTACTAGAGAAGGACAGACTCGTCTAATCAGAGATGGGGAG
     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
     GAACATCTTAATGAACCTGGTACTAGAGAAGGACAGACTCGTCTAATCAGAGATGGGGAG
           1030       1040       1050       1060       1070       1080

     910        920        930        940        950        960
PLAP AAAGTCGAAGCCTATCAGTGGAGTGTTAGTGAAGGGAGGTGGATAAAAATTGGTGATGTT
     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
     AAAGTCGAAGCCTATCAGTGGAGTGTTAGTGAAGGGAGGTGGATAAAAATTGGTGATGTT
           1090       1100       1110       1120       1130       1140

     970        980        990       1000       1010       1020
PLAP GTTGGCTCATCTGGTGCTAATCAGCAAACATCTGGAAAAGTTTTATATGAAGGGAAAGAA
     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
     GTTGGCTCATCTGGTGCTAATCAGCAAACATCTGGAAAAGTTTTATATGAAGGGAAAGAA
           1150       1160       1170       1180       1190       1200

    1030       1040       1050       1060       1070       1080
PLAP TTTGATTATGTTTTCTCAATTGATGTCAATGAAGGTGGACCATCATATAAATTGCCATAT
     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
     TTTGATTATGTTTTCTCAATTGATGTCAATGAAGGTGGACCATCATATAAATTGCCATAT
           1210       1220       1230       1240       1250       1260

    1090       1100       1110       1120       1130       1140
PLAP AATACCAGTGATGACCCTTGGTTAACTGCATACAACTTCTTACAGAAGAATGATTTGAAT
     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
     AATACCAGTGATGACCCTTGGTTAACTGCATACAACTTCTTACAGAAGAATGATTTGAAT
           1270       1280       1290       1300       1310       1320

    1150       1160       1170       1180       1190       1200
PLAP CCTATGTTTCTGGATCAAGTAGCTAAATTTATTATTGATAACACAAAAGGTCAAATGTTG
     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
     CCTATGTTTCTGGATCAAGTAGCTAAATTTATTATTGATAACACAAAAGGTCAAATGTTG
           1330       1340       1350       1360       1370       1380

    1210       1220       1230       1240       1250       1260
PLAP GGACTTGGGAATCCCAGCTTTTCAGATCCATTTACAGGTGGTGGTCGGTATGTTCCGGGC
     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
     GGACTTGGGAATCCCAGCTTTTCAGATCCATTTACAGGTGGTGGTCGGTATGTTCCGGGC
           1390       1400       1410       1420       1430       1440

    1270       1280       1290       1300       1310       1320
PLAP TCTTCGGGATCTTCTAACACACTACCCACAGCAGATCCTTTTACAGGTGCTGGTCGTTAT
     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
     TCTTCGGGATCTTCTAACACACTACCCACAGCAGATCCTTTTACAGGTGCTGGTCGTTAT
           1450       1460       1470       1480       1490       1500

    1330       1340       1350       1360       1370       1380
PLAP GTACCAGGTTCTGCAAGTATGGGAACTACCATGGCCGGAGTTGATCCATTTACAGGGAAT
     ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
     GTACCAGGTTCTGCAAGTATGGGAACTACCATGGCCGGAGTTGATCCATTTACAGGGAAT
           1510       1520       1530       1540       1550       1560
```

Figure 12C

```
    1390       1400       1410       1420       1430       1440
PLAP    AGTGCCTACCGATCAGCTGCATCTAAAACAATGAATATTTATTTCCCTAAAAAAGAGGCT
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        AGTGCCTACCGATCAGCTGCATCTAAAACAATGAATATTTATTTCCCTAAAAAAGAGGCT
              1570       1580       1590       1600       1610       1620

    1450       1460       1470       1480       1490       1500
PLAP    GTCACATTTGACCAAGCAAACCCTACACAAATATTAGGTAAACTGAAGGAACTTAATGGA
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        GTCACATTTGACCAAGCAAACCCTACACAAATATTAGGTAAACTGAAGGAACTTAATGGA
              1630       1640       1650       1660       1670       1680

    1510       1520       1530       1540       1550       1560
PLAP    ACTGCACCTGAAGAGAAGAAGTTAACTGAGGATGACTTGATACTTCTTGAGAAGATACTG
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        ACTGCACCTGAAGAGAAGAAGTTAACTGAGGATGACTTGATACTTCTTGAGAAGATACTG
              1690       1700       1710       1720       1730       1740

    1570       1580       1590       1600       1610       1620
PLAP    TCTCTAATATGTAATAGTTCTTCAGAAAAACCCACAGTCCAGCAACTTCAGATTTTGTGG
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        TCTCTAATATGTAATAGTTCTTCAGAAAAACCCACAGTCCAGCAACTTCAGATTTTGTGG
              1750       1760       1770       1780       1790       1800

    1630       1640       1650       1660       1670       1680
PLAP    AAAGCTATTAACTGTCCTGAAGATATTGTCTTTCCTGCACTTGACATTCTTCGGTTGTCA
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        AAAGCTATTAACTGTCCTGAAGATATTGTCTTTCCTGCACTTGACATTCTTCGGTTGTCA
              1810       1820       1830       1840       1850       1860

    1690       1700       1710       1720       1730       1740
PLAP    ATTAAACACCCCAGTGTGAATGAGAACTTCTGCAATGAAAAGGAAGGGGCTCAGTTCAGC
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        ATTAAACACCCCAGTGTGAATGAGAACTTCTGCAATGAAAAGGAAGGGGCTCAGTTCAGC
              1870       1880       1890       1900       1910       1920

    1750       1760       1770       1780       1790       1800
PLAP    AGTCATCTTATCAATCTTCTGAACCCTAAAGGAAAGCCAGCAAACCAGCTGCTTGCTCTC
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        AGTCATCTTATCAATCTTCTGAACCCTAAAGGAAAGCCAGCAAACCAGCTGCTTGCTCTC
              1930       1940       1950       1960       1970       1980

    1810       1820       1830       1840       1850       1860
PLAP    AGGACTTTTTGCAATTGTTTTGTTGGCCAGGCAGGACAAAAACTCATGATGTCCCAGAGG
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        AGGACTTTTTGCAATTGTTTTGTTGGCCAGGCAGGACAAAAACTCATGATGTCCCAGAGG
              1990       2000       2010       2020       2030       2040

    1870       1880       1890       1900       1910       1920
PLAP    GAATCACTGATGTCCCATGCAATAGAACTGAAATCAGGGAGCAATAAGAACATTCACATT
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        GAATCACTGATGTCCCATGCAATAGAACTGAAATCAGGGAGCAATAAGAACATTCACATT
              2050       2060       2070       2080       2090       2100
```

Figure 12D

```
    1930       1940       1950       1960       1970       1980
PLAP   GCTCTGGCTACATTGGCCCTGAACTATTCTGTTTGTTTTCATAAAGACCATAACATTGAA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GCTCTGGCTACATTGGCCCTGAACTATTCTGTTTGTTTTCATAAAGACCATAACATTGAA
             2110       2120       2130       2140       2150       2160

    1990       2000       2010       2020       2030       2040
PLAP   GGGAAAGCCCAATGTTTGTCACTAATTAGCACAATCTTGGAAGTAGTACAAGACCTAGAA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GGGAAAGCCCAATGTTTGTCACTAATTAGCACAATCTTGGAAGTAGTACAAGACCTAGAA
             2170       2180       2190       2200       2210       2220

    2050       2060       2070       2080       2090       2100
PLAP   GCCACTTTTAGACTTCTTGTGGCTCTTGGAACACTTATCAGTGATGATTCAAATGCTGTA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GCCACTTTTAGACTTCTTGTGGCTCTTGGAACACTTATCAGTGATGATTCAAATGCTGTA
             2230       2240       2250       2260       2270       2280

    2110       2120       2130       2140       2150       2160
PLAP   CAATTAGCCAAGTCTTTAGGTGTTGATTCTCAAATAAAAAAGTATTCCTCAGTATCAGAA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       CAATTAGCCAAGTCTTTAGGTGTTGATTCTCAAATAAAAAAGTATTCCTCAGTATCAGAA
             2290       2300       2310       2320       2330       2340

    2170       2180       2190       2200       2210
PLAP   CCAGCTAAAGTAAGTGAATGCTGTAGATTTATCCTAAATTTGCTGTAG
       :::::::::::::::::::::::::::::::::::::::::::::::
       CCAGCTAAAGTAAGTGAATGCTGTAGATTTATCCTAAATTTGCTGTAG
             2350       2360       2370       2380
```

Figure 12E

```
ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989)
> TANGO 243 a.a.                                795 aa vs.
> PLAP a.a.                                     738 aa
scoring matrix: pam120.mat, gap penalties: -12/-4
92.8% identity;         Global alignment score: 3651

               10        20        30        40        50        60
T243    MTSGATRYRLSCSLRGHELDVRGLVCCAYPPGAFVSVSRDRTTRLWAPDSPNRSFTEMHC
        :                                                         ::
        M-----------------------------------------------------------HC


               70        80        90       100       110       120
T243    MSGHSNFVSCVCIIPSSDIYPHGLIATGGNDHNICIFSLDSPMPLYILKGHKNTVCSLSS
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        MSGHSNFVSCVCIIPSSDIYPHGLIATGGNDHNICIFSLDSPMPLYILKGHKNTVCSLSS
             10        20        30        40        50        60

              130       140       150       160       170       180
T243    GKFGTLLSGSWDTTAKVWLNDKCMMTLQGHTAAVWAVKILPEQGLMLTGSADKTVKLWKA
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        GKFGTLLSGSWDTTAKVWLNDKCMMTLQGHTAAVWAVKILPEQGLMLTGSADKTVKLWKA
             70        80        90       100       110       120

              190       200       210       220       230       240
T243    GRCERTFSGHEDCVRGLAILSETEFLSCANDASIRRWQITGECLEVYYGHTNYIYSISVF
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        GRCERTFSGHEDCVRGLAILSETEFLSCANDASIRRWQITGECLEVYYGHTNYIYSISVF
            130       140       150       160       170       180

              250       260       270       280       290       300
T243    PNCRDFVTTAEDRSLRIWKHGECAQTIRLPAQSIWCCCVLDNGDIVVGASDGIIRVFTES
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        PNCRDFVTTAEDRSLRIWKHGECAQTIRLPAQSIWCCCVLDNGDIVVGASDGIIRVFTES
            190       200       210       220       230       240

              310       320       330       340       350       360
T243    EDRTASAEEIKAFEKELSHATIDSKTGDLGDINAEQLPGREHLNEPGTREGQTRLIRDGE
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        EDRTASAEEIKAFEKELSHATIDSKTGDLGDINAEQLPGREHLNEPGTREGQTRLIRDGE
            250       260       270       280       290       300

              370       380       390       400       410       420
T243    KVEAYQWSVSEGRWIKIGDVVGSSGANQQTSGKVLYEGKEFDYVFSIDVNEGGPSYKLPY
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        KVEAYQWSVSEGRWIKIGDVVGSSGANQQTSGKVLYEGKEFDYVFSIDVNEGGPSYKLPY
            310       320       330       340       350       360

              430       440       450       460       470       480
T243    NTSDDPWLTAYNFLQKNDLNPMFLDQVAKFIIDNTKGQMLGLGNPSFSDPFTGGGRYVPG
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
        NTSDDPWLTAYNFLQKNDLNPMFLDQVAKFIIDNTKGQMLGLGNPSFSDPFTGGGRYVPG
            370       380       390       400       410       420
```

Figure 13A

```
           490       500       510       520       530       540
T243   SSGSSNTLPTADPFTGAGRYVPGSASMGTTMAGVDPFTGNSAYRSAASKTMNIYFPKKEA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       SSGSSNTLPTADPFTGAGRYVPGSASMGTTMAGVDPFTGNSAYRSAASKTMNIYFPKKEA
           430       440       450       460       470       480

           550       560       570       580       590       600
T243   VTFDQANPTQILGKLKELNGTAPEEKKLTEDDLILLEKILSLICNSSSEKPTVQQLQILW
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       VTFDQANPTQILGKLKELNGTAPEEKKLTEDDLILLEKILSLICNSSSEKPTVQQLQILW
           490       500       510       520       530       540

           610       620       630       640       650       660
T243   KAINCPEDIVFPALDILRLSIKHPSVNENFCNEKEGAQFSSHLINLLNPKGKPANQLLAL
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       KAINCPEDIVFPALDILRLSIKHPSVNENFCNEKEGAQFSSHLINLLNPKGKPANQLLAL
           550       560       570       580       590       600

           670       680       690       700       710       720
T243   RTFCNCFVGQAGQKLMMSQRESLMSHAIELKSGSNKNIHIALATLALNYSVCFHKDHNIE
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       RTFCNCFVGQAGQKLMMSQRESLMSHAIELKSGSNKNIHIALATLALNYSVCFHKDHNIE
           610       620       630       640       650       660

           730       740       750       760       770       780
T243   GKAQCLSLISTILEVVQDLEATFRLLVALGTLISDDSNAVQLAKSLGVDSQIKKYSSVSE
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
       GKAQCLSLISTILEVVQDLEATFRLLVALGTLISDDSNAVQLAKSLGVDSQIKKYSSVSE
           670       680       690       700       710       720

           790
T243   PAKVSECCRFILNLL
       ::::::::::::::
       PAKVSECCRFILNLL
           730
```

Figure 13B

Input file mT228.seq; Output File mT228.pat
Sequence length 911

```
                              M   C   L   S   A   V   S   F   K   G   I   R   C     13
CGGCACAGGGCGACGGCCGCTCGCCA ATG TGC CTC TCT GCC GTT TCA TTC AAG GGA ATA AGA TGC     65

 W   L   D   K   L   L   L   W   A   L   T   I   S   I   T   L   Q   N   A   A     33
TGG CTG GAC AAA CTG TTA CTT TGG GCT CTT ACA ATT TCT ATC ACA CTT CAG AAT GCT GCA   125

 V   D   C   T   R   V   E   N   N   E   L   P   S   P   N   L   N   S   S   M     53
GTG GAT TGT ACG AGG GTG GAA AAT AAC GAA TTA CCT TCT CCA AAT CTG AAC TCA AGT ATG   185

 N   V   V   R   M   G   Q   N   V   S   L   S   C   S   T   K   N   T   S   V     73
AAC GTG GTC AGG ATG GGC CAA AAT GTA TCT CTG TCT TGT TCC ACC AAG AAC ACA TCA GTA   245

 D   I   T   Y   S   L   F   W   G   T   K   Y   L   E   S   K   R   R   R   G     93
GAC ATC ACC TAT TCG CTC TTC TGG GGT ACA AAA TAT CTA GAA AGC AAG AGA AGA CGA GGG   305

 G   A   V   D   F   H   L   R   I   S   N   A   N   E   S   G   P   Y   K   C    113
GGA GCT GTG GAT TTC CAC CTG AGG ATC TCC AAT GCC AAC GAG TCA GGC CCC TAC AAA TGC   365

 K   V   N   V   S   N   L   M   K   Y   S   Q   D   F   N   F   T   M   A   K    133
AAA GTC AAT GTT TCC AAC TTG ATG AAA TAC AGT CAG GAT TTC AAC TTC ACA ATG GCC AAA   425

 D   E   S   C   P   S   C   R   L   S   L   L   L   P   G   L   L   L   G   I    153
GAT GAG AGC TGC CCT TCA TGC CGG CTG TCA CTG TTG CTC CCA GGG CTG TTA CTG GGG ATA   485

 L   V   I   V   L   V   L   A   Y   L   I   H   L   K   Y   K   K   G   K   K    173
CTG GTA ATA GTC CTA GTT CTG GCT TAT TTG ATT CAT CTA AAA TAC AAA AAA GGA AAG AAG   545

 T   Q   R   E   D   Q   S   K   G   S   G   D   A   P   A   Q   D   E   L   Y    193
ACT CAG AGA GAG GAC CAG TCC AAG GGT TCT GGA GAT GCG CCT GCA CAG GAC GAG CTG TAT   605

 V   N   A   C   K   T   Q   T   E   Q   P   Q   E   I   H   Y   A   T   P   V    213
GTC AAC GCC TGC AAG ACT CAG ACA GAG CAA CCC CAG GAG ATA CAC TAT GCC ACT CCA GTC   665

 F   K   E   M   A   P   M   E   E   E   G   G   T   D   G   K   A   D   Y   I    233
TTC AAG GAG ATG GCA CCC ATG GAA GAA GAA GGT GGT ACG GAT GGA AAA GCT GAT TAC ATC   725

 Y   S   E   L   T   H   *                                                       240
TAC TCT GAA CTC ACC CAC TGA                                                     746

AGTGTGAAGAAACTGACTGTATCCCAGTGTAAAGACTTTCCAGTAAGCTGGTGTATGAGAAAATAGGAAAACTCACCTG  825

GCACTTAAGAGTTCCATTCTANGCTGANGCAGGAGGATCCTGAGTTTTGANGCCACTTGGGACTACATAGCAAGACCTG  904

GCTTAAA                                                                         911
```

Figure 14

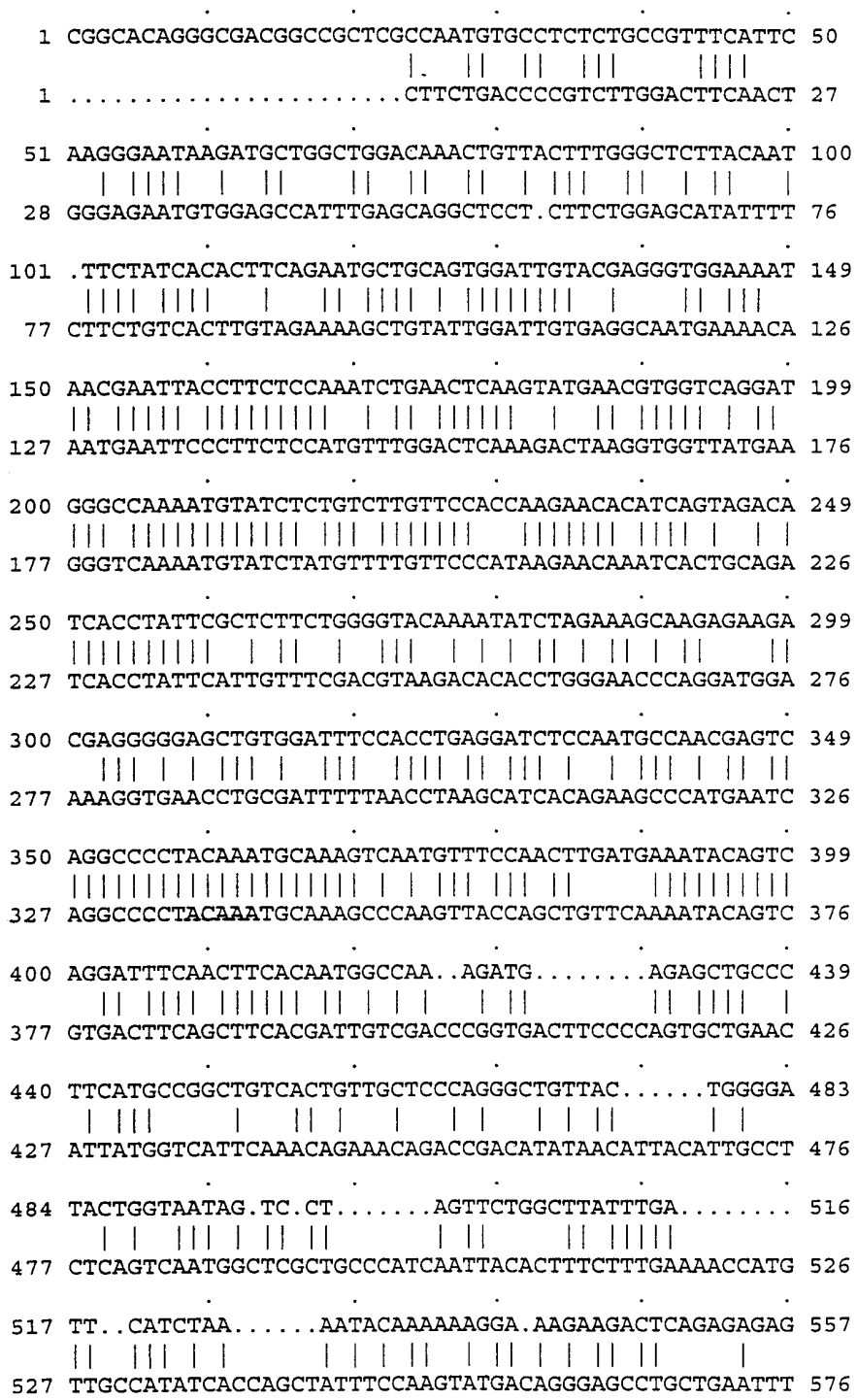
1 CGGCACAGGGCGACGGCCGCTCGCCAATGTGCCTCTCTGCCGTTTCATTC 50
1 .....CTTCTGACCCCGTCTTGGACTTCAACT 27
51 AAGGGAATAAGATGCTGGCTGGACAAACTGTTACTTTGGGCTCTTACAAT 100
28 GGGAGAATGTGGAGCCATTTGAGCAGGCTCCT.CTTCTGGAGCATATTTT 76
101 .TTCTATCACACTTCAGAATGCTGCAGTGGATTGTACGAGGGTGGAAAAT 149
77 CTTCTGTCACTTGTAGAAAAGCTGTATTGGATTGTGAGGCAATGAAAACA 126
150 AACGAATTACCTTCTCCAAATCTGAACTCAAGTATGAACGTGGTCAGGAT 199
127 AATGAATTCCCTTCTCCATGTTTGGACTCAAAGACTAAGGTGGTTATGAA 176
200 GGGCCAAAATGTATCTCTGTCTTGTTCCACCAAGAACACATCAGTAGACA 249
177 GGGTCAAAATGTATCTATGTTTTGTTCCCATAAGAACAAATCACTGCAGA 226
250 TCACCTATTCGCTCTTCTGGGGTACAAAATATCTAGAAAGCAAGAGAAGA 299
227 TCACCTATTCATTGTTTCGACGTAAGACACACCTGGGAACCCAGGATGGA 276
300 CGAGGGGGAGCTGTGGATTTCCACCTGAGGATCTCCAATGCCAACGAGTC 349
277 AAAGGTGAACCTGCGATTTTTAACCTAAGCATCACAGAAGCCCATGAATC 326
350 AGGCCCCTACAAATGCAAAGTCAATGTTTCCAACTTGATGAAATACAGTC 399
327 AGGCCCCTACAAATGCAAAGCCCAAGTTACCAGCTGTTCAAAATACAGTC 376
400 AGGATTTCAACTTCACAATGGCCAA..AGATG.....AGAGCTGCCC 439
377 GTGACTTCAGCTTCACGATTGTCGACCCGGTGACTTCCCCAGTGCTGAAC 426
440 TTCATGCCGGCTGTCACTGTTGCTCCCAGGGCTGTTAC.....TGGGGA 483
427 ATTATGGTCATTCAAACAGAAACAGACCGACATATAACATTACATTGCCT 476
484 TACTGGTAATAG.TC.CT.....AGTTCTGGCTTATTTGA..... 516
477 CTCAGTCAATGGCTCGCTGCCCATCAATTACACTTTCTTTGAAAACCATG 526
517 TT..CATCTAA.....AATACAAAAAAGGA.AAGAAGACTCAGAGAGAG 557
527 TTGCCATATCACCAGCTATTTCCAAGTATGACAGGGAGCCTGCTGAATTT 576

Figure 15A

```
                .         .         .         .         .
558 GACCAGTCC...AAGGGTTCTGGAGATGCG..CCTGCACAGG........ 594
     ||    ||   |||  | |||||||| | |     | | |||
577 AACTTAACCAAGAAGAATCCTGGAGAAGAGGAAGAGTATAGGTGTGAAGC 626
                .         .         .         .         .
595 .ACGAGCTGTATGTCAACGCCTGCAAGACTCAGACAGAGCAACCCCAGGA 643
     |  | | |  || | |    |||||  |  ||| ||      | |||  |
627 TAAAAACAGATTGCCTAACTATGCAACATACAGTCACCCTGTCACCATGC 676
                .         .         .         .         .
644 GAT..ACACTATGCCAC.....TCCAGTCTTCAAGGAGATGGCAC..CCA 684
      |  |||    |  ||     |||  |||    | || |   ||  |||
677 CCTCAACAGGCGGAGACAGCTGTCCTTTCTGTCTGAAGCTACTACTTCCA 726
                .         .         .         .         .
685 TGGAAGAAGAAGGTGGTACGGATGGAAAAGCTGATTACATC.TACTCTGA 733
     ||        | || |   | ||  ||  || |||    | |  |  |
727 GGGTTATTACTGTTGCTGGTGGTGATAATCCTAATTCTGGCTTTTTGGGT 776
                .         .         .         .         .
734 ACTCACCCACT.........GAAGTGTGAAGAAACTGACTGTATCC.... 770
    |||  || | |         |||  |  | || |   | |||  ||
777 ACTGCCCAAATACAAAACAAGAAAAGCTATGAGAAATAATGTGCCCAGGG 826
                .         .         .         .         .
771 .CAGTGTAAAGACTTTCCAGTAAGCTGGTGTATGAGAAAATA.....GGA 814
     | ||| | | ||   |  | ||| |||  | |  | |||||     | |
827 ACCGTGGAGACACAGCCATGGAAGTTGGAATCTATGCAAATATCCTTGAA 876
                .         .         .         .         .
815 AAACTCACCTGGCACTTAAGAGTTCCA.TTCTANGCT.GANGCAG.GAGG 861
    ||||   |   | |   |   || |||    | :| |  |:|| | | |
877 AAACAAGCAAAGGAGGAATCTGTGCCAGAAGTGGGATCCAGGCCGTGTGT 926
                .         .         .         .         .
862 ATCCTGAG....TTTTGANGCCA.......CTTGGGACTACA.TA.GCAA 898
     |||  ||       |||:||||       |  ||  ||||| || || |
927 TTCCACAGCCCAAGATGAGGCCAAACACTCCCAGGAGCTACAGTATGCCA 976
                .         .         .         .         .
899 GACCTGGCTTAAA..................................... 911
      || |  ||  |
977 CCCCCGTGTTCCAGGAGGTGGCACCAAGAGAGCAAGAAGCCTGTGATTCT 1026
```

Figure 15B

Input file huT240; Output File huT240.pat2
Sequence length 2165

```
   M   A   A   P   A   L   G   L   V   C   G   R   C   P   E   L   G   L   V      19
C ATG GCT GCG CCC GCA CTA GGG CTG GTG TGT GGA CGT TGC CCT GAG CTG GGT CTC GTC      58

 L   L   L   L   L   L   S   L   L   C   G   A   A   G   S   Q   E   A   G   T    39
CTC TTG CTG CTG CTG CTC TCG CTG CTG TGT GGA GCG GCA GGG AGC CAG GAG GCC GGG ACC   118

 G   A   G   A   G   S   L   A   G   S   C   G   C   G   T   P   Q   R   P   G    59
GGT GCG GGC GCG GGG TCC CTT GCG GGT TCT TGC GGC TGC GGC ACG CCC CAG CGG CCT GGC   178

 A   H   G   N   S   A   A   A   H   R   Y   S   R   E   A   N   A   P   G   P    79
GCC CAT GGC AAT TCG GCA GCC GCT CAC CGA TAC TCG CGG GAG GCT AAC GCT CCG GGC CCC   238

 V   P   G   E   R   Q   L   A   H   S   K   M   V   P   I   P   V   G   V   F    99
GTA CCC GGA GAG CGG CAA CTC GCG CAC TCA AAG ATG GTC CCC ATC CCT GTT GGA GTA TTT   298

 T   M   G   T   D   D   P   Q   I   K   Q   D   G   E   A   P   A   R   R   V   119
ACA ATG GGC ACA GAT GAT CCT CAG ATA AAG CAG GAT GGG GAA GCA CCT GCG AGG AGA GTT   358

 T   I   D   A   F   Y   M   D   A   Y   E   V   S   N   T   E   F   E   K   F   139
ACT ATT GAT GCC TTT TAC ATG GAT GCC TAT GAA GTC AGT AAT ACT GAA TTT GAG AAG TTT   418

 V   N   S   T   G   Y   L   T   E   A   E   K   F   G   D   S   F   V   F   E   159
GTG AAC TCA ACT GGC TAT TTG ACA GAG GCT GAG AAG TTT GGC GAC TCC TTT GTC TTT GAA   478

 G   M   L   S   E   Q   V   K   T   N   I   Q   Q   A   V   A   A   A   P   W   179
GGC ATG TTG AGT GAG CAA GTG AAG ACC AAT ATT CAA CAG GCA GTT GCA GCT GCT CCC TGG   538

 W   L   P   V   K   G   A   N   W   R   H   P   E   G   P   D   S   T   I   L   199
TGG TTA CCT GTG AAA GGC GCT AAC TGG AGA CAC CCA GAA GGG CCT GAC TCT ACT ATT CTG   598

 H   R   P   D   H   P   V   L   H   V   S   W   N   D   A   V   A   Y   C   T   219
CAC AGG CCG GAT CAT CCA GTT CTC CAT GTG TCC TGG AAT GAT GCG GTT GCC TAC TGC ACT   658

 W   A   G   K   R   L   P   T   E   A   E   W   E   Y   S   C   R   G   G   L   239
TGG GCA GGG AAG CGG CTG CCC ACG GAA GCT GAG TGG GAA TAC AGC TGT CGA GGA GGC CTG   718

 H   N   R   L   F   P   W   G   N   K   L   Q   P   K   G   Q   H   Y   A   N   259
CAT AAT AGA CTT TTC CCC TGG GGC AAC AAA CTG CAG CCC AAA GGC CAG CAT TAT GCC AAC   778

 I   W   Q   G   E   F   P   V   T   N   T   G   E   D   G   F   Q   G   T   A   279
ATT TGG CAG GGC GAG TTT CCG GTG ACC AAC ACT GGT GAG GAT GGC TTC CAA GGA ACT GCG   838

 P   V   D   A   F   P   P   N   G   Y   G   L   Y   N   I   V   G   N   A   W   299
CCT GTT GAT GCC TTC CCT CCC AAT GGT TAT GGC TTA TAC AAC ATA GTG GGG AAC GCA TGG   898

 E   W   T   S   D   W   W   T   V   H   H   S   V   E   E   T   L   N   P   K   319
GAA TGG ACT TCA GAC TGG TGG ACT GTT CAT CAT TCT GTT GAA GAA ACG CTT AAC CCA AAA   958

 G   P   P   S   G   K   D   R   V   K   K   G   G   S   Y   M   C   H   R   S   339
GGT CCC CCT TCT GGG AAA GAC CGA GTG AAG AAA GGT GGA TCC TAC ATG TGC CAT AGG TCT  1018
```

Figure 6A

```
                .          .          .          .          .
539 CCAAGGGTTCTGGAGATGCGCCTGCACAGGA......CGAGCTGTAT.GT 581
    |||||  |    | ||  |  |||.|    |      | ||  | ||  |
515 CCAAGTATGACAGGGAGCCTGCTGAATTTAACTTAACCAAGAAGAATCCT 564
                .          .          .          .          .
582 CAACGCCTGCAAGACTCAGACAGAGCAAC.CCCAGGAGAT....ACACTA 626
      |     | |||| |      | | | |    |  ||||      ||||
565 GGAGAAGAGGAAGAGTATAGGTGTGAAGCTAAAAACAGATTGCCTAACTA 614
                .          .          .          .          .
627 TGCCAC.TCCAGTCTTCAAG..GAGATGGCACCCATGGAAGAAGAAGGTG 673
    |||  ||  | |||||  |  |     |||  |  | |  |  | |||  |
615 TGCAACATACAGTCACCCTGTCACCATGCCCTCAACAGGCGGAGACAGCT 664
                .          .          .          .          .
674 GTACGGATGG..AAAAGCTGATTACATCTACTCTGA..ACTCACCCAC.. 717
    ||  |     |    ||||| | ||| ||  |   | |  |||    |
665 GTCCTTTCTGTCTGAAGCT.ACTACTTCCAGGGTTATTACTGTTGCTGGT 713
```

Figure 16B

```
          .         .         .         .         .
  1 MCLSAVSFKGIRCWLDKLLLWALTISITLQNAAVDCTRVENNELPSPNLN 50
            .   | :|| |.: _|:| . | .||  .. || ||| |.
  1 ..........MWSHLSRLLFWSIFSSVTCRKAVLDCEAMKTNEFPSPCLD 40
          .         .         .         .         .
 51 SSMNVVRMGQNVSLSCSTKNTSVDITYSLFWGTKYLESKRRRGGAVDFHL 100
    |   ||  |||||: || || |. ||||||    :| ..  :|    |.|
 41 SKTKVVMKGQNVSMFCSHKNKSLQITYSLFRRKTHLGTQDGKGEPAIFNL 90
          .         .         .         .         .
101 RISNANESGPYKCKVNVSNLMKYSQDFNFTMAKDESCPSCRLSLLLPGLL 150
     |. |.||||||||  |..  |||.||.||.    . |   : .:
 91 SITEAHESGPYKCKAQVTSCSKYSRDFSFTIVDPVTSPVLNIMVIQTETD 140
          .         .         .         .         .
151 LGILVIVLVLAYLIHLKYKKGKKTQREDQSKGSGD.APAQDELYVNACKT 199
      | .  | .   : : |   .       .    |  ||:  |
141 RHITLHCLSVNGSLPINYTFFENHVAISPAISKYDREPAEFNLTKKNPGE 190
          .         .         .         .         .
200 QTEQPQEIHYATPVFKEMAPMEEEGGTDGKADYIYSELTH.......... 239
    : |   |     | :   .       | | .     .|
191 EEEYRCEAKNRLPNYATYSHPVTMPSTGGDSCPFCLKLLLPGLLLLLVVI 240
```

Figure 17

Input file mT240.seq; Output File mT240.pat
Sequence length 2426

```
   M   A   A   P   A   R   E   P   A   L   R   C   C   I   R   L   A   R   V      19
C ATG GCT GCG CCC GCG CGA GAG CCG GCT CTC CGC TGC TGC ATC AGA CTG GCG CGA GTC      58

 F   L   L   L   V   L   A   C   E   V   A   G   S   D   E   A   E   A   R   E    39
TTC TTG CTG CTG GTG TTG GCG TGC GAG GTG GCG GGA AGC GAT GAG GCC GAG GCC AGG GAA   118

 G   A   A   S   L   A   G   S   C   G   C   G   T   P   Q   R   A   G   A   H    59
GGT GCG GCG TCC CTT GCG GGC TCG TGC GGC TGC GGA ACG CCC CAG AGG GCC GGG GCC CAT   178

 G   S   S   A   A   A   Q   R   Y   S   R   E   A   N   A   P   G   L   T   S    79
GGC AGC TCG GCG GCG GCG CAG CGC TAC TCC CGG GAG GCG AAC GCC CCG GGC CTG ACC TCA   238

 G   P   R   P   L   A   L   T   K   M   V   P   I   P   A   G   V   F   T   M    99
GGC CCG CGA CCG CTC GCG CTC ACC AAG ATG GTC CCC ATT CCT GCT GGA GTA TTC ACA ATG   298

 G   T   D   D   P   Q   I   R   Q   D   G   E   A   P   A   R   R   V   T   V   119
GGC ACT GAT GAT CCT CAG ATC AGG CAG GAT GGA GAA GCC CCT GCC AGG AGA GTC ACT GTT   358

 D   G   F   Y   M   D   A   Y   E   V   S   N   A   D   F   E   K   F   V   N   139
GAT GGC TTT TAC ATG GAC GCC TAT GAA GTC AGC AAT GCG GAT TTT GAG AAG TTT GTG AAC   418

 S   T   G   Y   L   T   E   A   E   K   F   G   D   S   F   V   F   E   G   M   159
TCG ACT GGC TAT TTG ACA GAG GCT GAG AAG TTT GGA GAC TCT TTC GTC TTT GAA GGC ATG   478

 L   S   E   Q   V   K   T   H   I   H   Q   A   V   A   A   A   P   W   W   L   179
TTG AGC GAG CAA GTG AAA ACG CAT ATC CAC CAG GCA GTT GCA GCT GCT CCA TGG TGG TTG   538

 P   V   K   G   A   N   W   R   H   P   E   G   P   D   S   S   I   L   H   R   199
CCT GTC AAG GGA GCT AAT TGG AGA CAC CCA GAG GGT CCG GAC TCC AGT ATT CTG CAC AGG   598

 S   N   H   P   V   L   H   V   S   W   N   D   A   V   A   Y   C   T   W   A   219
TCA AAT CAT CCG GTT CTC CAT GTT TCC TGG AAC GAT GCT GTT GCC TAC TGC ACA TGG GCG   658

 G   K   R   L   P   T   E   A   E   W   E   Y   S   C   R   G   G   L   Q   N   239
GGC AAG AGG TTG CCT ACT GAG GCA GAG TGG GAA TAC AGC TGT AGA GGA GGC CTG CAG AAC   718

 R   L   F   P   W   G   N   K   L   Q   P   K   G   Q   H   Y   A   N   I   W   259
AGG CTT TTC CCC TGG GGC AAC AAA CTG CAG CCC AAA GGA CAG CAT TAT GCC AAC ATC TGG   778

 Q   G   K   F   P   V   S   N   T   G   E   D   G   F   Q   G   T   A   P   V   279
CAG GGC AAG TTT CCT GTG AGC AAC ACT GGC GAG GAT GGC TTC CAA GGA ACT GCC CCC GTT   838

 D   A   F   P   P   N   G   Y   G   L   Y   N   I   V   G   N   V   W   E   W   299
GAT GCC TTT CCT CCC AAT GGC TAT GGC TTA TAC AAC ATA GTG GGG AAT GTG TGG GAG TGG   898

 T   S   D   W   W   T   V   H   H   S   V   E   E   T   F   N   P   K   G   P   319
ACC TCA GAC TGG TGG ACT GTT CAC CAT TCT GTT GAG GAA ACG TTC AAC CCA AAG GGT CCC   958
```

Figure 18A

```
 T   S   G   K   D   R   V   K   K   G   G   S   Y   M   C   H   K   S   Y   C    339
ACT TCT GGG AAA GAC CGA GTG AAG AAG GGT GGA TCC TAC ATG TGC CAT AAG TCC TAT TGC  1018

 Y   R   Y   R   C   A   A   R   S   Q   N   T   P   D   S   S   A   S   N   L    359
TAT AGG TAC CGC TGT GCA GCT CGA AGC CAG AAC ACA CCA GAT AGC TCT GCA TCC AAC CTG  1078

 G   F   R   C   A   A   D   H   L   P   T   A   D   *                          373
GGA TTC CGA TGT GCA GCC GAC CAC CTG CCC ACC GCA GAC TGA                          1120

CAGCCAAGAGGAGCTTTTCCAGATTCAAGAAGGCGTTTCTTACTCGCAGCTGGCCTCCCCTGGAAATCTGAACTGATCT  1199

CATGTAAAGTATTCCCATCTACGGAGGATTCCATGTCCACCCAGTGGCCAAAGGAACTGTCTCGCGTGACCAAATGGCT  1278

GGCGAGTGTCAGCACGTGTGCTTTATTGTGTGGTGTATCTTTGGGGATCATCGCCATGTTTTACTTTGAAAGCCTTTTG  1357

AAGAGGAGAGAGCCGAGAACCAGGAAGTCCTGGCCAGACTCTGCCACAGGGTCAGACCCTGGAGTCCAGCACCTTGTCT  1436

GCCTTGACCTCTGTCTCCTCATGAAATGAGGGATGGTCAACGTGATCTTTGAGGCTCTCTCCAACTCTATTTGAACTAG  1515

CAGATTCTATTCGAACTAGCAGAGTGTATTGTGATTGCATAGTGAGAATTTATGACAGATTATTTTTTAGCTATTTTTT  1594

TGCCATGTGTGAATCTTGAGTAATACTAATCATATAAGGCGAGAGTTATCTTACATATTATTTTCAGAAAAGGGTGGGG  1673

TTTGAGTCTTTTATATTCATACTGCACTTTGTTCTTTCAAGGAAATCAGTGTCTTTTACATTGTTGTGACAAATCCCAT  1752

TGGGACAGCGAGGGGACACTTAAGTTTGGAGTTCTGAACACACAGGAATGCCTGTGGAGTGACTCTACTGTCCTTTTTC  1831

TTTTGACATTAAGTGCCTTTGGCTCAGAGGGACAGTTTGAAGCCTTGTTTCCCCTTTGCCCCCAAGCCTTCAAAGAATG  1910

TGAAATATGTACTAATTAGGGAAACCATTTAATTCTAGGTCTTTGGGTGTTGAGGTTTTGTCAGATGGTATGAATTGTA  1989

TTGTAATGCTAAATCTGGTACCTGAAGGTCTAGGCCTGTGAGTGAATTCTCACATTTACAAGATTTTGTTGTGCAAACC  2068

TTGTTCCTTAATTTAAAACTATTGGTTAAATAAAATTGGCTACAGCCAATTACTGGAGGGATTAGAGGTAGGTGGGTTT  2147

TGAGTGTGGTTGGGTATGGAGAGAGAGAGGAGAGATCAAGGAGAGAAGCAGGAAGAAGAGGATAGGAGGAGCTGCCATG  2226

AAGAAGATGGACCATAAGCCTTTGTCCAGAGGAAACTCCCAAGTATCTGGGAACACCGCTGTGAGGCAACCAGGCCAGC  2305

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AAAAAAAAAAAAAAAAAAAAAAAAACAAAAAAAAAAAAAAA                                       2426
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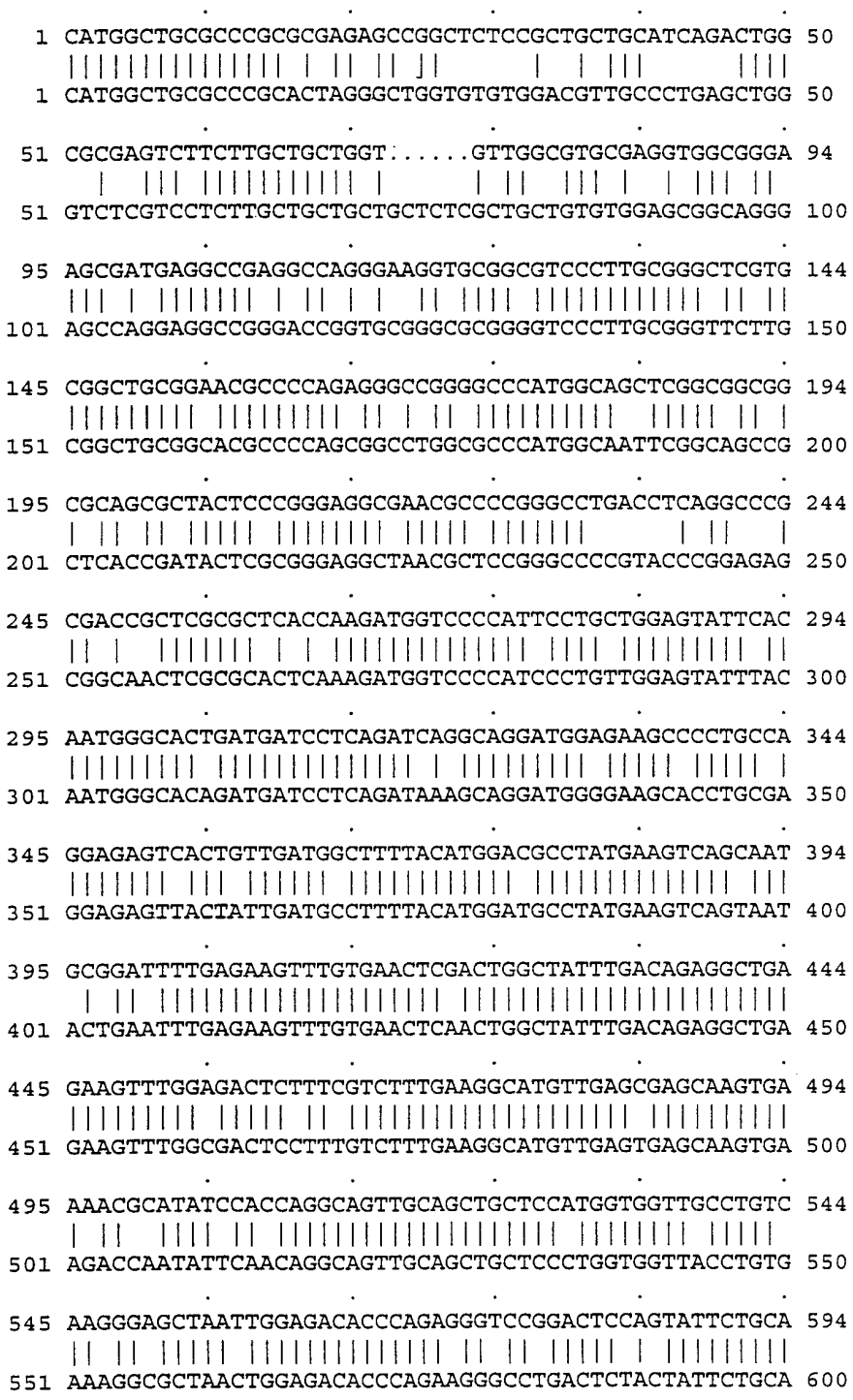
Figure 18B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1 CATGGCTGCGCCCGCACTAGGGCTGGTGTGTGGACGTTGCCCTGAGCTGG 50
51 CGCGAGTCTTCTTGCTGCTGGT.....GTTGGCGTGCGAGGTGGCGGGA 94
51 GTCTCGTCCTCTTGCTGCTGCTGCTCTCGCTGCTGTGTGGAGCGGCAGGG 100
95 AGCGATGAGGCCGAGGCCAGGGAAGGTGCGGCGTCCCTTGCGGGCTCGTG 144
101 AGCCAGGAGGCCGGGACCGGTGCGGGCGCGGGGTCCCTTGCGGGTTCTTG 150
145 CGGCTGCGGAACGCCCCAGAGGGCCGGGGCCCATGGCAGCTCGGCGGCGG 194
151 CGGCTGCGGCACGCCCCAGCGGCCTGGCGCCCATGGCAATTCGGCAGCCG 200
195 CGCAGCGCTACTCCCGGGAGGCGAACGCCCCGGGCCTGACCTCAGGCCCG 244
201 CTCACCGATACTCGCGGGAGGCTAACGCTCCGGGCCCCGTACCCGGAGAG 250
245 CGACCGCTCGCGCTCACCAAGATGGTCCCCATTCCTGCTGGAGTATTCAC 294
251 CGGCAACTCGCGCACTCAAAGATGGTCCCCATCCCTGTTGGAGTATTTAC 300
295 AATGGGCACTGATGATCCTCAGATCAGGCAGGATGGAGAAGCCCCTGCCA 344
301 AATGGGCACAGATGATCCTCAGATAAAGCAGGATGGGGAAGCACCTGCGA 350
345 GGAGAGTCACTGTTGATGGCTTTTACATGGACGCCTATGAAGTCAGCAAT 394
351 GGAGAGTTACTATTGATGCCTTTTACATGGATGCCTATGAAGTCAGTAAT 400
395 GCGGATTTTGAGAAGTTTGTGAACTCGACTGGCTATTTGACAGAGGCTGA 444
401 ACTGAATTTGAGAAGTTTGTGAACTCAACTGGCTATTTGACAGAGGCTGA 450
445 GAAGTTTGGAGACTCTTTCGTCTTTGAAGGCATGTTGAGCGAGCAAGTGA 494
451 GAAGTTTGGCGACTCCTTTGTCTTTGAAGGCATGTTGAGTGAGCAAGTGA 500
495 AAACGCATATCCACCAGGCAGTTGCAGCTGCTCCATGGTGGTTGCCTGTC 544
501 AGACCAATATTCAACAGGCAGTTGCAGCTGCTCCCTGGTGGTTACCTGTG 550
545 AAGGGAGCTAATTGGAGACACCCAGAGGGTCCGGACTCCAGTATTCTGCA 594
551 AAAGGCGCTAACTGGAGACACCCAGAAGGGCCTGACTCTACTATTCTGCA 600

Figure 19A

```
595 CAGGTCAAATCATCCGGTTCTCCATGTTTCCTGGAACGATGCTGTTGCCT 644
601 CAGGCCGGATCATCCAGTTCTCCATGTGTCCTGGAATGATGCGGTTGCCT 650

645 ACTGCACATGGGCGGGCAAGAGGTTGCCTACTGAGGCAGAGTGGGAATAC 694
651 ACTGCACTTGGGCAGGGAAGCGGCTGCCCACGGAAGCTGAGTGGGAATAC 700

695 AGCTGTAGAGGAGGCCTGCAGAACAGGCTTTTCCCCTGGGGCAACAAACT 744
701 AGCTGTCGAGGAGGCCTGCATAATAGACTTTTCCCCTGGGGCAACAAACT 750

745 GCAGCCCAAAGGACAGCATTATGCCAACATCTGGCAGGGCAAGTTTCCTG 794
751 GCAGCCCAAAGGCCAGCATTATGCCAACATTTGGCAGGGCGAGTTTCCGG 800

795 TGAGCAACACTGGCGAGGATGGCTTCCAAGGAACTGCCCCCGTTGATGCC 844
801 TGACCAACACTGGTGAGGATGGCTTCCAAGGAACTGCGCCTGTTGATGCC 850

845 TTTCCTCCCAATGGCTATGGCTTATACAACATAGTGGGGAATGTGTGGGA 894
851 TTCCCTCCCAATGGTTATGGCTTATACAACATAGTGGGGAACGCATGGGA 900

895 GTGGACCTCAGACTGGTGGACTGTTCACCATTCTGTTGAGGAAACGTTCA 944
901 ATGGACTTCAGACTGGTGGACTGTTCATCATTCTGTTGAAGAAACGCTTA 950

945 ACCCAAAGGGTCCCACTTCTGGGAAAGACCGAGTGAAGAAGGGTGGATCC 994
951 ACCCAAAAGGTCCCCCTTCTGGGAAAGACCGAGTGAAGAAAGGTGGATCC 1000

995 TACATGTGCCATAAGTCCTATTGCTATAGGTACCGCTGTGCAGCTCGAAG 1044
1001 TACATGTGCCATAGGTCTTATTGTTACAGGTATCGCTGTGCTGCTCGGAG 1050

1045 CCAGAACACACCAGATAGCTCTGCATCCAACCTGGGATTCCGATGTGCAG 1094
1051 CCAGAACACACCTGATAGCTCTGCTTCGAATCTGGGATTCCGCTGTGCAG 1100

1095 CCGACCACCTGCCCACCGCAGACTGACAGCCAAGAGGAGCTTTTCCAGAT 1144
1101 CCGACCGCCTGCCCACTATGGACTGACAACCAAGGAAAGTCTTCCCCAGT 1150
```

Figure 19B

```
1145 TCA...AGAAGGCGTTTCTTACTCGCAGCTGGCCTCCCCTGGAAATCTGA 1191
1151 CCAAGGAGCAGTCGTGTCTGACCTACATTGGGCTTTTCTCAGAACTTTGA 1200

1192 ACTGATCTCATGTAAAGTATTCCCA.TCTACGGAGGATTCCATGTCCACC 1240
1201 AC.GATCCCATGCAAAGAATTCCCACCCTGAGGTGGGTTACATACCTGCC 1249

1241 CAGTGGCCAAAGGAACTGTCTCGCGTGACCAAATGGCTGGCGAGTGTCAG 1290
1250 CAATGGCCAAAGGAACCGCCTTGTGAGACCAAATTGCTGACCTGGGTCAG 1299

1291 CACGTGTGCTTTATTGTGTGGTGTATCTTTGGGGATCATCGCCATGTTTT 1340
1300 TGCATGTGCTTTATGGTGTGGTGCATCTTTGGAGATCATCGCCATATTTT 1349

1341 AC.TTTGAAAGCCTTT..........TGAAGAGGAGAGAGCCGAGAAC.. 1377
1350 ACTTTTGAGAGTCTTTAAAGAGGAAGGGGAGTGGAGGGAACCCTGAGCTA 1399

1378 .....CAGGA......AGTCCTGGCCAGACTCTGCCACA.GGGTCAGACC 1415
1400 GGCTTCAGGAGGCCCGCGTCCTACGCAGGCTCTGCCACAGGGGTTAGACC 1449

1416 CTGGAGTCCAGCACCTTGTCTGCCTTGACCTC.TGTCTCCTC....ATGA 1460
1450 CCAG.GTCCGACGCTTGACCTTCCTGGGCCTCAAGTGCCCTCCCCTATCA 1498

1461 AATG.AGGGATGGTCAACGTGATCTTTGAGGCTCTCTCCAACTCTATTTG 1509
1499 AATGAAGGGATGGACAGCATGACCTCTGGGTGTCTCTCCAACTC...... 1542

1510 AACTAGCAGATTCTATTCGAACTAGCAGAGTGTATTGTGATTGCATAGTG 1559
1543 ....ACCAG.TTCTAAAAAGGGTATCAGATTCTATTGTGACTTCATAGTG 1587

1560 AGAATTTATGACAGATTATTTTTTAGCTATTTTTTTGCCATGTGTGAATC 1609
1588 AGAATTTATGATAGATTATTTTTTAGCTATTTTTT..CCATGTGTGAACC 1635

1610 TTGAGTAATACTAATCATATAAGGCGAGAGTTATCTTACATATTATTTTC 1659
1636 TTGAGTGATACTAATCATGTAAAGTAAGAGTTCTCTTATGTATTATTTTC 1685
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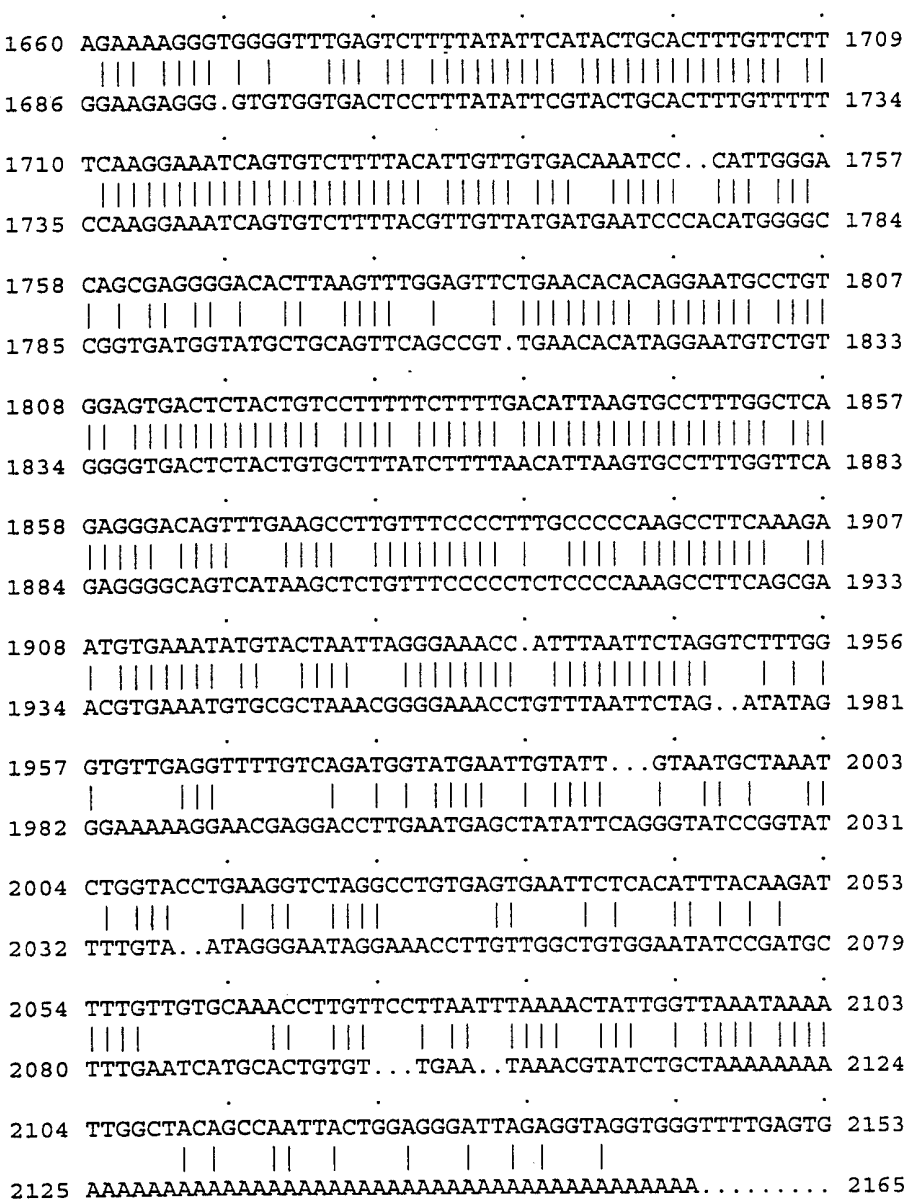
Figure 19C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1686 GGAAGAGGG.GTGTGGTGACTCCTTTATATTCGTACTGCACTTTGTTTTT 1734
1710 TCAAGGAAATCAGTGTCTTTTACATTGTTGTGACAAATCC..CATTGGGA 1757
1735 CCAAGGAAATCAGTGTCTTTTACGTTGTTATGATGAATCCCACATGGGGC 1784
1758 CAGCGAGGGGACACTTAAGTTTGGAGTTCTGAACACACAGGAATGCCTGT 1807
1785 CGGTGATGGTATGCTGCAGTTCAGCCGT.TGAACACATAGGAATGTCTGT 1833
1808 GGAGTGACTCTACTGTCCTTTTTCTTTTGACATTAAGTGCCTTTGGCTCA 1857
1834 GGGGTGACTCTACTGTGCTTTATCTTTTAACATTAAGTGCCTTTGGTTCA 1883
1858 GAGGGACAGTTTGAAGCCTTGTTTCCCCTTTGCCCCCAAGCCTTCAAAGA 1907
1884 GAGGGGCAGTCATAAGCTCTGTTTCCCCCTCTCCCCAAAGCCTTCAGCGA 1933
1908 ATGTGAAATATGTACTAATTAGGGAAACC.ATTTAATTCTAGGTCTTTGG 1956
1934 ACGTGAAATGTGCGCTAAACGGGGAAACCTGTTTAATTCTAG..ATATAG 1981
1957 GTGTTGAGGTTTTGTCAGATGGTATGAATTGTATT...GTAATGCTAAAT 2003
1982 GGAAAAAGGAACGAGGACCTTGAATGAGCTATATTCAGGGTATCCGGTAT 2031
2004 CTGGTACCTGAAGGTCTAGGCCTGTGAGTGAATTCTCACATTTACAAGAT 2053
2032 TTTGTA..ATAGGGAATAGGAAACCTTGTTGGCTGTGGAATATCCGATGC 2079
2054 TTTGTTGTGCAAACCTTGTTCCTTAATTTAAAACTATTGGTTAAATAAAA 2103
2080 TTTGAATCATGCACTGTGT...TGAA..TAAACGTATCTGCTAAAAAAAA 2124
2104 TTGGCTACAGCCAATTACTGGAGGGATTAGAGGTAGGTGGGTTTTGAGTG 2153
2125 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA..... 2165

Figure 19D

```
  1 ATGGCTGCGCCCGCGCGAGAGCCGGCTCTCCGCTGCTGCATCAGACTGGC 50
    ||||||||||||||| | || || ||      |  | |||      ||||
  1 ATGGCTGCGCCCGCACTAGGGCTGGTGTGTGGACGTTGCCCTGAGCTGGG 50

 51 GCGAGTCTTCTTGCTGCTGGT......GTTGGCGTGCGAGGTGGCGGGAA 94
     |  ||| ||||||||||| |      | ||  ||| |  | ||| || |
 51 TCTCGTCCTCTTGCTGCTGCTGCTCTCGCTGCTGTGTGGAGCGGCAGGGA 100

 95 GCGATGAGGCCGAGGCCAGGGAAGGTGCGGCGTCCCTTGCGGGCTCGTGC 144
    || | ||||||| | || | |  || |||| |||||||||||| || |||
101 GCCAGGAGGCCGGGACCGGTGCGGGCGCGGGGTCCCTTGCGGGTTCTTGC 150

145 GGCTGCGGAACGCCCCAGAGGGCCGGGGCCCATGGCAGCTCGGCGGCGGC 194
    |||||||| ||||||||| || | || ||||||||||  ||||| || ||
151 GGCTGCGGCACGCCCCAGCGGCCTGGCGCCCATGGCAATTCGGCAGCCGC 200

195 GCAGCGCTACTCCCGGGAGGCGAACGCCCCGGGCCTGACCTCAGGCCCGC 244
     || || ||||| |||||||| ||||| |||||||      | ||   ||
201 TCACCGATACTCGCGGGAGGCTAACGCTCCGGGCCCCGTACCCGGAGAGC 250

245 GACCGCTCGCGCTCACCAAGATGGTCCCCATTCCTGCTGGAGTATTCACA 294
    | |  ||||||| | | |||||||||||||| |||| ||||||||| |||
251 GGCAACTCGCGCACTCAAAGATGGTCCCCATCCCTGTTGGAGTATTTACA 300

295 ATGGGCACTGATGATCCTCAGATCAGGCAGGATGGAGAAGCCCCTGCCAG 344
    |||||||| |||||||||||||| | ||||||||| ||||| ||||| ||
301 ATGGGCACAGATGATCCTCAGATAAAGCAGGATGGGGAAGCACCTGCGAG 350

345 GAGAGTCACTGTTGATGGCTTTTACATGGACGCCTATGAAGTCAGCAATG 394
    |||||| ||| |||||| |||||||||||| |||||||||||||| |||
351 GAGAGTTACTATTGATGCCTTTTACATGGATGCCTATGAAGTCAGTAATA 400

395 CGGATTTTGAGAAGTTTGTGAACTCGACTGGCTATTTGACAGAGGCTGAG 444
    | || ||||||||||||||||||| |||||||||||||||||||||||||
401 CTGAATTTGAGAAGTTTGTGAACTCAACTGGCTATTTGACAGAGGCTGAG 450

445 AAGTTTGGAGACTCTTTCGTCTTTGAAGGCATGTTGAGCGAGCAAGTGAA 494
    |||||||| ||||| || |||||||||||||||||||| ||||||||||
451 AAGTTTGGCGACTCCTTTGTCTTTGAAGGCATGTTGAGTGAGCAAGTGAA 500

495 AACGCATATCCACCAGGCAGTTGCAGCTGCTCCATGGTGGTTGCCTGTCA 544
     ||  |||| || |||||||||||||||||||| |||||||| ||||| |
501 GACCAATATTCAACAGGCAGTTGCAGCTGCTCCCTGGTGGTTACCTGTGA 550
```

Figure 20A

```
545 AGGGAGCTAATTGGAGACACCCAGAGGGTCCGGACTCCAGTATTCTGCAC 594
551 AAGGCGCTAACTGGAGACACCCAGAAGGGCCTGACTCTACTATTCTGCAC 600

595 AGGTCAAATCATCCGGTTCTCCATGTTTCCTGGAACGATGCTGTTGCCTA 644
601 AGGCCGGATCATCCAGTTCTCCATGTGTCCTGGAATGATGCGGTTGCCTA 650

645 CTGCACATGGGCGGGCAAGAGGTTGCCTACTGAGGCAGAGTGGGAATACA 694
651 CTGCACTTGGGCAGGGAAGCGGCTGCCCACGGAAGCTGAGTGGGAATACA 700

695 GCTGTAGAGGAGGCCTGCAGAACAGGCTTTTCCCCTGGGGCAACAAACTG 744
701 GCTGTCGAGGAGGCCTGCATAATAGACTTTTCCCCTGGGGCAACAAACTG 750

745 CAGCCCAAAGGACAGCATTATGCCAACATCTGGCAGGGCAAGTTTCCTGT 794
751 CAGCCCAAAGGCCAGCATTATGCCAACATTTGGCAGGGCGAGTTTCCGGT 800

795 GAGCAACACTGGCGAGGATGGCTTCCAAGGAACTGCCCCCGTTGATGCCT 844
801 GACCAACACTGGTGAGGATGGCTTCCAAGGAACTGCGCCTGTTGATGCCT 850

845 TTCCTCCCAATGGCTATGGCTTATACAACATAGTGGGGAATGTGTGGGAG 894
851 TCCCTCCCAATGGTTATGGCTTATACAACATAGTGGGGAACGCATGGGAA 900

895 TGGACCTCAGACTGGTGGACTGTTCACCATTCTGTTGAGGAAACGTTCAA 944
901 TGGACTTCAGACTGGTGGACTGTTCATCATTCTGTTGAAGAAACGCTTAA 950

945 CCCAAAGGGTCCCACTTCTGGGAAAGACCGAGTGAAGAAGGGTGGATCCT 994
951 CCCAAAAGGTCCCCCTTCTGGGAAAGACCGAGTGAAGAAAGGTGGATCCT 1000

995 ACATGTGCCATAAGTCCTATTGCTATAGGTACCGCTGTGCAGCTCGAAGC 1044
1001 ACATGTGCCATAGGTCTTATTGTTACAGGTATCGCTGTGCTGCTCGGAGC 1050

1045 CAGAACACACCAGATAGCTCTGCATCCAACCTGGGATTCCGATGTGCAGC 1094
1051 CAGAACACACCTGATAGCTCTGCTTCGAATCTGGGATTCCGCTGTGCAGC 1100

1095 CGACCACCTGCCCACCGCAGAC 1116
1101 CGACCGCCTGCCCACTATGGAC 1122
```

Figure 20B

```
                .         .         .         .         .
  1 MAAPAREPALRCCIRLARVFLLLVLA..CEVAGSDEAEAREGAASLAGSC 48
    |||||       |  |  | |||.|.  |  ||| ||    || ||||||
  1 MAAPALGLVCGRCPELGLVLLLLLLSLLCGAAGSQEAGTGAGAGSLAGSC 50
                .         .         .         .         .
 49 GCGTPQRAGAHGSSAAAQRYSREANAPGLTSGPRPLALTKMVPIPAGVFT 98
    ||||||| ||||.|||| ||||||||||   | | || .|||||| ||||
 51 GCGTPQRPGAHGNSAAAHRYSREANAPGPVPGERQLAHSKMVPIPVGVFT 100
                .         .         .         .         .
 99 MGTDDPQIRQDGEAPARRVTVDGFYMDAYEVSNADFEKFVNSTGYLTEAE 148
    ||||||||:||||||||||||:| |||||||||| :|||||||||||||||
101 MGTDDPQIKQDGEAPARRVTIDAFYMDAYEVSNTEFEKFVNSTGYLTEAE 150
                .         .         .         .         .
149 KFGDSFVFEGMLSEQVKTHIHQAVAAAPWWLPVKGANWRHPEGPDSSILH 198
    |||||||||||||||||.| |||||||||||||||||||||||.|||
151 KFGDSFVFEGMLSEQVKTNIQQAVAAAPWWLPVKGANWRHPEGPDSTILH 200
                .         .         .         .         .
199 RSNHPVLHVSWNDAVAYCTWAGKRLPTEAEWEYSCRGGLQNRLFPWGNKL 248
    | .|||||||||||||||||||||||||||||||||| ||||||||||
201 RPDHPVLHVSWNDAVAYCTWAGKRLPTEAEWEYSCRGGLHNRLFPWGNKL 250
                .         .         .         .         .
249 QPKGQHYANIWQGKFPVSNTGEDGFQGTAPVDAFPPNGYGLYNIVGNVWE 298
    ||||||||||||.|||.|||||||||||||||||||||||||||||| ||
251 QPKGQHYANIWQGEFPVTNTGEDGFQGTAPVDAFPPNGYGLYNIVGNAWE 300
                .         .         .         .         .
299 WTSDWWTVHHSVEETFNPKGPTSGKDRVKKGGSYMCHKSYCYRYRCAARS 348
    |||||||||||||| ||||| ||||||||||||||:||||||||||||
301 WTSDWWTVHHSVEETLNPKGPPSGKDRVKKGGSYMCHRSYCYRYRCAARS 350
                .         .
349 QNTPDSSASNLGFRCAADHLPTAD 372
    |||||||||||||||| ||| |
351 QNTPDSSASNLGFRCAADRLPTMD 374
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Figure 21

Input file hT243.seq; Output File hT243.pat
Sequence length 2811

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  P   V   S   G   R   P   L   P   A   G   S   S   P   A   A   A   R   R   S   P    39
CCG GTC TCC GGC CGC CCC TTA CCT GCA GGC TCT TCT CCC GCC GCG GCC CGG CGC TCT CCG   119

  S   R   P   C   G   L   V   S   H   S   A   W   A   P   G   A   R   Q   T   L    59
AGT CGC CCC TGC GGA CTG GTC TCG CAC AGT GCC TGG GCA CCG GGC GCC AGA CAG ACA CTG   179

  A   M   T   S   G   A   T   R   Y   R   L   S   C   S   L   R   G   H   E   L    79
GCC ATG ACG AGC GGC GCA ACC AGG TAC CGG CTG AGC TGC TCG CTC CGG GGC CAC GAG CTG   239

  D   V   R   G   L   V   C   C   A   Y   P   P   G   A   F   V   S   V   S   R    99
GAC GTA CGG GGC CTG GTG TGC TGC GCC TAT CCG CCG GGA GCC TTT GTG TCC GTG TCC CGA   299

  D   R   T   T   R   L   W   A   P   D   S   P   N   R   S   F   T   E   M   H   119
GAC CGC ACC ACC CGC CTC TGG GCC CCA GAC AGT CCA AAC AGG AGC TTT ACA GAA ATG CAC   359

  C   M   S   G   H   S   N   F   V   S   C   V   C   I   I   P   S   S   D   I   139
TGT ATG AGT GGC CAT TCC AAT TTT GTA TCT TGT GTA TGC ATC ATA CCC TCA AGT GAC ATC   419

  Y   P   H   G   L   I   A   T   G   G   N   D   H   N   I   C   I   F   S   L   159
TAC CCT CAT GGC CTA ATT GCC ACC GGT GGA AAT GAC CAC AAT ATA TGC ATT TTC TCA CTG   479

  D   S   P   M   P   L   Y   I   L   K   G   H   K   N   T   V   C   S   L   S   179
GAC AGT CCA ATG CCA CTT TAT ATT CTA AAA GGC CAC AAA AAT ACT GTT TGT AGT CTA TCA   539

  S   G   K   F   G   T   L   L   S   G   S   W   D   T   T   A   K   V   W   L   199
TCT GGA AAA TTT GGG ACA TTA CTT AGT GGT TCA TGG GAC ACC ACT GCT AAA GTC TGG CTG   599

  N   D   K   C   M   M   T   L   Q   G   H   T   A   A   V   W   A   V   K   I   219
AAT GAC AAG TGC ATG ATG ACC TTG CAG GGT CAT ACA GCT GCA GTG TGG GCG GTA AAG ATC   659

  L   P   E   Q   G   L   M   L   T   G   S   A   D   K   T   V   K   L   W   K   239
TTA CCT GAA CAG GGC TTA ATG TTG ACT GGA TCA GCA GAC AAG ACT GTT AAA CTG TGG AAG   719

  A   G   R   C   E   R   T   F   S   G   H   E   D   C   V   R   G   L   A   I   259
GCT GGA AGA TGT GAG AGG ACT TTT TCA GGG CAT GAA GAC TGT GTA AGA GGT TTG GCA ATT   779

  L   S   E   T   E   F   L   S   C   A   N   D   A   S   I   R   R   W   Q   I   279
TTG AGT GAA ACA GAA TTT CTT TCC TGT GCA AAT GAT GCT AGT ATT AGA AGG TGG CAA ATC   839

  T   G   E   C   L   E   V   Y   Y   G   H   T   N   Y   I   Y   S   I   S   V   299
ACT GGC GAG TGT CTT GAA GTA TAT TAT GGA CAT ACA AAT TAT ATT TAT AGC ATA TCC GTT   899

  F   P   N   C   R   D   F   V   T   T   A   E   D   R   S   L   R   I   W   K   319
TTT CCA AAT TGT AGA GAC TTT GTG ACA ACA GCA GAG GAC AGA TCT CTG AGA ATC TGG AAA   959

  H   G   E   C   A   Q   T   I   R   L   P   A   Q   S   I   W   C   C   C   V   339
CAT GGG GAA TGT GCT CAA ACT ATC CGA CTT CCA GCT CAG TCT ATA TGG TGC TGC TGT GTG  1019
```

Figure 22A

```
 L   D   N   G   D   I   V   V   G   A   S   D   G   I   I   R   V   F   T   E   359
CTC GAC AAT GGT GAC ATT GTG GTT GGT GCG AGT GAT GGC ATT ATT AGA GTG TTT ACA GAA  1079

 S   E   D   R   T   A   S   A   E   E   I   K   A   F   E   K   E   L   S   H   379
TCA GAA GAT CGA ACA GCA AGT GCT GAA GAA ATC AAG GCT TTT GAA AAA GAA CTG TCT CAC  1139

 A   T   I   D   S   K   T   G   D   L   G   D   I   N   A   E   Q   L   P   G   399
GCA ACC ATT GAT TCT AAA ACT GGC GAT TTA GGG GAC ATC AAT GCT GAG CAG CTT CCT GGG  1199

 R   E   H   L   N   E   P   G   T   R   E   G   Q   T   R   L   I   R   D   G   419
AGG GAA CAT CTT AAT GAA CCT GGT ACT AGA GAA GGA CAG ACT CGT CTA ATC AGA GAT GGG  1259

 E   K   V   E   A   Y   Q   W   S   V   S   E   G   R   W   I   K   I   G   D   439
GAG AAA GTC GAA GCC TAT CAG TGG AGT GTT AGT GAA GGG AGG TGG ATA AAA ATT GGT GAT  1319

 V   V   G   S   S   G   A   N   Q   Q   T   S   G   K   V   L   Y   E   G   K   459
GTT GTT GGC TCA TCT GGT GCT AAT CAG CAA ACA TCT GGA AAA GTT TTA TAT GAA GGG AAA  1379

 E   F   D   Y   V   F   S   I   D   V   N   E   G   G   P   S   Y   K   L   P   479
GAA TTT GAT TAT GTT TTC TCA ATT GAT GTC AAT GAA GGT GGA CCA TCA TAT AAA TTG CCA  1439

 Y   N   T   S   D   D   P   W   L   T   A   Y   N   F   L   Q   K   N   D   L   499
TAT AAT ACC AGT GAT GAC CCT TGG TTA ACT GCA TAC AAC TTC TTA CAG AAG AAT GAT TTG  1499

 N   P   M   F   L   D   Q   V   A   K   F   I   I   D   N   T   K   G   Q   M   519
AAT CCT ATG TTT CTG GAT CAA GTA GCT AAA TTT ATT ATT GAT AAC ACA AAA GGT CAA ATG  1559

 L   G   L   G   N   P   S   F   S   D   P   F   T   G   G   G   R   Y   V   P   539
TTG GGA CTT GGG AAT CCC AGC TTT TCA GAT CCA TTT ACA GGT GGT GGT CGG TAT GTT CCG  1619

 G   S   S   G   S   S   N   T   L   P   T   A   D   P   F   T   G   A   G   R   559
GGC TCT TCG GGA TCT TCT AAC ACA CTA CCC ACA GCA GAT CCT TTT ACA GGT GCT GGT CGT  1679

 Y   V   P   G   S   A   S   M   G   T   T   M   A   G   V   D   P   F   T   G   579
TAT GTA CCA GGT TCT GCA AGT ATG GGA ACT ACC ATG GCC GGA GTT GAT CCA TTT ACA GGG  1739

 N   S   A   Y   R   S   A   A   S   K   T   M   N   I   Y   F   P   K   K   E   599
AAT AGT GCC TAC CGA TCA GCT GCA TCT AAA ACA ATG AAT ATT TAT TTC CCT AAA AAA GAG  1799

 A   V   T   F   D   Q   A   N   P   T   Q   I   L   G   K   L   K   E   L   N   619
GCT GTC ACA TTT GAC CAA GCA AAC CCT ACA CAA ATA TTA GGT AAA CTG AAG GAA CTT AAT  1859

 G   T   A   P   E   E   K   K   L   T   E   D   D   L   I   L   L   E   K   I   639
GGA ACT GCA CCT GAA GAG AAG AAG TTA ACT GAG GAT GAC TTG ATA CTT CTT GAG AAG ATA  1919

 L   S   L   I   C   N   S   S   S   E   K   P   T   V   Q   Q   L   Q   I   L   659
CTG TCT CTA ATA TGT AAT AGT TCT TCA GAA AAA CCC ACA GTC CAG CAA CTT CAG ATT TTG  1979

 W   K   A   I   N   C   P   E   D   I   V   F   P   A   L   D   I   L   R   L   679
TGG AAA GCT ATT AAC TGT CCT GAA GAT ATT GTC TTT CCT GCA CTT GAC ATT CTT CGG TTG  2039

 S   I   K   H   P   S   V   N   E   N   F   C   N   E   K   E   G   A   Q   F   699
TCA ATT AAA CAC CCC AGT GTG AAT GAG AAC TTC TGC AAT GAA AAG GAA GGG GCT CAG TTC  2099
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Figure 22B

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 S   S   H   L   I   N   L   L   N   P   K   G   K   P   A   N   Q   L   L   A    719
AGC AGT CAT CTT ATC AAT CTT CTG AAC CCT AAA GGA AAG CCA GCA AAC CAG CTG CTT GCT  2159

 L   R   T   F   C   N   C   F   V   G   Q   A   G   Q   K   L   M   M   S   Q    739
CTC AGG ACT TTT TGC AAT TGT TTT GTT GGC CAG GCA GGA CAA AAA CTC ATG ATG TCC CAG  2219

 R   E   S   L   M   S   H   A   I   E   L   K   S   G   S   N   K   N   I   H    759
AGG GAA TCA CTG ATG TCC CAT GCA ATA GAA CTG AAA TCA GGG AGC AAT AAG AAC ATT CAC  2279

 I   A   L   A   T   L   A   L   N   Y   S   V   C   F   H   K   D   H   N   I    779
ATT GCT CTG GCT ACA TTG GCC CTG AAC TAT TCT GTT TGT TTT CAT AAA GAC CAT AAC ATT  2339

 E   G   K   A   Q   C   L   S   L   I   S   T   I   L   E   V   V   Q   D   L    799
GAA GGG AAA GCC CAA TGT TTG TCA CTA ATT AGC ACA ATC TTG GAA GTA GTA CAA GAC CTA  2399

 E   A   T   F   R   L   L   V   A   L   G   T   L   I   S   D   D   S   N   A    819
GAA GCC ACT TTT AGA CTT CTT GTG GCT CTT GGA ACA CTT ATC AGT GAT GAT TCA AAT GCT  2459

 V   Q   L   A   K   S   L   G   V   D   S   Q   I   K   K   Y   S   S   V   S    839
GTA CAA TTA GCC AAG TCT TTA GGT GTT GAT TCT CAA ATA AAA AAG TAT TCC TCA GTA TCA  2519

 E   P   A   K   V   S   E   C   C   R   F   I   L   N   L   L   *                856
GAA CCA GCT AAA GTA AGT GAA TGC TGT AGA TTT ATC CTA AAT TTG CTG TAG              2570

CAGTGGGGAAGAGGGACGGATATTTTTAATTGATTAGTGTTTTTTTCCTCACATTTGACATGACTGATAACAGATAATT  2649

AAAAAAAGAGAATACGGTGGATTAAGTAAAATTTTACATCTTGTAAAGTGGTGGGGAGGGGAAACAGAAATAAAATTTT  2728

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AAAA                                                                             2811
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Figure 22C

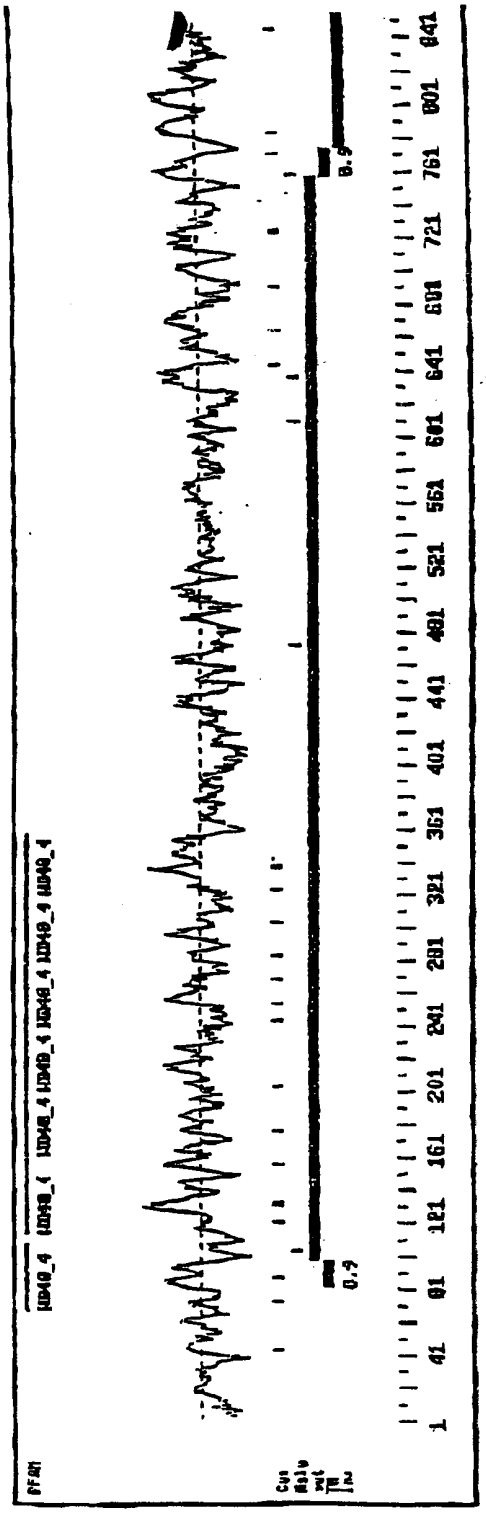

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Figure 23