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(54) METHODS FOR ISOLATING NOVEL ANTIMICROBIAL AGENTS FROM HYPERMUTABLE MAMMALIAN CELLS

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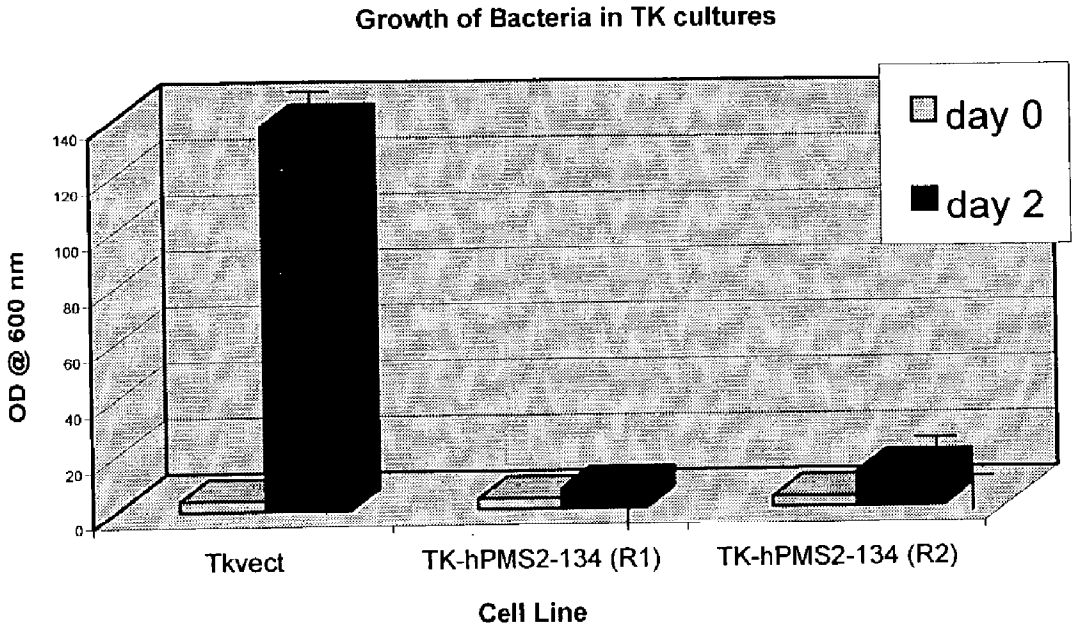
(57) **ABSTRACT**

Dominant-negative alleles of human mismatch repair genes can be used to generate hypermutable cells and organisms. By introducing these genes into mammalian cells new cell lines with novel and useful properties can be prepared more efficiently than by relying on the natural rate of mutation or introduction of mutations by chemical mutagens. These methods are useful for generating novel and highly active antimicrobial molecules as well as superior antimicrobial agents from pre-existing chemicals. These methods are also useful for generating cell lines expressing novel antimicrobials that are useful for pharmaceutical manufacturing.



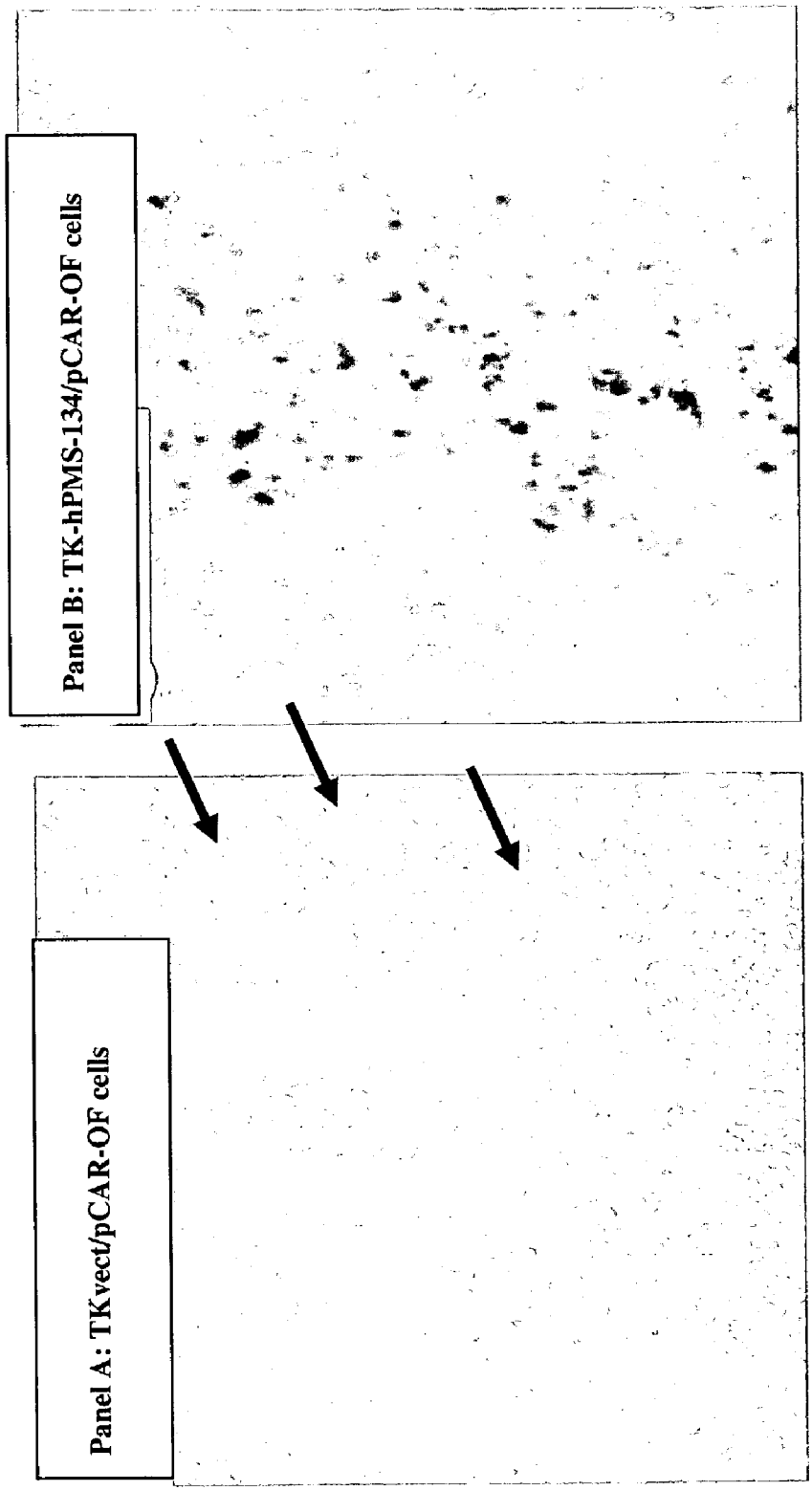
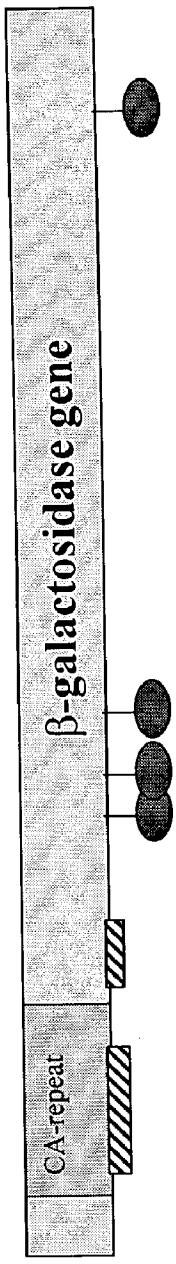


Figure 1



β-galactosidase gene

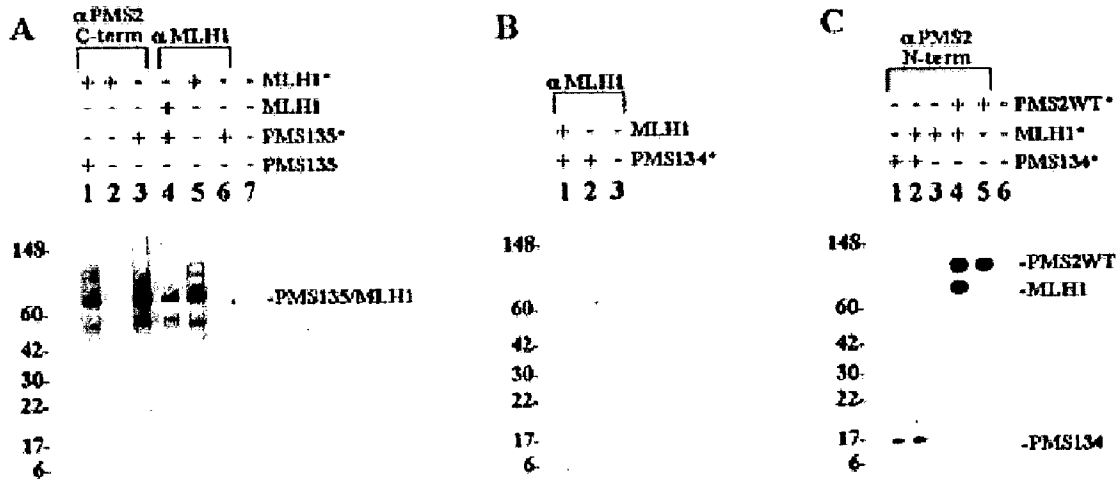
CA-repeat

= Insertion/Deletion alterations

= Point mutations

Figure 2

Figure 3


A
αPMS2 C-term
αMLH1
+ + - - + - - MLH1*
- - - + - - - MLH1
- - + + - + - PMS135*
+ - - - - - - PMS135
1 2 3 4 5 6 7
148
60
42
30
22
17
6
-PMS135/MLH1
B
αMLH1
+ - - MLH1
+ + - PMS134*
1 2 3
148
60
42
30
22
17
6
C
αPMS2 N-term
- - - + + - PMS2WT*
- + + + - - MLH1*
+ + - - - - PMS134*
1 2 3 4 5 6
148
60
42
30
22
17
6
-PMS2WT
-MLH1
-PMS134

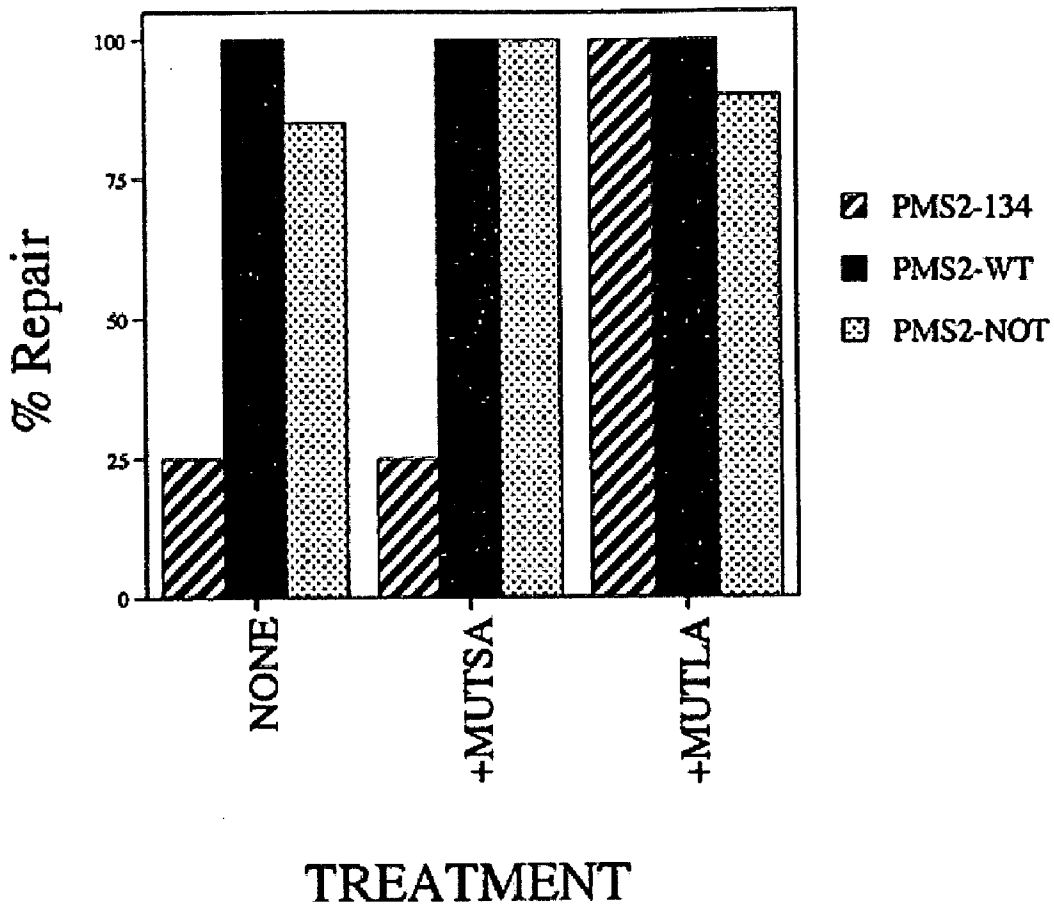

% Repair
100
75
50
25
0
NONE
+MUTSA
+MUTLA
TREATMENT
PMS2-134
PMS2-WT
PMS2-NOT

Figure 4

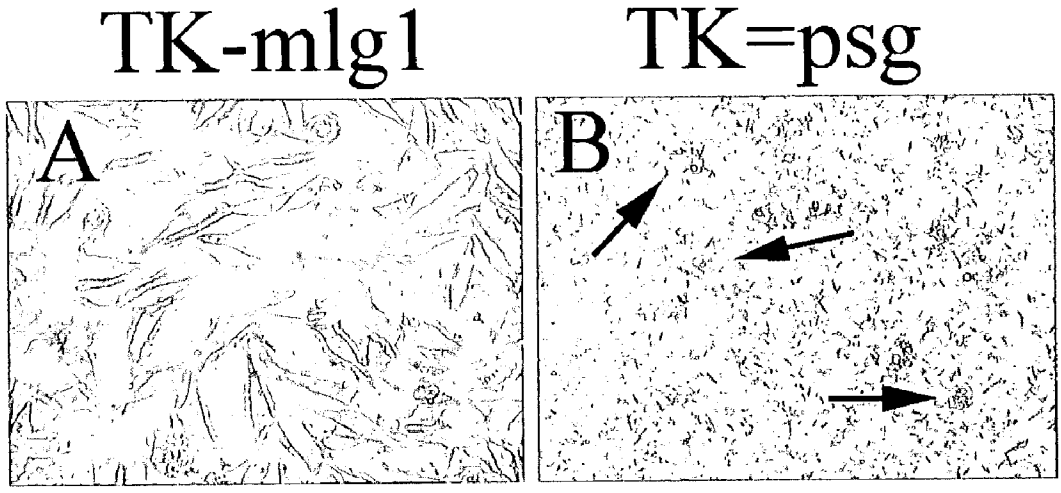


Figure 5

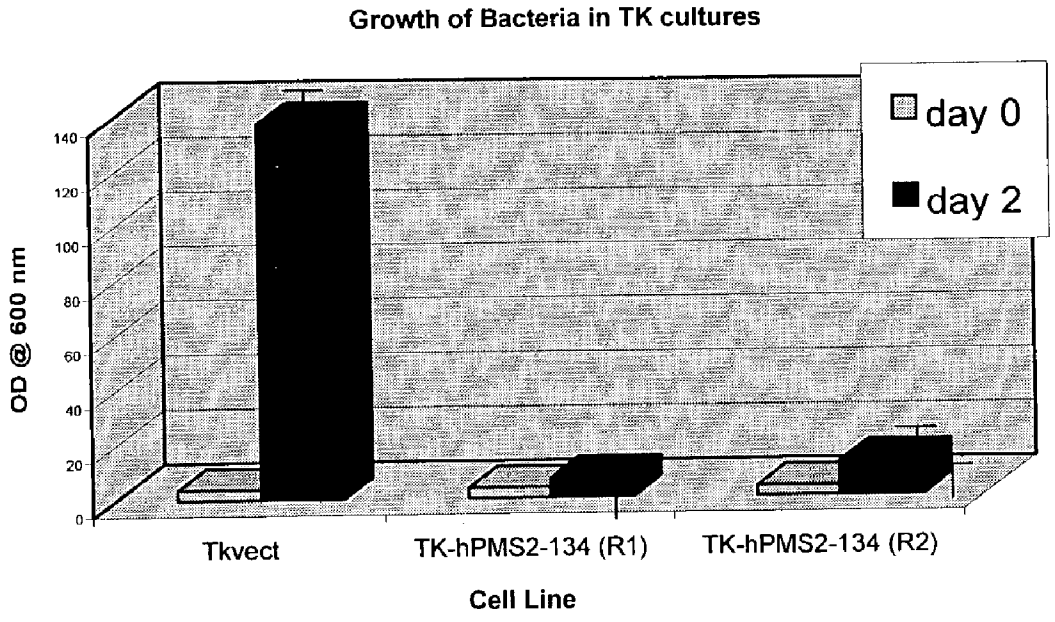

Growth of Bacteria in TK cultures
day 0
day 2
OD @ 600 nm
140
120
100
80
60
40
20
0
Tkvect
TK-hPMS2-134 (R1)
TK-hPMS2-134 (R2)
Cell Line

Figure 6

METHODS FOR ISOLATING NOVEL ANTIMICROBIAL AGENTS FROM HYPERMUTABLE MAMMALIAN CELLS

FIELD OF THE INVENTION

[0001] The present invention is related to the area of antimicrobial agents and cellular production of those agents. In particular, it is related to the field of identification of novel antimicrobial agents by placing mammalian cells under selection in the presence of the microbe.

BACKGROUND OF THE INVENTION

[0002] For as long as man has shared the planet with microorganisms there have been widespread outbreaks of infectious disease and subsequent widespread mortality associated with it. Although microorganisms and man frequently share a symbiotic relationship, microorganisms can, under some conditions, lead to sickness and death. The discovery, wide use and dissemination of antibiotics to treat microbial infection in both human and animal populations over the last one hundred or so years has done much to control, and in some instances, eradicate some microbes and associated infectious disease. However, microbes have a strong propensity to evolve and alter their genetic makeup when confronted with toxic substances that place them under life and death selective pressures. Therefore, emerging infectious diseases currently pose an important public health problem in both developed as well as developing countries. Not only have microbes evolved to evade and defeat current antibiotic therapeutics, but also there are novel and previously unrecognized and/or characterized bacterial, fungal, viral, and parasitic diseases that have emerged within the past two decades. Sass, Curr. Opin. in *Drug Discov. & Develop.* 2000, 3(5):646-654.

[0003] Since the accidental discovery of a penicillin-producing mold by Fleming there has been steady progress in synthesizing, isolating and characterizing new and more effective beta-lactam antibiotics. In addition to the great success of the beta-lactam family of antibiotics, the newer fluoroquinolones have a broad-spectrum of bactericidal activity as well as excellent oral bio-availability, tissue penetration and favorable safety and tolerability profiles. King et al., *Am. Fam. Physician,* 2000, 61, 2741-2748. A newly devised four-generation classification of the quinolone drugs accounts for the expanded antimicrobial spectrum of the more recently introduced fluoroquinolones and their clinical indications. The so-called first generation drugs, which include nalidixic acid, are capable of achieving minimal serum levels. The second-generation quinolones, such as ciprofloxacin, have an increased gram-negative and systemic activity. The third-generation drugs comprise pharmaceuticals such as levofloxacin and are have significant and expanded action against gram-positive bacteria and a typical pathogens. Finally, the fourth-generation quinolone drugs, which, to date, only includes trovofloxacin, are highly active against anaerobes in addition to the activity described for the third-generation drugs. Furthermore, the quinolone class of anti-microbial drugs can be divided based on their pharmacokinetic properties and bioavailability.

[0004] Mammalian epithelial surfaces are remarkable for their ability to provide critical physiologic functions in the face of frequent microbial challenges. The fact that these mucosal surfaces remain infection-free in the normal host suggests that highly effective mechanisms of host defense have evolved to protect these environmentally exposed tissues. Throughout the animal and plant kingdoms, endogenous genetically encoded antimicrobial peptides have been shown to be key elements in the response to epithelial compromise and microbial invasion. Zasloff, *Curr. Opin. Immunol.,* 1992, 4, 3-7; and Bevins, *Ciba Found Symp.,* 1994, 186, 250-69. In mammals, a variety of such peptides have been identified, including the well-characterized defensins and cathelicidins and others (andropin, magainin, tracheal antimicrobial peptide, and PR-39; see Bevins, *Ciba Found. Symp.,* 1994, 186, 250-69 and references therein). A major source of these host defense molecules is circulating phagocytic leukocytes. However, more recently, it has been shown that resident epithelial cells of the skin and respiratory, alimentary, and genitourinary tracts also synthesize and release antimicrobial peptides. Both in vitro and in vivo data support the hypothesis that these molecules are important contributors to intrinsic mucosal immunity. Alterations in their level of expression or biologic activity can predispose the organism to microbial infection. Huttner et al., *Pediatr. Res.,* 1999, 45, 785-94.

[0005] Across the evolutionary scale species from insects to mammals to plants defend themselves against invading pathogenic microorganisms by utilizing cationic antimicrobial peptides that rapidly kill microbes without exerting toxicity to the host. Physicochemical peptide-lipid interactions provide attractive mechanisms for innate immunity as discussed below. Many of these peptides form cationic amphipathic secondary structures, typically alpha-helices and beta-sheets, which can selectively interact with anionic bacterial membranes via electrostatic interactions. Rapid, peptide-induced membrane permeabilization and subsequent cellular lysis is the result. Matsuzaki, *Biochim. Biophys. Acta,* 1999, 1462, 1-10.

[0006] The primary structures of a large number of these host-defense peptides have been determined. While there is no primary structure homology, the peptides are characterized by a preponderance of cationic and hydrophobic amino acids. The secondary structures of many of the host-defense peptides have been determined by a variety of techniques. Sitaram et al., *Biochim, Biophys. Acta,* 1999, 1462, 29-54. The acyclic peptides tend to adopt helical conformation, especially in media of low dielectric constant, whereas peptides with more than one disulfide bridge adopt beta-structures.

[0007] As described above, one reason for the rise in microbial drug resistance to the first line antimicrobial therapies in standard use today is the inappropriate and over-use of prescription antibiotics. Although bacteria are the most common organisms to develop drug-resistance, there are numerous examples of demonstrated resistance in fungi, viruses, and parasites. The development of a resistant phenotype is a complex phenomenon that involves an interaction of the microorganism, the environment, and the patient, separately as well as in combination. Sitaram et al., *Biochim. Biophys. Acta,* 1999, 1462, 29-54. The microorganism in question may develop resistance while under antibiotic selection or it may be a characteristic of the microbe prior to exposure to a given agent. There are a number of mechanisms of resistance to antibiotics that have been described, including genes that encode antibiotic resis-

tance enzymes that are harbored on extrachromosomal plasmids as well as DNA elements (e.g. transposable elements) that can reside either extra-chromosomally or within the host genome.

[0008] Due to the ability of microorganisms to acquire the ability to develop resistance to antibiotics there is a need to continually develop novel antibiotics. Traditional methods to develop novel antibiotics have included medicinal chemistry approaches to modify existing antibiotics (Kang et al., *Bioorg. Med. Chem. Lett.,* 2000, 10, 95-99) as well as isolation of antibiotics from new organisms (Alderson et al., *Res. Microbiol.,* 1993, 144, 665-72). Each of these methods, however, has limitations. The traditional medicinal chemistry approach entails modification of an existing molecule to impart a more effective activity. The chemist makes a "best guess" as to which parts of the molecule to alter, must then devise a synthetic strategy, synthesize the molecule, and then have it tested. This approach is laborious, requires large numbers of medicinal chemists and frequently results in a molecule that is lower in activity than the original antibiotic. The second approach, isolation of novel antimicrobial agents, requires screening large numbers of diverse organisms for novel antimicrobial activity. Then, the activity must be isolated from the microorganism. This is not a small task, and frequently takes many years of hard work to isolate the active molecule. Even after the molecule is identified, it may not be possible for medicinal chemists to effectively devise a synthetic strategy due to the complexity of the molecule. Furthermore, the synthetic strategy must allow for a cost-effective synthesis. Therefore, a method that would allow for creation of more effective antibiotics from existing molecules or allow rapid isolation of novel antimicrobial agents is needed to combat the ever-growing list of antibiotic resistant organisms. The present invention described herein is directed to the use of random genetic mutation of a cell to produce novel antibiotics by blocking the endogenous mismatch repair activity of a host cell. The cell can be a mammalian cell that produces an antimicrobial agent naturally, or a cell that is placed under selective pressure to obtain a novel antimicrobial molecule that attacks a specific microbe. Moreover, the invention describes methods for obtaining enhanced antimicrobial activity of a cell line that produces an antimicrobial activity due to recombinant expression or as part of the innate capacity of the cell to harbor such activity.

[0009] In addition, the generation of genetically altered host cells that are capable of secreting an antimicrobial activity, which can be protein or non-protein based, will be valuable reagents for manufacturing the entity for clinical studies. An embodiment of the invention described herein is directed to the creation of genetically altered host cells with novel and/or increased antimicrobial production that are generated by a method that interferes with the highly ubiquitous and phylogenetically conserved process of mismatch repair.

[0010] The present invention facilitates the generation of novel antimicrobial agents and the production of cell lines that express elevated levels of antimicrobial activity. Advantages of the present invention are further described in the examples and figures described herein.

SUMMARY OF THE INVENTION

[0011] One embodiment of the present invention provides a method for generating genetically altered mammalian cells and placing the cells under direct microbial selection as a means to isolate novel antimicrobial agents. Another embodiment provides a method for identifying novel microbe-specific toxic molecules by altering the ability of the cell to correct natural defects that occur in the DNA during the process of DNA replication. Interference with this process, called mismatch repair, leads to genetically dissimilar sibling cells. These genetically dissimilar cells contain mutations, ranging from one mutation/genome to two or more mutations/genome, offer a rich population of cells from which to select for specific output traits, such as the novel ability to resist microbial insult. The genetically altered cell generated by manipulation of the mismatch repair process is then incubated with a microbe that is normally toxic to cells. Most of the cells will rapidly lose viability and die; however, a subset of resistant cells will have the capacity to resist the microbial insult. These cells express a molecule, protein or non-protein in structure, that imbues an antimicrobial activity to the newly selected mammalian clones. These newly created cells can be expanded in vitro and the new molecule isolated and characterized by standard methods that are well described it the art. The novel molecule(s) are then tested for their ability to kill or inhibit the growth of the microbe by standard microbial assays that are well described in the art. Finally, the novel cell line generated serves as an additional resource for large-scale production of the novel antimicrobial agent for use in clinical studies. The processes described herein are applicable to any mammalian cell and any microbe for which an antibiotic agent is sought.

[0012] The invention provides methods for rendering mammalian cells hypermutable as a means to generate antimicrobial agents.

[0013] The invention also provides methods for generating genetically altered cell lines that secrete enhanced amounts of a known or novel antimicrobial polypeptide.

[0014] The invention also provides methods for generating genetically altered cell lines that secrete enhanced amounts of a known or novel antimicrobial non-polypeptide based molecule.

[0015] The invention also provides methods for generating genetically altered cell lines that do not secrete enhanced amounts of an antimicrobial peptide or non-peptide molecule but rather have a cell-surface active molecule that detoxifies the microbe under test.

[0016] The invention also provides methods for producing an enhanced rate of genetic hypermutation in a mammalian cell and use of this as the basis to select for microbial-resistant cell lines.

[0017] The invention also provides methods of mutating a known antimicrobial encoding gene of interest in a mammalian cell as a means to obtain a molecule with enhanced bactericidal activity.

[0018] The invention also provides methods for creating genetically altered antimicrobial molecules in vivo.

[0019] The invention also provides methods for creating novel antimicrobial molecules from preexisting antimicrobial molecules by altering the innate enzymatic or binding ability of the molecules by altering the mismatch repair system within the host mammalian cell.

[0020] The invention also provides methods for creating a novel anti-microbial polypeptide or non-polypeptide based molecule that has the capacity to bind in an irreversible manner to a microbe and thereby block binding of the pathogenic microbe to a host target organism and result in loss of viability of the microbe.

[0021] The invention also provides methods for creating a novel antimicrobial polypeptide or non-polypeptide based small molecule that can block microbial cell growth and/or survival.

[0022] The invention also provides methods for creating a novel antimicrobial polypeptide or non-polypeptide based biochemical that are able to irreversibly bind to toxic chemicals produced by pathogenic microbes.

[0023] The invention also provides methods for creating genetically altered antimicrobial molecules, either peptide of non-peptide based, that have enhanced pharmacokinetic properties in host organisms.

[0024] The invention also provides methods for creating genetically altered cell lines that manufacture an antimicrobial molecules, either peptide of non-peptide based, for use in large-scale production of the antimicrobial agent for clinical studies.

[0025] These and other aspects of the invention are described in the embodiments below. In one embodiment of the invention described, a method for making a microbial-sensitive mammalian cell microbe resistant by rendering the cell line hypermutable is provided. A polynucleotide encoding a dominant negative allele of a mismatch repair gene is introduced into an mammalian cell. The cell becomes hypermutable as a result of the introduction of the gene.

[0026] In another embodiment of the invention, an isolated hypermutable cell is provided. The cell comprises a dominant negative allele of a mismatch repair gene. The cell exhibits an enhanced rate of hypermutation.

[0027] In another embodiment of the invention, an isolated hypermutable cell is provided. The cell comprises a dominant negative allele of a mismatch repair gene. The cell exhibits an enhanced rate of hypermutation. The populations of cells generated by introduction of the mismatch repair gene are grown in the presence of microbes that are toxic to the wild type non-mutant cells. Cells are selected that are resistant to the microbe and the novel molecule(s) isolated and characterized for antimicrobial activity by standard methods well described in the art.

[0028] In another embodiment of the invention, an isolated hypermutable cell is described to create a novel antimicrobial molecule from a pre-existing antimicrobial molecule by altering the innate enzymatic or binding ability of the molecule.

[0029] In another embodiment of the invention, a method of creating a novel antimicrobial polypeptide or non-polypeptide based molecule that has the capacity to bind in an irreversible manner to a microbe and thereby block binding of the pathogenic microbe to a host target organism and result in loss of viability of the microbe.

[0030] In another embodiment of the invention, a method of creating a novel antimicrobial polypeptide or non-polypeptide based small molecule that can block microbial cell growth and/or survival is described.

[0031] In another embodiment of the invention, a method of creating a novel antimicrobial polypeptide or non-polypeptide based biochemical that are able to irreversibly bind to toxic chemicals produced by pathogenic microbes is described.

[0032] In another embodiment of the invention, a method is provided for introducing a mutation into a known endogenous gene encoding for an antimicrobial polypeptide or a non-protein based antimicrobial molecule as a means to create a more efficacious antimicrobial. A polynucleotide encoding a dominant negative allele of a mismatch repair gene is introduced into a cell. The cell becomes hypermutable as a result of the introduction of the gene. The cell further comprises an antimicrobial gene(s) of interest. The cell is grown and tested to determine whether the gene encoding for an antimicrobial is altered and whether the novel molecule is more active by standard microbiology assays well known in the art.

[0033] In another embodiment of the invention, a gene or genes encoding for an antimicrobial molecule is introduced into a mammalian cell host that is mismatch repair defective. The cell is grown, and then clones are analyzed for enhanced antimicrobial characteristics.

[0034] In another embodiment of the invention, a method is provided for producing new phenotypes of a cell. A polynucleotide encoding a dominant negative allele of a mismatch repair gene is introduced into a cell. The cell becomes hypermutable as a result of the introduction of the gene. The cell is grown and tested for the expression of new phenotypes where the phenotype is enhanced secretion of a novel or known antimicrobial polypeptide.

[0035] In another embodiment of the invention, a method is provided for producing new phenotypes of a cell. A polynucleotide encoding a dominant negative allele of a mismatch repair gene is introduced into a cell. The cell becomes hypermutable as a result of the introduction of the gene. The cell is grown and tested for the expression of new phenotypes where the phenotype is enhanced secretion of a novel or known antimicrobial non-polypeptide based molecule.

[0036] In another embodiment of the invention, a method is provided for producing new phenotypes of a cell. A polynucleotide encoding a dominant negative allele of a mismatch repair gene is introduced into a cell. The cell becomes hypermutable as a result of the introduction of the gene. The cell is grown and tested for the expression of new phenotypes where the phenotype is enhanced antimicrobial activity of a novel or known antimicrobial polypeptide that is not secreted.

[0037] In another embodiment of the invention, a method is provided for producing new phenotypes of a cell. A polynucleotide encoding a dominant negative allele of a mismatch repair gene is introduced into a cell. The cell becomes hypermutable as a result of the introduction of the gene. The cell is grown and tested for the expression of new phenotypes where the phenotype is enhanced antimicrobial activity of a novel or known antimicrobial non-polypeptide based molecule that is not secreted.

[0038] In another embodiment of the invention, a method is provided for restoring genetic stability in a cell containing a polynucleotide encoding a dominant negative allele of a mismatch repair gene. The expression of the dominant negative mismatch repair gene is suppressed and the cell is restored to its former genetic stability.

[0039] In another embodiment of the invention, a method is provided for restoring genetic stability in a cell containing a polynucleotide encoding a dominant negative allele of a mismatch repair gene and a newly selected phenotype. The expression of the dominant negative mismatch repair gene is suppressed and the cell restores its genetic stability and the new phenotype is stable.

[0040] These and other embodiments of the invention provide the art with methods that generate enhanced mutability in cells and animals as well as providing cells and animals harboring potentially useful mutations and novel protein and non-protein based molecules.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] **FIG. 1** is a representative in situ β-galactosidase staining of TK-hPMS2-134 or TKvect cells to measure for cells containing genetically altered β-galactosidase genes; arrows indicate Blue (β-galactosidase positive) cells.

[0042] **FIG. 2** is a schematic representation of sequence of alterations of the β-galactosidase gene produced by expression of TK-hPMS2-134 host cells in TK cells.

[0043] **FIGS. 3A, 3B** and 3C show a representative immunoprecipitation of in vitro translated hPMS2 and hMLH1 proteins.

[0044] **FIG. 4** shows representative complementation of MMR activity in transduced SH cells.

[0045] **FIG. 5** is a representative photograph of Syrian hamster TK-ts 13 cells transfected with a eukaryotic expression vector that produces a novel anti-microbial polypeptide.

[0046] **FIG. 6** is a representative graph showing TK-HPMS-134 transfected TK cells can suppress the growth of bacteria in vitro.

[0047] The presented invention is directed to, in part, methods for developing hypermutable mammalian cells by taking advantage of the conserved mismatch repair process of host cells. Mismatched repair process is described in several references. Baker et al., *Cell,* 1995, 82, 309 319; Bronner et al., *Nature,* 1994, 368, 258 261; de Wind et al., *Cell,* 1995, 82, 321 330; and Drummond et al., *Science,* 1995, 268, 1909 1912. Dominant negative alleles of such genes, when introduced into cells or transgenic animals, increase the rate of spontaneous mutations by reducing the effectiveness of DNA repair and thereby render the cells or animals hypermutable. Hypermutable cells or animals can then be utilized to develop new mutations in a gene of interest or in a gene whose function has not been previously described. Blocking mismatch repair in cells such as, for example, mammalian cells or mammalian cells transfected with genes encoding for specific antimicrobial peptides or non-peptide based antimicrobials, can enhance the rate of mutation within these cells leading to clones that have novel or enhanced antimicrobial activity or production and/or cells that contain genetically altered antimicrobials with enhanced biochemical activity against a range of opportunistic microbes.

[0048] The process of mismatch repair, also called mismatch proofreading, is carried out by protein complexes in cells ranging from bacteria to mammalian cells. Modrich, *Science,* 1994, 266, 1959 1960. A mismatch repair gene is a gene that encodes for one of the proteins of such a mismatch repair complex. Baker et. al., Cell, 1995, 82, 309 319; Bronner et al., *Nature,* 1994, 368, 258 261; de Wind et al., *Cell,* 1995, 82, 321 330; Drummond et al., *Science,* 1995, 268, 1909 1912; and Modrich, *Science,* 1994, 266, 1959 1960. Although not wanting to be bound by any particular theory of mechanism of action, a mismatch repair complex is believed to detect distortions of the DNA helix resulting from non-complementary pairing of nucleotide bases. The non-complementary base on the newer DNA strand is excised, and the excised base is replaced with the appropriate base that is complementary to the older DNA strand. In this way, cells eliminate many mutations, which occur as a result of mistakes in DNA replication.

[0049] Dominant negative alleles cause a mismatch repair defective phenotype even in the presence of a wild-type allele in the same cell. An example of a dominant negative allele of a mismatch repair gene is the human gene hPMS2-134, which carries a truncation mutation at codon 134. Nicolaides et al., *Mol. Cell. Biol.,* 1998, 18, 1635-1641. The mutation causes the product of this gene to abnormally terminate at the position of the 134th amino acid, resulting in a shortened polypeptide containing the N-terminal 133 amino acids. Such a mutation causes an increase in the rate of mutations that accumulate in cells after DNA replication. Expression of a dominant negative allele of a mismatch repair gene results in impairment of mismatch repair activity, even in the presence of the wild-type allele. Any allele, which produces such effect, can be used in this invention.

[0050] Dominant negative alleles of a mismatch repair gene can be obtained from the cells of humans, animals, yeast, bacteria, or other organisms. Prolla et al., *Science,* 1994, 264, 1091 1093; Strand et al., *Nature,* 1993, 365, 274 276; and Su et al., *J. Biol. Chem.,* 1988, 263, 6829 6835. Screening cells for defective mismatch repair activity can identify such alleles. Cells from animals or humans with cancer can be screened for defective mismatch repair. Cells from colon cancer patients may be particularly useful. Parsons et al., *Cell,* 1993, 75, 1227 1236; and Papadopoulos et al., *Science,* 1993, 263, 1625 1629. Genomic DNA, cDNA, or mRNA from any cell encoding a mismatch repair protein can be analyzed for variations from the wild type sequence. Perucho, *Biol. Chem.,* 1996, 377, 675 684. Dominant negative alleles of a mismatch repair gene can also be created artificially, for example, by producing variants of the hPMS2-134 allele or other mismatch repair genes. Various techniques of site-directed mutagenesis can be used. The suitability of such alleles, whether natural or artificial, for use in generating hypermutable cells or animals can be evaluated by testing the mismatch repair activity caused by the allele in the presence of one or more wild-type alleles, to determine if it is a dominant negative allele.

[0051] A cell or an animal into which a dominant negative allele of a mismatch repair gene has been introduced will become hypermutable. This means that the spontaneous mutation rate of such cells or animals is elevated compared to cells or animals without such alleles. The degree of elevation of the spontaneous mutation rate can be at least

2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, 200-fold, 500-fold, or 1000-fold that of the normal cell or animal.

[0052] According to one aspect of the invention, a polynucleotide encoding a dominant negative form of a mismatch repair protein is introduced into a cell. The gene can be any dominant negative allele encoding a protein that is part of a mismatch repair complex, for example, PMS2, PMS1, MLH1, or MSH2. The dominant negative allele can be naturally occurring or made in the laboratory. The polynucleotide can be in the form of genomic DNA, cDNA, RNA, or a chemically synthesized polynucleotide.

[0053] The polynucleotide can be cloned into an expression vector containing a constitutively active promoter segment (such as but not limited to CMV, SV40, Elongation Factor or LTR sequences) or to inducible promoter sequences such as the steroid inducible pIND vector (InVitrogen), where the expression of the dominant negative mismatch repair gene can be regulated. The polynucleotide can be introduced into the cell by transfection.

[0054] Transfection is any process whereby a polynucleotide is introduced into a cell. The process of transfection can be carried out in a living animal, e.g., using a vector for gene therapy, or it can be carried out in vitro, e.g., using a suspension of one or more isolated cells in culture. The cell can be any type of eukaryotic cell, including, for example, cells isolated from humans or other primates, mammals or other vertebrates, invertebrates, and single celled organisms such as protozoa, yeast, or bacteria.

[0055] In general, transfection will be carried out using a suspension of cells, or a single cell, but other methods can also be applied as long as a sufficient fraction of the treated cells or tissue incorporates the polynucleotide so as to allow transfected cells to be grown and utilized. The protein product of the polynucleotide may be transiently or stably expressed in the cell. Techniques for transfection are well known and available techniques for introducing polynucleotides include but are not limited to electroporation, transduction, cell fusion, the use of calcium chloride, and packaging of the polynucleotide together with lipid for fusion with the cells of interest. Once a cell has been transfected with the mismatch repair gene, the cell can be grown and reproduced in culture. If the transfection is stable, such that the gene is expressed at a consistent level for many cell generations, then a cell line results.

[0056] An isolated cell is a cell obtained from a tissue of humans or animals by mechanically separating out individual cells and transferring them to a suitable cell culture medium, either with or without pretreatment of the tissue with enzymes, e.g., collagenase or trypsin. Such isolated cells are typically cultured in the absence of other types of cells. Cells selected for the introduction of a dominant negative allele of a mismatch repair gene may be derived from a eukaryotic organism in the form of a primary cell culture or an immortalized cell line, or may be derived from suspensions of single-celled organisms.

[0057] The invention described herein is useful for creating microbial-resistant mammalian cells that secrete new antimicrobial biochemical agents, either protein or non-protein in nature. Furthermore, the invention can be applied to cell lines that express known antimicrobial agents as a means to enhance the biochemical activity of the antimicrobial agent.

[0058] Once a transfected cell line has been produced, it can be used to generate new mutations in one or more gene(s) of interest or in genes that have not been previously described. A gene of interest can be any gene naturally possessed by the cell line or introduced into the cell line by standard methods known in the art. An advantage of using transfected cells or to induce, mutation(s) in a gene or genes of interest that encode antimicrobial activity is that the cell need not be exposed to mutagenic chemicals or radiation, which may have secondary harmful effects, both on the object of the exposure and on the workers. Furthermore, it has been demonstrated that chemical and physical mutagens are base pair specific in the way they alter the structure of DNA; the invention described herein results in mutations that are not dependent upon the specific nucleotide or a specific string of nucleotides and is a truly random genetic approach. Therefore, use of the present invention to obtain mutations in novel or known antimicrobial genes will be much more efficient and have a higher likelihood of success in contrast to conventional mutagenesis with chemical or irradiation. Once a new antimicrobial trait is identified in a sibling cell, the dominant negative allele can be removed from the cell by a variety of standard methods known in the art. For example, the gene can be directly knocked out the allele by technologies used by those skilled in the art or use of a inducible expression system; the dominant-negative allele is driven by a standard promoter that is regulated by inclusion of an inducer, withdrawal of the inducer results in attenuation of the expression of the dominant negative mismatch repair mutant and a normal DNA repair process will ensue.

[0059] New antimicrobial agents are selected from cells that have been exposed to the dominant negative mismatch repair process followed by incubating the mutant cells in the presence of the microbe for which an novel antimicrobial agent is sought. The novel antimicrobial agent is purified by standard methods known to those skilled in the art and characterized. The antimicrobial agents are re-screened to determine the specific activity of the novel antimicrobial as well as tested against a broad range of microbes to determine spectrum of activity. The gene(s) that encode the novel antimicrobial are isolated by standard well known methods to those in the art. The mutations can be detected by analyzing for alterations in the genotype of the cells by examining the sequence of genomic DNA, cDNA, messenger RNA, or amino acids associated with the gene of interest. A mutant polypeptide can be detected by identifying alterations in electrophoretic mobility, spectroscopic properties, or other physical or structural characteristics of a protein encoded by a mutant gene when the cell that has undergone alteration encodes a known antimicrobial that is altered by the means described in the current invention to obtain a more efficacious antimicrobial. Examples of mismatch repair proteins and nucleic acid sequences include the following:

PMS2 (mouse) (SEQ ID NO:7)

MEQTEGVSTE CAKAIKPIDG KSVHQICSGQ VILSLSTAVK ELIENSVDAG ATTIDLRLKD 60

YGVDLTEVSD NGCGVEEENF EGLALKHHTS KIQEFADLTQ VETFGERGEA LSSLCALSDV 120

TISTCHGSAS VGTRLVFDHN GKITQKTPYP RPKGTTVSVQ HLFYTLPVRY KEFQRNIKKE 180

YSKMVQVLQA YCIISAGVRV SCTNQLGQGK RHAVVCTSGT SGMKENIGSV FGQKQLQSLI 240

PFVQLPPSDA VCEEYGLSTS GRHKTFSTFR ASFESARTAP GGVQQTGSFS SSIRGPVTQQ 300

RSLSLSMRFY HMYNRHQYPF VVLNVSVDSE CVDINVTPDK RQILLQEEKL LLAVLKTSLI 360

GMFDSDANKL NVNQQPLLDV EGNLVKLHTA ELEKPVPGKQ DNSPSLKSTA DEKRVASISR 420

LREAFSLHPT KEIKSRGPET AELTRSFPSE KRGVLSSYPS DVISYRGLRG SQDKLVSPTD 480

SPGDCMDREK IEKDSGLSST SAGSEEEFST PEVASSFSSD YNVSSLEDRP SQETINCGDL 540

DCRPPGTGQS LKPEDHGYQC KALPLARLSP TNAKRFKTEE RPSNVNISQR LPGPQSTSAA 600

EVDVAIKMNK RIVLLEFSLS SLAKRMKQLQ HLKAQNKHEL SYRKFRAKIC PGENQAAEDE 660

LRKEISKSMF AEMEILGQFN LGFTVTKLKE DLFLVDQHAA DEKYNFEMLQ QHTVLQAQRL 720

ITPQTLNLTA VNEAVLIENL EIFRKNGFDF VTDEDAPVTE RAKLISLPTS KNWTFGPQDI 780

DELIFMLSDS PGVMCRPSRV RQMFASRACR KSVMIGTALN ASEMKKLITH MGEMDHPWNC 840

PHGRPTMRHV ANLDVISQN 859

PMS2 (mouse cDNA) (SEQ ID NO:8)

gaattccggt gaaggtcctg aagaatttcc agattcctga gtatcattgg aggagacaga 60

taacctgtcg tcaggtaacg atggtgtata tgcaacagaa atgggtgttc ctggagacgc 120

gtcttttccc gagagcggca ccgcaactct cccgcggtga ctgtgactgg aggagtcctg 180

catccatgga gcaaaccgaa ggcgtgagta cagaatgtgc taaggccatc aagcctattg 240

atgggaagtc agtccatcaa atttgttctg ggcaggtgat actcagttta agcaccgctg 300

tgaaggagtt gatagaaaat agtgtagatg ctggtgctac tactattgat ctaaggctta 360

aagactatgg ggtggacctc attgaagttt cagacaatgg atgtggggta gaagaagaaa 420

actttgaagg tctagctctg aaacatcaca catctaagat tcaagagttt gccgacctca 480

cgcaggttga aactttcggc tttcgggggg aagctctgag ctctctgtgt gcactaagtg 540

atgtcactat atctacctgc cacgggtctg caagcgttgg gactcgactg gtgtttgacc 600

ataatgggaa aatcacccag aaaactccct acccccgacc taaaggaacc acagtcagtg 660

tgcagcactt attttataca ctacccgtgc gttacaaaga gtttcagagg aacattaaaa 720

aggagtattc caaaatggtg caggtcttac aggcgtactg tatcatctca gcaggcgtcc 780

gtgtaagctg cactaatcag ctcggacagg ggaagcggca cgctgtggtg tgcacaagcg 840

gcacgtctgg catgaaggaa aatatcgggt ctgtgtttgg ccagaagcag ttgcaaagcc 900

tcattccttt tgttcagctg ccccctagtg acgctgtgtg tgaagagtac ggcctgagca 960

cttcaggacg ccacaaaacc ttttctacgt ttcgggcttc atttcacagt gcacgcacgg 1020

cgccgggagg agtgcaacag acaggcagtt tttcttcatc aatcagaggc cctgtgaccc 1080

agcaaaggtc tctaagcttg tcaatgaggt tttatcacat gtataaccgg catcagtacc 1140

catttgtcgt ccttaacgtt tccgttgact cagaatgtgt ggatattaat gtaactccag 1200

ataaaaggca aattctacta caagaagaga agctattgct ggccgtttta aagacctcct 1260

tgataggaat gtttgacagt gatgcaaaca agcttaatgt caaccagcag ccactgctag 1320

atgttgaagg taacttagta aagctgcata ctgcagaact agaaaagcct gtgccaggaa 1380

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agactgctga actgacacgg agttttccaa gtgagaaaag gggcgtgtta tcctcttatc 1560

cttcagacgt catctcttac agaggcctcc gtggctcgca ggacaaattg gtgagtccca 1620

cggacagccc tggtgactgt atggacagag agaaaataga aaaagactca gggctcagca 1680

gcacctcagc tggctctgag gaagagttca gcaccccaga agtggccagt agctttagca 1740

gtgactataa cgtgagctcc ctagaagaca gaccttctca ggaaaccata aactgtggtg 1800

acctggactg ccgtcctcca ggtacaggac agtccttgaa gccagaagac catggatatc 1860

aatgcaaagc tctacctcta gctcgtctgt cacccacaaa tgccaagcgc ttcaagacag 1920

aggaaagacc ctcaaatgtc aacatttctc aaagattgcc tggtcctcag agcacctcag 1980

cagctgaggt cgatgtagcc ataaaaatga ataagagaat cgtgctcctc gagttctctc 2040

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ttaacctggg atttatagta accaaactga aagaggacct cttcctggtg gaccagcatg 2280

ctgcggatga gaagtacaac tttgagatgc tgcagcagca cacggtgctc caggcgcaga 2340

ggctcatcac accccagact ctgaacttaa ctgctgtcaa tgaaqctgta ctgatagaaa 2400

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ctgaaagggc taaattgatt tccttaccaa ctagtaaaaa ctggaccttt ggaccccaag 2520

atatagatga actgatcttt atgttaagtg acagccctgg ggtcatgtgc cggccctcac 2580

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tcaatgcgag cgagatgaag aagctcatca cccacatggg tgagatggac caccctgga 2700

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actgacacac cccttgtagc atagagttta ttacagattg ttcggtttgc aaagagaagg 2820

ttttaagtaa tctgattatc gttgtacaaa aattagcatg ctgctttaat gtactggatc 2880

catttaaaag cagtgttaag gcaggcatga tggagtgttc ctctagctca gctacttggg 2940

tgatccggtg ggagctcatg tgagcccagg actttgagac cactccgagc cacattcatg 3000

agactcaatt caaggacaaa aaaaaaaaga tatttttgaa gccttttaaa aaaaaa 3056

PMS2 (human) (SEQ ID NO:9)

MERAESSSTE PAKAIKPIDR KSVHQICSGQ VVLSLSTAVK ELVENSLDAG ATNIDLKLKD 60

YGVDLIEVSD NGCGVEEENF EGLTLKHHTS KIQEFADLTQ VETFGFRGEA LSSLCALSDV 120

TISTCHASAK VGTRLMFDHN GKIIQKTPYP RPRGTTVSVQ QLFSTLPVRH KEFQRNIKKE 180

YAKMVQVLHA YCIISAGIRV SCTNQLGQGK RQPVVCTGGS PSIKENIGSV FGQKQLQSLI 240

PFVQLPPSDS VCEEYGLSCS DALHNLFYIS GFISQCTHGV GRSSTDRQFF FINRRPCDPA 300

KVCRLVNEVY HMYNRHQYPF VVLNISVDSE CVDINVTPDK RQILLQEEKL LLAVLKTSLI 360

GMFDSDVNKL NVSQQPLLDV EGNLIKMHAA DLEKPMVEKQ DQSPSLRTGE EKKDVSISRL 420

REAFSLRHTT ENKPHSPKTP EPRRSPLGQK RGMLSSSTSG AISDKGVLRP QKEAVSSSHG 480

PSDPTDRAEV EKDSGHGSTS VDSEGFSIPD TGSHCSSEYA ASSPGDRGSQ EHVDSQEKAP 540

ETDDSFSDVD CHSNQEDTGC KFRVLPQPTN LATPNTKRFK KEEILSSSDI CQKLVNTQDM 600

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SASQVDVAVK INKKVVPLDF SMSSLAKRIK QLHHEAQQSE GEQNYRKFRA KICPGENQAA 660

EDELRKEISK TMFAEMEIIG QFNLGFIITK LNEDIFIVDQ HATDEKYNFE MLQQHTVLQG 720

QRLIAPQTLN LTAVNEAVLI ENLETFRKNG FDFVIDENAP VTERAKLISL PTSKNWTFGP 780

QDVDELIFML SDSPGVMCRP SRVKQMFASR ACRKSVMIGT ALNTSEMKKL ITHMGEMDHP 840

WNCPHGRPTM RHIANLGVIS QN 862

PMS2 (human cDNA) (SEQ ID NO:10)

cgaggcggat cgggtgttgc atccatggag cgagctgaga gctcgagtac agaacctgct 60

aaggccatca aacctattga tcggaagtca gtccatcaga tttgctctgg gcaggtggta 120

ctgagtctaa gcactgcggt aaaggagtta gtagaaaaca gtctggatgc tggtgccact 180

aatattgatc taaagcttaa ggactatgga gtggatctta ttgaagtttc agacaatgga 240

tgtggggtag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt 300

caagagtttg ccgacctaac tcaggttgaa acttttggct ttcgggggga agctctgagc 360

tcactttgtg cactgagcga tgtcaccatt tctacctgcc acgcatcggc gaaggttgga 420

actcgactga tgtttgatca caatgggaaa attatccaga aaacccccta cccccgcccc 480

agagggacca cagtcagcgt gcagcagtta ttttccacac tacctgtgcg ccataaggaa 540

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atcatttcag caggcatccg tgtaagttgc accaatcagc ttggacaagg aaaacgacag 660

cctgtggtat gcacaggtgg aagccccagc ataaaggaaa atatcggctc tgtgtttggg 720

cagaagcagt tgcaaagcct cattcctttt gttcagctgc cccctagtga ctccgtgtgt 780

gaagagtacg gtttgagctg ttcggatgct ctgcataatc ttttttacat ctcaggtttc 840

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cagcagcaca ccgtgctcca ggggcagagg ctcatagcac ctcagactct caacttaact  2220

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tttgttatcg atgaaaatgc tccagtcact gaaagggcta aactgatttc cttgccaact  2340

agtaaaaact ggaccttcgg acaccaggac gtcgatgaac tgatcttcat gctgagcgac  2400

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cggaagtcgg tgatgattgg gactgctctt aacacaagcg agatgaagaa actgatcacc  2520

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cttttcaaac c                                                       2771
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PMS 1 (human) (SEQ ID NO:11)

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MKQLPAATVR LLSSSQIITS VVSVVKELIE NSLDAGATSV DVKLENYGFD KIEVRDNGEG  60

IKAVDAPVMA MKYYTSKINS HEDLENLTTY GERGEALGSI CCTAEVLITT RTAADNFSTQ  120

YVLDGSGHIL SQKPSHLGQG TTVTALRLFK NLPVRKQFYS TAKKCKDEIK KIQDLLMSFG  180

ILKPDLRIVF VHNKAVIWQK SRVSDHKMAL MSVLGTAVMN NMESFQYHSE ESQIYLSGFL  240

PKCDADHSFT SLSTPERSFI FINSRPVHQK DILKLIRHHY NLKCLKESTR LYPVFFLKID  300

VPTADVDVNL TPDKSQVLLQ NKESVLIALE NLMTTCYGPL PSTNSYENNK TDVSAADIVL  360

SKTAETDVLF NKVESSGKNY SNVDTSVIPF QNDMHNDESG KNTDDCLNHQ ISIGDFGYGH  420

CSSEISNIDK NTKNAFQDIS MSNVSWENSQ TEYSKTCFIS SVKHTQSENG NKDHIDESGE  480

NEEEAGLENS SEISADEWSR GNILKNSVGE NIEPVKILVP EKSLPCKVSN NNYPIPEQMN  540

LNEDSCNKKS NVIDNKSGKV TAYDLLSNRV IKKPMSASAL FVQDHRPQFL IENPKTSLED  600

ATLQIEELWK TLSEEEKLKY EEKATKDLER YNSQMKRAIE QESQMSLKDG RKKIKPTSAW  660

NLAQKHKLKT SLSNQPKLDE LLQSQIEKRR SQNIKMVQIP FSMKNLKINF KKQNKVDLEE  720

KDEPCLIHNL RFPDAWLMTS KTEVMLLNPY RVEEALLFKR LLENHKLPAE PLEKPIMLTE  780

SLFNGSHYLD VLYKMTADDQ RYSGSTYLSD PRLTANGFKI KLIPGVSITE NYLEIEGMAN  840

CLPFYGVADL KEILNAILNR NAKEVYECRP RKVISYLEGE AVRLSRQLPM YLSKEDIQDI  900

IYRMKHQFGN EIKECVHGRP FFHHLTYLPE TT                                932
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PMS 1 (human) (SEQ ID NO:12)

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ggcacgagtg gctgcttgcg gctagtggat qgtaattgcc tgcctcgcgc tagcagcaag  60

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gttctcagat catcacttcg gtggtcagtg ttgtaaaaga gcttattgaa aactccttgg  180

atgctggtgc cacaagcgta gatgttaaac tggagaacta tggatttgat aaaattgagg  240

tgcgagataa cggggagggt atcaaqgctg ttgatgcacc tgtaatggca atgaagtact  300

acacctcaaa aataaatagt catgaagatc ttgaaaattt gacaacttac ggttttcgtg  360

gagaagcctt ggggtcaatt tgttgtatag ctgaggtttt aattacaaca agaacggctg  420
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taagaaagca gttttactca actgcaaaaa aatgtaaaga tgaaataaaa aagatccaag  600

atctcctcat gagctttggt atccttaaac ctgacttaag gattgtcttt gtacataaca  660

aggcagttat ttggcagaaa agcagagtat cagatcacaa gatggctctc atgtcagttc  720

tggggactgc tgttatgaac aatatggaat cctttcagta ccactctgaa gaatctcaga  780

tttatctcaq tggatttctt ccaaagtgtg atgcagacca ctctttcact agtctttcaa  840

caccagaaag aagtttcatc ttcataaaca gtcgaccagt acatcaaaaa gatatcttaa  900

agttaatccg acatcattac aatctgaaat gcctaaagga atctactcgt ttgtatcctg  960

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cgacttgtta tggaccatta cctagtacaa attcttatga aaataataaa acagatgttt 1140

ccgcagctga catcgttctt agtaaaacag cagaaacaga tgtgcttttt aataaagtgg 1200

aatcatctgq aaagaattat tcaaatgttg atacttcagt cattccattc caaaatgata 1260

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gtgactttgg ttatggtcat tgtagtagtg aaatttctaa cattgataaa aacactaaga 1380

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ctgtgaaaat tttagtgcct gaaaaaagtt taccatgtaa agtaagtaat aataattatc 1680

caatccctga acaaatgaat cttaatgaag attcatgtaa caaaaaatca aatgtaatag 1740

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aagaggaaaa actgaaatat gaagagaagg ctactaaaga cttggaacga tacaatagtc 1980

aaatgaagag agccattgaa caggagtcac aaatgtcact aaaagatggc agaaaaaaga 2040

taaaacccac cagcgcatgg aatttggccc agaagcacaa gttaaaaacc tcattatcta 2100

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ttaaaatggt acagatcccc ttttctatga aaaacttaaa aataaatttt aagaaacaaa 2220

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aagccctgct atttaaaaga cttcttgaga atcataaact tcctgcagag ccactggaaa 2400

agccaattat gttaacagag agtctttta atggatctca ttatttagac gttttatata 2460

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ttaatgctat attaaacaga aatgcaaagg aagtttatga atgtagacct cgcaaagtga 2700

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tctggtttta aattatcttt gtattatgtg tcacatggtt atttttaaa tgaggattca 3000

ctgacttgtt tttatattga aaaaagttcc acgtattgta gaaaacgtaa ataaactaat 3060 3063

aac 3063

MSH2 (human) (SEQ ID NO:13)

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QGVIKYMGPA GAKNLQSVVL SKMNFESFVK DLLLVRQYRV EVYKNRAGNK ASKENDWYLA 120

YKASPGNLSQ FEDILFGNND MSASIGVVGV KMSAVDGQRQ VGVGYVDSIQ RKLGLGEFPD 180

NDQFSNLEAL LIQIGPKECV LPGGETAGDM GKLRQIIQRG GILITERKKA DFSTKDIYQD 240

LNRLLKGKKG EQMNSAVLPE MENQVAVSSL SAVIKFLELL SDDSNFGQFE LTTFDFSQYM 300

KLDIAAVRAL NLFQGSVEDT TGSQSLAALL NKCKTPQGQR LVNQWIKQPL MDKNRIEERL 360

NLVEAFVEDA ELRQTLQEDL LRRFPDLNRL AKKFQRQAAN LQDGYRLYQG INQLPNVIQA 420

LEKHEGKHQK LLLAVFVTPL TDLRSDFSKF QEMIETTLDM DQVENHEFLV KPSFDPNLSE 480

LREIMNDLEK KMQSTLTSAA RDLGLDPGKQ IKLDSSAQFG YYFRVTCKEE KVLRNNKNFS 540

TVDIQKNGVK FTNSKLTSLN EEYTKNKTEY EEAQDAIVKE IVNISSGYVE PMQTLNDVLA 600

QLDAVVSFAH VSNGAPVPYV RPAILEKGQG RIILKASRHA CVEVQDEIAF IPNDVYFEKD 660

KQMFHIITGP NMGGKSTYTR QTGVIVLMAQ IGCFVPCESA EVSIVDCILA RVGAGDSQLK 720

GVSTFMAEML ETASILRSAT KDSLITIDEL GRGTSTYDGF GLAWAISEYI ATKIGAFCMF 780

ATHFHELTAL ANQIPTVNNL HVTALTTEET LTMLYQVKKG VCDQSFGIHV AELANFPKHV 840

IECAKQKALE LEEFQYIGES QGYDIMEPAA KKCYLEREQG EKIIQEFLSK VKQMPFTEMS 900

EENITIKLKQ LKAEVIAKNN SFVNEIISRI KVTT 934

MSH2 (human cDNA) (SEQ ID NO:14)

ggcgggaaac agcttagtgg gtgtggggtc gcgcattttc ttcaaccagg aggtgaggag 60

gtttcgacat ggcggtgcag ccgaaggaga cgctgcagtt ggagagcgcg gccgaggtcg 120

gcttcgtgcg cttctttcag ggcatgccgg agaagccgac caccacagtg cgccttttcg 180

accggggcga cttctatacg gcgcacggcg aggacgcgct gctggccgcc cgggaggtgt 240

tcaagaccca gggggtgatc aagtacatgg ggccggcagg agcaaagaat ctgcagagtg 300

ttgtgcttag taaaatgaat tttgaatctt ttgtaaaaga tcttcttctg gttcgtcagt 360

atagagttga agtttataag aatagagctg gaaataaggc atccaaggag aatgattggt 420

atttggcata taaggcttct cctggcaatc tctctcagtt tgaagacatt ctctttggta 480

acaatgatat gtcagcttcc attggtgttg tgggtgttaa aatgtccgca gttgatggcc 540

agagacaggt tggagttggg tatgtggatt ccatacagag gaaactagga ctgtgtgaat 600

tccctgataa tgatcagttc tccaatcttg aggctctcct catccagatt ggaccaaagg 660

aatgtgtttt acccggagga gagactgctg gagacatggg gaaactgaga cagataattc 720

aaagaggagg aattctgatc acagaaagaa aaaaagctga cttttccaca aaagacattt 780

atcaggacct caaccggttg ttgaaaggca aaaagggaga gcagatgaat agtgctgtat 840

tgccagaaat ggagaatcag gttgcagttt catcactgtc tgcggtaatc aagtttttag 900

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aactcttatc agatgattcc aactttggac agtttgaact gactactttt gacttcagcc  960

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aagataccac tggctctcag tctctggctg ccttgctgaa taagtgtaaa acccctcaag  1080

gacaaagact tgttaaccag tggattaagc agcctctcat ggataagaac agaatagagg  1140

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tacaggctct ggaaaaacat gaaggaaaac accagaaatt attgttggca gtttttgtga  1380

ctcctcttac tgatcttcgt tctgacttct ccaagtttca ggaaatgata gaaacaactt  1440

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tcagtgaatt aagagaaata atgaatgact tggaaaagaa gatgcagtca acattaataa  1560

gtgcagccag agatcttggc ttggaccctg gcaaacagat taaactggat tccagtgcac  1620

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ttaaagaaat tgtcaatatt tcttcaggct atgtagaacc aatgcagaca ctcaatgatg  1860

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catatgtacg accagccatt ttggagaaag gacaaggaag aattatatta aaagcatcca  1980

ggcatgcttg tgttgaagtt caagatgaaa ttgcatttat tcctaatgac gtatactttg  2040

aaaaagataa acagatgttc cacatcatta ctggccccaa tatgggaggt aaatcaacat  2100

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agtcagcaga agtgtccatt gtggactgca tcttagcccg agtaggggct ggtgacagtc  2220

aattgaaagg agtctccacg ttcatggctg aaatgttgga aactgcttct atcctcaggt  2280

ctgcaaccaa agattcatta ataatcatag atgaattggg aagaggaact tctacctacg  2340

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gcatgtttgc aacccatttt catgaactta ctgccttggc caatcagata ccaactgtta  2460

ataatctaca tgtcacagca ctcaccactg aagagacctt aactatgctt tatcaggtga  2520

agaaaggtgt ctgtgatcaa agttttggga ttcatgttgc agagcttgct aatttcccta  2580

agcatgtaat agagtgtgct aaacagaaag ccctggaact tgaggagttt cagtatattg  2640

gagaatcgca aggatatgat atcatggaac cagcagcaaa gaagtgctat ctggaaagag  2700

agcaaggtga aaaaattatt caggagttcc tgtccaaggt gaaacaaatg ccctttactg  2760

aaatgtcaga agaaaacatc acaataaagt taaaacagct aaaagctgaa gtaatagcaa  2820

agaataatag ctttgtaaat gaaatcattt cacgaataaa agttactacg tgaaaaatcc  2880

cagtaatgga atgaaggtaa tattgataag ctattgtctg taatagtttt atattgtttt  2940

atattaaccc tttttccata gtgttaactg tcagtgccca tgggctatca acttaataag  3000

atatttagta atattttact ttgaggacat tttcaaagat ttttattttg aaaaatgaga  3060

gctgtaactg aggactgttt gcaattgaca taggcaataa taagtgatgt gctgaatttt  3120

ataaataaaa tcatgtagtt tgtgg                                       3145
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MLH1 (human) (SEQ ID NO:15)

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MSFVAGVIRR LDETVVNRIA AGEVIQRPAN AIKEMIENCL DAKSTSTQVI VKEGGLKLIQ 60

IQDNGTGIRK EDLDIVCERF TTSKLQSFED LASISTYGFR GEALASISHV AHVTIITTKTA 120

DGKCAYRASY SDGKLKAPPK PCAGNQGTQI TVEDLFYNIA TRRKALKNPS EEYGKILEVV 180

GRYSVHNAGI SFSVKKQGET VADVRTLPNA STVDNIRSIF GNAVSRELIE TGCEDKTLAF 240

KMNGYISNAN YSVKKCIFLL FINHRLVEST SLRKATETVY AAYLPKNTHP FLYLSLEISP 300

QNVDVNVHPT KHEVHFLHEE SILERVQQHI ESKLLGSNSS RMYFTQTLLP GLAGPSGEMV 360

KSTTSLTSSS TSGSSDKVYA HQMVRTDSRE QKLDAFLQPL SKPLSSQPQA IVTEDKTDIS 420

SGRARQQDEE MLELPAPAEV AAKNQSLEGD TTKGTSEMSE KRGPTSSNPR KRHREDSDVE 480

MVEDDSRKEM TAACTPRRRI INLTSVLSLQ EEINEQGHEV LREMLHNHSF VGCVNPQWAL 540

AQHQTKLYLL NTTKLSEELF YQILTYDFAN FGVLRLSEPA PLFDLAMLAL DSPESGWTEE 600

DGPKEGLAEY IVEFLKKKAE MLADYFSLEI DEEGNLIGLP LLIDNYVPPL EGLPIFILRL 660

ATEVNWDEEK ECFESLSKEC ANFYSIRKQY ISEESTLSGQ QSEVPGSIPN SWKWTVEHIV 720

YKALRSHILP PKHFTEDGNI LQLANLPDLY KVFERC 756

MLH1 (human) (SEQ ID NO:16)

cttggctctt ctggcgccaa aatgtcgttc gtggcagggg ttattcggcg gctggacgag 60

acagtggtga accgcatcgc ggcgggggaa gttatccagc ggccagctaa tgctatcaaa 120

gagatgattg agaactgttt agatgcaaaa tccacaagta ttcaagtgat tgttaaagag 180

ggaggcctga agttgattca gatccaagac aatggcaccg ggatcaggaa agaagatctg 240

gatattgtat gtgaaaggtt cactactagt aaactgcagt cctttgagga tttagccagt 300

atttctacct atggctttcg aggtgaggct ttggccagca taagccatgt ggctcatgtt 360

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aaactgaaag cccctcctaa accatgtgct ggcaatcaag ggacccagat cacggtggag 480

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gggaaaattt tggaagttgt tggcaggtat tcagtacaca atgcaggcat tagtttctca 600

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gacaatattc gctccatctt tggaaatgct gttagtcgag aactgataga aattggatgt 720

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aagaagtgca tcttcttact cttcatcaac catcgtctgg tagaatcaac ttccttgaga 840

aaagccatag aaacagtgta tgcagcctat ttgcccaaaa acacacaccc attcctgtac 900

ctcagtttag aaatcagtcc ccagaatgtg gatgttaatg tgcaccccac aaagcatgaa 960

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gggacttcag aaatgtcaga gaagagagga cctacttcca gcaaccccag aaagagacat 1440

cgggaagatt ctgatgtgga aatggtggaa gatgattccc gaaaggaaat gactgcagct 1500

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aatgagcagg gacatgaggt tctccgggag atgttgcata accactcctt cgtgggctgt  1620

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gagagtggct ggacagagga agatggtccc aaagaaggac ttgctgaata cattgttgag  1860

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cctatcttca ttcttcgact agccactgag gtgaattggg acgaagaaaa ggaatgtttt  2040

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tggactgtgg aacacattgt ctataaagcc ttgcgctcac acattctgcc tcctaaacat  2220

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cacttaagac ttatacttgc cttctgatag tattccttta tacacagtgg attgattata  2460

aataaataga tgtgtcttaa cata                                        2484

hPMS2-134 (human)                                    (SEQ ID NO:17)
MERAESSSTE PAKAIKPIDR KSVHQICSGQ VVLSLSTAVK ELVENSLDAG ATNIDLKLKD  60

YGVDLIEVSD NGCGVEEENF EGLTLKHHTS KIQEFADLTQ VETFGFRGEA LSSLCALSDV  120

TISTCHASAK VGT                                                     133

hPMS2-134 (human cDNA)                               (SEQ ID NO:18)
cgaggcggat cgggtgttgc atccatggag cgagctgaga gctcgagtac agaacctgct  60

aaggccatca aacctattga tcggaagtca gtccatcaga tttgctctgg gcaggtggta  120

ctgagtctaa gcactgcggt aaaggagtta gtagaaaaca gtctggatgc tggtgccact  180

aatattgatc taaagcttaa ggactatgga gtggatctta ttgaagtttc agacaatgga  240

tgtggggtag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt  300

caagagtttg ccgacctaac tcaggttgaa acttttggct ttcgggggga agctctgagc  360

tcactttgtg cactgagcga tgtcaccatt tctacctgcc acgcatcggc gaaggttgga  420

acttga                                                             426
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[0060] The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLES

Example 1

hPMS2-134 Encodes a Dominant Negative Mismatch Repair Protein

[0061] A profound defect in MMR was found in the normal cells of two HNPCC patients. That this defect was operative in vivo was demonstrated by the widespread presence of microsatellite instability in non neoplastic cells of such patients. One of the two patients had a germ line truncating mutation of the hPMS2 gene at codon 134 (the hPMS2 134 mutation), while the other patient had a small germ line deletion within the hMLH1 gene. Leach et al., *Cell,* 1993, 75, 1215 1225. These data contradicted the two hit model generally believed to explain the biochemical and biological features of HNPCC patients. The basis for this MMR deficiency in the normal cells of these patients was unclear, and several potential explanations were offered. For example, it was possible that the second allele of the relevant MMR gene was inactivated in the germ line of these patients through an undiscovered mechanism, or that unknown mutations of other genes involved in the MMR process were present that cooperated with the known germ line mutation.

It is clear from knock out experiments in mice that MMR deficiency is compatible with normal growth and development, supporting these possibilities. Edelmann et al., *Cell,* 1996, 85, 1125 1134. Alternatively, it was possible that the mutant alleles exerted a dominant-negative effect, resulting in MMR deficiency even in the presence of the wild type allele of the corresponding MMR gene and all other genes involved in the MMR process. To distinguish between these possibilities, the truncated polypeptide encoded by the hPMS2 134 mutation was expressed in an MMR proficient cell line its affect on MMR activity was analyzed. The results showed that this mutant could indeed exert a dominant-negative effect, resulting in biochemical and genetic manifestations of MMR deficiency. One embodiment of the present invention is demonstrated in Table 1, where a Syrian hamster fibroblast cell line (TK) was transfected with an expression vector containing the hPMS2-134 (TK-PMS2-134) or the empty expression vector (TKvect), which also contains the NEO gene as a selectable marker. TK-PMS2-134 cells were determined to be stably expressing the gene via western blot analysis (data not shown). Nuclear lysates from hPMS2-134 and control cells were measured for the ability to correct mismatched DNA substrates. As shown in Table 1, TK-PMS2-134 cells had a dramatic decrease in repair activity while TKvect control cells were able to repair mismatched DNA duplexes at a rate of ~4.0 fmol/15 minutes ($p<0.01$).

TABLE 1

Relative endogenous MMR activity of MMR-proficient cells expressing an ectopically expressed morphogene or an empty expression vector

Cell Lines	5' DNA Repair activity of G/T mismatch (fmol/15 minutes)
TKvect	
1	3.5
2	2.9
3	5.5
TK-PMS2-134	
1	0
2	0
3	0.5

[**0062**] These data show that the expression of the TK-PMS2-134 results in suppressed MMR of a host organism and allows for an enhanced mutation rate of genetic loci with each mitosis.

Example 2

hPMS2-134 Can Alter Genes In Vivo

[**0063**] An example of the ability to alter mismatch repair comes from experiments using manipulation of mismatch repair TK cells (described above) that expressed the TK-hPMS2-134 mutant were used by transfection of the mammalian expression construct containing a defective β-galactosidase gene (referred to as pCAR-OF) which was transfected into TK-hPMS2-134 or TKvect cells as described above. The pCAR OF vector consists of a β-galactosidase gene containing a 29-basepair poly-CA tract inserted at the 5' end of its coding region, which causes the wild-type reading frame to shift out-of-frame. This chimeric gene is cloned into the pCEP4, which contains the constitutively active cytomegalovirus (CMV) promoter upstream of the cloning site and also contains the hygromycin-resistance gene that allows for selection of cells containing this vector. The pCAR-OF reporter cannot generate β-galactosidase activity unless a frame-restoring mutation (i.e., insertion or deletion) arises following transfection into a host. Another reporter vector called pCAR-IF contains a β-galactosidase in which a 27-bp poly-CA repeat was cloned into the same site as the pCAR-OF gene, but it is biologically active because the removal of a single repeat restores the open reading frame and produces a functional chimeric β-galactosidase polypeptide (not shown). In these experiments, TK-hPMS2-134 and TKvect cells were transfected with the pCAR-OF reporter vector and selected for 17 days in neomycin plus hygromycin selection medium. After the 17 days, resistant colonies were stained for β-galactosidase production to determine the number of clones containing a genetically altered β-galactosidase gene. All conditions produced a relatively equal number of neomycin/hygromycin resistant cells, however, only the cells expressing the TK-hPMS2-134 contained a subset of clones that were positive for β-galactosidase activity. Representative results are shown in Table 2, which shows the data from these experiments where cell colonies were stained in situ for β-galactosidase activity and scored for activity. Cells were scored positive if the colonies turned blue in the presence of X-gal substrate and scored negative if colonies remained white. Analysis of triplicate experiments showed that a significant increase in the number of functional β-galactosidase positive cells was found in the TK-hPMS2-134 cultures, while no β-galactosidase positive cells were seen in the control TKvect cells.

TABLE 2

Number of TKmorph and TKvect cells containing functional β-galactosidase activity

Cells	White Colonies	Blue Colonies	% Clones with altered B-gal
Tkvect	65 +/- 9	0	0/65 = 0%
TK-PMS2-134	40 +/- 12	28 +/- 4	28/68 = 41%

[**0064**] TK-PMS2-134 40+/−12 28+/−4 28/68=41% TK-PMS2-134/pCAR-OF clones that were pooled and expanded also showed a number of cells that contained a functional β-galactosidase gene. No β-galactosidase positive cells were observed in TKvect cells transfected with the pCAR-OF vector. These data are shown in **FIG. 1** where the dark staining in panel B represent β-galactosidase positive cells present in the TK-PMS2-134/pCAR-OF cultures while none are found in the TKvect cells grown under similar conditions (panel A). These data demonstrate the ability of the mutant mismatch repair gene, hPMS2-134, to generate gene alterations in vivo, which allows for the rapid screening of clones with altered polypeptides exhibiting new biochemical features.

[**0065**] To confirm that alterations within the nucleotide sequences of the β-galactosidase gene was indeed responsible for the in vivo β-galactosidase activity present in TK-hPMS2-134 clones, RNA was isolated from TK-hPMS2-134/pCAR-OF and TKvect/pCAR-OF and the β-galactosidase mRNA primary structure was examined by

reverse transcriptase polymerase chain reaction (RT-PCR) amplification and sequencing. Sequence analysis of β-galactosidase message from TKvect cells found no structural alterations in the input gene sequence. Analysis of the β-galactosidase message from TK-hPMS-134 cells found several changes within the coding sequences of the gene. These sequence alterations included insertion and deletions of the poly CA tract in the amino terminus as expected. Other alterations included insertions of sequences outside of the polyCA repeat as well as a series of single base alterations contained throughout the length of the gene.

[0066] A summary of the genetic alterations are given in **FIG. 2** where a schematic representation of the β-galactosidase gene is shown with the regions and types of genetic alterations depicted below.

[0067] Plasmids. The full length wild type hPMS2 cDNA was obtained from a human Hela cDNA library as described in Strand et al., *Nature,* 1993, 365, 274 276, which is incorporated herein by reference in its entirety. An hPMS2 cDNA containing a termination codon at amino acid 134 was obtained via RT PCR from the patient in which the mutation was discovered. Nicolaides et al., *Mol. Cell. Biol.,* 1998, 18, 1635-1641, which is incorporated herein by reference in its entirety. The cDNA fragments were cloned into the BamHI site into the pSG5 vector, which contains an SV40 promoter followed by an SV40 polyadenylation signal. Nicolaides et al., *Genomics,* 1995, 29, 329 334, which is incorporated herein by reference in its entirety. The pCAR reporter vectors described in **FIG. 1** were constructed as described in Palombo et al., *Nature,* 1994, 36, 417, which is incorporated herein by reference in its entirety.

[0068] β-galactosidase assay. Seventeen days following transfection with pCAR, β-galactosidase assays were performed using 20 μg of protein in 45 mM 2 mercaptoethanol, 1 mM $MgCl_2$, 0.1 M $NaPO_4$ and 0.6 mg/ml Chlorophenol red β-D galatopyranoside (CPRG, Boehringer Mannheim). Reactions were incubated for 1 hour, terminated by the addition of 0.5 M Na_2CO_3, and analyzed by spectrophotometry at 576 nm. Nicolaides et al., *Mol. Cell. Biol.,* 1998, 18, 1635-1641. For in situ β-galactosidase staining, cells were fixed in 1% glutaraldehyde in PBS and incubated in 0.15 M NaCl, 1 mM $MgCl_2$, 3.3 mM $K_4Fe(CN)_6$, 3.3 mM $K_3Fe(CN)_6$, 0.2%×Gal for 2 hours at 37° C.

Example 3

hPMS2-134 Causes a Defect in MMR Activity

[0069] The differences in β-galactosidase activity between PMS2 WT and PMS2 134 transfected cells can be due to the PMS2 134 protein disturbing MMR activity resulting in a higher frequency of mutation within the pCAR OF reporter and re establishing the ORF. To directly test whether MMR was altered, a biochemical assay for MMR with the individual clones described in Example 1 was employed. Nuclear extracts were prepared from the clones and incubated with heteroduplex substrates containing either a /CA\ insertion deletion or a G/T mismatch under conditions described previously. The /CA\ and G/T heteroduplexes were used to test repair from the 3' and 5' directions, respectively. There was a dramatic difference between the PMS2-134 expressing clones and the other clones in these assays (Table 3).

TABLE 3

MMR activity of nuclear extracts from SH clones or pooled culturesa

Cell line	Amt of repaired substrate (fmol/15 min) 3'/CA\	3' G/T	5' G/T	3'/CTG\	5'/CTG\
SH clonesb PMS2-NOT					
Clone A	10.2	3.5			
Clone B	12.7	2.9			
Clone C	13.5	5.5			
PMS2-WT					
Clone A	2.8	2.2			
Clone B	5.7	4.8			
Clone c	4.7	2.9			
PMS2-134					
Clone A	2.5	0.0			
Clone B	2.4	0.0			
Clone C	5.0	0.5			
Pooled cultures					
PMS2-NOT		2.07 ± 0.09	2.37 ± 0.37	3.45 ± 1.35	2.77 ± 1.37
PMS2-WT		1.65 ± 0.94	1.86 ± 0.57	1.13 ± 0.23	1.23 ± 0.65
PMS2-134		0.14 ± 0.2	0.0 ± 0.0	1.31 ± 0.66	0.0 ± 0.0

aThe extracts were tested for MMR activity with 24 fmol of heteroduplex.
bThese data represent similar results derived from more than five independent experiments.

[0070] While all clones repaired substrates from the 3' direction (/CA\ heteroduplex), cells expressing the PMS2 134 polypeptide had very little 5' repair activity. A similar directional defect in mismatch repair was evident with pooled clones resulting from PMS2 134 transfection, or when the heteroduplex contained a 2 4 base pair loop, examples of which are shown in Table 3. A small decrease in MMR activity was observed in the 3'/CA\ PMS2-WT repair assays, perhaps a result of interference in the biochemical assays by over-expression of the PMS2 protein. No significant activity was caused by PMS2-WT in the in situ β-galactosidase assays, a result more likely to reflect the in vivo condition.

[0071] Biochemical assays for mismatch repair. MMR activity in nuclear extracts was performed as described, using 24 fmol of substrate, in Bronner et al., *Nature,* 1994, 368, 258 261 and Nicolaides et al., *Mol. Cell. Biol.,* 1998, 18, 1635-1641, each of which is incorporated herein by reference in its entirety. Complementation assays were done by adding ~100 ng of purified MutL" or MutS" components to 100 μg of nuclear extract, adjusting the final KCl concentration to 100 mM. Bevins, *Ciba Found. Symp.,* 1994, 186, 250-69 and Alderson et al., *Res. Microbiol.,* 1993, 144, 665-72. The substrates used in these experiments contain a strand break 181 nucleotides 5' or 125 nucleotides 3' to the mismatch. Values represent experiments performed at least in duplicate.

Example 4

C-Terminus of hPMS2 Mediates Interaction Between hPMS2 and hMLH1

[0072] To elucidate the mechanism by which hPMS2 134 affected MMR, the interaction between hPMS2 and hMLH1 was analyzed. Previous studies have shown that these two proteins dimerize to form a functionally active complex. Bronner et al., *Nature,* 1994, 368, 258 261. Proteins were synthesized in vitro using reticulocyte lysates, employing RNA generated from cloned templates. The full length hMLH1 and hPMS2 proteins bound to each other and were co precipitated with antibodies to either protein, as expected (data not shown). To determine the domain of hPMS2 that bound to hMLH1, the amino terminus (codons 1-134), containing the most highly conserved domain among mutL proteins (Su et al.,*J. Biol. Chem.,* 1988, 263, 6829 6835 and Edelmann et al., *Cell,* 1996, 85, 1125 1134), and the carboxyl terminus (codons 135-862) were separately cloned and proteins produced in vitro in coupled transcription translation reactions. **FIGS. 3A, 3B**, and **3**C show a representative immunoprecipitation of in vitro-translated hPMS2 and hMLH1 proteins. **FIG. 3A** shows labeled (indicated by an asterisk) or unlabelled proteins incubated with an antibody to the C-terminus of hPMS2 in lanes 1 to 3 and to hMLH1 in lanes 4 to 6. Lane 7 contains a nonprogrammed reticulocyte lysate. PMS2-135 contains codons 135 to 862 of hPMS2. The major translation products of hPMS2 and hMLH1 are indicated. **FIG. 3B** shows labeled hPMS2-134 (containing codons 1-134 of hPMS2) incubated in the presence or absence of unlabelled hMLH1 plus an antibody to hMLH1 (lanes 1 and 2, respectively). Lane 3 contains lysate from a nonprogrammed reticulolysate. **FIG. 3C** shows labeled proteins incubated with an antibody to the N terminus of hPMS2. Lane 6 contains a nonprogrammed reticulocyte lysate. In both panels A and B, autoradiographs of immunoprecipitated products are shown. When a 35S labelled, full-length hMLH1 protein (**FIG. 3**A, lane 5) was mixed with the unlabelled carboxyl terminal hPMS2 polypeptide, a monoclonal antibody (mAb) to the carboxyl terminus of hPMS2 efficiently immunoprecipitated the labeled hMLH1 protein (lane 1). No hMLH1 protein was precipitated in the absence of hPMS2 (lane 2). Conversely, when the 35S labelled carboxyl terminus of hPMS2 (lane 3) was incubated with unlabelled, full length hMLH1 protein, an anti hMLH1 mAb precipitated the hPMS2 polypeptide (lane 4). In the absence of the unlabelled hMLH1 protein, no hPMS2 protein was precipitated by this mAb (lane 6). The same antibody failed to immunoprecipitate the amino terminus of hPMS2 (amino acids 1 134) when mixed with unlabelled MLH1 protein (**FIG. 3**B, lane 1). This finding was corroborated by the converse experiment in which radiolabelled hPMS2-134 (**FIG. 3**C, lane 1) was unable to coprecipitate radiolabelled MLH1 when precipitations were done using an N terminal hPMS2 antibody (**FIG. 3**C, lane 2) while this antibody was shown to be able to coprecipitate MLH1 when mixed with wild type hPMS2 (**FIG. 3**C, lane 4).

[0073] The initial steps of MMR are dependent on two protein complexes, called MutS" and MutL". Drummond et al., *Science,* 1995, 268, 1909 1912. As the amino terminus of hPMS2 did not mediate binding of hPMS2 to hMLH1, it was of interest to determine whether it might instead mediate the interaction between the MutL" complex (comprised of hMLH1 and hPMS2) and the MutS" complex (comprised of MSH2 and GTBP). Because previous studies have demonstrated that MSH2 and the MutL" components do not associate in solution, direct hPMS2-134:MutS" interaction was unable to be assayed. A different approach was used to address this issue, and attempted to complement nuclear extracts from the various SH cell lines with MutS" or MutL". If the truncated protein present in the PMS2-134 expressing SH cells was binding to MutS" and lowering its effective concentration in the extract, then adding intact MutS" should rescue the MMR defect in such extracts. **FIG. 4** shows complementation of MMR activity in transduced SH cells. Lysates from pooled clones stably transduced with PMS2-NOT, PMS2-WT, or PMS2-134 were complemented with purified MutS" or MutL" MMR components by using the 5' G/T heteroduplex substrate. The values are presented as the percentage of repair activity in each case compared to that in lysates complemented with both purified MutL" and MutS" components to normalize for repair efficiency in the different lysate backgrounds. The values shown represent the average of at least three different determinations. Purified MutS" added to such extracts had no effect (**FIG. 4**). In contrast, addition of intact MutL" to the extract completely restored directional repair to the extracts from PMS2-134 cells (**FIG. 4**).

[0074] The results described above lead to several conclusions. First, expression of the amino terminus of hPMS2 results in an increase in microsatellite instability, consistent with a replication error (RER) phenotype. That this elevated microsatellite instability is due to MMR deficiency was proven by evaluation of extracts from stably transduced cells. Interestingly, the expression of PMS2-134 resulted in a polar defect in MMR, which was only observed using heteroduplexes designed to test repair from the 5' direction (no significant defect in repair from the 3' direction was observed in the same extracts). Interestingly, cells deficient in hMLH1 also have a polar defect in MMR, but in this case preferentially affecting repair from the 3' direction. Huttner et al., Pediatr. Res., 1999, 45, 785-94. It is known from previous studies in both prokaryotes and eukaryotes that the separate enzymatic components mediate repair from the two different directions. Our results indicate a model in which 5' repair is primarily dependent on hPMS2 while 3' repair is primarily dependent on hMLH1. It is easy to envision how the dimeric complex between PMS2 and MLH1 might set up this directionality. The combined results also demonstrate that a defect in directional MMR is sufficient to produce a RER+phenotype.

[0075] The dominant-negative function of the PMS2-134 polypeptide can result from its binding to MLH1 and consequent inhibition of MutL" function. This is based in part on the fact that the most highly conserved domain of the PMS2 gene is located in its amino terminus, and the only known biochemical partner for PMS2 is MLH1. The binding studies revealed, however, that the carboxyl terminus of PMS2, rather than the highly conserved amino terminus, actually mediated binding to MLH1. This result is consistent with those recently obtained in *S. cerevisciae*, in which the MLH 1 interacting domain of PMS1 (the yeast homolog of human PMS2) was localized to its carboxyl terminus. Leach et al., *Cell,* 1993, 75, 1215 1225. The add back experiments additionally showed that the hPMS2-134 mutant was not likely to mediate an interaction with the MutS" complex (**FIG. 4**). The hPMS2-134 polypeptide does not inhibit the

initial steps in MMR, but rather interacts with and inhibits a downstream component of the pathway, perhaps a nuclease required for repair from the 5' direction.

[0076] The demonstration that the hPMS2-134 mutation can confer a dominant-negative MMR defect to transfected cells helps to explain the phenotype of the kindred in which this mutant was discovered. Three individuals from this kindred were found to carry the mutation, a father and his two children. Both children exhibited microsatellite instability in their normal tissues and both developed tumors at an early age, while the father had no evidence of microsatellite instability in his normal cells and was completely healthy at age 35. The only difference known to us with respect to the MMR genes in this family is that the father's mutant allele was expressed at lower levels than the wild type allele as assessed by sequencing of RT PCR products of RNA from lymphocytes. The children expressed both alleles at approximately equal levels. The dominant negative attribute of the hPMS2-134 mutant may only be manifest when it is present at sufficient concentrations (at least equimolar) thus, explaining the absence of MMR deficiency in the father. The reason for the differential expression of the hPMS2-134 allele in this kindred is not clear, though imprinting is a possibility. Ascertainment of additional, larger kindreds with such mutations will facilitate the investigation of this issue.

[0077] Western blots. Equal number of cells were lysed directly in lysis buffer (60 mM Tris, pH 6.8, 2% SDS, 10% glycerol, 0.1 M 2 mercaptoethanol, 0.001% bromophenol blue) and boiled for 5 minutes. Lysate proteins were separated by electrophoresis on 4 12% Tris glycine gels (for analysis of full length hPMS2) or 4 20% Tris glycine gels (for analysis of hPMS2-134). Gels were electroblotted onto Immobilon P (Millipore) in 48 mM Tris base, 40 mM glycine, 0.0375% SDS, 20% methanol and blocked overnight at 4° C. in Tris buffered saline plus 0.05% Tween 20 and 5% condensed milk. Filters were probed with a polyclonal antibody generated against residues 2-20 of hPMS2 (Santa Cruz Biotechnology, Inc.) and a horseradish peroxidase conjugated goat anti rabbit secondary antibody, using chemiluminescence for detection (Pierce).

[0078] In vitro translation. Linear DNA fragments containing hPMS2 and hMLH1 cDNA sequences were prepared by PCR, incorporating sequences for in vitro transcription and translation in the sense primer. A full length hMLH1 fragment was prepared using the sense primer 5' ggatcctaatacgactcactatagggaga ccaccatgtcgttcgtggcaggg 3' (SEQ ID NO:1) (codons 1-6) and the antisense primer 5' taagtcttaagtgctaccaac 3' (SEQ ID NO:2) (located in the 3' untranslated region, nt 2411 2433), using a wild type HMLH1 cDNA clone as template. A full length hPMS2 fragment was prepared with the sense primer 5' ggatcctaatacgactcactatagggag accaccatggaacaattgcctgcgg 3' (SEQ ID NO:3) (codons 1-6) and the antisense primer 5' aggttagtgaagactctgtc 3' (SEQ ID NO:4) (located in 3' untranslated region, nt 2670 2690) using a cloned hPMS2 cDNA as template. A fragment encoding the amino terminal 134 amino acids of hPMS2 was prepared using the same sense primer and the antisense primer 5' agtcgagttccaaccttcg 3' (SEQ ID NO:5). A fragment containing codons 135-862 of hPMS135 was generated using the sense primer 5' ggatcctaatacgactcactatagggagaccaccatgatgtttgatcacaatgg 3' (SEQ ID NO:6) (codons 135-141) and the same antisense primer as that used for the full length hPMS2 protein. These fragments were used to produce proteins via the coupled transcription translation system (Promega). The reactions were supplemented with 35S labelled methionine or unlabelled methionine, as indicated in the text. The PMS135 and hMLH1 proteins could not be simultaneously radiolabelled and immunoprecipitated because of their similar molecular weights precluded resolution. Lower molecular weight bands are presumed to be degradation products and/or polypeptides translated from alternative internal methionines.

[0079] Immunoprecipitation. Immunoprecipitations were performed on in vitro translated proteins by mixing the translation reactions with 1 μg of the MLH1 specific monoclonal antibody (mAB) MLH14 (Oncogene Science, Inc.), a polyclonal antibody generated to codons 2-20 of hPMS2 described above, or a polyclonal antibody generated to codons 843-862 of hPMS2 (Santa Cruz Biotechnology, Inc.) in 400:1 of EBC buffer (50 mM Tris, pH 7.5, 0.1 M NaCl, 0.5% NP40). After incubation for 1 hour at 4° C., protein A sepharose (Sigma) was added to a final concentration of 10% and reactions were incubated at 4° C. for 1 hour. Proteins bound to protein A were washed five times in EBC and separated by electrophoresis on 4 20% Tris glycine gels, which were then dried and autoradiographed.

Example 5

Syrian Hamster Tk-ts-13 Cells Produce a Novel Anti-Microbial Polypeptide can Suppress the Growth of *Bacillus subtilis*

[0080] The feasibility of creating microbial-resistant mammalian cells is demonstrated as follows. Syrian Hamster TK fibroblasts were transfected with a mammalian expression vector containing a novel anti-microbial polypeptide called mlg1 or the empty expression vector called psg. When cells expressing the mlg polypeptide (referred to as TK-mlg1) were grown in the presence of *Bacillus subtilis*, these cells were able to suppress the growth of the microbes and allow the TK host to remain viable in contrast to TK cells transfected with the empty vector (TK=psg), which all died from the toxic effects that *Bacillus subtilis* has on mammalian cells. **FIG. 5** shows a photograph of TK-mlg1 and TK=psg cultures grown in the presence of Bacillus for 4 days. Syrian hamster Tk-ts 13 cells were transfected with a eukaryotic expression vector that produces a novel antimicrobial polypeptide referred to as mlg1 (Panel A) or the expression vector lacking an inserted cDNA for expression (TK=psg, Panel B). Cells were plated at a density of 5×10^5 cells/ml in a 10 cm falcon pyrogenic-free petri dish in growth medium for 24 hours and then inoculated with 10:1 of an exponentially growing culture of *Bacillus subtilis*. Cultures were then incubated for 4 days at which time Bacilli grow and begin to lyse the Tk-ts13 parental culture as shown in panel B (indicated by arrows), while cells expressing the anti-microbial mlglpolypeptide (Panel A) remain viable in the presence of Bacillus (small granular structures present in panels A and B). These data demonstrate the feasibility of cells to survive in the presence of Bacillus contamination when they produce an anti-microbial agent. These data show that antimicrobial producing mammalian cells are capable of growing and surviving in the presence of toxic microbes.

[0081] Cell lines and transfection. Syrian Hamster fibroblast Tk ts13 cells were obtained from ATCC and cultured

as described. Modrich, Science, 1994,266, 1959 1960. Stably transfected cell lines expressing hPMS2 were created by cotransfection of the PMS2 expression vectors and the pLHL4 plasmid encoding the hygromycin resistance gene at a ratio of 3:1 (pCAR:pLHL4) and selected with hygromycin. Stably transfected cell lines containing pCAR reporters were generated by co transfection of pCAR vectors together with either pNTK plasmid encoding the neomycin resistance plasmid or with pLHL4. All transfections were performed using calcium phosphate as previously described in Modrich, *Science,* 1994, 266, 1959 1960, which is incorporated herein by reference in its entirety.

Example 6

TK-hPMS2-134 Cells can Suppress the Growth of *Escherichia coli* In Vitro

[**0082**] While TK-hPMS2-134 TK-ts13 cells have been previously shown to be capable of altering genes in vivo (refer to Table 2 and **FIG. 1**), the ability to generate "naturally microbial-resistant" clones has not been reported in the literature. To generate microbial-resistant TK cells, TK-ts13 cells constitutively expressing the a dominant-negative mismatch repair gene, TK-hPMS2-134 or the empty vector (TKvect) that have been in culture for >3 months (~60 passages) were seeded at 5×10^5 cells/ml in Dulbelcco's Modified Eagles Medium (DMEM) plus 10% fetal bovine serum (FBS) and plated into 10 cm dishes (Falcon) in duplicate. These cells were grown overnight at 37° C. in 5%CO2 to allow cells to adhere to the plastic. The next day, TK cultures were inoculated with 10:1 of an exponentially growing culture of *Escherichia coli*. Cultures were then grown at 37° C. in 5%CO2 and observed on day 7 and 14 for microbial-resistant cell clones; these cells appear as clones of cells surrounded by "cleared" areas on the plate. At day 7, all cells in the control transfected TKvect culture were dead, while a subset of cells were viable in the TK-hPMS2-134 transfected cultures. At day 14, there were no clones in the control transfected TKvect cultures, while there were 34 and 40 *Escherichia coli*-resistant colonies formed in the TK-hPMS2-134 transfected cultures. Growing clones from each dish were then pooled as individual cultures and grown to confluence. These cultures were named TK-hPMS2-134 (R1) and TK-hPMS2-134 (R2). Cultures were cured of *Escherichia coli* by the addition of 1 mg/ml G418, in which the TK-hPMS2-134 cells are resistant due to expression of the neomycin-resistance gene contained on the mammalian expression vector used to generate the cells.

[**0083**] TKvect, TK-hPMS2-134 (R1) and TK-hPMS2-134 (R2) cells were plated at 5×10^5 cell/ml in 10 mls and plated into 10 cm dishes in duplicate. The next day, 10:1 of a logarithmic stage *Escherichia coli* culture was added to each TK culture and cultures were grown for 48 hours at 37° C. in 5% CO_2. An aliquot of supernatant from each culture was harvested immediately after inoculation to establish a baseline density of bacteria for each culture. After 48 hours, 2 ml of supernatant were harvested from each culture as well as from uninfected TK cultures. One ml of each supernatant was then analyzed by a spectrophotometer at an OD6% to measure for bacterial density. Supernatants from uninfected cultures were used as a blank to correct for background. As shown in **FIG. 6**, bacterial growth was significantly suppressed in TK-hPMS2-134 (R1) and TK-hPMS2-134 (R2) cultures in contrast to TKvect control cells. These data demonstrate the feasibility of using a dominant-negative mismatch repair mutant hPMS2-134 on mammalian cells to produce genetically altered clones capable of producing a molecule(s) that can suppress microbial growth.

[**0084**] As those skilled in the art will appreciate, numerous changes and modifications may be made to the preferred embodiments of the invention without departing from the spirit of the invention. It is intended that all such variations fall within the scope of the invention. In addition, the entire disclosure of each publication cited herein is hereby incorporated herein by reference.

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atctggaaat attcagaaag aatggctttg actttgtcat tgatgaggat gctccagtca 2460

ctgaaagggc taaattgatt tccttaccaa ctagtaaaaa ctggaccttt ggaccccaag 2520

atatagatga actgatcttt atgttaagtg acagccctgg ggtcatgtgc cggccctcac 2580

gagtcagaca gatgtttgct tccagagcct gtcggaagtc agtgatgatt ggaacggcgc 2640

tcaatgcgag cgagatgaag aagctcatca cccacatggg tgagatggac cacccctgga 2700

actgccccca cggcaggcca accatgaggc acgttgccaa tctggatgtc atctctcaga 2760

actgacacac cccttgtagc atagagttta ttacagattg ttcggtttgc aaagagaagg 2820

ttttaagtaa tctgattatc gttgtacaaa aattagcatg ctgctttaat gtactggatc 2880

catttaaaag cagtgttaag gcaggcatga tggagtgttc ctctagctca gctacttggg 2940

tgatccggtg ggagctcatg tgagcccagg actttgagac cactccgagc cacattcatg 3000

agactcaatt caaggacaaa aaaaaaaaga tatttttgaa gccttttaaa aaaaaa 3056

<210> SEQ ID NO 9
<211> LENGTH: 862
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

Met Glu Arg Ala Glu Ser Ser Ser Thr Glu Pro Ala Lys Ala Ile Lys
1 5 10 15

Pro Ile Asp Arg Lys Ser Val His Gln Ile Cys Ser Gly Gln Val Val
20 25 30

Leu Ser Leu Ser Thr Ala Val Lys Glu Leu Val Glu Asn Ser Leu Asp
35 40 45

Ala Gly Ala Thr Asn Ile Asp Leu Lys Leu Lys Asp Tyr Gly Val Asp
50 55 60

Leu Ile Glu Val Ser Asp Asn Gly Cys Gly Val Glu Glu Glu Asn Phe
65 70 75 80

Glu Gly Leu Thr Leu Lys His His Thr Ser Lys Ile Gln Glu Phe Ala
85 90 95

Asp Leu Thr Gln Val Glu Thr Phe Gly Phe Arg Gly Glu Ala Leu Ser
100 105 110

Ser Leu Cys Ala Leu Ser Asp Val Thr Ile Ser Thr Cys His Ala Ser
115 120 125

Ala Lys Val Gly Thr Arg Leu Met Phe Asp His Asn Gly Lys Ile Ile
130 135 140

Gln Lys Thr Pro Tyr Pro Arg Pro Arg Gly Thr Thr Val Ser Val Gln
145 150 155 160

Gln Leu Phe Ser Thr Leu Pro Val Arg His Lys Glu Phe Gln Arg Asn
165 170 175

Ile Lys Lys Glu Tyr Ala Lys Met Val Gln Val Leu His Ala Tyr Cys
180 185 190

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Ile Ile Ser Ala Gly Ile Arg Val Ser Cys Thr Asn Gln Leu Gly Gln
        195                 200                 205

Gly Lys Arg Gln Pro Val Val Cys Thr Gly Gly Ser Pro Ser Ile Lys
    210                 215                 220

Glu Asn Ile Gly Ser Val Phe Gly Gln Lys Gln Leu Gln Ser Leu Ile
225                 230                 235                 240

Pro Phe Val Gln Leu Pro Pro Ser Asp Ser Val Cys Glu Glu Tyr Gly
                245                 250                 255

Leu Ser Cys Ser Asp Ala Leu His Asn Leu Phe Tyr Ile Ser Gly Phe
            260                 265                 270

Ile Ser Gln Cys Thr His Gly Val Gly Arg Ser Ser Thr Asp Arg Gln
        275                 280                 285

Phe Phe Phe Ile Asn Arg Arg Pro Cys Asp Pro Ala Lys Val Cys Arg
    290                 295                 300

Leu Val Asn Glu Val Tyr His Met Tyr Asn Arg His Gln Tyr Pro Phe
305                 310                 315                 320

Val Val Leu Asn Ile Ser Val Asp Ser Glu Cys Val Asp Ile Asn Val
                325                 330                 335

Thr Pro Asp Lys Arg Gln Ile Leu Leu Gln Glu Glu Lys Leu Leu Leu
            340                 345                 350

Ala Val Leu Lys Thr Ser Leu Ile Gly Met Phe Asp Ser Asp Val Asn
        355                 360                 365

Lys Leu Asn Val Ser Gln Gln Pro Leu Leu Asp Val Glu Gly Asn Leu
    370                 375                 380

Ile Lys Met His Ala Ala Asp Leu Glu Lys Pro Met Val Glu Lys Gln
385                 390                 395                 400

Asp Gln Ser Pro Ser Leu Arg Thr Gly Glu Glu Lys Lys Asp Val Ser
                405                 410                 415

Ile Ser Arg Leu Arg Glu Ala Phe Ser Leu Arg His Thr Thr Glu Asn
            420                 425                 430

Lys Pro His Ser Pro Lys Thr Pro Glu Pro Arg Arg Ser Pro Leu Gly
        435                 440                 445

Gln Lys Arg Gly Met Leu Ser Ser Ser Thr Ser Gly Ala Ile Ser Asp
    450                 455                 460

Lys Gly Val Leu Arg Pro Gln Lys Glu Ala Val Ser Ser Ser His Gly
465                 470                 475                 480

Pro Ser Asp Pro Thr Asp Arg Ala Glu Val Glu Lys Asp Ser Gly His
                485                 490                 495

Gly Ser Thr Ser Val Asp Ser Glu Gly Phe Ser Ile Pro Asp Thr Gly
            500                 505                 510

Ser His Cys Ser Ser Glu Tyr Ala Ala Ser Ser Pro Gly Asp Arg Gly
        515                 520                 525

Ser Gln Glu His Val Asp Ser Gln Glu Lys Ala Pro Glu Thr Asp Asp
    530                 535                 540

Ser Phe Ser Asp Val Asp Cys His Ser Asn Gln Glu Asp Thr Gly Cys
545                 550                 555                 560

Lys Phe Arg Val Leu Pro Gln Pro Thr Asn Leu Ala Thr Pro Asn Thr
                565                 570                 575

Lys Arg Phe Lys Lys Glu Glu Ile Leu Ser Ser Ser Asp Ile Cys Gln
            580                 585                 590

Lys Leu Val Asn Thr Gln Asp Met Ser Ala Ser Gln Val Asp Val Ala
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595 600 605

Val Lys Ile Asn Lys Lys Val Val Pro Leu Asp Phe Ser Met Ser Ser
610 615 620

Leu Ala Lys Arg Ile Lys Gln Leu His His Glu Ala Gln Gln Ser Glu
625 630 635 640

Gly Glu Gln Asn Tyr Arg Lys Phe Arg Ala Lys Ile Cys Pro Gly Glu
645 650 655

Asn Gln Ala Ala Glu Asp Glu Leu Arg Lys Glu Ile Ser Lys Thr Met
660 665 670

Phe Ala Glu Met Glu Ile Ile Gly Gln Phe Asn Leu Gly Phe Ile Ile
675 680 685

Thr Lys Leu Asn Glu Asp Ile Phe Ile Val Asp Gln His Ala Thr Asp
690 695 700

Glu Lys Tyr Asn Phe Glu Met Leu Gln Gln His Thr Val Leu Gln Gly
705 710 715 720

Gln Arg Leu Ile Ala Pro Gln Thr Leu Asn Leu Thr Ala Val Asn Glu
725 730 735

Ala Val Leu Ile Glu Asn Leu Glu Ile Phe Arg Lys Asn Gly Phe Asp
740 745 750

Phe Val Ile Asp Glu Asn Ala Pro Val Thr Glu Arg Ala Lys Leu Ile
755 760 765

Ser Leu Pro Thr Ser Lys Asn Trp Thr Phe Gly Pro Gln Asp Val Asp
770 775 780

Glu Leu Ile Phe Met Leu Ser Asp Ser Pro Gly Val Met Cys Arg Pro
785 790 795 800

Ser Arg Val Lys Gln Met Phe Ala Ser Arg Ala Cys Arg Lys Ser Val
805 810 815

Met Ile Gly Thr Ala Leu Asn Thr Ser Glu Met Lys Lys Leu Ile Thr
820 825 830

His Met Gly Glu Met Asp His Pro Trp Asn Cys Pro His Gly Arg Pro
835 840 845

Thr Met Arg His Ile Ala Asn Leu Gly Val Ile Ser Gln Asn
850 855 860

<210> SEQ ID NO 10
<211> LENGTH: 2771
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

cgaggcggat cgggtgttgc atccatggag cgagctgaga gctcgagtac agaacctgct 60

aaggccatca aacctattga tcggaagtca gtccatcaga tttgctctgg gcaggtggta 120

ctgagtctaa gcactgcggt aaaggagtta gtagaaaaca gtctggatgc tggtgccact 180

aatattgatc taaagcttaa ggactatgga gtggatctta ttgaagtttc agacaatgga 240

tgtggggtag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt 300

caagagtttg ccgacctaac tcaggttgaa acttttggct ttcgggggga agctctgagc 360

tcactttgtg cactgagcga tgtcaccatt tctacctgcc acgcatcggc gaaggttgga 420

actcgactga tgtttgatca caatgggaaa attatccaga aaacccccta cccccgcccc 480

agagggacca cagtcagcgt gcagcagtta ttttccacac tacctgtgcg ccataaggaa 540

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tttcaaagga atattaagaa ggagtatgcc aaaatggtcc aggtcttaca tgcatactgt 600

atcatttcag caggcatccg tgtaagttgc accaatcagc ttggacaagg aaaacgacag 660

cctgtggtat gcacaggtgg aagccccagc ataaaggaaa atatcggctc tgtgtttggg 720

cagaagcagt tgcaaagcct cattcctttt gttcagctgc cccctagtga ctccgtgtgt 780

gaagagtacg gtttgagctg ttcggatgct ctgcataatc ttttttacat ctcaggtttc 840

atttcacaat gcacgcatgg agttggaagg agttcaacag acagacagtt tttctttatc 900

aaccggcggc cttgtgaccc agcaaaggtc tgcagactcg tgaatgaggt ctaccacatg 960

tataatcgac accagtatcc atttgttgtt cttaacattt ctgttgattc agaatgcgtt 1020

gatatcaatg ttactccaga taaaaggcaa attttgctac aagaggaaaa gcttttgttg 1080

gcagttttaa agacctcttt gataggaatg tttgatagtg atgtcaacaa gctaaatgtc 1140

agtcagcagc cactgctgga tgttgaaggt aacttaataa aaatgcatgc agcggatttg 1200

gaaaagccca tggtagaaaa gcaggatcaa tccccttcat taaggactgg agaagaaaaa 1260

aaagacgtgt ccatttccag actgcgagag gccttttctc ttcgtcacac aacagagaac 1320

aagcctcaca gcccaaagac tccagaacca agaaggagcc ctctaggaca gaaaaggggt 1380

atgctgtctt ctagcacttc aggtgccatc tctgacaaag gcgtcctgag acctcagaaa 1440

gaggcagtga gttccagtca cggacccagt gaccctacgg acagagcgga ggtggagaag 1500

gactcggggc acggcagcac ttccgtggat tctgaggggt tcagcatccc agacacgggc 1560

agtcactgca gcagcgagta tgcggccagc tccccagggg acaggggctc gcaggaacat 1620

gtggactctc aggagaaagc gcctgaaact gacgactctt tttcagatgt ggactgccat 1680

tcaaaccagg aagataccgg atgtaaattt cgagttttgc ctcagccaac taatctcgca 1740

accccaaaca caaagcgttt taaaaaagaa gaaattcttt ccagttctga catttgtcaa 1800

aagttagtaa atactcagga catgtcagcc tctcaggttg atgtagctgt gaaaattaat 1860

aagaaagttg tgcccctgga cttttctatg agttctttag ctaaacgaat aaagcagtta 1920

catcatgaag cacagcaaag tgaaggggaa cagaattaca ggaagtttag ggcaaagatt 1980

tgtcctggag aaaatcaagc agccgaagat gaactaagaa aagagataag taaaacgatg 2040

tttgcagaaa tggaaatcat tggtcagttt aacctgggat ttataataac caaactgaat 2100

gaggatatct tcatagtgga ccagcatgcc acggacgaga agtataactt cgagatgctg 2160

cagcagcaca ccgtgctcca ggggcagagg ctcatagcac ctcagactct caacttaact 2220

gctgttaatg aagctgttct gatagaaaat ctggaaatat ttagaaagaa tggctttgat 2280

tttgttatcg atgaaaatgc tccagtcact gaaagggcta aactgatttc cttgccaact 2340

agtaaaaact ggaccttcgg accccaggac gtcgatgaac tgatcttcat gctgagcgac 2400

agccctgggg tcatgtgccg gccttcccga gtcaagcaga tgtttgcctc cagagcctgc 2460

cggaagtcgg tgatgattgg gactgctctt aacacaagcg agatgaagaa actgatcacc 2520

cacatggggg agatggacca cccctggaac tgtccccatg gaaggccaac catgagacac 2580

atcgccaacc tgggtgtcat ttctcagaac tgaccgtagt cactgtatgg aataattggt 2640

tttatcgcag atttttatgt tttgaaagac agagtcttca ctaacctttt ttgttttaaa 2700

atgaaacctg ctacttaaaa aaaatacaca tcacacccat ttaaaagtga tcttgagaac 2760

cttttcaaac c 2771

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<210> SEQ ID NO 11
<211> LENGTH: 932
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

Met Lys Gln Leu Pro Ala Ala Thr Val Arg Leu Leu Ser Ser Ser Gln
1 5 10 15

Ile Ile Thr Ser Val Val Ser Val Val Lys Glu Leu Ile Glu Asn Ser
20 25 30

Leu Asp Ala Gly Ala Thr Ser Val Asp Val Lys Leu Glu Asn Tyr Gly
35 40 45

Phe Asp Lys Ile Glu Val Arg Asp Asn Gly Glu Gly Ile Lys Ala Val
50 55 60

Asp Ala Pro Val Met Ala Met Lys Tyr Tyr Thr Ser Lys Ile Asn Ser
65 70 75 80

His Glu Asp Leu Glu Asn Leu Thr Thr Tyr Gly Phe Arg Gly Glu Ala
85 90 95

Leu Gly Ser Ile Cys Cys Ile Ala Glu Val Leu Ile Thr Thr Arg Thr
100 105 110

Ala Ala Asp Asn Phe Ser Thr Gln Tyr Val Leu Asp Gly Ser Gly His
115 120 125

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

Ala Leu Arg Leu Phe Lys Asn Leu Pro Val Arg Lys Gln Phe Tyr Ser
145 150 155 160

Thr Ala Lys Lys Cys Lys Asp Glu Ile Lys Lys Ile Gln Asp Leu Leu
165 170 175

Met Ser Phe Gly Ile Leu Lys Pro Asp Leu Arg Ile Val Phe Val His
180 185 190

Asn Lys Ala Val Ile Trp Gln Lys Ser Arg Val Ser Asp His Lys Met
195 200 205

Ala Leu Met Ser Val Leu Gly Thr Ala Val Met Asn Asn Met Glu Ser
210 215 220

Phe Gln Tyr His Ser Glu Glu Ser Gln Ile Tyr Leu Ser Gly Phe Leu
225 230 235 240

Pro Lys Cys Asp Ala Asp His Ser Phe Thr Ser Leu Ser Thr Pro Glu
245 250 255

Arg Ser Phe Ile Phe Ile Asn Ser Arg Pro Val His Gln Lys Asp Ile
260 265 270

Leu Lys Leu Ile Arg His His Tyr Asn Leu Lys Cys Leu Lys Glu Ser
275 280 285

Thr Arg Leu Tyr Pro Val Phe Phe Leu Lys Ile Asp Val Pro Thr Ala
290 295 300

Asp Val Asp Val Asn Leu Thr Pro Asp Lys Ser Gln Val Leu Leu Gln
305 310 315 320

Asn Lys Glu Ser Val Leu Ile Ala Leu Glu Asn Leu Met Thr Thr Cys
325 330 335

Tyr Gly Pro Leu Pro Ser Thr Asn Ser Tyr Glu Asn Asn Lys Thr Asp
340 345 350

Val Ser Ala Ala Asp Ile Val Leu Ser Lys Thr Ala Glu Thr Asp Val
355 360 365

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Leu Phe Asn Lys Val Glu Ser Ser Gly Lys Asn Tyr Ser Asn Val Asp
    370                 375                 380

Thr Ser Val Ile Pro Phe Gln Asn Asp Met His Asn Asp Glu Ser Gly
385                 390                 395                 400

Lys Asn Thr Asp Asp Cys Leu Asn His Gln Ile Ser Ile Gly Asp Phe
                405                 410                 415

Gly Tyr Gly His Cys Ser Ser Glu Ile Ser Asn Ile Asp Lys Asn Thr
            420                 425                 430

Lys Asn Ala Phe Gln Asp Ile Ser Met Ser Asn Val Ser Trp Glu Asn
        435                 440                 445

Ser Gln Thr Glu Tyr Ser Lys Thr Cys Phe Ile Ser Ser Val Lys His
    450                 455                 460

Thr Gln Ser Glu Asn Gly Asn Lys Asp His Ile Asp Glu Ser Gly Glu
465                 470                 475                 480

Asn Glu Glu Glu Ala Gly Leu Glu Asn Ser Ser Glu Ile Ser Ala Asp
                485                 490                 495

Glu Trp Ser Arg Gly Asn Ile Leu Lys Asn Ser Val Gly Glu Asn Ile
            500                 505                 510

Glu Pro Val Lys Ile Leu Val Pro Glu Lys Ser Leu Pro Cys Lys Val
        515                 520                 525

Ser Asn Asn Asn Tyr Pro Ile Pro Glu Gln Met Asn Leu Asn Glu Asp
    530                 535                 540

Ser Cys Asn Lys Lys Ser Asn Val Ile Asp Asn Lys Ser Gly Lys Val
545                 550                 555                 560

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

Ala Ser Ala Leu Phe Val Gln Asp His Arg Pro Gln Phe Leu Ile Glu
            580                 585                 590

Asn Pro Lys Thr Ser Leu Glu Asp Ala Thr Leu Gln Ile Glu Glu Leu
        595                 600                 605

Trp Lys Thr Leu Ser Glu Glu Glu Lys Leu Lys Tyr Glu Glu Lys Ala
    610                 615                 620

Thr Lys Asp Leu Glu Arg Tyr Asn Ser Gln Met Lys Arg Ala Ile Glu
625                 630                 635                 640

Gln Glu Ser Gln Met Ser Leu Lys Asp Gly Arg Lys Lys Ile Lys Pro
                645                 650                 655

Thr Ser Ala Trp Asn Leu Ala Gln Lys His Lys Leu Lys Thr Ser Leu
            660                 665                 670

Ser Asn Gln Pro Lys Leu Asp Glu Leu Leu Gln Ser Gln Ile Glu Lys
        675                 680                 685

Arg Arg Ser Gln Asn Ile Lys Met Val Gln Ile Pro Phe Ser Met Lys
    690                 695                 700

Asn Leu Lys Ile Asn Phe Lys Lys Gln Asn Lys Val Asp Leu Glu Glu
705                 710                 715                 720

Lys Asp Glu Pro Cys Leu Ile His Asn Leu Arg Phe Pro Asp Ala Trp
                725                 730                 735

Leu Met Thr Ser Lys Thr Glu Val Met Leu Leu Asn Pro Tyr Arg Val
            740                 745                 750

Glu Glu Ala Leu Leu Phe Lys Arg Leu Leu Glu Asn His Lys Leu Pro
        755                 760                 765
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Ala Glu Pro Leu Glu Lys Pro Ile Met Leu Thr Glu Ser Leu Phe Asn
    770                 775                 780

Gly Ser His Tyr Leu Asp Val Leu Tyr Lys Met Thr Ala Asp Asp Gln
785                 790                 795                 800

Arg Tyr Ser Gly Ser Thr Tyr Leu Ser Asp Pro Arg Leu Thr Ala Asn
                805                 810                 815

Gly Phe Lys Ile Lys Leu Ile Pro Gly Val Ser Ile Thr Glu Asn Tyr
            820                 825                 830

Leu Glu Ile Glu Gly Met Ala Asn Cys Leu Pro Phe Tyr Gly Val Ala
        835                 840                 845

Asp Leu Lys Glu Ile Leu Asn Ala Ile Leu Asn Arg Asn Ala Lys Glu
    850                 855                 860

Val Tyr Glu Cys Arg Pro Arg Lys Val Ile Ser Tyr Leu Glu Gly Glu
865                 870                 875                 880

Ala Val Arg Leu Ser Arg Gln Leu Pro Met Tyr Leu Ser Lys Glu Asp
                885                 890                 895

Ile Gln Asp Ile Ile Tyr Arg Met Lys His Gln Phe Gly Asn Glu Ile
            900                 905                 910

Lys Glu Cys Val His Gly Arg Pro Phe Phe His His Leu Thr Tyr Leu
        915                 920                 925

Pro Glu Thr Thr
    930
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<210> SEQ ID NO 12
<211> LENGTH: 3063
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

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ggcacgagtg gctgcttgcg gctagtggat ggtaattgcc tgcctcgcgc tagcagcaag     60

ctgctctgtt aaaagcgaaa atgaaacaat tgcctgcggc aacagttcga ctcctttcaa    120

gttctcagat catcacttcg gtggtcagtg ttgtaaaaga gcttattgaa aactccttgg    180

atgctggtgc cacaagcgta gatgttaaac tggagaacta tggatttgat aaaattgagg    240

tgcgagataa cggggagggt atcaaggctg ttgatgcacc tgtaatggca atgaagtact    300

acacctcaaa aataaatagt catgaagatc ttgaaaattt gacaacttac ggttttcgtg    360

gagaagcctt ggggtcaatt tgttgtatag ctgaggtttt aattacaaca agaacggctg    420

ctgataattt tagcacccag tatgttttag atggcagtgg ccacatactt tctcagaaac    480

cttcacatct tggtcaaggt acaactgtaa ctgctttaag attatttaag aatctacctg    540

taagaaagca gttttactca actgcaaaaa aatgtaaaga tgaaataaaa aagatccaag    600

atctcctcat gagctttggt atccttaaac ctgacttaag gattgtcttt gtacataaca    660

aggcagttat ttggcagaaa agcagagtat cagatcacaa gatggctctc atgtcagttc    720

tggggactgc tgttatgaac aatatggaat cctttcagta ccactctgaa gaatctcaga    780

tttatctcag tggatttctt ccaaagtgtg atgcagacca ctctttcact agtctttcaa    840

caccagaaag aagtttcatc ttcataaaca gtcgaccagt acatcaaaaa gatatcttaa    900

agttaatccg acatcattac aatctgaaat gcctaaagga atctactcgt ttgtatcctg    960

ttttctttct gaaaatcgat gttcctacag ctgatgttga tgtaaattta acaccagata   1020

aaagccaagt attattacaa aataaggaat ctgttttaat tgctcttgaa aatctgatga   1080
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cgacttgtta tggaccatta cctagtacaa attcttatga aaataataaa acagatgttt 1140

ccgcagctga catcgttctt agtaaaacag cagaaacaga tgtgcttttt aataaagtgg 1200

aatcatctgg aaagaattat tcaaatgttg atacttcagt cattccattc caaaatgata 1260

tgcataatga tgaatctgga aaaaacactg atgattgttt aaatcaccag ataagtattg 1320

gtgactttgg ttatggtcat tgtagtagtg aaatttctaa cattgataaa aacactaaga 1380

atgcatttca ggacatttca atgagtaatg tatcatggga gaactctcag acggaatata 1440

gtaaaacttg ttttataagt tccgttaagc acacccagtc agaaaatggc aataaagacc 1500

atatagatga gagtggggaa aatgaggaag aagcaggtct tgaaaactct tcggaaattt 1560

ctgcagatga gtggagcagg ggaaatatac ttaaaaattc agtgggagag aatattgaac 1620

ctgtgaaaat tttagtgcct gaaaaaagtt taccatgtaa agtaagtaat aataattatc 1680

caatccctga acaaatgaat cttaatgaag attcatgtaa caaaaaatca aatgtaatag 1740

ataataaatc tggaaaagtt acagcttatg atttacttag caatcgagta atcaagaaac 1800

ccatgtcagc aagtgctctt tttgttcaag atcatcgtcc tcagtttctc atagaaaatc 1860

ctaagactag tttagaggat gcaacactac aaattgaaga actgtggaag acattgagtg 1920

aagaggaaaa actgaaatat gaagagaagg ctactaaaga cttggaacga tacaatagtc 1980

aaatgaagag agccattgaa caggagtcac aaatgtcact aaaagatggc agaaaaaaga 2040

taaaacccac cagcgcatgg aatttggccc agaagcacaa gttaaaaacc tcattatcta 2100

atcaaccaaa acttgatgaa ctccttcagt cccaaattga aaaaagaagg agtcaaaata 2160

ttaaaatggt acagatcccc ttttctatga aaaacttaaa aataaatttt aagaaacaaa 2220

acaaagttga cttagaagag aaggatgaac cttgcttgat ccacaatctc aggtttcctg 2280

atgcatggct aatgacatcc aaaacagagg taatgttatt aaatccatat agagtagaag 2340

aagccctgct atttaaaaga cttcttgaga atcataaact tcctgcagag ccactggaaa 2400

agccaattat gttaacagag agtcttttta atggatctca ttatttagac gttttatata 2460

aaatgacagc agatgaccaa agatacagtg gatcaactta cctgtctgat cctcgtctta 2520

cagcgaatgg tttcaagata aaattgatac caggagtttc aattactgaa aattacttgg 2580

aaatagaagg aatggctaat tgtctcccat tctatggagt agcagattta aaagaaattc 2640

ttaatgctat attaaacaga aatgcaaagg aagtttatga atgtagacct cgcaaagtga 2700

taagttattt agagggagaa gcagtgcgtc tatccagaca attacccatg tacttatcaa 2760

aagaggacat ccaagacatt atctacagaa tgaagcacca gtttggaaat gaaattaaag 2820

agtgtgttca tggtcgccca ttttttcatc atttaaccta tcttccagaa actacatgat 2880

taaatatgtt taagaagatt agttaccatt gaaattggtt ctgtcataaa acagcatgag 2940

tctggtttta aattatcttt gtattatgtg tcacatggtt atttttaaa tgaggattca 3000

ctgacttgtt tttatattga aaaaagttcc acgtattgta gaaaacgtaa ataaactaat 3060

aac 3063

<210> SEQ ID NO 13
<211> LENGTH: 934
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

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Met Ala Val Gln Pro Lys Glu Thr Leu Gln Leu Glu Ser Ala Ala Glu
  1               5                  10                  15

Val Gly Phe Val Arg Phe Phe Gln Gly Met Pro Glu Lys Pro Thr Thr
            20                  25                  30

Thr Val Arg Leu Phe Asp Arg Gly Asp Phe Tyr Thr Ala His Gly Glu
         35                  40                  45

Asp Ala Leu Leu Ala Ala Arg Glu Val Phe Lys Thr Gln Gly Val Ile
     50                  55                  60

Lys Tyr Met Gly Pro Ala Gly Ala Lys Asn Leu Gln Ser Val Val Leu
 65                  70                  75                  80

Ser Lys Met Asn Phe Glu Ser Phe Val Lys Asp Leu Leu Leu Val Arg
                85                  90                  95

Gln Tyr Arg Val Glu Val Tyr Lys Asn Arg Ala Gly Asn Lys Ala Ser
            100                 105                 110

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

Ser Gln Phe Glu Asp Ile Leu Phe Gly Asn Asn Asp Met Ser Ala Ser
    130                 135                 140

Ile Gly Val Val Gly Val Lys Met Ser Ala Val Asp Gly Gln Arg Gln
145                 150                 155                 160

Val Gly Val Gly Tyr Val Asp Ser Ile Gln Arg Lys Leu Gly Leu Cys
                165                 170                 175

Glu Phe Pro Asp Asn Asp Gln Phe Ser Asn Leu Glu Ala Leu Leu Ile
            180                 185                 190

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

Asp Met Gly Lys Leu Arg Gln Ile Ile Gln Arg Gly Gly Ile Leu Ile
    210                 215                 220

Thr Glu Arg Lys Lys Ala Asp Phe Ser Thr Lys Asp Ile Tyr Gln Asp
225                 230                 235                 240

Leu Asn Arg Leu Leu Lys Gly Lys Lys Gly Glu Gln Met Asn Ser Ala
                245                 250                 255

Val Leu Pro Glu Met Glu Asn Gln Val Ala Val Ser Ser Leu Ser Ala
            260                 265                 270

Val Ile Lys Phe Leu Glu Leu Leu Ser Asp Asp Ser Asn Phe Gly Gln
        275                 280                 285

Phe Glu Leu Thr Thr Phe Asp Phe Ser Gln Tyr Met Lys Leu Asp Ile
    290                 295                 300

Ala Ala Val Arg Ala Leu Asn Leu Phe Gln Gly Ser Val Glu Asp Thr
305                 310                 315                 320

Thr Gly Ser Gln Ser Leu Ala Ala Leu Leu Asn Lys Cys Lys Thr Pro
                325                 330                 335

Gln Gly Gln Arg Leu Val Asn Gln Trp Ile Lys Gln Pro Leu Met Asp
            340                 345                 350

Lys Asn Arg Ile Glu Glu Arg Leu Asn Leu Val Glu Ala Phe Val Glu
        355                 360                 365

Asp Ala Glu Leu Arg Gln Thr Leu Gln Glu Asp Leu Leu Arg Arg Phe
    370                 375                 380

Pro Asp Leu Asn Arg Leu Ala Lys Lys Phe Gln Arg Gln Ala Ala Asn
385                 390                 395                 400
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-continued

```
Leu Gln Asp Cys Tyr Arg Leu Tyr Gln Gly Ile Asn Gln Leu Pro Asn
                405                 410                 415

Val Ile Gln Ala Leu Glu Lys His Glu Gly Lys His Gln Lys Leu Leu
            420                 425                 430

Leu Ala Val Phe Val Thr Pro Leu Thr Asp Leu Arg Ser Asp Phe Ser
        435                 440                 445

Lys Phe Gln Glu Met Ile Glu Thr Thr Leu Asp Met Asp Gln Val Glu
    450                 455                 460

Asn His Glu Phe Leu Val Lys Pro Ser Phe Asp Pro Asn Leu Ser Glu
465                 470                 475                 480

Leu Arg Glu Ile Met Asn Asp Leu Glu Lys Lys Met Gln Ser Thr Leu
                485                 490                 495

Ile Ser Ala Ala Arg Asp Leu Gly Leu Asp Pro Gly Lys Gln Ile Lys
            500                 505                 510

Leu Asp Ser Ser Ala Gln Phe Gly Tyr Tyr Phe Arg Val Thr Cys Lys
        515                 520                 525

Glu Glu Lys Val Leu Arg Asn Asn Lys Asn Phe Ser Thr Val Asp Ile
    530                 535                 540

Gln Lys Asn Gly Val Lys Phe Thr Asn Ser Lys Leu Thr Ser Leu Asn
545                 550                 555                 560

Glu Glu Tyr Thr Lys Asn Lys Thr Glu Tyr Glu Glu Ala Gln Asp Ala
                565                 570                 575

Ile Val Lys Glu Ile Val Asn Ile Ser Ser Gly Tyr Val Glu Pro Met
            580                 585                 590

Gln Thr Leu Asn Asp Val Leu Ala Gln Leu Asp Ala Val Val Ser Phe
        595                 600                 605

Ala His Val Ser Asn Gly Ala Pro Val Pro Tyr Val Arg Pro Ala Ile
    610                 615                 620

Leu Glu Lys Gly Gln Gly Arg Ile Ile Leu Lys Ala Ser Arg His Ala
625                 630                 635                 640

Cys Val Glu Val Gln Asp Glu Ile Ala Phe Ile Pro Asn Asp Val Tyr
                645                 650                 655

Phe Glu Lys Asp Lys Gln Met Phe His Ile Ile Thr Gly Pro Asn Met
            660                 665                 670

Gly Gly Lys Ser Thr Tyr Ile Arg Gln Thr Gly Val Ile Val Leu Met
        675                 680                 685

Ala Gln Ile Gly Cys Phe Val Pro Cys Glu Ser Ala Glu Val Ser Ile
    690                 695                 700

Val Asp Cys Ile Leu Ala Arg Val Gly Ala Gly Asp Ser Gln Leu Lys
705                 710                 715                 720

Gly Val Ser Thr Phe Met Ala Glu Met Leu Glu Thr Ala Ser Ile Leu
                725                 730                 735

Arg Ser Ala Thr Lys Asp Ser Leu Ile Ile Ile Asp Glu Leu Gly Arg
            740                 745                 750

Gly Thr Ser Thr Tyr Asp Gly Phe Gly Leu Ala Trp Ala Ile Ser Glu
        755                 760                 765

Tyr Ile Ala Thr Lys Ile Gly Ala Phe Cys Met Phe Ala Thr His Phe
    770                 775                 780

His Glu Leu Thr Ala Leu Ala Asn Gln Ile Pro Thr Val Asn Asn Leu
785                 790                 795                 800

His Val Thr Ala Leu Thr Thr Glu Glu Thr Leu Thr Met Leu Tyr Gln
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               805                 810                 815

Val Lys Lys Gly Val Cys Asp Gln Ser Phe Gly Ile His Val Ala Glu
           820                 825                 830

Leu Ala Asn Phe Pro Lys His Val Ile Glu Cys Ala Lys Gln Lys Ala
       835                 840                 845

Leu Glu Leu Glu Glu Phe Gln Tyr Ile Gly Glu Ser Gln Gly Tyr Asp
   850                 855                 860

Ile Met Glu Pro Ala Ala Lys Lys Cys Tyr Leu Glu Arg Glu Gln Gly
865                 870                 875                 880

Glu Lys Ile Ile Gln Glu Phe Leu Ser Lys Val Lys Gln Met Pro Phe
               885                 890                 895

Thr Glu Met Ser Glu Glu Asn Ile Thr Ile Lys Leu Lys Gln Leu Lys
           900                 905                 910

Ala Glu Val Ile Ala Lys Asn Asn Ser Phe Val Asn Glu Ile Ile Ser
       915                 920                 925

Arg Ile Lys Val Thr Thr
   930
```

<210> SEQ ID NO 14
<211> LENGTH: 3145
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

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gtttcgacat ggcggtgcag ccgaaggaga cgctgcagtt ggagagcgcg gccgaggtcg    120

gcttcgtgcg cttctttcag ggcatgccgg agaagccgac caccacagtg cgccttttcg    180

accggggcga cttctatacg gcgcacggcg aggacgcgct gctggccgcc cgggaggtgt    240

tcaagaccca gggggtgatc aagtacatgg ggccggcagg agcaaagaat ctgcagagtg    300

ttgtgcttag taaaatgaat tttgaatctt ttgtaaaaga tcttcttctg gttcgtcagt    360

atagagttga agtttataag aatagagctg gaaataaggc atccaaggag aatgattggt    420

atttggcata taaggcttct cctggcaatc tctctcagtt tgaagacatt ctctttggta    480

acaatgatat gtcagcttcc attggtgttg tgggtgttaa aatgtccgca gttgatggcc    540

agagacaggt tggagttggg tatgtggatt ccatacagag gaaactagga ctgtgtgaat    600

tccctgataa tgatcagttc tccaatcttg aggctctcct catccagatt ggaccaaagg    660

aatgtgtttt acccggagga gagactgctg gagacatggg gaaactgaga cagataattc    720

aaagaggagg aattctgatc acagaaagaa aaaaagctga cttttccaca aaagacattt    780

atcaggacct caaccggttg ttgaaaggca aaaagggaga gcagatgaat agtgctgtat    840

tgccagaaat ggagaatcag gttgcagttt catcactgtc tgcggtaatc aagtttttag    900

aactcttatc agatgattcc aactttggac agtttgaact gactactttt gacttcagcc    960

agtatatgaa attggatatt gcagcagtca gagcccttaa ccttttttcag ggttctgttg   1020

aagataccac tggctctcag tctctggctg ccttgctgaa taagtgtaaa acccctcaag   1080

gacaaagact tgttaaccag tggattaagc agcctctcat ggataagaac agaatagagg   1140

agagattgaa tttagtggaa gcttttgtag aagatgcaga attgaggcag actttacaag   1200

aagatttact tcgtcgattc ccagatctta accgacttgc caagaagttt caaagacaag   1260
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cagcaaactt acaagattgt taccgactct atcagggtat aaatcaacta cctaatgtta 1320

tacaggctct ggaaaaacat gaaggaaaac accagaaatt attgttggca gtttttgtga 1380

ctcctcttac tgatcttcgt tctgacttct ccaagtttca ggaaatgata gaaacaactt 1440

tagatatgga tcaggtggaa aaccatgaat tccttgtaaa accttcattt gatcctaatc 1500

tcagtgaatt aagagaaata atgaatgact tggaaaagaa gatgcagtca acattaataa 1560

gtgcagccag agatcttggc ttggaccctg gcaaacagat taaactggat tccagtgcac 1620

agtttggata ttactttcgt gtaacctgta aggaagaaaa agtccttcgt aacaataaaa 1680

actttagtac tgtagatatc cagaagaatg gtgttaaatt taccaacagc aaattgactt 1740

ctttaaatga agagtatacc aaaaataaaa cagaatatga agaagcccag gatgccattg 1800

ttaaagaaat tgtcaatatt tcttcaggct atgtagaacc aatgcagaca ctcaatgatg 1860

tgttagctca gctagatgct gttgtcagct ttgctcacgt gtcaaatgga gcacctgttc 1920

catatgtacg accagccatt ttggagaaag gacaaggaag aattatatta aaagcatcca 1980

ggcatgcttg tgttgaagtt caagatgaaa ttgcatttat tcctaatgac gtatactttg 2040

aaaaagataa acagatgttc cacatcatta ctggccccaa tatgggaggt aaatcaacat 2100

atattcgaca aactggggtg atagtactca tggcccaaat tgggtgtttt gtgccatgtg 2160

agtcagcaga agtgtccatt gtggactgca tcttagcccg agtaggggct ggtgacagtc 2220

aattgaaagg agtctccacg ttcatggctg aaatgttgga aactgcttct atcctcaggt 2280

ctgcaaccaa agattcatta ataatcatag atgaattggg aagaggaact tctacctacg 2340

atggatttgg gttagcatgg gctatatcag aatacattgc aacaaagatt ggtgcttttt 2400

gcatgtttgc aacccatttt catgaactta ctgccttggc caatcagata ccaactgtta 2460

ataatctaca tgtcacagca ctcaccactg aagagacctt aactatgctt tatcaggtga 2520

agaaaggtgt ctgtgatcaa agttttggga ttcatgttgc agagcttgct aatttcccta 2580

agcatgtaat agagtgtgct aaacagaaag ccctggaact tgaggagttt cagtatattg 2640

gagaatcgca aggatatgat atcatggaac cagcagcaaa gaagtgctat ctggaaagag 2700

agcaaggtga aaaaattatt caggagttcc tgtccaaggt gaaacaaatg ccctttactg 2760

aaatgtcaga agaaaacatc acaataaagt taaaacagct aaaagctgaa gtaatagcaa 2820

agaataatag ctttgtaaat gaaatcattt cacgaataaa agttactacg tgaaaaatcc 2880

cagtaatgga atgaaggtaa tattgataag ctattgtctg taatagtttt atattgtttt 2940

atattaaccc tttttccata gtgttaactg tcagtgccca tgggctatca acttaataag 3000

atatttagta atattttact ttgaggacat tttcaaagat ttttattttg aaaaatgaga 3060

gctgtaactg aggactgttt gcaattgaca taggcaataa taagtgatgt gctgaatttt 3120

ataaataaaa tcatgtagtt tgtgg 3145

<210> SEQ ID NO 15
<211> LENGTH: 756
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

Met Ser Phe Val Ala Gly Val Ile Arg Arg Leu Asp Glu Thr Val Val
1 5 10 15

Asn Arg Ile Ala Ala Gly Glu Val Ile Gln Arg Pro Ala Asn Ala Ile

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            20                  25                  30

Lys Glu Met Ile Glu Asn Cys Leu Asp Ala Lys Ser Thr Ser Ile Gln
         35                  40                  45

Val Ile Val Lys Glu Gly Gly Leu Lys Leu Ile Gln Ile Gln Asp Asn
     50                  55                  60

Gly Thr Gly Ile Arg Lys Glu Asp Leu Asp Ile Val Cys Glu Arg Phe
 65                  70                  75                  80

Thr Thr Ser Lys Leu Gln Ser Phe Glu Asp Leu Ala Ser Ile Ser Thr
                85                  90                  95

Tyr Gly Phe Arg Gly Glu Ala Leu Ala Ser Ile Ser His Val Ala His
            100                 105                 110

Val Thr Ile Thr Thr Lys Thr Ala Asp Gly Lys Cys Ala Tyr Arg Ala
        115                 120                 125

Ser Tyr Ser Asp Gly Lys Leu Lys Ala Pro Pro Lys Pro Cys Ala Gly
    130                 135                 140

Asn Gln Gly Thr Gln Ile Thr Val Glu Asp Leu Phe Tyr Asn Ile Ala
145                 150                 155                 160

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

Leu Glu Val Val Gly Arg Tyr Ser Val His Asn Ala Gly Ile Ser Phe
            180                 185                 190

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

Asn Ala Ser Thr Val Asp Asn Ile Arg Ser Ile Phe Gly Asn Ala Val
    210                 215                 220

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

Lys Met Asn Gly Tyr Ile Ser Asn Ala Asn Tyr Ser Val Lys Lys Cys
                245                 250                 255

Ile Phe Leu Leu Phe Ile Asn His Arg Leu Val Glu Ser Thr Ser Leu
            260                 265                 270

Arg Lys Ala Ile Glu Thr Val Tyr Ala Ala Tyr Leu Pro Lys Asn Thr
        275                 280                 285

His Pro Phe Leu Tyr Leu Ser Leu Glu Ile Ser Pro Gln Asn Val Asp
    290                 295                 300

Val Asn Val His Pro Thr Lys His Glu Val His Phe Leu His Glu Glu
305                 310                 315                 320

Ser Ile Leu Glu Arg Val Gln Gln His Ile Glu Ser Lys Leu Leu Gly
                325                 330                 335

Ser Asn Ser Ser Arg Met Tyr Phe Thr Gln Thr Leu Leu Pro Gly Leu
            340                 345                 350

Ala Gly Pro Ser Gly Glu Met Val Lys Ser Thr Thr Ser Leu Thr Ser
        355                 360                 365

Ser Ser Thr Ser Gly Ser Ser Asp Lys Val Tyr Ala His Gln Met Val
    370                 375                 380

Arg Thr Asp Ser Arg Glu Gln Lys Leu Asp Ala Phe Leu Gln Pro Leu
385                 390                 395                 400

Ser Lys Pro Leu Ser Ser Gln Pro Gln Ala Ile Val Thr Glu Asp Lys
                405                 410                 415

Thr Asp Ile Ser Ser Gly Arg Ala Arg Gln Gln Asp Glu Glu Met Leu
            420                 425                 430
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```
Glu Leu Pro Ala Pro Ala Glu Val Ala Ala Lys Asn Gln Ser Leu Glu
        435                 440                 445

Gly Asp Thr Thr Lys Gly Thr Ser Glu Met Ser Glu Lys Arg Gly Pro
    450                 455                 460

Thr Ser Ser Asn Pro Arg Lys Arg His Arg Glu Asp Ser Asp Val Glu
465                 470                 475                 480

Met Val Glu Asp Asp Ser Arg Lys Glu Met Thr Ala Ala Cys Thr Pro
                485                 490                 495

Arg Arg Arg Ile Ile Asn Leu Thr Ser Val Leu Ser Leu Gln Glu Glu
            500                 505                 510

Ile Asn Glu Gln Gly His Glu Val Leu Arg Glu Met Leu His Asn His
        515                 520                 525

Ser Phe Val Gly Cys Val Asn Pro Gln Trp Ala Leu Ala Gln His Gln
    530                 535                 540

Thr Lys Leu Tyr Leu Leu Asn Thr Thr Lys Leu Ser Glu Glu Leu Phe
545                 550                 555                 560

Tyr Gln Ile Leu Ile Tyr Asp Phe Ala Asn Phe Gly Val Leu Arg Leu
                565                 570                 575

Ser Glu Pro Ala Pro Leu Phe Asp Leu Ala Met Leu Ala Leu Asp Ser
            580                 585                 590

Pro Glu Ser Gly Trp Thr Glu Glu Asp Gly Pro Lys Glu Gly Leu Ala
        595                 600                 605

Glu Tyr Ile Val Glu Phe Leu Lys Lys Lys Ala Glu Met Leu Ala Asp
    610                 615                 620

Tyr Phe Ser Leu Glu Ile Asp Glu Glu Gly Asn Leu Ile Gly Leu Pro
625                 630                 635                 640

Leu Leu Ile Asp Asn Tyr Val Pro Pro Leu Glu Gly Leu Pro Ile Phe
                645                 650                 655

Ile Leu Arg Leu Ala Thr Glu Val Asn Trp Asp Glu Glu Lys Glu Cys
            660                 665                 670

Phe Glu Ser Leu Ser Lys Glu Cys Ala Met Phe Tyr Ser Ile Arg Lys
        675                 680                 685

Gln Tyr Ile Ser Glu Glu Ser Thr Leu Ser Gly Gln Gln Ser Glu Val
    690                 695                 700

Pro Gly Ser Ile Pro Asn Ser Trp Lys Trp Thr Val Glu His Ile Val
705                 710                 715                 720

Tyr Lys Ala Leu Arg Ser His Ile Leu Pro Pro Lys His Phe Thr Glu
                725                 730                 735

Asp Gly Asn Ile Leu Gln Leu Ala Asn Leu Pro Asp Leu Tyr Lys Val
            740                 745                 750

Phe Glu Arg Cys
        755
```

<210> SEQ ID NO 16
<211> LENGTH: 2484
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

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acagtggtga accgcatcgc ggcgggggaa gttatccagc ggccagctaa tgctatcaaa    120
```

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gagatgattg agaactgttt agatgcaaaa tccacaagta ttcaagtgat tgttaaagag 180

ggaggcctga agttgattca gatccaagac aatggcaccg ggatcaggaa agaagatctg 240

gatattgtat gtgaaaggtt cactactagt aaactgcagt cctttgagga tttagccagt 300

atttctacct atggctttcg aggtgaggct ttggccagca taagccatgt ggctcatgtt 360

actattacaa cgaaaacagc tgatggaaag tgtgcataca gagcaagtta ctcagatgga 420

aaactgaaag cccctcctaa accatgtgct ggcaatcaag ggacccagat cacggtggag 480

gacctttttt acaacatagc cacgaggaga aaagctttaa aaaatccaag tgaagaatat 540

gggaaaattt tggaagttgt tggcaggtat tcagtacaca atgcaggcat tagtttctca 600

gttaaaaaac aaggagagac agtagctgat gttaggacac tacccaatgc ctcaaccgtg 660

gacaatattc gctccatctt tggaaatgct gttagtcgag aactgataga aattggatgt 720

gaggataaaa ccctagcctt caaaatgaat ggttacatat ccaatgcaaa ctactcagtg 780

aagaagtgca tcttcttact cttcatcaac catcgtctgg tagaatcaac ttccttgaga 840

aaagccatag aaacagtgta tgcagcctat ttgcccaaaa acacacaccc attcctgtac 900

ctcagtttag aaatcagtcc ccagaatgtg gatgttaatg tgcaccccac aaagcatgaa 960

gttcacttcc tgcacgagga gagcatcctg gagcgggtgc agcagcacat cgagagcaag 1020

ctcctgggct ccaattcctc caggatgtac ttcacccaga ctttgctacc aggacttgct 1080

ggcccctctg gggagatggt taaatccaca acaagtctga cctcgtcttc tacttctgga 1140

agtagtgata aggtctatgc ccaccagatg gttcgtacag attcccggga acagaagctt 1200

gatgcatttc tgcagcctct gagcaaaccc ctgtccagtc agccccaggc cattgtcaca 1260

gaggataaga cagatatttc tagtggcagg gctaggcagc aagatgagga gatgcttgaa 1320

ctcccagccc ctgctgaagt ggctgccaaa aatcagagct tggaggggga tacaacaaag 1380

gggacttcag aaatgtcaga gaagagagga cctacttcca gcaaccccag aaagagacat 1440

cgggaagatt ctgatgtgga aatggtggaa gatgattccc gaaaggaaat gactgcagct 1500

tgtacccccc ggagaaggat cattaacctc actagtgttt tgagtctcca ggaagaaatt 1560

aatgagcagg gacatgaggt tctccgggag atgttgcata accactcctt cgtgggctgt 1620

gtgaatcctc agtgggcctt ggcacagcat caaaccaagt tataccttct caacaccacc 1680

aagcttagtg aagaactgtt ctaccagata ctcatttatg attttgccaa ttttggtgtt 1740

ctcaggttat cggagccagc accgctcttt gaccttgcca tgcttgcctt agatagtcca 1800

gagagtggct ggacagagga agatggtccc aaagaaggac ttgctgaata cattgttgag 1860

tttctgaaga agaaggctga gatgcttgca gactatttct ctttggaaat tgatgaggaa 1920

gggaacctga ttggattacc ccttctgatt gacaactatg tgcccccttt ggagggactg 1980

cctatcttca ttcttcgact agccactgag gtgaattggg acgaagaaaa ggaatgtttt 2040

gaaagcctca gtaaagaatg cgctatgttc tattccatcc ggaagcagta catatctgag 2100

gagtcgaccc tctcaggcca gcagagtgaa gtgcctggct ccattccaaa ctcctggaag 2160

tggactgtgg aacacattgt ctataaagcc ttgcgctcac acattctgcc tcctaaacat 2220

ttcacagaag atggaaatat cctgcagctt gctaacctgc ctgatctata caaagtcttt 2280

gagaggtgtt aaatatggtt atttatgcac tgtgggatgt gttcttcttt ctctgtattc 2340

cgatacaaag tgttgtatca aagtgtgata tacaaagtgt accaacataa gtgttggtag 2400

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cacttaagac ttatacttgc cttctgatag tattccttta tacacagtgg attgattata 2460

aataaataga tgtgtcttaa cata 2484

<210> SEQ ID NO 17
<211> LENGTH: 133
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

Met Glu Arg Ala Glu Ser Ser Ser Thr Glu Pro Ala Lys Ala Ile Lys
1 5 10 15

Pro Ile Asp Arg Lys Ser Val His Gln Ile Cys Ser Gly Gln Val Val
20 25 30

Leu Ser Leu Ser Thr Ala Val Lys Glu Leu Val Glu Asn Ser Leu Asp
35 40 45

Ala Gly Ala Thr Asn Ile Asp Leu Lys Leu Lys Asp Tyr Gly Val Asp
50 55 60

Leu Ile Glu Val Ser Asp Asn Gly Cys Gly Val Glu Glu Glu Asn Phe
65 70 75 80

Glu Gly Leu Thr Leu Lys His His Thr Ser Lys Ile Gln Glu Phe Ala
85 90 95

Asp Leu Thr Gln Val Glu Thr Phe Gly Phe Arg Gly Glu Ala Leu Ser
100 105 110

Ser Leu Cys Ala Leu Ser Asp Val Thr Ile Ser Thr Cys His Ala Ser
115 120 125

Ala Lys Val Gly Thr
130

<210> SEQ ID NO 18
<211> LENGTH: 426
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

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aaggccatca aacctattga tcggaagtca gtccatcaga tttgctctgg gcaggtggta 120

ctgagtctaa gcactgcggt aaaggagtta gtagaaaaca gtctggatgc tggtgccact 180

aatattgatc taaagcttaa ggactatgga gtggatctta ttgaagtttc agacaatgga 240

tgtggggtag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt 300

caagagtttg ccgacctaac tcaggttgaa acttttggct ttcgggggga agctctgagc 360

tcactttgtg cactgagcga tgtcaccatt tctacctgcc acgcatcggc gaaggttgga 420

acttga 426

What is claimed is:

1. A method for making a mammalian cell hypermutable, comprising:

introducing into said mammalian cell a polynucleotide comprising a dominant-negative allele of a mismatch repair gene, whereby said cell becomes hypermutable.

2. The method of claim 1 wherein said polynucleotide is introduced into said cell by transfection in vitro.

3. The method of claim 1, wherein said polynucleotide is introduced into said cell by transfection of an adherent cell in vitro.

4. The method of claim 1 wherein said mismatch repair gene is PMS2.

5. The method of claim 1 wherein said mismatch repair gene is human PMS2.

6. The method of claim 1 wherein said mismatch repair gene is human MLH1.

7. The method of claim 1 wherein said mismatch repair gene is human PMS1.

8. The method of claim 1 wherein said mismatch repair gene is human MSH2.

9. The method of claim 4 wherein said allele comprises a truncation mutation.

10. The method of claim 4 wherein said allele comprises a truncation mutation at codon 134.

11. The method of claim 9 wherein said truncation mutation is a thymidine at nucleotide 424 of wild-type PMS2.

12. A homogeneous composition comprising a cultured, hypermutable, mammalian cell comprising a dominant negative allele of a mismatch repair gene.

13. The composition of claim 12 wherein said mismatch repair gene is PMS2.

14. The composition of claim 12 wherein said mismatch repair gene is human PMS2.

15. The composition of claim 12 wherein said mismatch repair gene is human MLH1.

16. The composition of claim 12 wherein said mismatch repair gene is human PMS1.

17. The composition of claim 12 wherein said mismatch repair gene is human MSH2.

18. The composition of claim 12 wherein said cell expresses a protein consisting of the first 133 amino acids of hPMS2.

19. A method for obtaining a mammalian cell that is resistant to a selected microbe comprising:

growing a culture of mammalian cells wherein said cells have a dominant-negative allele of a mismatch repair gene;

exposing said cells to said selected microbe; and

selecting said mammalian cell that is resistant to said selected microbe.

20. The method of claim 19 wherein said hypermutable cell is selected for resistance to a gram-negative microbe.

21. The method of claim 19 wherein said hypermutable cell is selected for resistance to a gram-positive microbe.

22. The method of claim 19 wherein said hypermutable cell is selected for resistance to a protozoan.

23. The method of claim 19 wherein said hypermutable cell is selected for resistance to a bacteria.

24. The method of claim 19 wherein said hypermutable cell is selected for resistance to a fungi.

25. The method of claim 19 wherein said step of selecting for microbial resistance comprises isolating and testing conditioned medium from said hypermutable cell.

26. A method for obtaining a cell comprising a mutation in a gene encoding an antimicrobial activity comprising:

growing a culture of mammalian cells having said gene encoding said antimicrobial activity, and a dominant negative allele of a mismatch repair gene;

selecting a cell comprising said antimicrobial activity; and

determining whether said gene comprises a mutation.

27. The method of claim 26 wherein said hypermutable cell is selected for resistance to a gram-negative microbe.

28. The method of claim 26 wherein said hypermutable cell is selected for resistance to a gram-positive microbe.

29. The method of claim 26 wherein said hypermutable cell is selected for resistance to a protozoan.

30. The method of claim 26 wherein said hypermutable cell is selected for resistance to a bacteria.

31. The method of claim 26 wherein said hypermutable cell is selected for resistance to a fungi.

32. The method of claim 26 wherein said step of selecting a cell for antimicrobial activity comprises isolating and testing conditioned medium from said hypermutable cell.

33. The method of claim 26 wherein said step of examining said cell to determine whether said gene comprises a mutation comprises analyzing a nucleotide sequence of said gene.

34. The method of claim 26 wherein said step of examining said cell to determine whether said gene comprises a mutation comprises analyzing mRNA transcribed from said gene.

35. The method of claim 26 wherein said step of examining said cell to determine whether said gene comprises a mutation comprises analyzing a protein encoded by said gene.

36. The method of claim 26 wherein said step of examining said cell to determine whether said gene comprises a mutation comprises analyzing the phenotype of said gene.

* * * * *