US 20050003463A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2005/0003463 A1**

Adorjan et al. (43) **Pub. Date: Jan. 6, 2005**

(54) **METHOD AND NUCLEIC ACIDS FOR THE ANALYSIS OF COLORECTAL CELL PROLIFERATIVE DISORDERS**

(76) Inventors: **Peter Adorjan**, Berlin (DE); **Matthias Burger**, Berlin (DE); **Sabine Maier**, Berlin (DE); **Ralf Lesche**, Berlin (DE); **Susan Cottrell**, Seattle, WA (US); **Theo De Vos**, Seattle, WA (US)

Correspondence Address:
DAVIS WRIGHT TREMAINE, LLP
2600 CENTURY SQUARE
1501 FOURTH AVENUE
SEATTLE, WA 98101-1688 (US)

(21) Appl. No.: **10/482,823**

(22) PCT Filed: **Feb. 13, 2003**

(86) PCT No.: **PCT/EP03/01457**

(30) **Foreign Application Priority Data**

Feb. 27, 2002 (EP) 02004551.4

Publication Classification

(51) **Int. Cl.**[7] **C12Q 1/68**; G01N 33/574

(52) **U.S. Cl.** **435/7.23**

(57) **ABSTRACT**

The invention provides methods and nucleic acids for detecting, differentiating or distinguishing between colon cell proliferative disorders as well as therapy thereof by analysis of the gene EYA4 and its promoter and regulatory sequences. The invention further provides novel nucleic acid sequences useful for the cell proliferative disorder specific analysis of said gene as well as methods, assays and kits thereof.

FIGURE 1

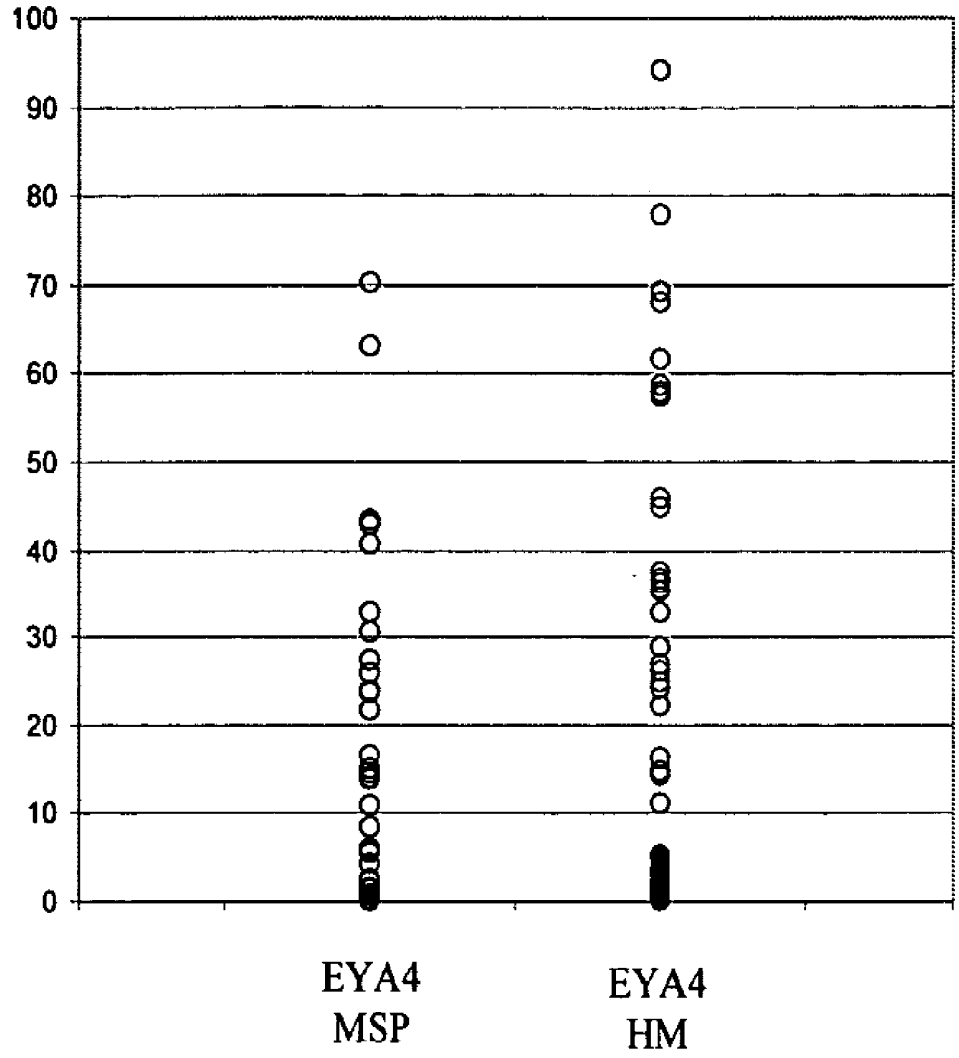


FIGURE 2

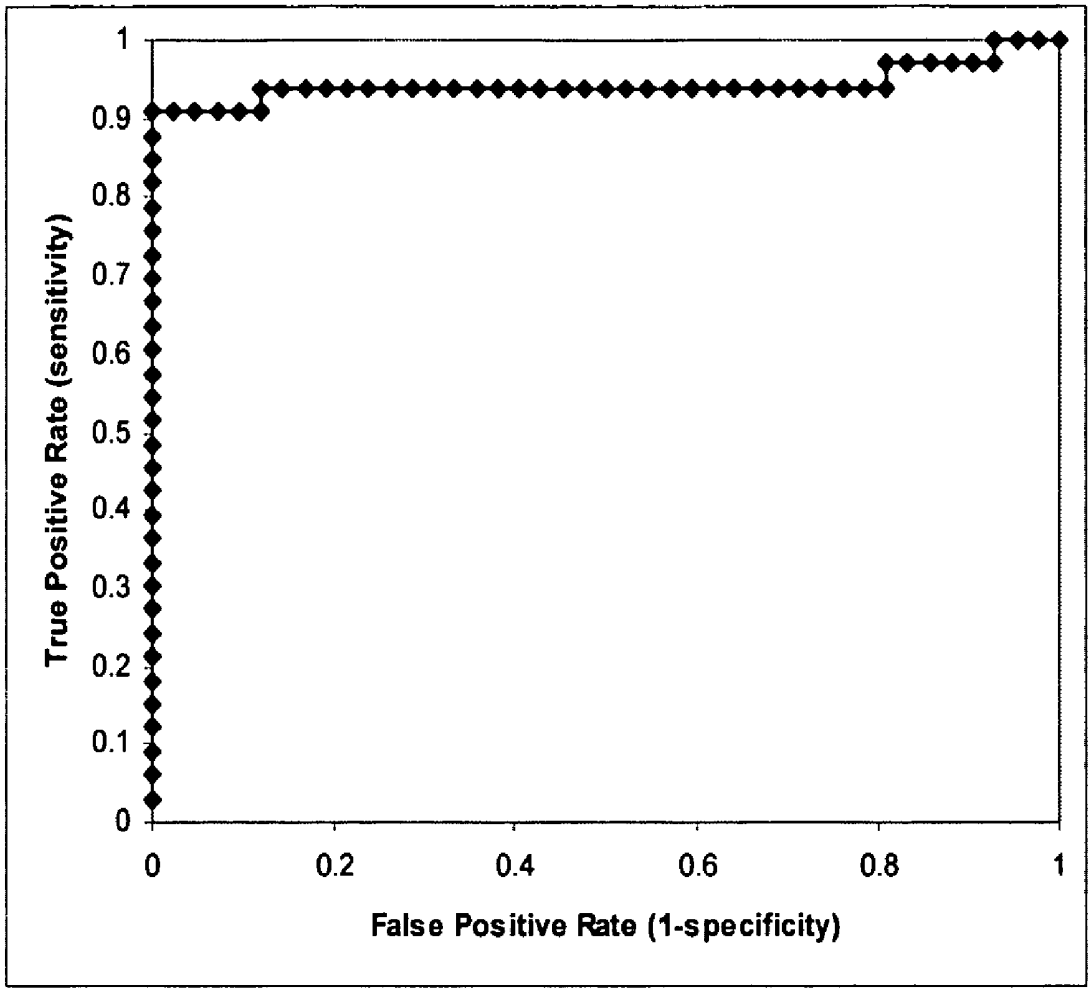

True Positive Rate (sensitivity)
False Positive Rate (1-specificity)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
0.2
0.4
0.6
0.8
1

FIGURE 3

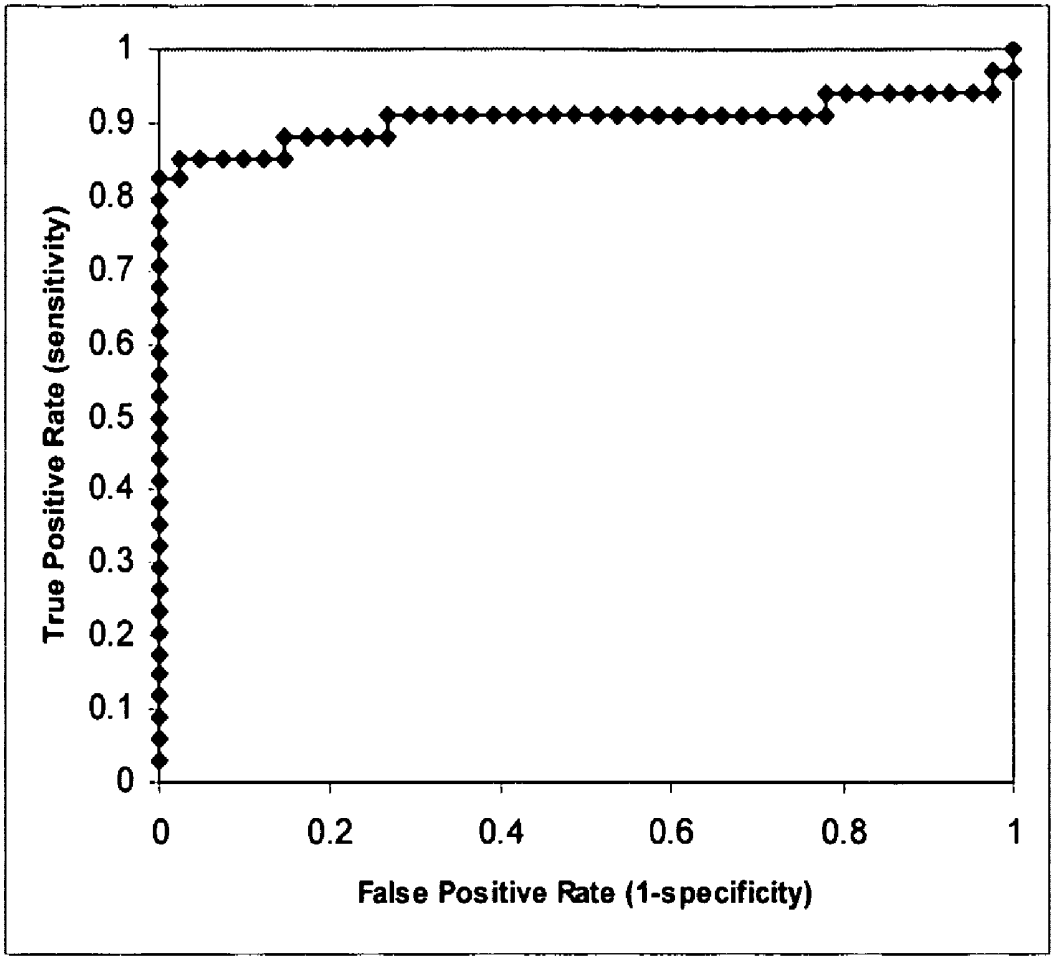

True Positive Rate (sensitivity)
False Positive Rate (1-specificity)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
0.2
0.4
0.6
0.8
1

FIGURE 4

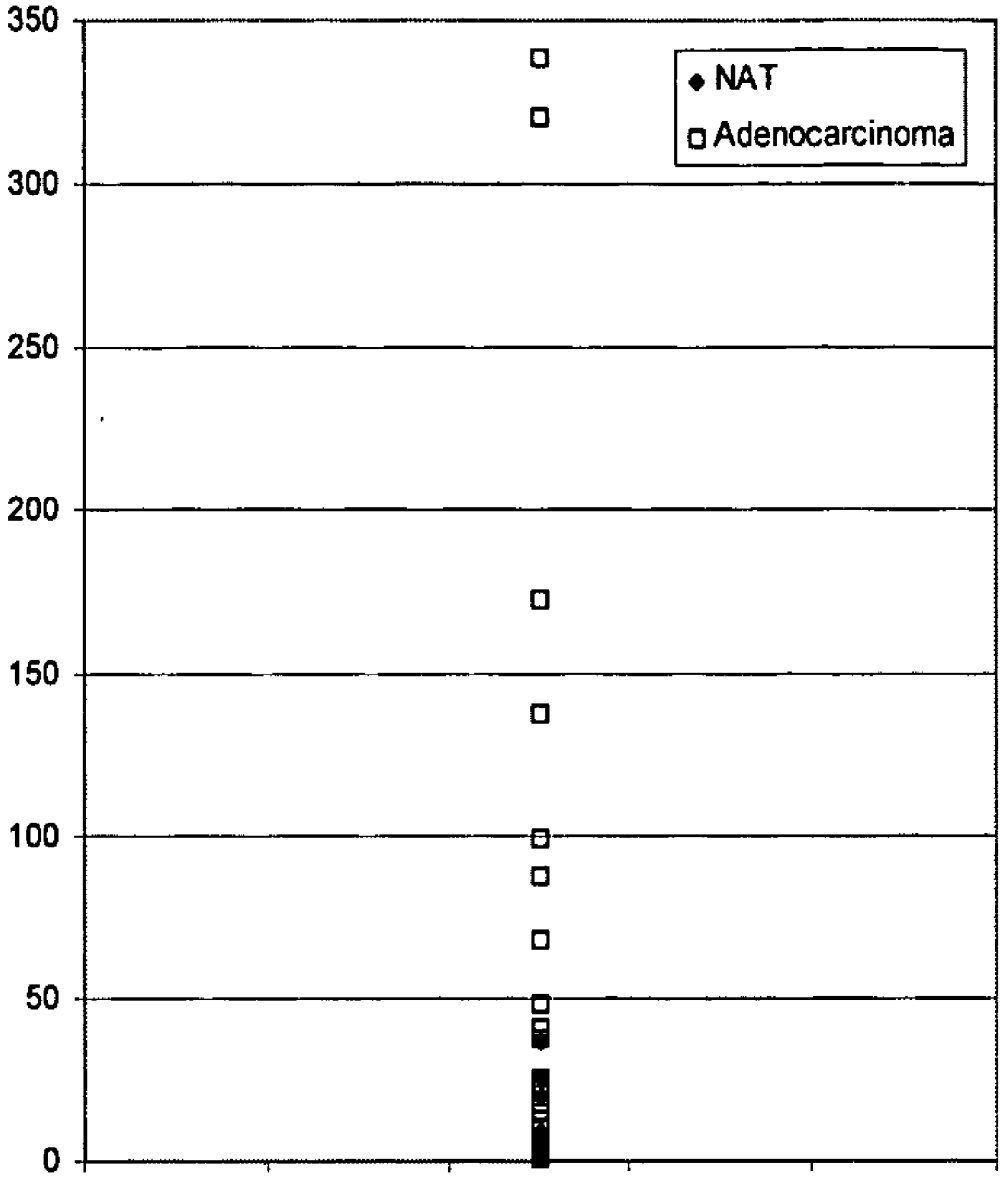


FIGURE 5

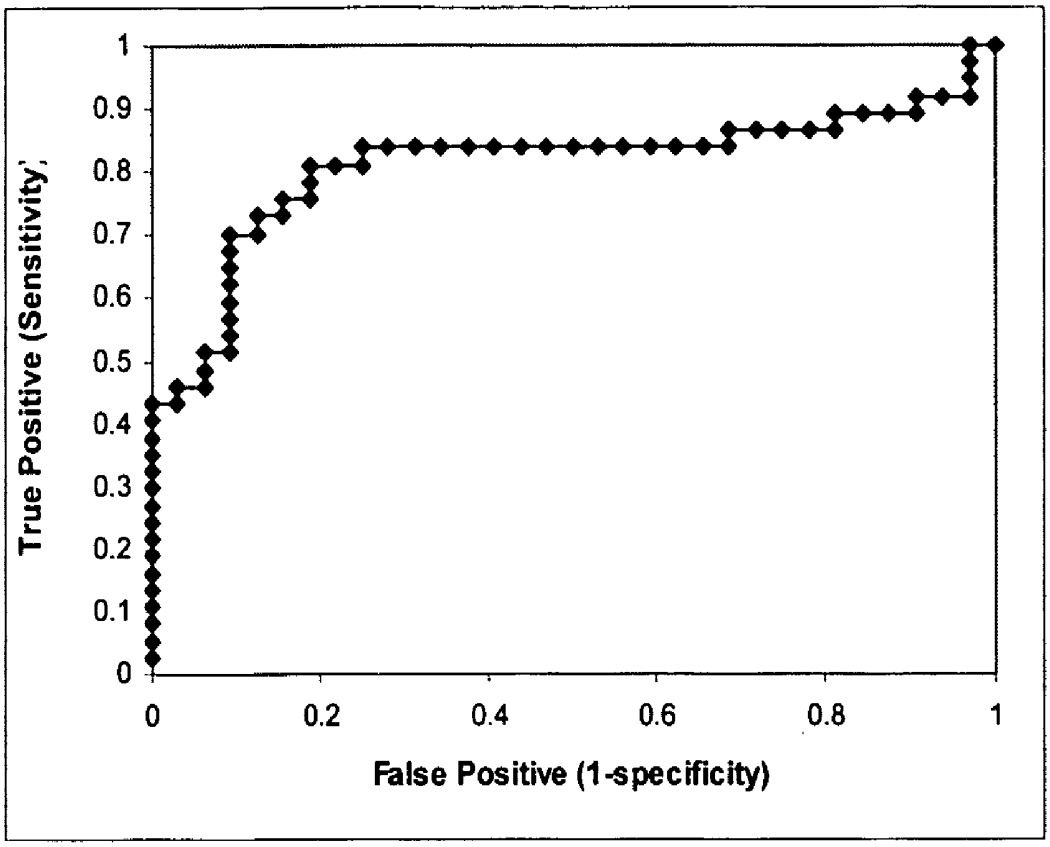

True Positive (Sensitivity)
False Positive (1-specificity)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
0.2
0.4
0.6
0.8
1

FIGURE 6

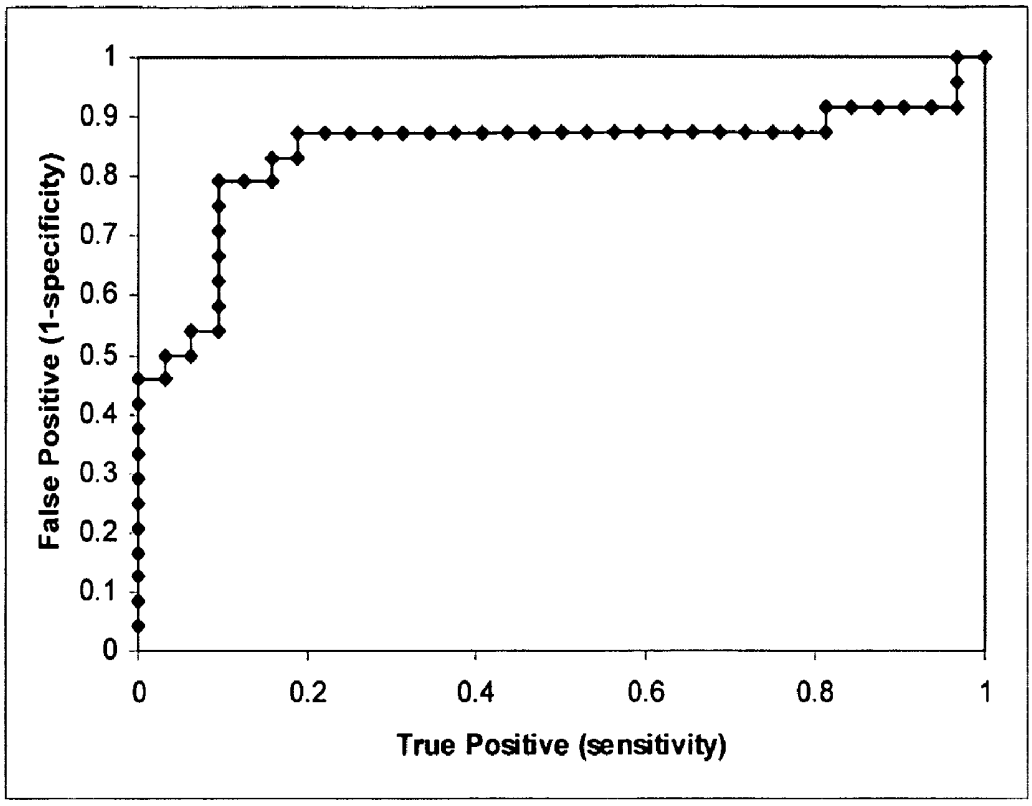

False Positive (1-specificity)
True Positive (sensitivity)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
0.2
0.4
0.6
0.8
1

FIGURE 7

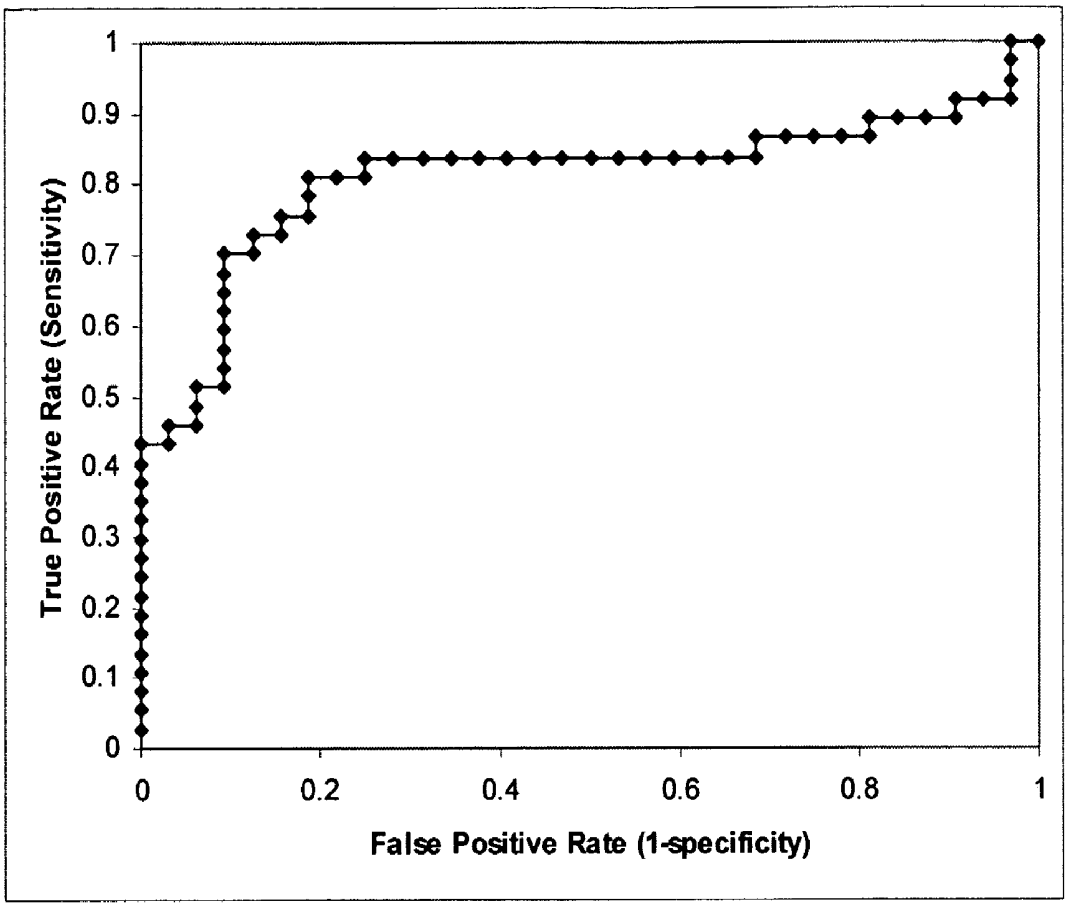

True Positive Rate (Sensitivity)
False Positive Rate (1-specificity)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
0.2
0.4
0.6
0.8
1

FIGURE 8

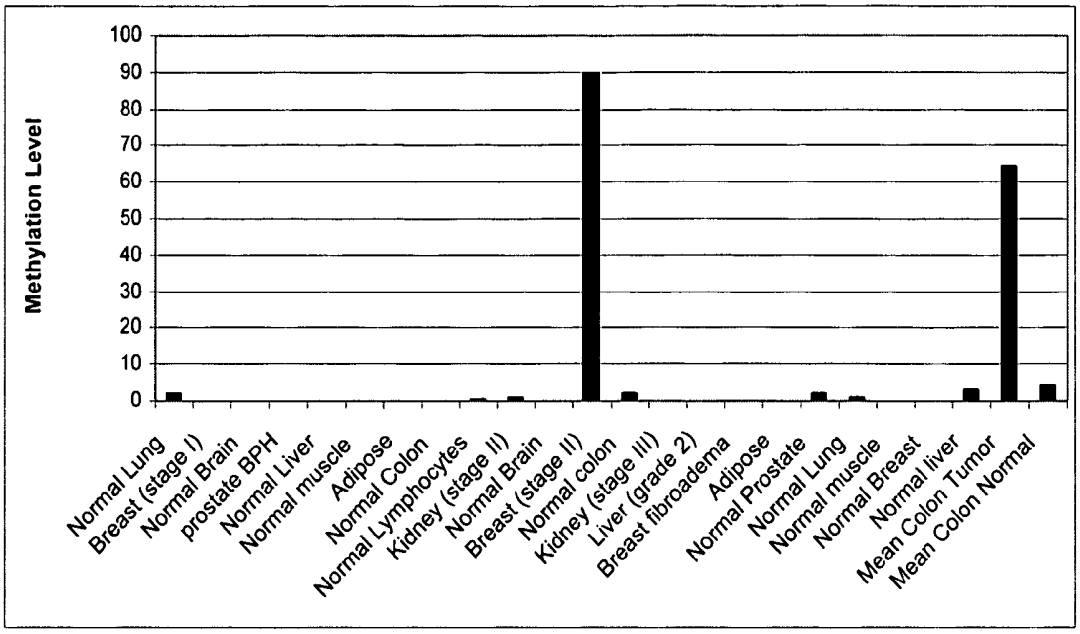

Methylation Level
100
90
80
70
60
50
40
30
20
10
0
Normal Lung
Breast (stage I)
Normal Brain
prostate BPH
Normal Liver
Normal muscle
Adipose
Normal Colon
Normal Lymphocytes
Kidney (stage II)
Normal Brain
Breast (stage II)
Normal colon
Kidney (stage III)
Liver (grade 2)
Breast fibroadema
Adipose
Normal Prostate
Normal Lung
Normal muscle
Normal Breast
Normal liver
Mean Colon Tumor
Mean Colon Normal

FIGURE 9

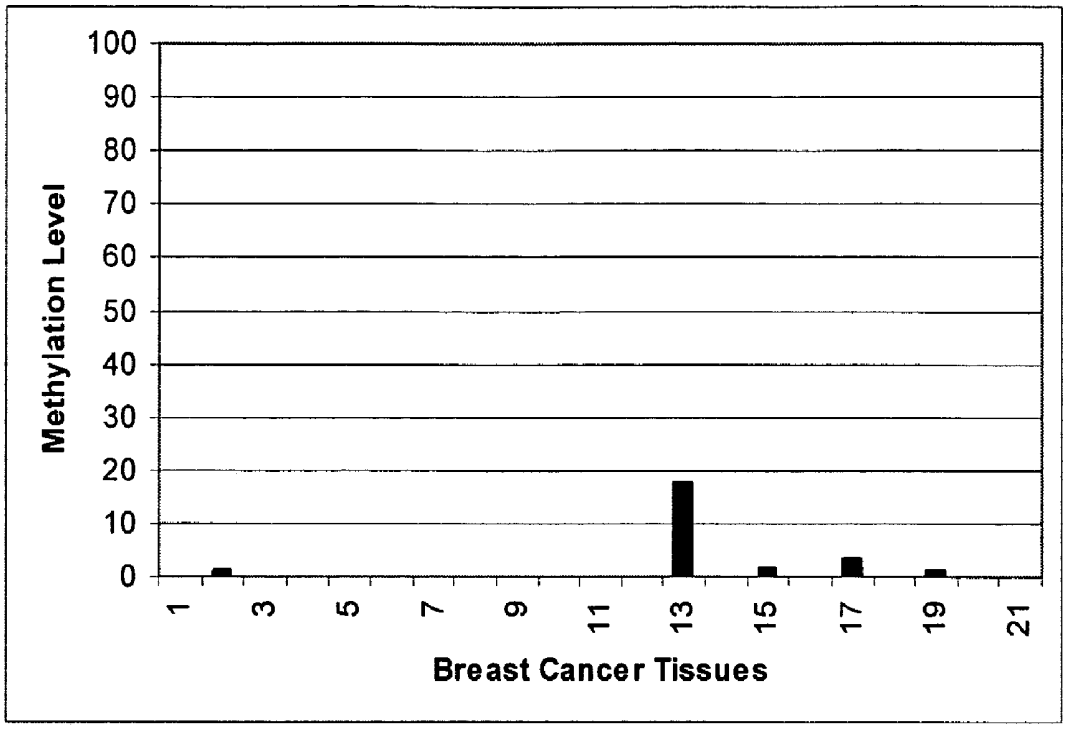
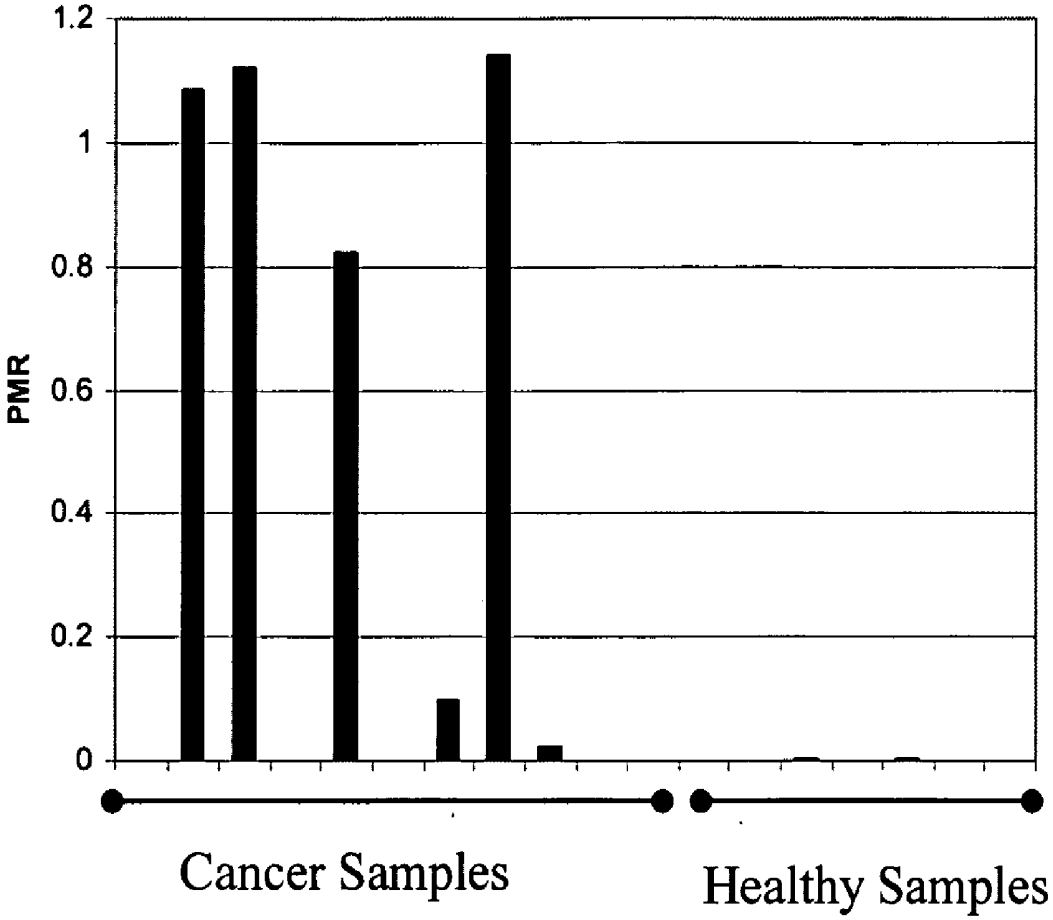

Methylation Level
100
90
80
70
60
50
40
30
20
10
0
1
3
5
7
9
11
13
15
17
19
21
Breast Cancer Tissues

FIGURE 10



METHOD AND NUCLEIC ACIDS FOR THE ANALYSIS OF COLORECTAL CELL PROLIFERATIVE DISORDERS

FIELD OF THE INVENTION

[0001] Colorectal cancer is the fourth leading cause of cancer mortality in men and women. The 5-year survival rate is 61% over all stages with early detection being a prerequisite for curative therapy of the disease. Up to 95% of all colorectal cancers are adenocarcinomas of varying differentiation grades.

[0002] Sporadic colon cancer develops in a multistep process starting with the pathological transformation of normal colonic epithelium to an adenoma which consecutively progresses to invasive cancer. The progression rate of colonic adenomas is currently predicted based on their histological appearance, location, degree of spread and extent of bowel involvement. For example, tubular-type benign adenomas rarely progress to malignant tumours, whereas villous benign adenomas, particularly if larger than 2 cm in diameter, have a significant malignant potential.

[0003] During progression from benign proliferative lesions to malignant neoplasms several genetic and epigenetic alterations are known to occur. Somatic mutation of the APC gene seems to be one of the earliest events in 75 to 80% of colorectal adenomas and carcinomas. Activation of K-RAS is thought to be a critical step in the progression towards a malignant phenotype. Consecutively, mutations in other oncogenes as well as alterations leading to inactivation of tumour suppressor genes accumulate.

[0004] Aberrant DNA methylation within CpG islands is among the earliest and most common alterations in human cancers leading to abrogation or overexpression of a broad spectrum of genes. In addition, abnormal methylation has been shown to occur in CpG rich regulatory elements in intronic and coding parts of genes for certain tumours. In contrast to the specific hypermethylation of tumour suppressor genes, an overall hypomethylation of DNA can be observed in tumour cells. This decrease in global methylation can be detected early, far before the development of frank tumour formation. Also, correlation between hypomethylation and increased gene expression was reported for many oncogenes. In colon cancer, aberrant DNA methylation constitutes one of the most prominent alterations and inactivates many tumour suppressor genes such as p14ARF, p161NK4a, THBS1, MINT2, and MINT31 and DNA mismatch repair genes such as hMLH1.

[0005] In the molecular evolution of colorectal cancer, DNA methylation errors have been suggested to play two distinct roles. In normal colonic mucosal cells, methylation errors accumulate as a function of age or as time-dependent events predisposing these cells to neoplastic transformation. For example, hypermethylation of several loci could be shown to be already present in adenomas, particularly in the tubulovillous and villous subtype. At later stages, increased DNA methylation of CpG islands plays an important role in a subset of tumours affected by the so called CpG island methylator phenotype (CIMP). Most CIMP+ tumours, which constitute about 15% of all sporadic colorectal cancers, are characterised by microsatellite instability (MIN) due to hypermethylation of the hMLHI promoter and other DNA mismatch repair genes. By contrast, CIMP— colon cancers evolve along a more classic genetic instability pathway (CIN), with a high rate of p53 mutations and chromosomal changes.

[0006] However, the molecular subtypes do not only show varying frequencies regarding molecular alterations. According to the presence of either micro satellite instability or chromosomal aberrations, colon cancer can be subclassified into two classes, which also exhibit significant clinical differences. Almost all MIN tumours originate in the proximal colon (ascending and transversum), whereas 70% of CIN tumours are located in the distal colon and rectum. This has been attributed to the varying prevalence of different carcinogens in different sections of the colon. Methylating carcinogens, which constitute the prevailing carcinogen in the proximal colon have been suggested to play a role in the pathogenesis of MIN cancers, whereas CIN tumours are thought to be more frequently caused by adduct-forming carcinogens, which occur more frequently in distal parts of the colon and rectum. Moreover, MIN tumours have a better prognosis than do tumours with a CIN phenotype and respond better to adjuvant chemotherapy.

[0007] The identification of markers for the differentiation of colon carcinoma as well as for early detection are main goals of current research.

[0008] EYA4 is the most recently identified member of the vertebrate Eya (eyes-absent) gene family, a group of four transcriptional activators that interact with other proteins in a conserved regulatory hierarchy to ensure normal embryologic development. The EYA4 gene is mapped to 6q22.3 and encodes a 640 amino acid protein. The structure of EYA4 conforms to the basic pattern established by EYA1-3, and includes a highly conserved 271 amino acid C-terminus called the eya-homologous region (eyaHR; alternatively referred to as the eya domain or eya homology domain 1) and a more divergent proline-serine-threonine (PST)-rich (34-41%) transactivation domain at the N-terminus (Borsani G, et al., EYA4, a novel vertebrate gene related to Drosophila eyes absent. Hum Mol Genet 1999 January;8(1):11-23). EYA proteins interact with members of the SIX and DACH protein families during early embryonic development. Mutations in the EYA4 gene are responsible for postlingual, progressive, autosomal dominant hearing loss at the DFNA10 locus (Wayne S, Robertson NG, DeClau F, Chen N, Verhoeven K, Prasad S, Tranebjarg L, Morton CC, Ryan A F, Van Camp G, Smith R J: Mutations in the transcriptional activator EYA4 cause late-onset deafness at the DFNA10 locus. Hum Mol Genet 2001 Feb. 1;10(3):195-200 with further references). A link between the Methylation of Cytosine positions in the EYA 4 gene and cancer has not yet been established.

[0009] 5-methylcytosine is the most frequent covalent base modification in the DNA of eukaryotic cells. It plays a role, for example, in the regulation of the transcription, in genetic imprinting, and in tumorigenesis. Therefore, the identification of 5-methylcytosine as a component of genetic information is of considerable interest. However, 5-methylcytosine positions cannot be identified by sequencing since 5-methylcytosine has the same base pairing behaviour as cytosine. Moreover, the epigenetic information carried by 5-methylcytosine is completely lost during PCR amplification.

[0010] A relatively new and currently the most frequently used method for analysing DNA for 5-methylcytosine is

based upon the specific reaction of bisulfite with cytosine which, upon subsequent alkaline hydrolysis, is converted to uracil which corresponds to thymidine in its base pairing behaviour. However, 5-methylcytosine remains unmodified under these conditions. Consequently, the original DNA is converted in such a manner that methylcytosine, which originally could not be distinguished from cytosine by its hybridisation behaviour, can now be detected as the only remaining cytosine using "normal" molecular biological techniques, for example, by amplification and hybridisation or sequencing. All of these techniques are based on base pairing which can now be fully exploited. In terms of sensitivity, the prior art is defined by a method which encloses the DNA to be analysed in an agarose matrix, thus preventing the diffusion and renaturation of the DNA (bisulfite only reacts with single-stranded DNA), and which replaces all precipitation and purification steps with fast dialysis (Olek A, Oswald J, Walter J. A modified and improved method for bisulphite based cytosine methylation analysis. Nucleic Acids Res. 1996 Dec. 15;24(24):5064-6). Using this method, it is possible to analyse individual cells, which illustrates the potential of the method. However, currently only individual regions of a length of up to approximately 3000 base pairs are analysed, a global analysis of cells for thousands of possible methylation events is not possible. However, this method cannot reliably analyse very small fragments from small sample quantities either. These are lost through the matrix in spite of the diffusion protection.

[**0011**] An overview of the further known methods of detecting 5-methylcytosine may be gathered from the following review article: Rein, T., DePamphilis, M. L., Zorbas, H., Nucleic Acids Res. 1998, 26, 2255.

[**0012**] To date, barring few exceptions (e.g., Zeschnigk M, Lich C, Buiting K, Doerfler W, Horsthemke B. A single-tube PCR test for the diagnosis of Angelman and Prader-Willi syndrome based on allelic methylation differences at the SNRPN locus. Eur J Hum Genet. 1997 March-April;5(2):94-8) the bisulfite technique is only used in research. Always, however, short, specific fragments of a known gene are amplified subsequent to a bisulfite treatment and either completely sequenced (Olek A, Walter J. The pre-implantation ontogeny of the H19 methylation imprint. Nat Genet. 1997 November;17(3):275-6) or individual cytosine positions are detected by a primer extension reaction (Gonzalgo ML, Jones P A. Rapid quantitation of methylation differences at specific sites using methylation-sensitive single nucleotide primer extension (Ms-SNuPE). Nucleic Acids Res. 1997 Jun. 15;25(12):2529-31, WO 95/00669) or by enzymatic digestion (Xiong Z, Laird P W. COBRA: a sensitive and quantitative DNA methylation assay. Nucleic Acids Res. 1997 Jun. 15;25(12):2532-4). In addition, detection by hybridisation has also been described (Olek et al., WO 99/28498).

[**0013**] Further publications dealing with the use of the bisulfite technique for methylation detection in individual genes are: Grigg G, Clark S. Sequencing 5-methylcytosine residues in genomic DNA. Bioessays. 1994 June;16(6):431-6, 431; Zeschnigk M, Schmitz B, Dittrich B, Buiting K, Horsthemke B, Doerfler W. Imprinted segments in the human genome: different DNA methylation patterns in the Prader-Willi/Angelman syndrome region as determined by the genomic sequencing method. Hum Mol Genet. 1997 March;6(3):387-95; Feil R, Charlton J, Bird A P, Walter J, Reik W. Methylation analysis on individual chromosomes: improved protocol for bisulphite genomic sequencing. Nucleic Acids Res. 1994 Feb. 25;22(4):695-6; Martin V, Ribieras S, Song-Wang X, Rio M C, Dante R. Genomic sequencing indicates a correlation between DNA hypomethylation in the 5' region of the pS2 gene and its expression in human breast cancer cell lines. Gene. 1995 May 19;157(1-2):261-4; WO 97/46705 and WO 95/15373.

[**0014**] An overview of the Prior Art in oligomer array manufacturing can be gathered from a special edition of Nature Genetics (Nature Genetics Supplement, Volume 21, January 1999), published in January 1999, and from the literature cited therein.

[**0015**] Fluorescently labelled probes are often used for the scanning of immobilised DNA arrays. The simple attachment of Cy3 and Cy5 dyes to the 5'-OH of the specific probe are particularly suitable for fluorescence labels. The detection of the fluorescence of the hybridised probes may be carried out, for example via a confocal microscope. Cy3 and Cy5 dyes, besides many others, are commercially available.

[**0016**] Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-TOF) is a very efficient development for the analysis of biomolecules (Karas M, Hillenkamp F. Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. Anal Chem. 1988 Oct. 15;60(20):2299-301). An analyte is embedded in a light-absorbing matrix. The matrix is evaporated by a short laser pulse thus transporting the analyte molecule into the vapour phase in an unfragmented manner. The analyte is ionised by collisions with matrix molecules. An applied voltage accelerates the ions into a field-free flight tube. Due to their different masses, the ions are accelerated at different rates. Smaller ions reach the detector sooner than bigger ones.

[**0017**] MALDI-TOF spectrometry is excellently suited to the analysis of peptides and proteins. The analysis of nucleic acids is somewhat more difficult (Gut I G, Beck S. DNA and Matrix Assisted Laser Desorption Ionization Mass Spectrometry. Current Innovations and Future Trends. 1995, 1; 147-57). The sensitivity to nucleic acids is approximately 100 times worse than to peptides and decreases disproportionally with increasing fragment size. For nucleic acids having a multiply negatively charged backbone, the ionisation process via the matrix is considerably less efficient. In MALDI-TOF spectrometry, the selection of the matrix plays an eminently important role. For the desorption of peptides, several very efficient matrixes have been found which produce a very fine crystallisation. There are now several responsive matrixes for DNA, however, the difference in sensitivity has not been reduced. The difference in sensitivity can be reduced by chemically modifying the DNA in such a manner that it becomes more similar to a peptide. Phosphorothioate nucleic acids in which the usual phosphates of the backbone are substituted with thiophosphates can be converted into a charge-neutral DNA using simple alkylation chemistry (Gut IG, Beck S. A procedure for selective DNA alkylation and detection by mass spectrometry. Nucleic Acids Res. 1995 Apr. 25;23(8):1367-73). The coupling of a charge tag to this modified DNA results in an increase in sensitivity to the same level as that found for peptides. A further advantage of charge tagging is the

increased stability of the analysis against impurities which make the detection of unmodified substrates considerably more difficult.

[0018] Genomic DNA is obtained from DNA of cell, tissue or other test samples using standard methods. This standard methodology is found in references such as Sambrook, Fritsch and Maniatis eds., Molecular Cloning: A Laboratory Manual, 1989.

DESCRIPTION

[0019] The present invention discloses novel methods for the detection of cell proliferative disorders. Said invention discloses the use of the gene EYA4, as well as its promoter and regulatory elements as a marker for colon cell proliferative disorders. More specifically, the disclosed matter shows the applicability of said gene to the detection of colon cell proliferative disorders, distinguishing between different classes of colon cell proliferative disorders as well as the differentiation of colon cell proliferative disorders from cell proliferative disorders originating from other tissues.

[0020] In one aspect of the invention, the disclosed matters provides novel nucleic acid sequences useful for the analysis of methylation within said gene, other aspects provide novel uses of the gene and the gene product as well as methods, assays and kits directed to detecting, differentiating and distinguishing colon cell proliferative disorders, as well as therapeutic and diagnostic methods thereof.

[0021] In one embodiment the method discloses the use of the gene EYA4 as a marker for the differentiation, detection and distinguishing of colon cell proliferative disorders. Said use of the gene may be enabled by means of any analysis of the expression of the gene, by means of mRNA expression analysis or protein expression analysis. However, in the most preferred embodiment of the invention, the detection, differentiation and distinguishing of colon cell proliferative disorders is enabled by means of analysis of the methylation status of the gene EYA4 and its promoter or regulatory elements.

[0022] To detect the presence of mRNA encoding EYA4 in a detection system for colon cancer, a sample is obtained from a patient. The sample can be a tissue biopsy sample or a sample of blood, plasma, serum or the like. The sample may be treated to extract the nucleic acids contained therein. The resulting nucleic acid from the sample is subjected to gel electrophoresis or other separation techniques. Detection involves contacting the nucleic acids and in particular the mRNA of the sample with a DNA sequence serving as a probe to form hybrid duplexes. The stringency of hybridisation is determined by a number of factors during hybridisation and during the washing procedure, including temperature, ionic strength, length of time and concentration of formamide. These factors are outlined in, for example, Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2d ed., 1989). Detection of the resulting duplex is usually accomplished by the use of labelled probes. Alternatively, the probe may be unlabeled, but may be detectable by specific binding with a ligand which is labelled, either directly or indirectly. Suitable labels and methods for labelling probes and ligands are known in the art, and include, for example, radioactive labels which may be incorporated by known methods (e.g., nick translation or kinasing), biotin, fluorescent groups, chemiluminescent groups (e.g., dioxetanes, particularly triggered dioxetanes), enzymes, antibodies, and the like.

[0023] In order to increase the sensitivity of the detection in a sample of mRNA encoding EYA4, the technique of reverse transcription/polymerisation chain reaction can be used to amplify cDNA transcribed from mRNA encoding EYA4. The method of reverse transcription/PCR is well known in the art (for example, see Watson and Fleming, supra).

[0024] The reverse transcription/PCR method can be performed as follows. Total cellular RNA is isolated by, for example, the standard guanidium isothiocyanate method and the total RNA is reverse transcribed. The reverse transcription method involves synthesis of DNA on a template of RNA using a reverse transcriptase enzyme and a 3' end primer. Typically, the primer contains an oligo(dT) sequence. The cDNA thus produced is then amplified using the PCR method and EYA4 specific primers. (Belyavsky et al, Nucl Acid Res 17:2919-2932, 1989; Krug and Berger, Methods in Enzymology, Academic Press, N.Y., Vol.152, pp. 316-325, 1987 which are incorporated by reference)

[0025] The present invention may also be described in certain embodiments as a kit for use in detecting a colon cancer disease state through testing of a biological sample. A representative kit may comprise one or more nucleic acid segments as described above that selectively hybridise to EYA4 mRNA and a container for each of the one or more nucleic acid segments. In certain embodiments the nucleic acid segments may be combined in a single tube. In further embodiments, the nucleic acid segments may also include a pair of primers for amplifying the target mRNA. Such kits may also include any buffers, solutions, solvents, enzymes, nucleotides, or other components for hybridisation, amplification or detection reactions. Preferred kit components include reagents for reverse transcription-PCR, in situ hybridisation, Northern analysis and/or RPA

[0026] The present invention further provides for methods to detect the presence of the polypeptide, EYA4, in a sample obtained from a patient. Any method known in the art for detecting proteins can be used. Such methods include, but are not limited to immunodiffusion, immunoelectrophoresis, immunochemical methods, binder-ligand assays, immunohistochemical techniques, agglutination and complement assays. (for example see Basic and Clinical Immunology, Sites and Terr, eds., Appleton & Lange, Norwalk, Conn. pp 217-262, 1991 which is incorporated by reference). Preferred are binder-ligand immunoassay methods including reacting antibodies with an epitope or epitopes of EYA4 and competitively displacing a labelled EYA4 protein or derivative thereof.

[0027] Certain embodiments of the present invention comprise the use of antibodies specific to the polypeptide encoded by the EYA4 gene. Such antibodies may be useful for diagnostic and prognostic applications in detecting the disease state, by comparing a patient's levels of colon disease marker expression to expression of the same markers in normal individuals. In certain embodiments production of monoclonal or polyclonal antibodies can be induced by the use of the EYA4 polypeptide as antigene. Such antibodies may in turn be used to detect expressed proteins as markers for human disease states. The levels of such proteins present

in the peripheral blood or prostate tissue sample of a patient may be quantified by conventional methods. Antibody-protein binding may be detected and quantified by a variety of means known in the art, such as labelling with fluorescent or radioactive ligands. The invention further comprises kits for performing the above-mentioned procedures, wherein such kits contain antibodies specific for the EYA4 polypeptides.

[0028] Numerous competitive and non-competitive protein binding immunoassays are well known in the art. Antibodies employed in such assays may be unlabeled, for example as used in agglutination tests, or labelled for use a wide variety of assay methods. Labels that can be used include radionuclides, enzymes, fluorescers, chemiluminescers, enzyme substrates or co-factors, enzyme inhibitors, particles, dyes and the like for use in radioimmunoassay (RIA), enzyme immunoassays, e.g., enzyme-linked immunosorbent assay (ELISA), fluorescent immunoassays and the like. Polyclonal or monoclonal antibodies to EYA4 or an epitope thereof can be made for use in immunoassays by any of a number of methods known in the art. One approach for preparing antibodies to a protein is the selection and preparation of an amino acid sequence of all or part of the protein, chemically synthesising the sequence and injecting it into an appropriate animal, usually a rabbit or a mouse (Milstein and Kohler Nature 256:495-497, 1975; Gulfre and Milstein, Methods in Enzymology: Immunochemical Techniques 73:1-46, Langone and Banatis eds., Academic Press, 1981 which are incorporated by reference). Methods for preparation of EYA4 or an epitope thereof include, but are not limited to chemical synthesis, recombinant DNA techniques or isolation from biological samples.

[0029] The invention provides significant improvements over the state of the art in that there are currently no markers used to detect colon cancer from body fluid samples. Current methods used to detect and diagnose colon cell proliferative disorders include colonoscopy, sigmoidoscopy, and fecal occult blood colon cancer. In comparison to these methods, the disclosed invention is much less invasive than colonoscopy, and as, if not more sensitive than sigmoidoscopy and FOBT. Compared to the previous descriptions of these markers in the literature, the described invention provides significant advantages in terms of sensitivity and specificity due to the advantageous combination of using highly sensitive assay techniques.

[0030] The objective of the invention can be achieved by analysis of the methylation state of the CpG dinucleotides within the genomic sequence according to SEQ ID NO: 1 and sequences complementary thereto. SEQ ID NO: 1 discloses the gene EYA4 and its promoter and regulatory elements, wherein said fragment comprises CpG dinucleotides exhibiting a disease specific methylation pattern. The methylation pattern of the gene EYA4 and its promoter and regulatory elements have heretofore not been analysed with regard to cell proliferative disorders. Due to the degeneracy of the genetic code, the sequence as identified in SEQ ID NO: 1 should be interpreted so as to include all substantially similar and equivalent sequences upstream of the promoter region of a gene which encodes a polypeptide with the biological activity of that encoded by EYA4.

[0031] In a preferred embodiment of the method, the objective of the invention is achieved by analysis of a nucleic acid comprising a sequence of at least 18 bases in length according to one of SEQ ID NO: 2 to SEQ ID NO: 5 and sequences complementary thereto.

[0032] The sequences of SEQ ID NOS: 2 to 5 provide modified versions of the nucleic acid according to SEQ ID NO: 1, wherein the conversion of said sequence results in the synthesis of a nucleic acid having a sequence that is unique and distinct from SEQ ID NO: 1 as follows. (see also the following TABLE 1): SEQ ID NO: 1, sense DNA strand of EYA4 gene and its promoter and regulatory elements; SEQ ID NO: 2, converted SEQ ID NO: 1, wherein "C" or "T," but "cp." remains "cp." (i.e., corresponds to case where, for SEQ ID NO: 1, all "C" residues of cp. dinucleotide sequences are methylated and are thus not converted); SEQ ID NO: 3, complement of SEQ ID NO: 1, wherein "C" to "T," but "cp." remains "cp." (i.e., corresponds to case where, for the complement (antisense strand) of SEQ ID NO: 1, all "C" residues of cp. dinucleotide sequences are methylated and are thus not converted); SEQ ID NO: 4, converted SEQ ID NO: 1, wherein "C" to "T" for all "C" residues, including those of "cp." dinucleotide sequences (i.e., corresponds to case where, for SEQ ID NO: 1, all "C" residues of cp. dinucleotide sequences are unmethylated); SEQ ID NO: 5, complement of SEQ ID NO: 1, wherein "C" to "T" for all "C" residues, including those of "CpG" dinucleotide sequences (i.e., corresponds to case where, for the complement (antisense strand) of SEQ ID NO: 1, all "C" residues of CpG dinucleotide sequences are unmethylated).

TABLE 1

Description of SEQ ID NOS: 1 to 5

SEQ ID NO	Relationship to SEQ ID NO: 1	Nature of cytosine base conversion
SEQ ID NO: 1	Sense strand (EYA4 gene including promoter and regulatory elements)	None; untreated sequence
SEQ ID NO: 2	Converted sense strand	"C" to "T," but "CpG" remains "CpG" (all "C" residues of CpGs are methylated)
SEQ ID NO: 3	Converted antisense strand	"C." to "T," but "CpG" remains "CpG" (all "C" residues of CpGs are methylated)
SEQ ID NO: 4	Converted sense strand	"C." to "T" for all "C" residues (all "C" residues of CpGs are unmethylated)
SEQ ID NO: 5	Converted antisense strand	"C" to "T" for all "C" residues (all "C" residues of CpGs are unmethylated)

[0033] Significantly, heretofore, the nucleic acid sequences and molecules according to SEQ ID NO: 1 to SEQ ID NO: 5 were not implicated in or connected with the ascertainment of colon cell proliferative disorders.

[0034] The described invention further disclose an oligonucleotide or oligomer for detecting the cytosine methylation state within pretreated DNA, according to SEQ ID NO: 2 to SEQ ID NO: 5. Said oligonucleotide or oligomer comprising a nucleic acid sequence having a length of at least nine (9) nucleotides which hybridises, under moderately stringent or stringent conditions (as defined herein above), to a pretreated nucleic acid sequence according to SEQ ID NO: 2 to SEQ ID NO: 5 and/or sequences complementary thereto.

[0035] Thus, the present invention includes nucleic acid molecules (e.g., oligonucleotides and peptide nucleic acid (PNA) molecules (PNA-oligomers)) that hybridise under moderately stringent and/or stringent hybridisation conditions to all or a portion of the sequences of SEQ ID NOS: 2 to 5, or to the complements thereof. The hybridising portion of the hybridising nucleic acids is typically at least 9, 15, 20, 25, 30 or 35 nucleotides in length. However, longer molecules have inventive utility, and are thus within the scope of the present invention.

[0036] Preferably, the hybridising portion of the inventive hybridising nucleic acids is at least 95%, or at least 98%, or 100% identical to the sequence, or to a portion thereof of SEQ ID NOS: 2 to 5, or to the complements thereof.

[0037] Hybridising nucleic acids of the type described herein can be used, for example, as a primer (e.g., a PCR primer), or a diagnostic and/or prognostic probe or primer. Preferably, hybridisation of the oligonucleotide probe to a nucleic acid sample is performed under stringent conditions and the probe is 100% identical to the target sequence. Nucleic acid duplex or hybrid stability is expressed as the melting temperature or Tm, which is the temperature at which a probe dissociates from a target DNA. This melting temperature is used to define the required stringency conditions.

[0038] For target sequences that are related and substantially identical to the corresponding sequence of SEQ ID NO: 1 (such as EYA41 allelic variants and SNPs), rather than identical, it is useful to first establish the lowest temperature at which only homologous hybridisation occurs with a particular concentration of salt (e.g., SSC or SSPE). Then, assuming that 1% mismatching results in a 1° C. decrease in the Tm, the temperature of the final wash in the hybridisation reaction is reduced accordingly (for example, if sequences having >95% identity with the probe are sought, the final wash temperature is decreased by 5° C.). In practice, the change in Tm can be between 0.5° C. and 1.5° C. per 1% mismatch.

[0039] Examples of inventive oligonucleotides of length X (in nucleotides), as indicated by polynucleotide positions with reference to, e.g., SEQ ID NO: 1, include those corresponding to sets of consecutively overlapping oligonucleotides of length X, where the oligonucleotides within each consecutively overlapping set (corresponding to a given X value) are defined as the finite set of Z oligonucleotides from nucleotide positions:

[0040] n to (n+(X−1));

[0041] where n=1, 2, 3, . . . (Y−(X−1));

[0042] where Y equals the length (nucleotides or base pairs) of SEQ ID NO: 1;

[0043] where X equals the common length (in nucleotides) of each oligonucleotide in the set (e.g., X=20 for a set of consecutively overlapping 20-mers); and

[0044] where the number (Z) of consecutively overlapping oligomers of length X for a given SEQ ID NO of length Y is equal to Y−(X−1). For example Z=2,785−19=2,766 for either sense or antisense sets of SEQ ID NO: 1, where X=20.

[0045] Preferably, the set is limited to those oligomers that comprise at least one CpG, TpG or CpA dinucleotide.

[0046] The present invention encompasses, for each of SEQ ID NOS: 2 to 5 (sense and antisense), multiple consecutively overlapping sets of oligonucleotides or modified oligonucleotides of length X, where, e.g., X=9, 10, 17, 20, 22, 23, 25, 27, 30 or 35 nucleotides.

[0047] The oligonucleotides or oligomers according to the present invention constitute effective tools useful to ascertain genetic and epigenetic parameters of the genomic sequence corresponding to SEQ ID NO: 1. Preferred sets of such oligonucleotides or modified oligonucleotides of length X are those consecutively overlapping sets of oligomers corresponding to SEQ ID NOS: 1-5 (and to the complements thereof). Preferably, said oligomers comprise at least one CpG, TpG or CpA dinucleotide. Included in these preferred sets are the preferred oligomers corresponding to SEQ ID NO: 11 to SEQ ID NO: 15.

[0048] Particularly preferred oligonucleotides or oligomers according to the present invention are those in which the cytosine of the CpG dinucleotide (or of the corresponding converted TpG or CpA dinculeotide) sequences is within the middle third of the oligonucleotide; that is, where the oligonucleotide is, for example, 13 bases in length, the CpG, TpG or CpA dinucleotide is positioned within the fifth to ninth nucleotide from the 5'-end.

[0049] The oligonucleotides of the invention can also be modified by chemically linking the oligonucleotide to one or more moieties or conjugates to enhance the activity, stability or detection of the oligonucleotide. Such moieties or conjugates include chromophores, fluorophors, lipids such as cholesterol, cholic acid, thioether, aliphatic chains, phospholipids, polyamines, polyethylene glycol (PEG), palmityl moieties, and others as disclosed in, for example, U.S. Pat. Nos. 5,514,758, 5,565,552, 5,567,810, 5,574,142, 5,585,481, 5,587,371, 5,597,696 and 5,958,773. The probes may also exist in the form of a PNA (peptide nucleic acid) which has particularly preferred pairing properties. Thus, the oligonucleotide may include other appended groups such as peptides, and may include hybridisation-triggered cleavage agents (Krol et al., *BioTechniques* 6:958-976, 1988) or intercalating agents (Zon, *Pharm. Res.* 5:539-549, 1988). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a chromophore, fluorophor, peptide, hybridisation-triggered cross-linking agent, transport agent, hybridisation-triggered cleavage agent, etc.

[0050] The oligonucleotide may also comprise at least one art-recognized modified sugar and/or base moiety, or may comprise a modified backbone or non-natural internucleoside linkage.

[0051] The oligomers according to the present invention are normally used in so called "sets" which contain at least

one oligomer for analysis of each of the CpG dinucleotides of a genomic sequence comprising SEQ ID NO: 1 and sequences complementary thereto or to their corresponding CG, TG or CA dinucleotide within the pretreated nucleic acids according to SEQ ID NO: 2 to SEQ ID NO: 5 and sequences complementary thereto. Preferred is a set which contains at least one oligomer for each of the CpG dinucleotides within the gene EYA4 and it's promoter and regulatory elements in both the pretreated and genomic versions of said gene, SEQ ID NO: 2 to 5 and SEQ ID NO: 1, respectively. However, it is anticipated that for economic or other factors it may be preferable to analyse a limited selection of the CpG dinucleotides within said sequences and the contents of the set of oligonucleotides should be altered accordingly. Therefore, the present invention moreover relates to a set of at least 3 n (oligonucleotides and/or PNA-oligomers) used for detecting the cytosine methylation state in pretreated genomic DNA (SEQ ID NO: 2 to SEQ ID NO: 5 and sequences complementary thereto) and genomic DNA (SEQ ID NO: 1 and sequences complementary thereto). These probes enable diagnosis and/or therapy of genetic and epigenetic parameters of cell proliferative disorders. The set of oligomers may also be used for detecting single nucleotide polymorphisms (SNPs) in pretreated genomic DNA (SEQ ID NO: 2 to SEQ ID NO: 5, and sequences complementary thereto) and genomic DNA (SEQ ID NO: 1, and sequences complementary thereto).

[0052] Moreover, the present invention makes available a set of at least two oligonucleotides which can be used as so-called "primer oligonucleotides" for amplifying DNA sequences of one of SEQ ID NO: 1 to SEQ ID NO: 5 and sequences complementary thereto, or segments thereof.

[0053] In the case of the sets of oligonucleotides according to the present invention, it is preferred that at least one and more preferably all members of the set of oligonucleotides is bound to a solid phase.

[0054] According to the present invention, it is preferred that an arrangement of different oligonucleotides and/or PNA-oligomers (a so-called "array") made available by the present invention is present in a manner that it is likewise bound to a solid phase. This array of different oligonucleotide- and/or PNA-oligomer sequences can be characterised in that it is arranged on the solid phase in the form of a rectangular or hexagonal lattice. The solid phase surface is preferably composed of silicon, glass, polystyrene, aluminium, steel, iron, copper, nickel, silver, or gold. However, nitrocellulose as well as plastics such as nylon which can exist in the form of pellets or also as resin matrices may also be used.

[0055] Therefore, a further subject matter of the present invention is a method for manufacturing an array fixed to a carrier material for analysis in connection with cell proliferative disorders, in which method at least one oligomer according to the present invention is coupled to a solid phase. Methods for manufacturing such arrays are known, for example, from U.S. Pat. No. 5,744,305 by means of solid-phase chemistry and photolabile protecting groups.

[0056] A further subject matter of the present invention relates to a DNA chip for the analysis of cell proliferative disorders. DNA chips are known, for example, in U.S. Pat. No. 5,837,832.

[0057] The described invention further provides a composition of matter useful for detecting, differentiation and distinguishing between colon cell proliferative disorders. Said composition comprising at least one nucleic acid 18 base pairs in length of a segment of the nucleic acid sequence disclosed in SEQ ID NO: 2 to 5, and one or more substances taken from the group comprising:

[0058] 1-5 mM Magnesium Chloride, 100-500 μM dNTP, 0.5-5 units of taq polymerase, bovine serum albumen, an oligomer in particular an oligonucleotide or peptide nucleic acid (PNA)-oligomer, said oligomer comprising in each case at least one base sequence having a length of at least 9 nucleotides which is complementary to, or hybridises under moderately stringent or stringent conditions to a pretreated genomic DNA according to one of the SEQ ID NO: 2 to SEQ ID NO: 5 and sequences complementary thereto. It is preferred that said composition of matter comprises a buffer solution appropriate for the stabilisation of said nucleic acid in an aqueous solution and enabling polymerase based reactions within said solution. Suitable buffers are known in the art and commercially available.

[0059] The present invention further provides a method for conducting an assay in order to ascertain genetic and/or epigenetic parameters of the gene EYA4 and its promoter and regulatory elements. Most preferably the assay according to the following method is used in order to detect methylation within the gene EYA4 wherein said methylated nucleic acids are present in a solution further comprising an excess of background DNA, wherein the background DNA is present in between 100 to 1000 times the concentration of the DNA to be detected. Said method comprising contacting a nucleic acid sample obtained from said subject with at least one reagent or a series of reagents, wherein said reagent or series of reagents, distinguishes between methylated and non-methylated CpG dinucleotides within the target nucleic acid.

[0060] Preferably, said method comprises the following steps: In the first step, a sample of the tissue to be analysed is obtained. The source may be any suitable source, preferably, the source of the sample is selected from the group consisting of histological slides, biopsies, paraffin-embedded tissue, bodily fluids, plasma, serum, stool, urine, blood, and combinations thereof. Preferably, the source is biopsies, bodily fluids, urine, or blood.

[0061] The DNA is then isolated from the sample. Extraction may be by means that are standard to one skilled in the art, including the use of detergent lysates, sonification and vortexing with glass beads. Once the nucleic acids have been extracted, the genomic double stranded DNA is used in the analysis.

[0062] In the second step of the method, the genomic DNA sample is treated in such a manner that cytosine bases which are unmethylated at the 5'-position are converted to uracil, thymine, or another base which is dissimilar to cytosine in terms of hybridisation behaviour. This will be understood as 'pretreatment' herein.

[0063] The above described treatment of genomic DNA is preferably carried out with bisulfite (hydrogen sulfite, disulfite) and subsequent alkaline hydrolysis which results in a conversion of non-methylated cytosine nucleobases to uracil or to another base which is dissimilar to cytosine in terms of base pairing behaviour. Enclosing the DNA to be analysed in an agarose matrix, thereby preventing the diffusion and

renaturation of the DNA (bisulfite only reacts with single-stranded DNA), and replacing all precipitation and purification steps with fast dialysis (Olek A, et al., A modified and improved method for bisulfite based cytosine methylation analysis, *Nucleic Acids Res.* 24:5064-6, 1996). It is further preferred that the bisulfite treatment is carried out in the presence of a radical trap or DNA denaturing agent.

[**0064**] In the third step of the method, fragments of the pretreated DNA are amplified. Wherein the source of the DNA is free DNA from serum, or DNA extracted from paraffin it is particularly preferred that the size of the amplificate fragment is between 100 and 200 base pairs in length, and wherein said DNA source is extracted from cellular sources (e.g. tissues, biopsies, cell lines) it is preferred that the amplificate is between 100 and 350 base pairs in length. It is particularly preferred that said amplificates comprise at least one 20 base pair sequence comprising at least three CpG dinucleotides. Said amplification is carried out using sets of primer oligonucleotides according to the present invention, and a preferably heat-stable polymerase. The amplification of several DNA segments can be carried out simultaneously in one and the same reaction vessel, in one embodiment of the method preferably six or more fragments are amplified simultaneously. Typically, the amplification is carried out using a polymerase chain reaction (PCR). The set of primer oligonucleotides includes at least two oligonucleotides whose sequences are each reverse complementary, identical, or hybridise under stringent or highly stringent conditions to an at least 18-base-pair long segment of the base sequences of SEQ ID NO: 2 to SEQ ID NO: 5 and sequences complementary thereto.

[**0065**] In an alternate embodiment of the method, the methylation status of preselected CpG positions within the nucleic acid sequences comprising SEQ ID NO: 2 to SEQ ID NO: 5 may be detected by use of methylation-specific primer oligonucleotides. This technique (MSP) has been described in U.S. Pat. No. 6,265,171 to Herman. The use of methylation status specific primers for the amplification of bisulfite treated DNA allows the differentiation between methylated and unmethylated nucleic acids. MSP primers pairs contain at least one primer which hybridises to a bisulfite treated CpG dinucleotide. Therefore, the sequence of said primers comprises at least one CpG, TpG or CpA dinucleotide. MSP primers specific for non-methylated DNA contain a "T" at the 3' position of the C position in the CpG. Preferably, therefore, the base sequence of said primers is required to comprise a sequence having a length of at least 18 nucleotides which hybridises to a pretreated nucleic acid sequence according to SEQ ID NO: 2 to SEQ ID NO: 5 and sequences complementary thereto, wherein the base sequence of said oligomers comprises at least one CpG, TpG or CpA dinucleotide. In this embodiment of the method according to the invention it is particularly preferred that the MSP primers comprise between 2 and 4 CpG, TpG or CpA dinucleotides. It is further preferred that said dinucleotides are located within the 3' half of the primer e.g. wherein a primer is 18 bases in length the specified dinucleotides are located within the first 9 bases form the 3'end of the molecule. In addition to the CpG, TpG or CpA dinucleotides it is further preferred that said primers should further comprise several bisulfite converted bases (i.e. cytosine converted to thymine, or on the hybridising strand, guanine converted to adenosine). In a further preferred embodiment said primers are designed so as to comprise no more than 2 cytosine or guanine bases.

[**0066**] In one embodiment of the method the primers may be selected form the group consisting to SEQ ID NO: 6 to SEQ ID NO: 10.

[**0067**] The fragments obtained by means of the amplification can carry a directly or indirectly detectable label. Preferred are labels in the form of fluorescence labels, radionuclides, or detachable molecule fragments having a typical mass which can be detected in a mass spectrometer. Where said labels are mass labels, it is preferred that the labelled amplificates have a single positive or negative net charge, allowing for better detectability in the mass spectrometer. The detection may be carried out and visualised by means of, e.g., matrix assisted laser desorption/ionisation mass spectrometry (MALDI) or using electron spray mass spectrometry (ESI).

[**0068**] Matrix Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-TOF) is a very efficient development for the analysis of biomolecules (Karas & Hillenkamp, *Anal Chem.,* 60:2299-301, 1988). An analyte is embedded in a light-absorbing matrix. The matrix is evaporated by a short laser pulse thus transporting the analyte molecule into the vapour phase in an unfragmented manner. The analyte is ionised by collisions with matrix molecules. An applied voltage accelerates the ions into a field-free flight tube. Due to their different masses, the ions are accelerated at different rates. Smaller ions reach the detector sooner than bigger ones. MALDI-TOF spectrometry is well suited to the analysis of peptides and proteins. The analysis of nucleic acids is somewhat more difficult (Gut & Beck, *Current Innovations and Future Trends,* 1:147-57, 1995). The sensitivity with respect to nucleic acid analysis is approximately 100-times less than for peptides, and decreases disproportionally with increasing fragment size. Moreover, for nucleic acids having a multiply negatively charged backbone, the ionisation process via the matrix is considerably less efficient. In MALDI-TOF spectrometry, the selection of the matrix plays an eminently important role. For the desorption of peptides, several very efficient matrixes have been found which produce a very fine crystallisation. There are now several responsive matrixes for DNA, however, the difference in sensitivity between peptides and nucleic acids has not been reduced. This difference in sensitivity can be reduced, however, by chemically modifying the DNA in such a manner that it becomes more similar to a peptide. For example, phosphorothioate nucleic acids, in which the usual phosphates of the backbone are substituted with thiophosphates, can be converted into a charge-neutral DNA using simple alkylation chemistry (Gut & Beck, *Nucleic Acids Res.* 23: 1367-73, 1995). The coupling of a charge tag to this modified DNA results in an increase in MALDI-TOF sensitivity to the same level as that found for peptides. A further advantage of charge tagging is the increased stability of the analysis against impurities, which makes the detection of unmodified substrates considerably more difficult.

[**0069**] In a particularly preferred embodiment of the method the amplification of step three is carried out in the presence of at least one species of blocker oligonucleotides. The use of such blocker oligonucleotides has been described by Yu et al., *BioTechniques* 23:714-720, 1997. The use of

blocking oligonucleotides enables the improved specificity of the amplification of a subpopulation of nucleic acids. Blocking probes hybridised to a nucleic acid suppress, or hinder the polymerase mediated amplification of said nucleic acid. In one embodiment of the method blocking oligonucleotides are designed so as to hybridise to background DNA. In a further embodiment of the method said oligonucleotides are designed so as to hinder or suppress the amplification of unmethylated nucleic acids as opposed to methylated nucleic acids or vice versa.

[0070] Blocking probe oligonucleotides are hybridised to the bisulfite treated nucleic acid concurrently with the PCR primers. PCR amplification of the nucleic acid is terminated at the 5' position of the blocking probe, such that amplification of a nucleic acid is suppressed where the complementary sequence to the blocking probe is present. The probes may be designed to hybridise to the bisulfite treated nucleic acid in a methylation status specific manner. For example, for detection of methylated nucleic acids within a population of unmethylated nucleic acids, suppression of the amplification of nucleic acids which are unmethylated at the position in question would be carried out by the use of blocking probes comprising a 'TpG' at the position in question, as opposed to a 'CpG.' In one embodiment of the method the sequence of said blocking oligonucleotides should be identical or complementary to molecule is complementary or identical to a sequence at least 18 base pairs in length selected from the group consisting of SEQ ID NOS: 2 to 5, preferably comprising one or more CpG, TpG or CpA dinucleotides. In one embodiment of the method the sequence of said oligonucleotides is selected from the group consisting SEQ ID NO: 15 and SEQ ID NO: 16 and sequences complementary thereto.

[0071] For PCR methods using blocker oligonucleotides, efficient disruption of polymerase-mediated amplification requires that blocker oligonucleotides not be elongated by the polymerase. Preferably, this is achieved through the use of blockers that are 3'-deoxyoligonucleotides, or oligonucleotides derivitised at the 3' position with other than a "free" hydroxyl group. For example, 3'-O-acetyl oligonucleotides are representative of a preferred class of blocker molecule.

[0072] Additionally, polymerase-mediated decomposition of the blocker oligonucleotides should be precluded. Preferably, such preclusion comprises either use of a polymerase lacking 5'-3' exonuclease activity, or use of modified blocker oligonucleotides having, for example, thioate bridges at the 5'-termini thereof that render the blocker molecule nuclease-resistant. Particular applications may not require such 5' modifications of the blocker. For example, if the blocker- and primer-binding sites overlap, thereby precluding binding of the primer (e.g., with excess blocker), degradation of the blocker oligonucleotide will be substantially precluded. This is because the polymerase will not extend the primer toward, and through (in the 5'-3' direction) the blocker—a process that normally results in degradation of the hybridised blocker oligonucleotide.

[0073] A particularly preferred blocker/PCR embodiment, for purposes of the present invention and as implemented herein, comprises the use of peptide nucleic acid (PNA) oligomers as blocking oligonucleotides. Such PNA blocker oligomers are ideally suited, because they are neither decomposed nor extended by the polymerase.

[0074] In one embodiment of the method, the binding site of the blocking oligonucleotide is identical to, or overlaps with that of the primer and thereby hinders the hybridisation of the primer to its binding site. In a further preferred embodiment of the method, two or more such blocking oligonucleotides are used. In a particularly preferred embodiment, the hybridisation of one of the blocking oligonucleotides hinders the hybridisation of a forward primer, and the hybridisation of another of the probe (blocker) oligonucleotides hinders the hybridisation of a reverse primer that binds to the amplificate product of said forward primer.

[0075] In an alternative embodiment of the method, the blocking oligonucleotide hybridises to a location between the reverse and forward primer positions of the treated background DNA, thereby hindering the elongation of the primer oligonucleotides.

[0076] It is particularly preferred that the blocking oligonucleotides are present in at least 5 times the concentration of the primers.

[0077] In the fourth step of the method, the amplificates obtained during the third step of the method are analysed in order to ascertain the methylation status of the CpG dinucleotides prior to the treatment.

[0078] In embodiments where the amplificates were obtained by means of MSP amplification and/or blocking oligonucleotides, the presence or absence of an amplificate is in itself indicative of the methylation state of the CpG positions covered by the primers and or blocking oligonucleotide, according to the base sequences thereof. All possible known molecular biological methods may be used for this detection, including, but not limited to gel electrophoresis, sequencing, liquid chromatography, hybridisations, real time PCR analysis or combinations thereof. This step of the method further acts as a qualitative control of the preceding steps.

[0079] In the fourth step of the method amplificates obtained by means of both standard and methylation specific PCR are further analysed in order to determine the CpG methylation status of the genomic DNA isolated in the first step of the method. This may be carried out by means of based-based methods such as, but not limited to, array technology and probe based technologies as well as by means of techniques such as sequencing and template directed extension.

[0080] In one embodiment of the method, the amplificates synthesised in step three are subsequently hybridised to an array or a set of oligonucleotides and/or PNA probes. In this context, the hybridisation takes place in the following manner: the set of probes used during the hybridisation is preferably composed of at least 2 oligonucleotides or PNA-oligomers; in the process, the amplificates serve as probes which hybridise to oligonucleotides previously bonded to a solid phase; the non-hybridised fragments are subsequently removed; said oligonucleotides contain at least one base sequence having a length of at least 9 nucleotides which is reverse complementary or identical to a segment of the base sequences specified in the SEQ ID NO: 2 to SEQ ID NO: 5; and the segment comprises at least one CpG, TpG or CpA dinucleotide.

[0081] In a preferred embodiment, said dinucleotide is present in the central third of the oligomer. For example,

wherein the oligomer comprises one CpG dinucleotide, said dinucleotide is preferably the fifth to ninth nucleotide from the 5'-end of a 13-mer. One oligonucleotide exists for the analysis of each CpG dinucleotide within the sequence according to SEQ ID NO: 1, and the equivalent positions within SEQ ID NOS: 2 to 5. Said oligonucleotides may also be present in the form of peptide nucleic acids. The non-hybridised amplificates are then removed. The hybridised amplificates are detected. In this context, it is preferred that labels attached to the amplificates are identifiable at each position of the solid phase at which an oligonucleotide sequence is located.

[0082] In yet a further embodiment of the method, the genomic methylation status of the CpG positions may be ascertained by means of oligonucleotide probes that are hybridised to the bisulfite treated DNA concurrently with the PCR amplification primers (wherein said primers may either be methylation specific or standard).

[0083] A particularly preferred embodiment of this method is the use of fluorescence-based Real Time Quantitative PCR (Heid et al., *Genome Res.* 6:986-994, 1996; also see U.S. Pat. No. 6,331,393). There are two preferred embodiments of utilising this method. One embodiment, known as the TaqMan™ assay employs a dual-labelled fluorescent oligonucleotide probe. The TaqMan™ PCR reaction employs the use of a non-extendible interrogating oligonucleotide, called a TaqMan™ probe, which is designed to hybridise to a GpC-rich sequence located between the forward and reverse amplification primers. The TaqMan™ probe further comprises a fluorescent "reporter moiety" and a "quencher moiety" covalently bound to linker moieties (e.g., phosphoramidites) attached to the nucleotides of the TaqMan™ oligonucleotide. Hybridised probes are displaced and broken down by the polymerase of the amplification reaction thereby leading to an increase in fluorescence. For analysis of methylation within nucleic acids subsequent to bisulfite treatment, it is required that the probe be methylation specific, as described in U.S. Pat. No. 6,331,393, (hereby incorporated by reference in its entirety) also known as the MethylLight assay. The second preferred embodiment of this technology is the use of dual-probe technology (Lightcycler®), each carrying donor or recipient fluorescent moieties, hybridisation of two probes in proximity to each other is indicated by an increase or fluorescent amplification primers. Both these techniques may be adapted in a manner suitable for use with bisulfite treated DNA, and moreover for methylation analysis within CpG dinucleotides.

[0084] In a further preferred embodiment of the method, the fourth step of the method comprises the use of template-directed oligonucleotide extension, such as MS-SNuPE as described by Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997. In said embodiment it is preferred that the Ms-SNuPE primer is identical or complementary to a sequence at least nine but preferably no more than twenty five nucleotides in length of one or more of the sequences taken from the group of SEQ ID NO: 2 to SEQ ID NO: 5.

[0085] In yet a further embodiment of the method, the fourth step of the method comprises sequencing and subsequent sequence analysis of the amplificate generated in the third step of the method (Sanger F., et al., *Proc Natl Acad Sci USA* 74:5463-5467, 1977).

[0086] Additional embodiments of the invention provide a method for the analysis of the methylation status of genomic DNA according to the invention (SEQ ID NO: 1) without the need for pretreatment.

[0087] In the first step of such additional embodiments, the genomic DNA sample is isolated from tissue or cellular sources. Preferably, such sources include cell lines, histological slides, body fluids, or tissue embedded in paraffin. Extraction may be by means that are standard to one skilled in the art, including but not limited to the use of detergent lysates, sonification and vortexing with glass beads. Once the nucleic acids have been extracted, the genomic double-stranded DNA is used in the analysis.

[0088] In a preferred embodiment, the DNA may be cleaved prior to the treatment, and this may be by any means standard in the state of the art, in particular with methylation-sensitive restriction endonucleases.

[0089] In the second step, the DNA is then digested with one or more methylation sensitive restriction enzymes. The digestion is carried out such that hydrolysis of the DNA at the restriction site is informative of the methylation status of a specific CpG dinucleotide.

[0090] In the third step, which is optional but a preferred embodiment, the restriction fragments are amplified. This is preferably carried out using a polymerase chain reaction, and said amplificates may carry suitable detectable labels as discussed above, namely fluorophore labels, radionuclides and mass labels.

[0091] In the final step the amplificates are detected. The detection may be by any means standard in the art, for example, but not limited to, gel electrophoresis analysis, hybridisation analysis, incorporation of detectable tags within the PCR products, DNA array analysis, MALDI or ESI analysis.

[0092] The present invention enables diagnosis and/or prognosis of events which are disadvantageous to patients or individuals in which important genetic and/or epigenetic parameters within the EYA4 gene and its promoter or regulatory elements may be used as markers. Said parameters obtained by means of the present invention may be compared to another set of genetic and/or epigenetic parameters, the differences serving as the basis for a diagnosis and/or prognosis of events which are disadvantageous to patients or individuals.

[0093] Specifically, the present invention provides for diagnostic and/or prognostic cancer assays based on measurement of differential methylation of EYA4 CpG dinucleotide sequences. Preferred gene sequences useful to measure such differential methylation are represented herein by SEQ ID NOS: 1 to 5. Typically, such assays involve obtaining a tissue sample from a test tissue, performing an assay to measure the methylation status of at least one of the inventive EYA4-specific CpG dinucleotide sequences derived from the tissue sample, relative to a control sample, and making a diagnosis or prognosis based thereon.

[0094] In particular preferred embodiments, inventive oligomers are used to assess EYA4 specific CpG dinucleotide methylation status, such as those based on SEQ ID NOS: 1 to 5, including the representative preferred oligomers corresponding to SEQ ID NOS: 11 to 15, or arrays thereof, as

well as a kit based thereon are useful for the diagnosis and/or prognosis of cancer and/or other prostate cell proliferative disorders.

[0095] The present invention moreover relates to a diagnostic agent and/or therapeutic agent for the diagnosis and/or therapy colon cell proliferative disorders, the diagnostic agent and/or therapeutic agent being characterised in that at least one primer or probe based on SEQ ID NOS: 1 to 5 is used for manufacturing it, possibly together with suitable additives and ancillary agents. In one embodiment, the EYA4 polypeptide or a fragment or derivative thereof may be administered to a subject to treat or prevent colon cancers.

[0096] In another embodiment, a vector capable of expressing EYA4, or a fragment or a derivative thereof, may also be administered to a subject to treat or prevent colon cancers.

[0097] In another embodiment, agonists which are specific for EYA4 may be used to stimulate or prolong the activity of EYA4 and may be administered to a subject to treat or prevent colon cancers.

[0098] In other embodiments, any of the therapeutic proteins or vectors described above may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of colon cancer. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

[0099] Expression vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids may be used for delivery of nucleotide sequences to the targeted organ, tissue or cell population.

[0100] An additional embodiment of the invention relates to the administration of a pharmaceutical composition, in conjunction with a pharmaceutically acceptable carrier. Such pharmaceutical compositions may consist of EYA4 or agonists of EYA4. The compositions may be administered alone or in combination with at least one other agent, such as stabilising compound, which may be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs or hormones.

[0101] The pharmaceutical compositions utilised in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

[0102] In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.).

[0103] Moreover, an additional aspect of the present invention is a kit comprising, for example: a bisulfite-containing reagent as well as at least one oligonucleotide whose sequences in each case correspond, are complementary, or hybridise under stringent or highly stringent conditions to a 18-base long segment of the sequences SEQ ID NOS: 1 to 5. Said kit may further comprise instructions for carrying out and evaluating the described method. In a further preferred embodiment, said kit may further comprise standard reagents for performing a CpG position-specific methylation analysis, wherein said analysis comprises one or more of the following techniques: MS-SNuPE, MSP, MethyLight™, HeavyMethyl™, COBRA, and nucleic acid sequencing. However, a kit along the lines of the present invention can also contain only part of the aforementioned components.

[0104] Typical reagents (e.g., as might be found in a typical COBRA-based kit) for COBRA analysis may include, but are not limited to: PCR primers for specific gene (or methylation-altered DNA sequence or CpG island); restriction enzyme and appropriate buffer; gene-hybridisation oligo; control hybridisation oligo; kinase labelling kit for oligo probe; and radioactive nucleotides. Additionally, bisulfite conversion reagents may include: DNA denaturation buffer; sulfonation buffer; DNA recovery reagents or kits (e.g., precipitation, ultrafiltration, affinity column); desulfonation buffer; and DNA recovery components.

[0105] Typical reagents (e.g., as might be found in a typical MethyLight®-based kit) for MethyLight® analysis may include, but are not limited to: PCR primers for specific gene (or methylation-altered DNA sequence or CpG island); TaqMan® probes; optimised PCR buffers and deoxynucleotides; and Taq polymerase.

[0106] Typical reagents (e.g., as might be found in a typical Ms-SNuPE-based kit) for Ms-SNuPE analysis may include, but are not limited to: PCR primers for specific gene (or methylation-altered DNA sequence or CpG island); optimised PCR buffers and deoxynucleotides; gel extraction kit; positive control primers; Ms-SNuPE primers for specific gene; reaction buffer (for the Ms-SNuPE reaction); and radioactive nucleotides. Additionally, bisulfite conversion reagents may include: DNA denaturation buffer; sulfonation buffer; DNA recovery regents or kit (e.g., precipitation, ultrafiltration, affinity column); desulfonation buffer; and DNA recovery components.

[0107] Typical reagents (e.g., as might be found in a typical MSP-based kit) for MSP analysis may include, but are not limited to: methylated and unmethylated PCR primers for specific gene (or methylation-altered DNA sequence or CpG island), optimised PCR buffers and deoxynucleotides, and specific probes.

[0108] Definitions:

[0109] In the context of the present invention, the term "CpG island" refers to a contiguous region of genomic DNA that satisfies the criteria of (1) having a frequency of CpG dinucleotides corresponding to an "Observed/Expected Ratio">0.6, and (2) having a "GC Content">0.5. CpG islands are typically, but not always, between about 0.2 to about 1 kb in length.

[0110] In the context of the present invention, the term "methylation state" or "methylation status" refers to the presence or absence of 5-methylcytosine ("5-mCyt") at one or a plurality of CpG dinucleotides within a DNA sequence. Methylation states at one or more particular palindromic CpG methylation sites (each having two CpG CpG dinucleotide sequences) within a DNA sequence include "unmethylated,""fully-methylated" and "hemi-methylated."

[0111] In the context of the present invention, the term "hemi-methylation" or "hemimethylation" refers to the methylation state of a palindromic CpG methylation site, where only a single cytosine in one of the two CpG dinucleotide sequences of the palindromic CpG methylation site is methylated (e.g., 5'-CCMGG-3' (top strand): 3'-GGCC-5' (bottom strand)).

[0112] In the context of the present invention, the term "hypermethylation" refers to the average methylation state corresponding to an increased presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample.

[0113] In the context of the present invention, the term "hypomethylation" refers to the average methylation state corresponding to a decreased presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample.

[0114] In the context of the present invention, the term "microarray" refers broadly to both "DNA microarrays," and 'DNA chip(s),' as recognised in the art, encompasses all art-recognised solid supports, and encompasses all methods for affixing nucleic acid molecules thereto or synthesis of nucleic acids thereon.

[0115] "Genetic parameters" are mutations and polymorphisms of genes and sequences further required for their regulation. To be designated as mutations are, in particular, insertions, deletions, point mutations, inversions and polymorphisms and, particularly preferred, SNPs (single nucleotide polymorphisms).

[0116] "Epigenetic parameters" are, in particular, cytosine methylations. Further epigenetic parameters include, for example, the acetylation of histones which, however, cannot be directly analysed using the described method but which, in turn, correlate with the DNA methylation.

[0117] In the context of the present invention, the term "bisulfite reagent" refers to a reagent comprising bisulfite, disulfite, hydrogen sulfite or combinations thereof, useful as disclosed herein to distinguish between methylated and unmethylated CpG dinucleotide sequences.

[0118] In the context of the present invention, the term "Methylation assay" refers to any assay for determining the methylation state of one or more CpG dinucleotide sequences within a sequence of DNA.

[0119] In the context of the present invention, the term "MS.AP-PCR" (Methylation-Sensitive Arbitrarily-Primed Polymerase Chain Reaction) refers to the art-recognised technology that allows for a global scan of the genome using CG-rich primers to focus on the regions most likely to contain CpG dinucleotides, and described by Gonzalgo et al., *Cancer Research* 57:594-599, 1997.

[0120] In the context of the present invention, the term "MethyLight" refers to the art-recognised fluorescence-based real-time PCR technique described by Eads et al., *Cancer Res.* 59:2302-2306, 1999.

[0121] In the context of the present invention, the term "HeavyMethyl™" assay, in the embodiment thereof implemented herein, refers to a HeavyMethyl™ MethylLight assay, which is a variation of the MethylLight assay, wherein the MethylLight assay is combined with methylation specific blocking probes covering CpG positions between the amplification primers.

[0122] The term "Ms-SNuPE" (Methylation-sensitive Single Nucleotide Primer Extension) refers to the art-recognized assay described by Gonzalgo & Jones, *Nucleic Acids Res.* 25:2529-2531, 1997.

[0123] The term "MSP" (Methylation-specific PCR) refers to the art-recognised methylation assay described by Herman et al. *Proc. Natl. Acad. Sci. USA* 93:9821-9826, 1996, and by U.S. Pat. No. 5,786,146.

[0124] The term "COBRA" (Combined Bisulfite Restriction Analysis) refers to the art-recognized methylation assay described by Xiong & Laird, *Nucleic Acids Res.* 25:2532-2534, 1997.

[0125] The term "hybridisation" is to be understood as a bond of an oligonucleotide to a complementary sequence along the lines of the Watson-Crick base pairings in the sample DNA, forming a duplex structure.

[0126] "Stringent hybridisation conditions," as defined herein, involve hybridising at 68° C. in 5×SSC/5× Denhardt's solution/1.0% SDS, and washing in 0.2×SSC/0.1% SDS at room temperature, or involve the art-recognised equivalent thereof (e.g., conditions in which a hybridisation is carried out at 60° C. in 2.5×SSC buffer, followed by several washing steps at 37° C. in a low buffer concentration, and remains stable). Moderately stringent conditions, as defined herein, involve including washing in 3×SSC at 42° C., or the art-recognised equivalent thereof. The parameters of salt concentration and temperature can be varied to achieve the optimal level of identity between the probe and the target nucleic acid. Guidance regarding such conditions is available in the art, for example, by Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y.; and Ausubel et al. (eds.), 1995, Current Protocols in Molecular Biology, (John Wiley & Sons, N.Y.) at Unit 2.10.

[0127] "Background DNA" as used herein refers to any nucleic acids which originate from sources other than colon cells.

[0128] The invention will now be described in more detail based on the following examples, SEQ IDs, and Figures, without being limited thereto. In the sequence protocol and the Figures,

[0129] SEQ ID NO: 1 shows the sequence of the human gene EYA4,

[0130] SEQ ID NOS: 2 to 5 show chemically pretreated sequences of the gene EYA4,

[0131] SEQ ID NOS: 6 to 10 show the sequences of primers used in the examples, and

[0132] SEQ ID NOS: 11 to 15 show sequences of probes used in the examples.

[0133] **FIG. 1** shows the level of methylation determined by a MSP MethyLight assay and by a HeavyMethyl MethyLight assay according to examples 1 and 2. The Y-axis shows the degree of methylation within the region of the EYA4 gene investigated. Tumour samples are represented by white points, and normal colon tissue samples by white black points. A significantly higher degree of methylation was observed in tumour samples than in healthy tissue samples. The level of significance as measured using a t-test was p=0.00000312 (MSP-ML, Example 1) and p=0.000000326 (HM-ML, Example 2).

[0134] **FIG. 2** shows the Receiver Operating Characteristic curve (ROC curve) of the MSP-Methyl-Light-Assay for Adenocarcinomas according to Example 1. A ROC is a plot of the true positive rate against the false positive rate for the different possible cutpoints of a diagnostic test. It shows the tradeoff between sensitivity and specificity depending on the selected cutpoint (any increase in sensitivity will be accompanied by a decrease in specificity). The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test (the larger the area the better, optimum is 1, a random test would have a ROC curve lying on the diagonal with an area of 0.5) The AUC for the MSP-Methyl-Light-Assay is: 0.94.

[0135] **FIG. 3** shows the Receiver Operating Characteristic curve (ROC curve) of the HM-Methyl-Light-Assay for Adenocarcinoma according to Example 2. The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test. The AUC for the HM-Methyl-Light-Assay is: 0.91.

[0136] **FIG. 4** shows the level of methylation determined by a HeavyMethyl MethyLight assay according to example 2, testing an additional set of colon samples (25 adenocarcinoma, 33 normals, and 13 adenomas). The Y-axis shows the degree of methylation within the region of the EYA4 gene investigated. Adenocarcinoma samples are represented by white squares, and normal colon tissue samples by black diamonds. A significantly higher degree of methylation was observed in tumour samples than in healthy tissue samples. The level of significance as measured using a t-test was 0.00424.

[0137] **FIG. 5** shows the Receiver Operating Characteristic curve (ROC curve) of the HM-Methyl-Light-Assay for Adenocarcinoma and Adenoma according to Example 2 (additional sets of samples). The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test. The AUC for the HM-Methyl-Light-Assay is 0.81.

[0138] **FIG. 6** shows the Receiver Operating Characteristic curve (ROC curve) of the HM-Methyl-Light-Assay for Adenocarcinoma only according to Example 2 (additional sets of samples). The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test. The AUC for the HM-Methyl-Light-Assay is: 0.844.

[0139] **FIG. 7** shows the Receiver Operating Characteristic curve (ROC curve) of the HM-Methyl-Light-Assay for Adenenomas according to Example 2 (additional sets of samples). The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test. The AUC for the HM-Methyl-Light-Assay is: 0.748.

[0140] **FIG. 8** shows the level of methylation in different tumour and healthy tissues determined by a HeavyMethyl MethyLight assay according to example 3. The Y-axis shows the degree of methylation within the region of the EYA4 gene investigated. Besides the colon cancer samples only one of the two breast cancer tissues were methylated.

[0141] **FIG. 9** shows the level of methylation in different breast cancer tissues determined by a HeavyMethyl MethyLight assay according to example 3. Only one tissue was methylated.

[0142] **FIG. 10** shows the level of methylation in serum samples determined by a HeavyMethyl MethyLight assay according to example 4. The Y-axis shows the degree of methylation within the region of the EYA4 gene investigated.

EXAMPLE 1

[0143] Analysis of Methylation within Colon Cancer Using an MSP—MethyLight Assay

[0144] DNA was extracted from 33 colon adenocarcinoma samples and 43 colon normal adjacent tissues using a Qiagen® extraction kit. The DNA from each sample was treated using a bisulfite solution (hydrogen sulfite, disulfite) according to the agarose-bead method (Olek et al 1996). The treatment is such that all non methylated cytosines within the sample are converted to thymidine. Conversely, 5-methylated cytosines within the sample remain unmodified.

[0145] The methylation status was determined with a MSP-MethyLight assay designed for the CpG island of interest and a control fragment from the beta actin gene (Eads et al., 2001). The CpG island assay covers CpG sites in both the primers and the taqman style probe, while the control gene does not. The control gene is used as a measure of total DNA concentration, and the CpG island assay (methylation assay) determines the methylation levels at that site.

[0146] Methods: The EYA4 gene CpG island assay was performed using the following primers and probes:

```
Forward Primer:
CGGAGGGTACGGAGATTACG;            (SEQ ID NO: 6)

Reverse Primer:
CGACGACGCGCGAAA;                 (SEQ ID NO: 7)

and

Probe:
CGAAACCCTAAATATCCCGAATAACGCCG.   (SEQ ID NO: 12)
```

[0147] The corresponding control assay was performed using the following primers and probes:

```
Primer:
TGGTGATGGAGGAGGTTTAGTAAGT;       (SEQ ID NO: 8)

Primer:
AACCAATAAAACCTACTCCTCCCTTAA;     (SEQ ID NO: 9)
```

-continued

and

Probe:
ACCACCACCCAACACACAATAACAAACACA (SEQ ID NO: 13)

[0148] The reactions were run in triplicate on each DNA sample with the following assay conditions: Reaction solution: (900 nM primers; 300 nM probe; 3.5 mM Magnesium Chloride; 1 unit of taq polymerase; 200 μM dNTPs; 7 μl of DNA, in a final reaction volume of 20 μl);

[0149] Cycling conditions: (95° C. for 10 minutes; then 50 cycles of: 95° C. for 15 seconds; 60° C. for 1 minute). The data was analysed using a PMR calculation previously described in the literature (Eads et al 2001).

[0150] Results. The mean PMR for normal samples was 0.15, with a standard deviation of 0.18. The mean PMR for tumour samples was 17.98, with a standard deviation of 18.18. The overall difference in methylation levels between tumour and normal samples is significant in a t-test (p=0.00000312). The results are shown in **FIG. 1**.

[0151] A Receiver Operating Characteristic curve (ROC curve) of the assay was also determined. A ROC is a plot of the true positive rate against the false positive rate for the different possible cutpoints of a diagnostic test. It shows the tradeoff between sensitivity and specificity depending on the selected cutpoint (any increase in sensitivity will be accompanied by a decrease in specificity). The area under an ROC curve (AUC) is a measure for the accuracy of a diagnostic test (the larger the area the better, optimum is 1, a random test would have a ROC curve lying on the diagonal with an area of 0.5; for reference: J. P. Egan. Signal Detection Theory and ROC Analysis, Academic Press, New York, 1975). The AUC for the MSP-Methyl-Light-Assay is: 0.94 (**FIG. 2**).

EXAMPLE 2

[0152] Methylation within Colon Cancer was Analyzed using a HeavyMethyl MethyLight□ Assay.

[0153] The same DNA samples were also used to analyse methylation of the CpG island with a HeavyMethyl MethyLight (or HM MethyLight) assay, also referred to as the HeavyMethyl assay. The methylation status was determined with a HM MethyLight assay designed for the CpG island of interest and the same control gene assay described above. The CpG island assay covers CpG sites in both the blockers and the taqman style probe, while the control gene does not.

[0154] Methods. The CpG island assay (methylation assay) was performed using the following primers and probes:

Forward Primer: GGTGATTGTTTATTGTTATGGTTTG (SEQ ID NO: 10)

Reverse Primer: CCCCTCAACCTAAAAACTACAAC (SEQ ID NO: 11)

Forward Blocker: GTTATGGTTTGTGATTTTGTGTGGG (SEQ ID NO: 15)

-continued

Reverse Blocker: AAACTACAACCACTCAAATCAACCCA (SEQ ID NO: 16)

Probe: AAAATTACGACGACGCCACCCGAAA (SEQ ID NO: 14)

[0155] The reactions were each run in triplicate on each DNA sample with the following assay conditions:

[0156] Reaction solution: (400 nM primers; 400 nM probe; 10 μM both blockers; 3.5 mM magnesium chloride; 1×ABI Taqman buffer; 1 unit of ABI TaqGold polymerase; 200 μM dNTPs; and 7 μl of DNA, in a final reaction volume of 20 μl);

[0157] Cycling conditions: (95° C. for 10 minutes); (95° C. for 15 seconds, 64° C. for 1 minute (2 cycles)); (95° C. for 15 seconds, 62° C. for 1 minute (2 cycles); (95° C. for 15 seconds, 60° C. for 1 minute (2 cycles)); and (95° C. for 15 seconds, 58° C. for 1 minute, 60° C. for 40 seconds (41 cycles)).

[0158] Results. The mean PMR for normal samples was 1.12 with a standard deviation of 1.45. The mean PMR for tumour samples was 38.23 with a standard deviation of 33.22. The overall difference in methylation levels between tumour and normal samples is significant in a t-test (p=0.000000326). The results are shown in **FIG. 1**. A ROC curve of the assay was also determined. The AUC for the MSP-Methyl-Light-Assay is 0.91 (**FIG. 3**)

[0159] The assay was tested on an additional set of colon samples (25 adenocarcinoma, 33 normals, and 13 adenomas). The results showed a significant difference again (**FIG. 4**). The ROC are shown in **FIG. 5-7**.

EXAMPLE 3

[0160] The HeavyMethyl-MethyLight-assay was also tested against a panel of other tissues (**FIG. 8**). Besides the colon cancer samples only one of the two breast cancer tissues were methylated. However, on a panel of 21 additional breast tumours (different stages), only one was methylated (**FIG. 9**). So the marker is specific for colon tumour samples. All primers, probes, blockers and reaction conditions were identical to those used in the analysis of the colon cancer samples (Example 2).

EXAMPLE 4

[0161] Twelve of the colon tissues analysed by real-time PCR also had paired serum taken before surgery. We extracted DNA from 1 ml of that serum using a Qiagen UltraSens® DNA extraction kit, bisulfite treated the DNA sample, and ran the HeavyMethyl-MethyLight-assay on those samples. The control gene did not amplify for three of the cancer serum samples and three of the normal serum samples, so we can conclude that the sample preparation did not work in these cases. In the other cases, there was evidence of higher methylation in the cancer samples than the normal samples (**FIG. 10**).

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 15

<210> SEQ ID NO 1
<211> LENGTH: 29993
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 1

ctggtttttc ccttaaggtt gcttttaaat gaaattaaaa caaaaattat gttttggatt 60

atgagctcct agagcgcttt gtttaatttg acttatacaa aatctaatgc caagacagtg 120

ccatgcacgt ttgaaaaata aacaaatagt aaatggtaac tgcagaggca tcccattcca 180

gaggagttgg ttaagggaat tgctaataag tgaatcttat atttccactg atttacccta 240

taaagtctta tatttttat ctccccactc ctcccaaaac acatcccttt tcagactgag 300

ttgaacatgt acaaatattc tgagagcctg actcatgtca tgcagggccc actggtgttt 360

ttgaatcact tcaagaatct ggcagcaggt ctctgacttc aatttccccc atgtgtcttt 420

ctaggccctt tgcgtttctc ccactgttct actttccctc tcttcttcat tgtctgtact 480

gtattgcact actgggctaa tagatgctag ggaagggtaa tgagtaaccg gtacaatatt 540

tcaggaaagt gatttgcttt ttaattgtgc atttatgaat tgtttgcatt ttaccacaaa 600

taagatagta agtaaaacaa taaataatac atgaatttta aaaagacgcc tcaacttcag 660

agacttcaag tatattttag acaagaaaag caataattaa ataagcacag aatttaaaaa 720

gtaatacctc aaagtaaatt ggacacaaca ttttgttccg gttatgaaaa ggggaggctt 780

gaaaatatct cttggtgagc ctgagggaca tatggaatgt aattctttaa accaatgttt 840

ctcaaactgt atgtgagata attttaggtg gtacatgaga aataactttt atttctacaa 900

tataggttcc tttgcaagat catcttctat tcatggcggg ttttattagt tttccattta 960

tggtgactat aaaaaatgtc ctttatcaga taaacttatt taataaaaat agattattta 1020

aagatacata ttaagtcaat aatataggcg atatggctat aattataaaa gtgatattca 1080

atgtaaatgc tggaaataag gcctcattta agcaaacaat acatttttgc ctttataacc 1140

ctaaaattct gtttcctatg ttaaacttat ttcaaatgtg tactgaaaat tgacgtttta 1200

tctgacacct agtggtgtcc atgttaatat atgtatattt aagacaacta ttagaaggta 1260

gtaatatgaa gtcaaataac taatatatta tcctggattt taaaaataat ttctgtacat 1320

gtattcttat ctagatgtac aaaaaactat gcaggagcaa agtgaccttg aaagcaaggt 1380

cagtgtaact gaaaaatctg actttagaag ggtacaaaga aacatgctaa gaaaacccac 1440

cctatattta tgttaacaat atgctaaagt atgaattttt attttattca aatcatacat 1500

ctcccacctt tatttgtagg tgaaactagc acataatgtt ttatgccaaa atgaaattca 1560

aaatgttgtt gccttttgag gacagtatta aattcatcat tgtacttaat ccttacatga 1620

tatttgctat agccacagaa cattcttaga cactatgatt cacaaaacat ttaaattgca 1680

tatttggctt gtaatatatt acatatttaa aagaattact atttttctga ataccattaa 1740

tactaataat agcatcacag tgactaagag tggacttaaa taaaggctgg cttgaaactc 1800

aggtctgcca tttattagca agcttctaaa aattctgagc ccttagtttt ctcacatgtg 1860

aaatggagaa aataaatctg caaaattaat taaatctgtc cattcatatt tttctctcca 1920

-continued

gtgtatatta actggcattc ctcgttaggc cagaatgtgc tctcaaccat gctccaaatc 1980

cgctttgtgc caaccccact gccagaaccc tttctacctt gagaaccaga aaaggaaaca 2040

ttatgcctgg caatgcctac accctccaaa ataaatctgc aggaaagaac acccagtaag 2100

tgatgagagc agcaacgact gcctttatca ttttaaattt acaacaccac cttttctaga 2160

gcctcttaag cattgtagat aattccccac tcattaaaaa ataaattgta accataagta 2220

ttcagggttg atactgcttt tgaattagac agtgctcata tcagttgcat aagaccaacc 2280

taaagtagag gatgaaatct tttttctgaa ccttttttcag aacgtaactt agtgaatata 2340

ttaaaactaa actttctttg aatgggagta atttctacgg attaatctgt aatctcttag 2400

accacaccta aggtaatgta gaggttgttg tatcataggc tttgtgatta gagaccactg 2460

gatttgtctt tggaaaagtc actcatgatt ctttgggctt tggtttcctc atcttttata 2520

ccggcttaat aatgaccacc gtagagttgt catggaagtt aaatgaactc atgtaccata 2580

agtgaccaat acaatgattg acactcagtg atttatcaat aaattaaaac atttattatc 2640

aatatgacag agaaggtgcc gctaaaatag acaataggtt tttggaagag gtgattaaat 2700

ggatgcaaaa tttatggatt gtttattccg tctacctttg ctgtgtcccc tggttgtggc 2760

atacacacgt gtgggtataa aatcgtaaat cctatgtagt cgcgtagtgc atgcgcagaa 2820

ggcttagaca cgaaatgtca tttcagcaat gtgcctagag aagctctgac gccgccttgg 2880

aagtaagtcg ttgctgcctg acctttgggc gtctgggacg gatgcctata cctgcaccca 2940

gcagcactgg aaggggccca ggcccttcgc agcacagcct atccccagac cgcttagtcc 3000

ttcataacat atatctccac ggaaaagggt atttcctccc gtcagaaaaa gcgccccagt 3060

ctggtctggg ttggttttta tttcacgttg ttgcaagtag gcgaagtccc ttctgtctcc 3120

tcccttgggg taagtggaaa ggagtccggc agggggcccg cagtggcctg cacaggggaa 3180

ctgggtagcg agagagttcc aggcaattcc gggggctgcc ccacagaagc aggtggggat 3240

cgacagtggc tctccggccc agggaggaga gcgcggtcgc gggtccctcc cctcagcctg 3300

gaggctgcag ccgctcgagt cggcccgggt gggggcgggg tgggggcggc gcggagggca 3360

cggagattac ggcggcgcca cccgggacat ccagggcccc gaggccctgg gcggtcccca 3420

cgcgagatcg caaaccatga caataggcag tcacccgagg tcaaataaaa acggagtggg 3480

tcccccgcgc gccgccgccc cccgcgtccc tggcggcctc ccccgaggcc cccggcggcc 3540

tcacgagccc gcagtagccg gtggcgacgt cgcccccgcc ccacctccct gcgcaagtgc 3600

gaggctgccg gcagcgcggc gcacgctccg gccgttcccg gcttccgcgc aaaacttcca 3660

tcctgtccac gtgaagttgt cgctgcctta gagaggggga aagagctgcg ggaaaagccg 3720

gggagtgacg actgcggcgg ctgggcgcgc tctctcattt tcttttcttc tcctttcccc 3780

cctgtcgcag tccggagttt tggctcctct cctttcctcc tccccctcgg agccggcttc 3840

tccctccgcc ccgcttctcc cccgcttgtg tacgctattt gttgtggggt ggccgaaggg 3900

gatgtcctgt tttcaccaga ggcacagcgc gaaggggaaa cttcgacact ggaaggaacg 3960

agaataaata cttaattacg gacgcactga accgcggctg ggacagacac ttcgggaacc 4020

cgaggcggac cgggcgacga ggtgagtgac cccttcttcc aaccccgcc ccagggctcc 4080

cgggggagcc tgagttgaga gaaccccaa actttccggg aaagtgcgcg aggctccgcc 4140

ggggacgccg agcgctgggt actgaggacg cgcagctgga cggtgcgtgg gcgcctgcgt 4200

-continued

ccccgggggg cgcttggagg ccgggtgccc cacgcctgag ggcccgggcc gctcggaccg 4260

cagcggtgct ctctgcccta gaagacgtcc ccaagcccca agggtccctt ccgagcctgc 4320

ctgtcccttc cggggtcggc gcggagcctg cgcgtaacgg agttcatcca gcagtccagc 4380

gcgcggcttc tacctgcacc ccgcctccac ctggcagagg cgcgagcatc ggggtctccc 4440

ccacatcttt cttatgacgt gtattacttt ctgatgaccc cctagatggt ccaggcgcga 4500

ggatgctgac ccagagtcct tcggagggtc acaggcgcct gggctttccc ggtgccgggt 4560

gcgtgtgtac tttaaaggct cgcgttctaa tctccaggca ctgatcgggc ttttcaactg 4620

cggcgatccc actttaatag tttttatgtg gcgtggactg aatgtctcct gcagtttgcc 4680

agggtcggtg aaattagagg cgccttgtca gagcagtcgc gttcattggc tcgagtagcg 4740

ggtgccatgg aaggcttata acttctccaa aggaagggac ctggctgggt agagcaggtt 4800

tttctctcct tccaagcctg ctgggtctgg ggaggcagtg gaacttgaaa tggctcggat 4860

ttttagcgtg gtgaagcgag gtttggaagt agacgtgtgt gtgcttgttt tattctgcgc 4920

cgcacagcaa cccgaacttt cgtttggtag cacttgaaag agttttctcc ctttgtttgc 4980

gagattctga acagctcgga gcgattaggg aatttgcgga ccgagtccgg tggcagagct 5040

ggggcgaaag cagagagcgc aatttaattt ttgtcatctc ttccctgctt gggaggatag 5100

tgtttccctt caccaccacc cctctttctc cttcctatga agacaacgga tttgcgcctg 5160

gggtgagagt gtgtgcggga gagtggtgtg gagactgtcc tctctcaccg cgtctcctgc 5220

gcctctcccc gccatcccga gcgggcctag agagtcattc atgaatctta acctgagggc 5280

aggggaggaa ggtgcaggtc cctctgccct ttctgccaag gtgcagaata gcgcccgggc 5340

gtgtgttttg gttccagagc agttccacgt ggagcaactt cgtgtgtgtg tgtgtgtgtg 5400

tgtgtgtgtg ttgtgtactt gatctgtgag gaggtaacag gactctggtg ttcaaaccca 5460

gtgggccgtt ggccattagt ttgctttcct ggctgtcatt acagacactt ccaaaatctg 5520

atacctaaga gaaccaacag gttaggtttc acattaaggg ctcatactta acagttttct 5580

ttctccctta ccccttttgg cttggcaccc tgggatcaac gtaattgttg gagcgaaata 5640

cacctcctgg aatatggcat tttgttcctc ttctcatctg tggccacttt gtgaaccctc 5700

gggtgtttgt cagtttcagt gcgggctcct gcgggattta ggtgggagtc ttaggagcgt 5760

ttaacaaccg cgggctcccc atcagcagct tctgaagttt cacttacacg taggtgactg 5820

acaggattga aagttgacga tggtttttttt gtttgttgcg ttgtttttgt ttttaaaacc 5880

ttagggaagg gattgtactt gaattccttt cccgggtacg gtttggtttt aagcagaatc 5940

agtgcctttt ttttttttt tgtcttttaa aatattattg gcaagcttaa acctgaagaa 6000

ccaaaaacta gaggggggtgg ggagagaatc cccccaaaaa atatttgata cgtgatacgg 6060

agcgttttag gagactgcat tcaaagacat ttgtgtattt ttaaaaataa cattatccca 6120

agaaacaaaa agcagtagta acaagagaca gattgttttg tgtggagcaa gactgccaga 6180

atctgatttt tatggcaaca atatcgaaag cagacataac tacacccaca tttattgtta 6240

taaaccgtaa aaatagtttg ttccacctga ttaaaagttg taagtcattc aaagttaaac 6300

ccgtatttag gaatgactgt acaagaatgt taaaaccttt gacagctaca gctttgaaag 6360

caacataatt agatttgttg aaaatgtctc ctttctctga ttattagtga tatgtacttg 6420

tctttcataa taagtaaagg tctacaatca atatggtttt caaaagcctg ttttcctttg 6480

-continued

taaatttcct gaaaatagta tgtttcaata tttaaagacc attgtaacta ccggttggtt 6540

cacaattata actaaaataa gatttactca gttgttttct ttttattagt atttgttgaa 6600

tatttgaaat tgaatttctc atttccaaaa taaaacgtct cacatatatg tatgtactca 6660

caattaacac ttcttttaag tagtaggctt aagttttaaa atttttaaaa tcttaagatt 6720

tgtataaaaa ggagtgattg tataaaaagg aataataatt attaattgtt aagaaaaata 6780

gatgctgaat ttcagagggt ttctagaagc tggagaaaaa aattgcatag aagtttcttt 6840

tttgacgggt gactggcaga tgagttactt gtcattttgc tataatttat ttttttcttt 6900

taataaggct ctttgggtta aaaaaaaaaa aggaaaaaca agtactccat ttctaagagt 6960

ttccttttta tttatgttgt actttggggt gtaaataaat tttacatagc cacgggaaat 7020

agagtatcca atttcatgtt ttcagtctct gttgcaagct ctaaatgact gatgtcgtgt 7080

ctaaaaatat atttatatgg cttgatattt cattttaaca ttttatgcac tggaattaga 7140

ttttttagga tttttagaaa gatagaatga cagaaagacg atgactattg ttataaaaag 7200

ttagaatgtt aggaagttca cattctaact ttcaaataat caaacttatt ttgccccaca 7260

aaaactcaca gtttttatac agatttcaag aagaggggtg ataatctttt tcactactta 7320

gttaagtgtt aagtaattta agaatgcaag tatgtattat gctttctcat tatcattttt 7380

ttctgttagg taaatgcaag aacactggac attctgtaaa aacaggcccc tctttataag 7440

gagtttattc accatagttc tgtattgcag ctgctgatca tatttcatgg agctgttaaa 7500

gcacttaaaa cctaaaatta ggtactgtct ggttgtaaat atttcagatc acttatttaa 7560

gaaataaata gaaaagtgtg ctatcaaaag taggagacgt tttgaatcct ctcattgaag 7620

agctgaacaa acctctatca aaacaccttc ttctttttc agtgagaata taatcttgac 7680

agtttctttt tctaaatgga tattattctt acatgtacta aatgctaaac tcttataacg 7740

tgcctccctg tagagtatct taaactaatt atattcagaa atacagttgg gggatttat 7800

tacaatggtt actaggtgaa ggaaatcaac accaggggaa tggggtggca ttgcagtgta 7860

cctgcttctc tcatgaattt ttcctgctaa actaagaaat gacatgctgt ttcaggcatt 7920

tgccctggag atgggtgaga tgcaatatgt gtaatgctgc atcttatagt tagatgtgtt 7980

ttaatgaagg ggacactgca tagtcattaa atcatttttg gagccaaact tggcgtcatt 8040

tagcttgaac ttaagtggaa gaaaatgaac aagagttaca cattcaaaag aagtacaagc 8100

aactttgatt gcttttaaga ggtttgaaga ctttgtaaac attactgtca ctcaatattg 8160

cttgtggagc tgtacattaa tatatgcttt ggtgatatgt cattttacgc ttgaaatttg 8220

tcattcttag tgtttctcca tccatctttt tattagtaaa gagatactga aaatgaacac 8280

tactattctt actcccctaa ccccttttcac acctccagaa aaagagatga aactgattaa 8340

tttaaaatag aaaccatttt gtgttatcaa aaccacattt atatagtgat ttgagacagt 8400

ttcagagagt gcacctctga gtctcactgt aacctttttt gtcattgaaa ggtgctaatt 8460

gatcttaggg tactgacaca atagtatagt ttgatatttg aaacctttca gagttggtct 8520

ggccctttc ttaccctgag atttcagtgc atggatgatg aagaaagaca ccattctaaa 8580

ataccagaaa ttctcatttt tttccaatat gaaatgtttt aatacagtat gttcatattt 8640

ttaaagcttt tatttacata cagtaagtaa atttatttta acgtactttt ggacagtagg 8700

agaaagacct atatgttcta tcgtgttaga atttttagt ttttttttt ctgcacaggt 8760

-continued

agtttattta ggttataatt tttaggcaaa gtctgattcc tattatcaca tgaatatttt 8820

caaagtgaaa ttgcgttaaa ccaatgtgga atagcttttg tatcaccaag gcatatatta 8880

atgtagatgt caaatatgag agcatatttt cttgagtata tttatatcct aaagtgtatt 8940

tttaaataaa agtggtcact gtagtcttta gataattaca atttggctgt cattattact 9000

ataatattaa tcactatcac catcataaca tcatagctag catttactca ggaatttcgt 9060

gcaaaacact gttttaagca tttatatgga ttagctaatt ttaatcttct taactatgcc 9120

gtaaattagg tacttttgtt attcctattt tacagataag gaagctgaga cccagtcatg 9180

cagcacagtg gagccaggat cctaactccc cagtatgagg ccagcaccct tatccttaag 9240

cgtgtgctgg gctcttgctt ccatcagtca tataccacca tttttaggtg gtacctgaac 9300

attttgtttt aataatactt atattttatg taaggataac tagatattag aaaaatttgt 9360

taaattttgt attaaacctg taacttcatg ggcaatattg tttgagacaa gaccaaacaa 9420

agtattgaag tcaaagaaaa aaaattaagt atctgaagaa acgtattaag taacagtgaa 9480

caagaatatg gcctaaataa ccacagtcat gaaggctgga cagcaaatga ctgaatttgg 9540

agaaatgctg tattttgtaa tgtttcccaa ttatcaagaa cttatgacca gatcttttaa 9600

atatttaact aacatgtgga atcttccttt gctcttcaag ccttatccaa attggttaaa 9660

tgttatcaac tttgtatttt ctttgttttt gtttttttgtt gttttaatgc tggttttgaa 9720

tctcaaatct gcacatttat gttgaaccaa ctaataaggc ttgaagagtt aaagagtgca 9780

tgatggactt ctggaggcag gtttaaatta taatggagct gcccatattt tggaaatata 9840

ttcaatttat ctggttatcg catgtgtaag gttttctgta ggtaaatact tttgctgtta 9900

ataacctgtt ttacaatttt atagtatttt tccactgaag cagtggtttt catttttta 9960

tttacttata tactcagcca cggatccttt catttaaatg gaaactgatg atatgcccag 10020

tagagaaatg tgccactgct ctaggtgaag caggatgtag gtagcctgag aatgactcac 10080

caacagtacc ctctcacggt ggccgccgcc tctggagttc tctctaagtg tcttcaatgt 10140

atggaaactg ctgcaaaaaa attcaagtct tctgacaaaa ggggttaatt cagagtacct 10200

gccctaacat gttcatgtgg catcactaaa aaacagactg tcagatacgg taaaatatct 10260

cccagtgtga catatcagca gaaaggtgtg tctactctcc cttctataac ttgagtatcc 10320

gtattaacca gtcttcaaat tcgatttagc actgagaaaa ttaaaactga tcaaaatgtt 10380

ccctgtgtgt agttacaggt ctgaatgagg cacaaaggac ttgtacctgc aaaggttgac 10440

tttattaatt agaacatctt tcctccttta aagactgtaa gaagaaacac cagcagtggc 10500

ctaacttgac atgactttag attttcacgt aaattattgc tactatttct gttatccttt 10560

ccccctttct ttttaaaatg aaagggacat ttcttgtgaa agactacaat taaatcataa 10620

aaatttacat tcatgtgcca ttaagtttaa ttctactcac aaaagcaaca gtacagagtt 10680

tgaaattcta tccctaatca agtaggtgta ccacataccg ggagggctca ttatgcacaa 10740

ggtcatatat acaattcaca gacctctgca tatacccaac ggagtgatct attcattaca 10800

tttcacctct gactttgaac tccctaatgt taaaagattt gaaaagaacc gaatgttctg 10860

attaagagat tgaatatttc taacttaatg ttttcagtat gttgaaagtg atgatgactt 10920

gggggaatca gcagatctct acattaccta attcttttct cttacatttg aatgcaaatg 10980

tatattcatg tgcggttatg actcaagtca ttcttgctaa atttaatgac gttgtaggtg 11040

-continued

```
aatcacattc agatttcctt ttgcaggttt tccagtaatc taaaacaatg cttctagtag  11100
gtaacttaag catgcaaacc tcaataaacc tgtcaagaac ggcaattcta ctgttttatt  11160
ttgttttttt ttttgtttgt ttgtttttgc attaacttta gttgataaga tgatggtact  11220
gttatttttc ttagttgact catgaagaat tttaatttag gtctagtttt ttcccttaat  11280
tgttgacttt agtttttaaa ggtttcgttc atgaaaatgg ttagcaaagt tgtgggtact  11340
tggtaaatgc ttgttaaatg ctttttcctt atcagtgttg ctgaagactt gcaaaattag  11400
agtgggatgg atagatttct tttctactct gcatggcttt gaagactttg gagcttttat  11460
tgtattctta tatttttaca taccattccc aggaatattt agagagagaa tcattgtaac  11520
caaggtcaca ggtctaatcc tcaggtattc aaattagctt taggtggaca attgtcctat  11580
acacacttgt tcgtattaat gttgtcataa caaatatagt tataatatct tgatgcctct  11640
cctgggggcc cattctggac tgtgttgaag ctgtctctaa cactctctca cgtccctaag  11700
atattcaacc acatttgttc atgatttta tgaggtccct cctggactta aaattcctta  11760
aaaaatttgc ctgctgccta tagggcaaat tccaaaattc tctgcaggga agataaccct  11820
tcatactgag aaccttgctc aggttgctct tctcatctct cactccccac tcacaacaac  11880
tctcttttcc agctgtatgg aaaactgcag ttctcaaata caccttggac atttccactt  11940
catttctttg tgcgtggaat gctttttctc tggaaaacat cgcccctctc cccctgcaaa  12000
tatcttttct actgcttttt caatacttaa ggtgcaaaat gttagctcct ctgataagtt  12060
cctctgacct ccagagtctg agttgaacgt ttctttcttg ttactcccaa agcatccagc  12120
ttctacattt aacatagccc ttgccacatt gaaatgtaat agattacatt agtctaaatc  12180
ctgctttaag ctgagaattt actggaagga ttagctgtat ttgtcatttc tgtatcgcta  12240
atgcacagtg cactgctgga catatagtca gtaggtagtt gttatattcc cattgaatga  12300
atgagtagag gtactgggaa tgagagcaga gattgtgaca ctgaacatcc cctccttgag  12360
actgggaaca gtgagaaggg cagtgactat tacaaggtgg gactacctca ataacaacct  12420
cagggagttt tggtataaac ccagtagaat tgcagctgtg aatccaggct taggttatat  12480
gtatgtatac gtgaatgtag aaatgtgttt atttcttacc actgggaagt cagtgatctg  12540
gtggataagc caaggatcct gaaaatctct ggagatacgg taatttcata gtacttgaaa  12600
tctgagagac tcagttctta tagcacagtg agtaagtaga aagaatatgg gggctgggcg  12660
cggtggctca cacttgtaat cccagcactt tgggaggccg aggcggatgg atcacctgaa  12720
gtcaggagtt cgagaccagc ctgaccaaaa tggcaaaacc ccgtctctac taaaaataca  12780
aaaattagct gggcgtggtg gtgggcgcct gtaatcccag ctactcggga ggctgaggca  12840
ggagaatcac ttgaacccag aaggcagagg ttgcagtgag ctgagattgc accattgcac  12900
tccagcctgg acaacagagt gagactccat ctcaaaaata aataaataaa taaaaaggaa  12960
gaagaatatg gagccaggca cttgggttca gacctcagct ctaacattga tgagtttgta  13020
acatcaaaca cattactgaa acagattttg cttcagtttc tcatgtgtaa cagaacatgc  13080
ctcatggggt tctgatgaga attcaatgag taaatatatg taaatatatt tagagtagtg  13140
cttgatatga gtatcagttg ttattactat gattgggtat ctttcattag attaccccca  13200
aatgttccag agatatttag agctgaaggt ctttttttggg gggctatgga ttggtgacca  13260
taaatggagt gtccagtagt ccaagtataa attagatact gtgggtcatc ttggcaaatc  13320
```

-continued

tgaggttgta tttggagcct taagagaaga cactataggt gctcaacagt tggcattggc 13380

catttacgtt gtgcagtaat atttctttta gtaaatattt ccgcatacag taatctagat 13440

tgttttagag ctttaatctg tgtggttcct taactacttt aagcaattat aagcacgttg 13500

tataatggta gtactttata gccaatattt aagtttcctc agttgctttt ttttcagccg 13560

taaggtacca attatgagaa tttgtataat tttcaatagt cagtgggcct gatgaactca 13620

atgtaaactt tttaaaaggt agctttacat attgctatga ttcttacctt tagagagaca 13680

gtaggaggta attagtacct ctacttacag aatttatcac ttggattcag atataatggc 13740

tatgtggcag gatggggctg aaggaaaaag gaaagttaaa atattaattg tcaaggtctc 13800

atttttacag cttgtctgga actgctacca ggtgtatctg tatagtttta aaaatgataa 13860

cgttgtacct gaattcttca gtatatttaa ataagagttt ttcaagctct ggttattgac 13920

attttgggct ggataattta ttcaggggat actgtcctgt gcctggtagg atgcttagtg 13980

gcatcgctgg atgccaggga ccctccctcc cctggtaatg atggtgtctc cagacattgt 14040

caggtgatct ctgggagcaa aattgcctct tcttgagaac ccataaccta agcagatctt 14100

aatatcattt gatatagtca aaaaactccc agggctctgc ataagaggat tgttctattt 14160

ccagtcaagt gtggagaatc ctaatacctt cctcccaagt ttaaacatga agtcaaacaa 14220

ttctcattag tctgtgttga tagattaact ttgcacagag gggatttaca gatacgcttc 14280

acacagattg accatctcca gcatattttc cttttctgga aataatatat gagtgggagt 14340

aacagaatac ctgagagaga gtatgtagga agtaaatatt tatttgaatc atctatgtgt 14400

cttttcttcc ggttatttgt cagtagatag tttggattta tttttaaata atgaccattg 14460

cctttccatt tctgtgggta aatattcagt aataagaaac ttttatttta tttagtctgt 14520

agtgttagaa aaggtaaagt tactgataat cacaactgct gaagattaaa atacttagtg 14580

agttaaatta tttgtttgta atgagaaatt ttcaaaagaa ttatgtgtag ctttcagttg 14640

taaccacaag ttcaagatct tgagttaata acatttgtac agctagaaga aagtaaaaat 14700

aaatatatta catgtaaaaa ctctcttttg tgaattctca gatccaaact tttaagttct 14760

cattctatgg gtttgatgtt gaccaataca ttttatctat gaaatgatat ataattgatg 14820

taagtaaatt tgactgttgt ttgggcacca cccttacata atttaagcat atatgaaaca 14880

catttcaaaa atcacttgaa caaaatggga acaataatgt tcacataaag tgagagagga 14940

taacttttcc catatgagtt ttgggttgaa tttcaaaatt attctgaaat atgaaacatt 15000

aatattataa atatatgatt agactaattt tcttaagagt tctaaacagt agataatttt 15060

tatcttatta gaaattgcaa agatacttga aattccacac ttatgcattg tctaatttag 15120

tggttgtact ttaattgtat taggtagata atcatggaag cagtcagact aacctggaga 15180

tttcacatca tttactaatt gatgcctaag taggcagggt agaggtactt gaaaacacac 15240

acacacacac acacacacac acacacacac acacacacac actcactctc tctctctcac 15300

ttcctttgat ttgtgcttta attttgaaat gtgtgcttag gtggcaaagg gctgcaaata 15360

cagcatactg tgtttgacca aaatattttt ctgtttccta ggtggagaag gaccctgcat 15420

agctccttgt tagcagagag ataaagtggg tgctgggcaa cccaatgaca ggtggaggcc 15480

gttctcgggg agtgttgcgg agaatttgag acaggggttt aaatcacaga tagtggagga 15540

gagttctgct tcattcatgg acaggaagtc caaagaaggc acagcgaacc acaggtccat 15600

-continued

```
ccaggcactt tatgccagat attgaacggg aagcctagta gccctttagt tactggagaa  15660
cggccaaaaa cccagaggtt ctatttttcc aaatgagcac ttcctgggtt cataaatcaa  15720
ggaaccccac agagtattca agtcacatat ctcctttctc aggttgattc tttgtatagt  15780
gggtcctaat gtggtaagac ttacttatga acattttatt tcttataatg ccgtcagtca  15840
cttccttttt tctccctggc atattctcac cacccttatc ctccatggcc ccaactccct  15900
taacctactt gcctagtgct cttttgctgc ctacctggac aaaataataa taataataat  15960
aatctactca tctttcaagg cttagctcaa atgtcacctc ctctgtgaag ccttccttaa  16020
ggctcaggca gaattagttc ttttctctgt gtaataccat agcacttttt ttggactgtt  16080
aatataacag cagattgtgt tggggctcat tgtatgtgtc tgtcttctag agagactatg  16140
aactcacaga ggggagagat catatctttt tccctccgta acctcaacac ctggagatgt  16200
taatggaaat taagtgactg cactattcca tgcatatctt taaagggcat taggtcccaa  16260
accagacatc tgcaaaggat gaatcctgtt aacttttgg aaaactggtt ctcttttgct  16320
tgtggtcatg aaagctggtt tattggttat tttctactcc aacttattta aagcctcatt  16380
tgtaccaaag tattactgat tttctagtgg aaaaacaggt atgtccggcg tacattggta  16440
ttttcaataa actgacattt aaaagacaga tttctcgttg aggtttatgg tcttcataat  16500
tctagttttc tccagaaaca gaccttgatc tctttggttg ccttctgcta agatcgattt  16560
catgttattt tgaagaattt ttaaccctca gaattataga tttcatttga gagaaagcat  16620
gttctgtatt tgtttgtgtc agtgtttcaa aatgtgagga ccaaaaatcc cagatgtatc  16680
tattaaaaat atacgtggcc ttttgctgag ggaaattaca tctttttttt caggcacaat  16740
tgaggaagta aaaaaatttt gtttagtttt tagtggtttg tttttaggca ttttccatac  16800
aaagatgagc aagacccttc aaaaaccacc aatttgctta tttaggggggg aaagtcttcc  16860
tatgtccaga aataacatta aattaagatt attgtttcca atagtttcaa aaattgtggt  16920
tttatttttct tgtatgagtc atttttttagc atagtgcagt acatgattgc actacatcat  16980
gacaaagcat atttgctctg tgtttcagtg ggtcacttta tttatcagcg tcagatgatc  17040
agatcagaag agagatctag cctaaccctt ccattttgca gatgagatgt tgtattatgt  17100
gaccttaaga ccttaagtca tttactcaga acaaaacaga aaaagagaac ttcatgctgg  17160
ggacacttca tgctcccaac tctacatctt accactttat ttttatttgt ttgtttctga  17220
tgaggagttg agaatgctgg aatcagacta cctttgtgtg tatccaggct ctgccactca  17280
tcagcgatgt gactttggtc atgtaatttt ctgagtggct gtaagccata gttccagcat  17340
gtgtacatgg ggaataataa cagtactcgc ctcatggagt tgtaaggaat aattgaattc  17400
atgcaggaaa gacatttggt tcagtgcctg gcccactata aaagctcagg ttatagtttg  17460
aattgatatc ttggaataaa atgctgagtg tatttgatat tcagagaagg aaattcatgc  17520
tgctaaagca agtttttttt tttaatctgt tacttaaaaa atacaaataa gtcatacttt  17580
gtataacata ttaaaacaga gatgatagag gttacttttg agaggaatga ccagagggag  17640
caagagagag gctttggaat gctgattatg tcttttatct tggtttggtt gctggtcatg  17700
tgaaagtgtt tattcaataa aaattcatca gtgatcaact tttgatttat gtattttatt  17760
ttactgtgca tatgttatac tttcttaaaa agttttaaaa atacggatat atttaaagta  17820
aaaagtggat ttactcattc ttattctctg caaatcagga taagttgtta tacttagatg  17880
```

-continued

tgcatctttt caggcatttt tttttctata tatgtgtaaa tgtaataact atgtgaggat 17940

gtaatttatt ttcattttac atttttaact ccaataggat cattgatttc cttaaaactt 18000

gcttttgatc tgctgaatct tgaatatttt tccacatcag tacatatagt tttgggtgct 18060

tctatttaat gtatgcagat atttcataat ttcactgttc ccctcttggt gtatgtttac 18120

attgcttcca tatttttact attactaagt atgttgcact gatgattttt atgtctcttc 18180

gtacatctgc caaaaatatc tgtagataaa actctagatg caatattaat agataaatat 18240

gtatgtgcat tttccataga aaataccaaa atgcttttta aaaagattta tgaatcttgc 18300

atctttaagg taatattctc atctttctca caaaaaccag gtgcaaaaat ttcaacttaa 18360

ttgaaccttg taattatttg tgctctggtt aagaaaaatg gttatagtag taaattcctt 18420

gaaactcata tagtaatcgg aatactccac tatgattagg ataaataatt ctattaacga 18480

tatatattta atatatacat ataatttata taattagttt ctcacttaag aatatttagt 18540

attcattttt atgaatgcat attatattta cttatggttc gtaagcccaa atgttaggaa 18600

gaaataaatc agtaatatta tattcactac attttttttt acttagtacc atcaaatatt 18660

taccagaatt attaggagaa atggttctaa ctctcatgtg attggtgaga acttatgagg 18720

aatgatatat tgttattcta gtaagagcct actctgtgcc aggcacagtt ctaagcacct 18780

tacatatatt acgatagctt tttaacatag agatgaaagg tatagacatt gtataagtaa 18840

taaatgacat tactccatta taaatgtgtt gataaatatt attatacatg tcacaatatc 18900

agttgtgtca tatcaatgac taatttataa atctgttact tattataatc tgtttaatgt 18960

ggaaataaaa gttaatgcat caaaatccag aaaattgcta ctcaaatgct ggctaaagta 19020

atctgataga tacagtttta aaatgaagta tattaatcag ttgattttgt tgttcgattt 19080

ttatatgtag aaaattctgt ctccagaata ttcagttctt gtagtttttg gttaagattt 19140

tgattaatct tcaatggtta tctagtgcct ttaaaaagta gtccaactca gaatagctaa 19200

tgtattcctc ttgcattgaa taaatatggc tatcaatttt tgtggggttt tttttcctgc 19260

agaatttcct gttattctaa gggattcaat aggacttaca catataaaac tgaaaattat 19320

attactatgg ggaagctgct ttgtgccttt cagaaggaac tctcttgcta acttaagtac 19380

tgtatgtgtg tagaatagct aatattttcc tcccaagaaa tttcatgtat gcagttaaaa 19440

cactcttaaa ttgattaagg atttgttata tatttccaga agcactcaag agtacttta 19500

tggttataag agtagagtgc attagaatgc cagaaaccaa tgaatacgct agaggccttc 19560

aaaactgtgc ccaacattcc tgactttacc atataaacat gtggggtagc ctggaaaaac 19620

aaaacaactc tccttccccc aacgtaggtg aggcctgtga tgataacact tcaactggct 19680

tgaatattaa tttgatactt ttctgttaga agtaattttt attacttagc aaatgaaatg 19740

gagaggtaat aaatatgact gatatttatt tcttgagtaa agtattattt tacatcagat 19800

tcgaccagtt acctttatcc tttttggaaa atgtattata caaactacca cagtattttg 19860

cctattaatc agggaagcta aaaaacgttt tataaacgtt gaatcaaaac tctcactgct 19920

gtgagggaat taaatttcaa gcaatatact ccttttatag ggtgagaaac tgaagtatgg 19980

agaaattaca aggttttccc aaagctatac actgagtcat ggcagacata gaaatctaca 20040

attctgtttg cctgattcaa tctgaaactt catgaaacgt aatttatcca tttcctgaca 20100

tgtgctttag aagtatattt ccttatttgc ttatttatgc tctcattcat tcattcatgc 20160

-continued

atgcatttat gcactattga ctatttatgc actcattcac tcattcatgc attttaaaag 20220

aatcggtgtt tttgtatgat acagtcttga tttagggact atgaatattc tgaatttata 20280

tatcttagat atttcttcaa agaaactcat tgcatttttt tcacgatcat gaaaaagaaa 20340

ttaagacatt aagaaaacca agctggattg tccaagtgtg ggccatagca gtgtctatgg 20400

gcagcacctg aagctaattc aggagtgaat ctagtttgtt caagtgcttg aacaagaact 20460

tggctgaagc cctggaggag atgcctgggg catagagtgg ggacgaggaa aaaccatagc 20520

actcttagag agtactgtaa ggactagtat ccacatctct accacctgat gtctttacat 20580

ttggttcagt caagagagag ggggcaaatt attctgcttc tcattctgcc atcttcctga 20640

tttcagttct cacgctttaa attctttttt attgcgatct ttttcttact agtttaacca 20700

gtattttccc gcgctttctt caccaaattc gtttctttcc atcagcttgc ggagttatat 20760

tttttgatcg aactgctggg gttacaagta atttgaaatg aggaaagtgt ctagcaactt 20820

ccctcagtcc agtaccctgt aacagagact tcagagtacg tagtgatgac acctgccctc 20880

ctccccttct atagagagtt tagtgggcaa ctttttggc ttgttctctt gttcttttct 20940

tgcagcccca agacttagga ctcagacttg gtttctgctt gtatggctct ccatctcttc 21000

ccttggaaga gttctttccc gggggctttt taggtccaag agcatttgga cccctgggag 21060

atgaacttgt agattctaca aagaacatcc aagtaccagg caaaccacat cttcctgtag 21120

cttaaacgtt agtctttttt ccccccttcaa ttttactgaa gaataattta gtctccaaca 21180

aacaagcaaa cagaacccta gggtctttct caaaggtgca ttcttgctgc tttgaagatt 21240

atcatcatta atttctattt cagctaattg gttttccttt acatttttat ttgggtataa 21300

atgtgatact ttggtataca ttaatgttga tctatattaa tacgtcaatt tataattgag 21360

tgatttaaaa ataatgatgc actatgacct agtttatatc tgccttccac ttaatgcatt 21420

tcctaagcaa ttatgctttt gtggctgact gaattccctt ataattccct gttgggaaac 21480

aaagattttt ttttaaattg aagaagtggt cttgttaacc aggggcaccg tacttaactt 21540

gagagaaaaa gattaggaat gacaggaatg acagcatact cctaatatct aacagcctgc 21600

catacatgtg aaagcaggac acatagcagg gaagcataac gttgagttga ctcaaaacac 21660

caaatgtatt tgatacatta ttttctgaat tcttacaact tcagcccttt taaaggtctt 21720

tggatttta gaagctataa gggcagtttt tggtaaaggt ggccctactt ctaattcagt 21780

tacagagtca gtggaatgat ttttatattc aaatgctaca ggggaattta ttgagaggaa 21840

aaagggtact caaactttcc tggatgcctc aattaaaaat ctggatcaat aaaatttcct 21900

caagtgtccc aaatttaagg aaagcaaaca gcatcttatt taattacatt cttaattagt 21960

attcactata gacacaattc aaatcagtaa cttggcatta gatgaatcag atttattgcc 22020

atattatata actctcatgt ttactttgtt gtgtttggga cttctccctc cccataataa 22080

agaatatgac tcacaggtgt cacaccttga ttcctgagaa attaatgtta tagaaaagtt 22140

gtttggaaag atataattgt gattttgtta ttgtttttgt tgtcattatt ttcttttatg 22200

gggatgggca cacacttctt gagatttacc agatttacct tgtagttcat aaattatttc 22260

tttagatgta tctaatttgc tgtttagttt gtttcttgta aaaaaattca gtgaatatat 22320

ttttaaaaac atttttatgg ttctcttcca ggtttgtgtg tatatttttt tcgtgtgtgg 22380

cttttcaatt ttatttctgt ttcttttgtg tctgttttaa gtatacctat ctagtttttt 22440

-continued

```
ccacattgct ctgtagtttc acattcttag attcctaatt tgttgttgtg gctactctct  22500
actgtgtttt gtttctttat gtagcttata attttttttt tctttttgag atggagttgt  22560
gctctgttgc ccaggctgga gtgcagtggc acgatcttgg cttactgcaa tctctgcctc  22620
ctgggttcag gcgattctcc tgcctcagcc tcccgaatag ctgggatcac aggtgcccac  22680
caccatgccc agctaacttt tgtatttta gtagaaaagg ggtttcgcca tgttagccag  22740
gctggcctca aactcctgac ctcaagtgat ctgcctgcct gagcctccca atgtgctggg  22800
attacaggca tgagccaccg cacctcgcct gtagtttata acttttggtg gatttctctc  22860
ttagaaccct ttgtactctg tggagcaatt ttgcattccc ctggagtcta gggatttcaa  22920
acctctaggt cagtctttat tttattttat ttttttgtct tgaggtttcc ataccacaga  22980
tgcagtaaac atttagaatt tacatcctgc aaggctgaag ttttgattta tctcactcaa  23040
gaaattattt tcttttattc tcttcccaaa ggctccatca taggacaggc ttctttgctg  23100
aatctgatgg tgggccgagt ttttctatcc cccctccaat aaatgtgaca aacttttag  23160
actccttcct ctgtggattg cctcttgctt ttgcttcctg cccatcgcca taaaagaaga  23220
taatgacaac aaaacgataa caacacaaat gtaaacgtac cttttcctct taacttttcc  23280
atatcatcaa tttctcatga attaaggtgt gatacctgaa agtcacattt gccttatcag  23340
ttatattcat atagttcctt tgtcttattg atgtatctct catattatcc ctcatatcca  23400
cttctccagg ccttttcca ccccatgtca gtctagattc tcttggcttc tcacttggag  23460
cgtcacagca gcctttaagt tgccacttaa ctccacttct tgttcatctt gtttgtcaga  23520
aatctgaagt agatgtgttg ttgcaatgat atcacatgtg ccattctttg ttttgggggt  23580
tttaaggctt ttaaggttat caaaatattg taataaccaa ttgtcttaat tgtattttgt  23640
acttattctc ctgcttttgc caacttaaaa tgtcttaaca tcctgttaat actgaatgct  23700
gatgctttcc tattcccttt tctttcacat gtccgtctat aattgtttat tgattatatc  23760
agtctctcaa cactaaaggc accagtcaca actttgatgt tatacagaat gacaactaaa  23820
aggtgacaaa tgctaatgac ttaatcgagg agttgttcat ttttacaagc ttacctgagt  23880
tcagagaatt ggtaaaagac tggtgacttt tggctactat ctaaatataa ttggttaatg  23940
tagctcctgg agatcagtgg ttagtataat actcttagtt gagcagggtt aaaaataatt  24000
acatttgttt aagttgcaga tactactcta ctgtacagca aagaatgtta aaatgtttta  24060
attcagtata tgaagagcaa aatataaaaa caggttttct tccactctca ctagcaagca  24120
aagtggtctg tatttttgaa atatattttt tgtttaatca taacaaaata taatacaaat  24180
gttattaaaa gccctatttt atggatgaag acagtgatat ttagagatat taattagctt  24240
ttccaagatc atacatttat ccagtagtaa aagcaagttt tcacaagctg atctatttta  24300
ttccagtctt tatttggaat cactaaactt acattccctt tagcaaaact atcgtttaca  24360
tttctagggt ttcaagagtt caatgctaaa cttgggagat ctattcttaa attctctcaa  24420
agtgcaacac attttcctca ggatgatcat aaagtcatca gatgtgtgtt aagcaatata  24480
tatagtcata tactcttgat caaaaaagat ttccaggtta cacagaagag agatttatgg  24540
ctgagaaaaa ggcagattgc ttctaagaaa ggcttcaaat atattctctt ctgacaggct  24600
acaggagagc cagctagatg aaccattaca gtgtgtacaa aatgctcagc agaataattt  24660
ctcagtactg gtagggaaga gcaataggag taacacaaga gaaggaaagt tggtctgcag  24720
```

-continued

agtctgcctt agtatccgta ggggtgatga ggttgcttgg gctaagaatt ctttcacatg 24780

tctccctggg acaattcact cagaattact tctgtgtttt ctaggctatc taggcaacca 24840

tcaccaatta gcaaacacag acaaaataga acaccttgtt tggacctgct tgtcaaaaat 24900

caaatgatca taatttataa ttccaggaag acttgccatc aaatgaactt atgacaaatg 24960

acccaaatga ttctcacaaa gtagagcttg ttgtaacttt tagcatgaga agtatgaacc 25020

ccattatcat tttttaaaca ttttgagtta aatggaccgt tttgcaaagt ccatgttcta 25080

tctttataga cagctcaaaa atagctcagc atctagtttg taggttggct ccatttaagg 25140

agcttgaagt caggcaaggt gctcattact attcatccag atctgtttct tggacacctc 25200

agctgttttt cttttctacg gccagggagg agagtcatgt gcatagtcca agaatgtgca 25260

tctccatgtc tctgaacagt tggccataga attggagcta ggtgttcttt gtgccattgg 25320

ctttcctctc tggcatgctt cccgggtggt tggagccagc cagtgcctga tccagcttca 25380

cagacccagt aatgaaattt ggagggcaga gtaggtaact aggtacacaa gaattgtaaa 25440

gcaatgccca tctggacagt tgatcacaac tataacttct caataatgta acaggaatat 25500

aaactgttag acttcctata ccattgcaat ataaagaaga taattgaagg ccgtgaagat 25560

agctcatgaa atgcaaggaa gaattggaca actaagcaac aggaagagca gagaaacagt 25620

gagactttgg gagtcacaag atctgggtgc ttaaattctg ccaggacttt cttgccatct 25680

tcatttttgc tcctctttct cggtcagttc tcttctgtct ttccctacag accagctttt 25740

ttccacatgg atatgaaacg tttccaagtt ttatatccta ggggttttgc cactgaagga 25800

ggactagcct gatattttcc cagactcata tcctaaggaa gatgaatcat tagatccaag 25860

taatttcgtg caactggaga agacttgcag accctaccta atgggttaag ctggatatgc 25920

atctgtggga gggagatatg ttttgtgggt ataatacaca tgcaagcaaa ccttaatatt 25980

gtacaagata cgcttctgaa aatttacttg cagtttgaaa atctggaagc caaacagtag 26040

accaactgtt gtgagtgtta gaatttagtg taatggcact ttctgtgacc caggcaactc 26100

tgcataggca gaaagattct tgaaagcccc acccaggttg gaaagggtct gtgtcagagg 26160

tcatgagtgg gactcttcag gcacctgttt ccccatccca aggtctgcac atggactgga 26220

acaaaagttt tggcagctgc aatttttaaa tcttttccca agggtatcta gggatttaaa 26280

gcagtaggga atgtactccc ccactttccc ttgttttcct tttcactgca gcaagaggca 26340

agtatagccc tcagccctgc ttgtaccctc acaggagact catgtacagg tcccgggctc 26400

tggctgcagg acagtgtgag gctgctgaca gggcgagaga atggcgagaa caatggaaca 26460

ggactgaggg agaaggaaga ggaatcgtcc ccagaactac tacttgggac ggtggtccca 26520

gggagcaaaa aacacccctg gagccccttc aaggggcctt ttggcagagc ggtactaccg 26580

ctagacactg ccatggctgc aggtaggact gattaaagaa tttctgattt cttctgagga 26640

cagggtaaag ctctgaattg tttttagaga ctgcattcag atctatattt cggaaccatc 26700

actctggttg cactgtgaac ggtaacgtcc agggagcagg actggaggga aggagaccat 26760

tacgtggctg ttgctcctgc ccaagcaggg aacaatgatg gcggaacgcc tgtactggca 26820

gagaggagag agaggaggga tttggcgaac ttttggaggt attggtagga cttggtaata 26880

tattgaatgt ggagagcaaa tgggcaggga gtcagtgaca aagcctaggt gtttggcttt 26940

gatgactggc tatgcctgtg ccggttgtgt acagaatcca gaggtttagc gtttccatgt 27000

-continued

gcagcgtcgg tcctcattcc gcggcagcct ggtgtggagt tgagatgtgc cccctccact 27060

ccatgttcac gtttctttag ccagagcaaa gcatgcctgg acaacctagg tttctttctt 27120

tctttctttc ttttctctag agagagaggg tctccgctct gtcacctaga agaagtgcag 27180

tgtcgtgatc atagctcact gcagccttga actcctggcc tcaagtgatc agaggattag 27240

gtgtacttct ctacaaactt ctcaaacctc cttcagcatc tcattcctta agggacctta 27300

cagtctctcc cacctggacg gcgccatggt gcctcattcc atttctgaga attgtcttaa 27360

aactttatat agaaacttgc tattatgtat ttaaatgaga agaaatgttg aatttgtaat 27420

tctaattttc taagacaaga gtttgtagct cgcttctagc agagttaaag aaaaagatat 27480

gatgaatggt ctatatattg aaaactgcag aagaaataat ctaatatgga atgggtgagg 27540

ttgctacagg tggtgcaaaa ataattgcgg tttttgctgt tactttcagt ggcaaaaacc 27600

gcaattgttt ttgcacaaac ctaataatac tgtttgaaat tcatattttc tggtggaaca 27660

gtcttgagat agagcgggaa ctagattatt ggctcataaa acagcatgca gttactgtta 27720

ttccttaatc ttatatcttt attatgaggt aatgcttctc ttacttgatt tggagaatgt 27780

tctcatcttt ttctcatata tttgtgaaaa ctaaatgtta aaggaattaa cataacaacc 27840

attgctaaat tatgatgtaa aataatactg tgtctgcaat tagcctaggt agttttactt 27900

ggttcaataa tataaaatca ctaggctcat aatattttag taccttagtg tgattggcca 27960

agaaaaaaat tcacgttacc ataaaaagtc aaaccaagta acaattaatc taaatttcaa 28020

ggaaatataa agcaaagtat tatgttgtaa catggaacat taatgtaaat aactccccaa 28080

cagttcatga aatagattct gaactggcag ttgacaaaga aaggctagtt ttggcttaat 28140

tctcttatgc tccaatatct ttcctgaagt cttatttaat ttcacttgcg ttagcaacag 28200

tactctgaga tggtggtgca tctggaaaat tatcacttct ttcctgacaa tcagtatctt 28260

taatttaatt cttacatatg ctttctggaa atattacatt catactttga tgacgtctta 28320

aaaataattt caatgtaata attatacatg tgtggtgagt aaattgtgtt ccgtaaaaga 28380

tacttttaaa tcctaatgtc tgtatttggg aatgtgacct catttggaaa cagggtcttt 28440

gcaggtatga tcaagttaaa atgagatttt actggattag gatgggtcct aatacaatga 28500

ctgttgtact taagaggaaa atttgtacag agacatacac acacacaaaa gaatgccatg 28560

taacgttagg cacggagaac atcatgtgac aatgaaggca gagattggag tgatgcatct 28620

atgagccaag gatggccagc aacccccaga agctggggag agacaaagga tgcttccctа 28680

ggatctccag agcaagcata aatctgccag caccttgatt taggacttct ttcctccaga 28740

actgtgagaa tagatttcag ttctaagcca cccagtttgt ggtactttgt tataggagtc 28800

ctaggaaact aatgcaacat gctatgttgt aaataaacag tagttctaat tggtataaat 28860

aataatttta agtatatttt attattaggt caggtgagta ggaaattgcc tggtagacta 28920

attatcaatg ttgtattttc tgaaaagtta aaatattccc attcagtaaa taatacttgt 28980

ctaaaagttt ctatctgtat tgtcctaaaa caaagcaaaa attaaaatgg agtcagttat 29040

gtacctgaaa aatgtgaaaa aggaaagaaa aatcttttag gaaagatttt tcctaaaatg 29100

gctatacatt tgagaaagaa ttttaaaact taaatcatct ggctgtttat tccatttttt 29160

tgtttacatt atcagataca ttatgtatga tttcatacat tatgtatgtt acattcaatc 29220

aatatttatt aaatacctaa aatttgctag gccttgagct aggtattgta gataggtaaa 29280

-continued

taagacaaac attcccttcc cctaaagaga ttattttggc atgaaattta atctgtagaa 29340

gaagtgaaag aggccatttg cagagtctac catgaaaatt cattttcact atctgctgta 29400

gttttacctg atcctgtgaa accattttag catcatttgg ttttactttg tgcttctgta 29460

ttatattgga gaaaataaaa tttacttatt gcacagacaa cataaaatta attaaagtat 29520

gtgaaaccaa aaatgtaatt gtttatcaaa ttttttttag tgtttcccat tcatcagtat 29580

ccaaatacat tcttagttgt gcatccatgt tagcataact aaaatgtggt gttcttattt 29640

tttcatctaa ttcacaatca acaatgttca cttttccact ttttttttt gagacagagt 29700

ctcactctgt cacacccagg ctggagtgca gtggcatgat cttggcacac tgcaacctcc 29760

gcctcctggg ttcaagcgat tctcctgcct cagcctcctg agtaattggg attacaggcg 29820

cccgccacca tgcccggcta attttttgta ttttagtaga gacagggttt caccatgttg 29880

gtcaggctgg tctcgaactc ctgacctcag gtgatccgcc cacctcggcc tcccaaagtg 29940

ctgggattac aggcatgaac caccacgcct ggcccctcat catttttata att 29993

<210> SEQ ID NO 2
<211> LENGTH: 29993
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: chemically treated genomic DNA (Homo sapiens)

<400> SEQUENCE: 2

ttggtttttt ttttaaggtt gtttttaaat gaaattaaaa taaaaattat gttttggatt 60

atgagttttt agagcgtttt gtttaatttg atttatataa aatttaatgt taagatagtg 120

ttatgtacgt ttgaaaaata aataaatagt aaatggtaat tgtagaggta ttttatttta 180

gaggagttgg ttaagggaat tgttaataag tgaattttat atttttattg atttatttta 240

taaagtttta tatttttat ttttttattt tttttaaaat atatttttt ttagattgag 300

ttgaatatgt ataaatattt tgagagtttg atttatgtta tgtagggttt attggtgttt 360

ttgaattatt ttaagaattt ggtagtaggt ttttgatttt aatttttttt atgtgttttt 420

ttaggttttt tgcgtttttt ttattgtttt attttttttt ttttttttat tgtttgtatt 480

gtattgtatt attgggttaa tagatgttag ggaagggtaa tgagtaatcg gtataatatt 540

ttaggaaagt gatttgtttt ttaattgtgt atttatgaat tgtttgtatt ttattataaa 600

taagatagta agtaaaataa taaataatat atgaatttta aaaagacgtt ttaattttag 660

agattttaag tatattttag ataagaaaag taataattaa ataagtatag aatttaaaaa 720

gtaatatttt aaagtaaatt ggatataata ttttgtttcg gttatgaaaa ggggaggttt 780

gaaaatattt tttggtgagt ttgagggata tatggaatgt aatttttaa attaatgttt 840

tttaaattgt atgtgagata attttaggtg gtatatgaga aataattttt atttttataa 900

tataggtttt tttgtaagat tatttttat ttatggcggg ttttattagt tttttattta 960

tggtgattat aaaaaatgtt ttttattaga taaatttatt taataaaaat agattattta 1020

aagatatata ttaagttaat aatataggcg atatggttat aattataaaa gtgatattta 1080

atgtaaatgt tggaaataag gttttattta agtaaataat atatttttgt ttttataatt 1140

ttaaaatttt gtttttttatg ttaaatttat tttaaatgtg tattgaaaat tgacgtttta 1200

tttgatattt agtggtgttt atgttaatat atgtatattt aagataatta ttagaaggta 1260

-continued

gtaatatgaa gttaaataat taatatatta ttttggattt taaaaataat ttttgtatat 1320

gtatttttat ttagatgtat aaaaaattat gtaggagtaa agtgattttg aaagtaaggt 1380

tagtgtaatt gaaaaatttg attttagaag ggtataaaga aatatgttaa gaaaatttat 1440

tttatattta tgttaataat atgttaaagt atgaattttt attttattta aattatatat 1500

tttttatttt tatttgtagg tgaaattagt atataatgtt ttatgttaaa atgaaattta 1560

aaatgttgtt gttttttgag gatagtatta aatttattat tgtatttaat ttttatatga 1620

tatttgttat agttatagaa tatttttaga tattatgatt tataaaatat ttaaattgta 1680

tatttggttt gtaatatatt atatatttaa aagaattatt atttttttga atattattaa 1740

tattaataat agtattatag tgattaagag tggatttaaa taaaggttgg tttgaaattt 1800

aggtttgtta tttattagta agtttttaaa aattttgagt ttttagtttt tttatatgtg 1860

aaatggagaa aataaatttg taaaattaat taaatttgtt tatttatatt tttttttta 1920

gtgtatatta attggtattt ttcgttaggt tagaatgtgt ttttaattat gttttaaatt 1980

cgttttgtgt taattttatt gttagaattt tttttatttt gagaattaga aaaggaaata 2040

ttatgtttgg taatgtttat atttttaaa ataaatttgt aggaaagaat atttagtaag 2100

tgatgagagt agtaacgatt gtttttatta ttttaaattt ataatattat tttttttaga 2160

gtttttaag tattgtagat aatttttat ttattaaaaa ataaattgta attataagta 2220

tttagggttg atattgtttt tgaattagat agtgtttata ttagttgtat aagattaatt 2280

taaagtagag gatgaaattt ttttttgaa ttttttttag aacgtaattt agtgaatata 2340

ttaaaattaa attttttttg aatgggagta atttttacgg attaatttgt aatttttag 2400

attatattta aggtaatgta gaggttgttg tattataggt tttgtgatta gagattattg 2460

gatttgtttt tggaaaagtt atttatgatt ttttgggttt tggttttttt atttttata 2520

tcggtttaat aatgattatc gtagagttgt tatggaagtt aaatgaattt atgtattata 2580

agtgattaat ataatgattg atatttagtg atttattaat aaattaaaat atttattatt 2640

aatatgatag agaaggtgtc gttaaaatag ataataggtt tttggaagag gtgattaaat 2700

ggatgtaaaa tttatggatt gtttatttcg tttatttttg ttgtgttttt tggttgtggt 2760

atatatacgt gtgggtataa aatcgtaaat tttatgtagt cgcgtagtgt atgcgtagaa 2820

ggtttagata cgaaatgtta ttttagtaat gtgtttagag aagttttgac gtcgttttgg 2880

aagtaagtcg ttgttgtttg atttttgggc gtttgggacg gatgtttata tttgtattta 2940

gtagtattgg aaggggttta ggtttttcgt agtatagttt atttttagat cgtttagttt 3000

tttataatat atatttttac ggaaaagggt attttttttc gttagaaaaa gcgttttagt 3060

ttggtttggg ttggttttta ttttacgttg ttgtaagtag gcgaagtttt ttttgttttt 3120

ttttttgggg taagtggaaa ggagttcggt aggggttcg tagtggtttg tataggggaa 3180

ttgggtagcg agagagtttt aggtaatttc gggggttgtt ttatagaagt aggtggggat 3240

cgatagtggt ttttcggttt agggaggaga gcgcggtcgc gggtttttt ttttagtttg 3300

gaggttgtag tcgttcgagt cggttcgggt gggggcgggg tgggggcggc gcggagggta 3360

cggagattac ggcggcgtta ttcgggatat ttagggtttc gaggttttgg gcggttttta 3420

cgcgagatcg taaattatga taataggtag ttattcgagg ttaaataaaa acggagtggg 3480

tttttcgcgc gtcgtcgttt ttcgcgtttt tggcggtttt tttcgaggtt ttcggcggtt 3540

-continued

ttacgagttc gtagtagtcg gtggcgacgt cgttttcgtt ttatttttttt gcgtaagtgc 3600

gaggttgtcg gtagcgcggc gtacgtttcg gtcgttttcg gttttcgcgt aaaattttta 3660

ttttgtttac gtgaagttgt cgttgtttta gagaggggga aagagttgcg ggaaaagtcg 3720

gggagtgacg attgcggcgg ttgggcgcgt ttttttattt ttttttttt ttttttttt 3780

tttgtcgtag ttcggagttt tggttttttt ttttttttt ttttttcgg agtcggtttt 3840

ttttttcgtt tcgttttttt ttcgtttgtg tacgttattt gttgtggggt ggtcgaaggg 3900

gatgttttgt ttttattaga ggtatagcgc gaaggggaaa tttcgatatt ggaaggaacg 3960

agaataaata tttaattacg gacgtattga atcgcggttg ggatagatat ttcgggaatt 4020

cgaggcggat cgggcgacga ggtgagtgat ttttttttt aattttcgtt ttagggtttt 4080

cgggggagtt tgagttgaga gaatttttaa atttttcggg aaagtgcgcg aggtttcgtc 4140

ggggacgtcg agcgttgggt attgaggacg cgtagttgga cggtgcgtgg gcgtttgcgt 4200

tttcgggggg cgtttggagg tcgggtgttt tacgtttgag ggttcgggtc gttcggatcg 4260

tagcggtgtt ttttgtttta gaagacgttt ttaagtttta agggtttttt tcgagtttgt 4320

ttgttttttt cggggtcggc gcggagtttg cgcgtaacgg agtttattta gtagtttagc 4380

gcgcggtttt tatttgtatt tcgtttttat ttggtagagg cgcgagtatc ggggtttttt 4440

ttatattttt tttatgacgt gtattatttt ttgatgattt tttagatggt ttaggcgcga 4500

ggatgttgat ttagagtttt tcggagggtt ataggcgttt gggtttttttc ggtgtcgggt 4560

gcgtgtgtat tttaaaggtt cgcgttttaa tttttaggta ttgatcgggt tttttaattg 4620

cggcgatttt attttaatag tttttatgtg gcgtggattg aatgtttttt gtagtttgtt 4680

agggtcggtg aaattagagg cgttttgtta gagtagtcgc gtttattggt tcgagtagcg 4740

ggtgttatgg aaggtttata atttttttaa aggaagggat ttggttgggt agagtaggtt 4800

ttttttttt tttaagtttg ttgggtttgg ggaggtagtg gaatttgaaa tggttcggat 4860

ttttagcgtg gtgaagcgag gtttggaagt agacgtgtgt gtgtttgttt tattttgcgt 4920

cgtatagtaa ttcgaatttt cgtttggtag tatttgaaag agtttttttt ttttgtttgc 4980

gagattttga atagttcgga gcgattaggg aatttgcgga tcgagttcgg tggtagagtt 5040

ggggcgaaag tagagagcgt aatttaattt ttgttatttt ttttttgttt gggaggatag 5100

tgttttttt tattattatt ttttttttt ttttttatga agataacgga tttgcgtttg 5160

gggtgagagt gtgtgcggga gagtggtgtg gagattgttt ttttttatcg cgttttttgc 5220

gttttttttc gttatttcga gcgggtttag agagttattt atgaatttta atttgagggt 5280

aggggaggaa ggtgtaggtt tttttgtttt ttttgttaag gtgtagaata gcgttcgggc 5340

gtgtgttttg gttttagagt agttttacgt ggagtaattt cgtgtgtgtg tgtgtgtgtg 5400

tgtgtgtgtg ttgtgtattt gatttgtgag gaggtaatag gattttggtg tttaaattta 5460

gtgggtcgtt ggttattagt ttgtttttttt ggttgttatt atagatattt ttaaaatttg 5520

atatttaaga gaattaatag gttaggtttt atattaaggg tttatattta atagtttttt 5580

ttttttttta ttttttttgg tttggtattt tgggattaac gtaattgttg gagcgaaata 5640

tatttttgg aatatggtat tttgtttttt tttttatttg tggttatttt gtgaattttc 5700

gggtgtttgt tagttttagt gcgggttttt gcgggattta ggtgggagtt ttaggagcgt 5760

ttaataatcg cgggtttttt attagtagtt tttgaagttt tatttatacg taggtgattg 5820

-continued

ataggattga aagttgacga tggtttttttt gtttgttgcg ttgtttttgt ttttaaaatt 5880

ttagggaagg gattgtattt gaatttttttt ttcgggtacg gtttggtttt aagtagaatt 5940

agtgtttttt ttttttttttt tgtttttttaa aatattattg gtaagtttaa atttgaagaa 6000

ttaaaaatta gagggggtgg ggagagaatt tttttaaaaa atatttgata cgtgatacgg 6060

agcgttttag gagattgtat ttaaagatat ttgtgtattt ttaaaaataa tattatttta 6120

agaaataaaa agtagtagta ataagagata gattgttttg tgtggagtaa gattgttaga 6180

atttgatttt tatggtaata atatcgaaag tagatataat tatatttata tttattgtta 6240

taaatcgtaa aaatagtttg ttttatttga ttaaaagttg taagttattt aaagttaaat 6300

tcgtatttag gaatgattgt ataagaatgt taaaattttt gatagttata gttttgaaag 6360

taatataatt agatttgttg aaaatgtttt tttttttga ttattagtga tatgtatttg 6420

ttttttataa taagtaaagg tttataatta atatggtttt taaaagtttg ttttttttttg 6480

taaatttttt gaaaatagta tgttttaata tttaaagatt attgtaatta tcggttggtt 6540

tataattata attaaaataa gatttattta gttgtttttt ttttattagt atttgttgaa 6600

tatttgaaat tgaatttttt atttttaaaa taaaacgttt tatatatatg tatgtattta 6660

taattaatat ttttttttaag tagtaggttt aagttttaaa atttttaaaa ttttaagatt 6720

tgtataaaaa ggagtgattg tataaaaagg aataataatt attaattgtt aagaaaaata 6780

gatgttgaat tttagagggt ttttagaagt tggagaaaaa aattgtatag aagtttttttt 6840

tttgacgggt gattggtaga tgagttattt gttattttgt tataatttat ttttttttttt 6900

taataaggtt ttttgggtta aaaaaaaaaa aggaaaaata agtattttat ttttaagagt 6960

ttttttttta tttatgttgt attttggggt gtaaataaat tttatatagt tacgggaaat 7020

agagtattta attttatgtt tttagttttt gttgtaagtt ttaaatgatt gatgtcgtgt 7080

ttaaaaatat atttatatgg tttgatattt tattttaata ttttatgtat tggaattaga 7140

ttttttagga tttttagaaa gatagaatga tagaaagacg atgattattg ttataaaaag 7200

ttagaatgtt aggaagttta tattttaatt tttaaataat taaatttatt ttgttttata 7260

aaaatttata gtttttatat agattttaag aagaggggtg ataatttttt ttattattta 7320

gttaagtgtt aagtaattta agaatgtaag tatgtattat gtttttttat tattattttt 7380

ttttgttagg taaatgtaag aatattggat attttgtaaa aataggtttt tttttataag 7440

gagtttattt attatagttt tgtattgtag ttgttgatta tattttatgg agttgttaaa 7500

gtatttaaaa tttaaaatta ggtattgttt ggttgtaaat attttagatt atttatttaa 7560

gaaataaata gaaaagtgtg ttattaaaag taggagacgt tttgaatttt tttattgaag 7620

agttgaataa atttttatta aaatatttttt ttttttttttt agtgagaata taattttgat 7680

agtttttttt tttaaatgga tattattttt atatgtatta aatgttaaat ttttataacg 7740

tgtttttttg tagagtattt taaattaatt atatttagaa atatagttgg gggattttat 7800

tataatggtt attaggtgaa ggaaattaat attaggggaa tggggtggta ttgtagtgta 7860

tttgtttttt ttatgaattt tttttgttaa attaagaaat gatatgttgt tttaggtatt 7920

tgttttggag atgggtgaga tgtaatatgt gtaatgttgt attttatagt tagatgtgtt 7980

ttaatgaagg ggatattgta tagttattaa attatttttg gagttaaatt tggcgttatt 8040

tagtttgaat ttaagtggaa gaaaatgaat aagagttata tatttaaaag aagtataagt 8100

-continued

aattttgatt gtttttaaga ggtttgaaga ttttgtaaat attattgtta tttaatattg 8160

tttgtggagt tgtatattaa tatatgtttt ggtgatatgt tattttacgt ttgaaatttg 8220

ttatttttag tgtttttta tttatttttt tattagtaaa gagatattga aaatgaatat 8280

tattattttt atttttttaa tttttttat atttttagaa aaagagatga aattgattaa 8340

tttaaaatag aaattatttt gtgttattaa aattatattt atatagtgat ttgagatagt 8400

tttagagagt gtatttttga gttttattgt aatttttttt gttattgaaa ggtgttaatt 8460

gattttaggg tattgatata atagtatagt ttgatatttg aaatttttta gagttggttt 8520

ggtttttttt ttattttgag attttagtgt atggatgatg aagaaagata ttattttaaa 8580

atattagaaa tttttatttt tttttaatat gaaatgtttt aatatagtat gtttatattt 8640

ttaaagtttt tatttatata tagtaagtaa atttatttta acgtattttt ggatagtagg 8700

agaaagattt atatgtttta tcgtgttaga atttttttagt ttttttttttt ttgtataggt 8760

agtttattta ggttataatt tttaggtaaa gtttgatttt tattattata tgaatatttt 8820

taaagtgaaa ttgcgttaaa ttaatgtgga atagtttttg tattattaag gtatatatta 8880

atgtagatgt taaatatgag agtatatttt tttgagtata tttatatttt aaagtgtatt 8940

tttaaataaa agtggttatt gtagttttta gataattata atttggttgt tattattatt 9000

ataatattaa ttattattat tattataata ttatagttag tatttattta ggaatttcgt 9060

gtaaaatatt gttttaagta tttatatgga ttagttaatt ttaatttttt taattatgtc 9120

gtaaattagg tatttttgtt atttttattt tatagataag gaagttgaga tttagttatg 9180

tagtatagtg gagttaggat tttaattttt tagtatgagg ttagtatttt tatttttaag 9240

cgtgtgttgg gtttttgttt ttattagtta tatattatta tttttaggtg gtatttgaat 9300

attttgtttt aataatattt atattttatg taaggataat tagatattag aaaaatttgt 9360

taaattttgt attaaatttg taattttatg ggtaatattg tttgagataa gattaaataa 9420

agtattgaag ttaaagaaaa aaaattaagt atttgaagaa acgtattaag taatagtgaa 9480

taagaatatg gtttaaataa ttatagttat gaaggttgga tagtaaatga ttgaatttgg 9540

agaaatgttg tattttgtaa tgtttttaa ttattaagaa tttatgatta gatttttaa 9600

atatttaatt aatatgtgga attttttttt gttttttaag ttttatttaa attggttaaa 9660

tgttattaat tttgtatttt ttttgttttt gttttttgtt gttttaatgt tggttttgaa 9720

ttttaaattt gtatatttat gttgaattaa ttaataaggt ttgaagagtt aaagagtgta 9780

tgatggattt ttggaggtag gtttaaatta taatggagtt gtttatattt tggaaatata 9840

tttaatttat ttggttatcg tatgtgtaag gttttttgta ggtaaatatt tttgttgtta 9900

ataatttgtt ttataatttt atagtatttt tttattgaag tagtggtttt tatttttta 9960

tttatttata tatttagtta cggatttttt tatttaaatg gaaattgatg atatgtttag 10020

tagagaaatg tgttattgtt ttaggtgaag taggatgtag gtagtttgag aatgatttat 10080

taatagtatt tttttacggt ggtcgtcgtt tttggagttt tttttaagtg tttttaatgt 10140

atggaaattg ttgtaaaaaa atttaagttt tttgataaaa ggggttaatt tagagtattt 10200

gttttaatat gtttatgtgg tattattaaa aaatagattg ttagatacgg taaaatattt 10260

tttagtgtga tatattagta gaaaggtgtg tttatttttt ttttttataat ttgagtattc 10320

gtattaatta gtttttaaat tcgatttagt attgagaaaa ttaaaattga ttaaaatgtt 10380

-continued

```
ttttgtgtgt agttataggt ttgaatgagg tataaaggat ttgtatttgt aaaggttgat  10440
tttattaatt agaatatttt ttttttttta aagattgtaa gaagaaatat tagtagtggt  10500
ttaatttgat atgattttag atttttacgt aaattattgt tattattttt gttatttttt  10560
ttttttttt ttttaaaatg aaagggatat tttttgtgaa agattataat taaattataa  10620
aaatttatat ttatgtgtta ttaagtttaa ttttatttat aaaagtaata gtatagagtt  10680
tgaaatttta tttttaatta agtaggtgta ttatatatcg ggagggttta ttatgtataa  10740
ggttatatat ataatttata gatttttgta tatatttaac ggagtgattt atttattata  10800
ttttattttt gattttgaat tttttaatgt taaaagattt gaaaagaatc gaatgttttg  10860
attaagagat tgaatatttt taatttaatg tttttagtat gttgaaagtg atgatgattt  10920
gggggaatta gtagattttt atattattta attttttttt tttatatttg aatgtaaatg  10980
tatatttatg tgcggttatg atttaagtta tttttgttaa atttaatgac gttgtaggtg  11040
aattatattt agattttttt ttgtaggttt tttagtaatt taaaataatg tttttagtag  11100
gtaatttaag tatgtaaatt ttaataaatt tgttaagaac ggtaatttta ttgttttatt  11160
ttgttttttt ttttgtttgt ttgtttttgt attaatttta gttgataaga tgatggtatt  11220
gttatttttt ttagttgatt tatgaagaat tttaatttag gtttagtttt tttttttaat  11280
tgttgatttt agtttttaaa ggtttcgttt atgaaaatgg ttagtaaagt tgtgggtatt  11340
tggtaaatgt ttgttaaatg tttttttttt attagtgttg ttgaagattt gtaaaattag  11400
agtgggatgg atagattttt tttttatttt gtatggtttt gaagattttg gagtttttat  11460
tgtattttta tatttttata tattattttt aggaatattt agagagagaa ttattgtaat  11520
taaggttata ggtttaattt ttaggtattt aaattagttt taggtggata attgttttat  11580
atatatttgt tcgtattaat gttgttataa taaatatagt tataatattt tgatgttttt  11640
tttgggggtt tattttggat tgtgttgaag ttgtttttaa tatttttta cgtttttaag  11700
atatttaatt atatttgttt atgattttta tgaggttttt tttggattta aaatttttta  11760
aaaaatttgt ttgttgttta tagggtaaat tttaaaattt tttgtaggga agataatttt  11820
ttatattgag aattttgttt aggttgtttt ttttattttt tatttttat ttataataat  11880
ttttttttt agttgtatgg aaaattgtag tttttaaata tattttggat atttttattt  11940
tatttttttg tgcgtggaat gttttttttt tggaaaatat cgttttttt tttttgtaaa  12000
tatttttttt attgtttttt taatatttaa ggtgtaaaat gttagttttt ttgataagtt  12060
tttttgattt ttagagtttg agttgaacgt ttttttttg ttatttttaa agtatttagt  12120
ttttatattt aatatagttt ttgttatatt gaaatgtaat agattatatt agtttaaatt  12180
ttgttttaag ttgagaattt attggaagga ttagttgtat ttgttatttt tgtatcgtta  12240
atgtatagtg tattgttgga tatatagtta gtaggtagtt gttatatttt tattgaatga  12300
atgagtagag gtattgggaa tgagagtaga gattgtgata ttgaatattt ttttttgag  12360
attgggaata gtgagaaggg tagtgattat tataaggtgg gattatttta ataataattt  12420
tagggagttt tggtataaat ttagtagaat tgtagttgtg aatttaggtt taggttatat  12480
gtatgtatac gtgaatgtag aaatgtgttt atttttttatt attgggaagt tagtgatttg  12540
gtggataagt taaggatttt gaaaatttttt ggagatacgg taattttata gtatttgaaa  12600
tttgagagat ttagttttta tagtatagtg agtaagtaga aagaatatgg gggttgggcg  12660
```

-continued

cggtggttta tatttgtaat tttagtattt tgggaggtcg aggcggatgg attatttgaa 12720

gttaggagtt cgagattagt ttgattaaaa tggtaaaatt tcgtttttat taaaaatata 12780

aaaattagtt gggcgtggtg gtgggcgttt gtaattttag ttattcggga ggttgaggta 12840

ggagaattat ttgaatttag aaggtagagg ttgtagtgag ttgagattgt attattgtat 12900

tttagtttgg ataatagagt gagattttat tttaaaaata aataaataaa taaaaaggaa 12960

gaagaatatg gagttaggta tttgggttta gattttagtt ttaatattga tgagtttgta 13020

atattaaata tattattgaa atagattttg ttttagtttt ttatgtgtaa tagaatatgt 13080

tttatggggt tttgatgaga atttaatgag taaatatatg taaatatatt tagagtagtg 13140

tttgatatga gtattagttg ttattattat gattgggtat tttttattag attattttta 13200

aatgttttag agatatttag agttgaaggt tttttttggg gggttatgga ttggtgatta 13260

taaatggagt gtttagtagt ttaagtataa attagatatt gtgggttatt ttggtaaatt 13320

tgaggttgta tttggagttt taagagaaga tattataggt gtttaatagt tggtattggt 13380

tatttacgtt gtgtagtaat attttttttа gtaaatattt tcgtatatag taatttagat 13440

tgttttagag ttttaatttg tgtggttttt taattatttt aagtaattat aagtacgttg 13500

tataatggta gtattttata gttaatattt aagtttttтt agttgttttt tttttagtcg 13560

taaggtatta attatgagaa tttgtataat ttttaatagt tagtgggttt gatgaattta 13620

atgtaaattt tttaaaaggt agttttatat attgttatga tttttatttt tagagagata 13680

gtaggaggta attagtattt ttatttatag aatttattat ttggatttag atataatggt 13740

tatgtggtag gatggggttg aaggaaaaag gaaagttaaa atattaattg ttaaggtttt 13800

atttttatag tttgtttgga attgttatta ggtgtatttg tatagtttta aaaatgataa 13860

cgttgtattt gaatttttta gtatatttaa ataagagttt tttaagtttt ggttattgat 13920

attttgggtt ggataattta tttaggggat attgttttgt gtttggtagg atgtttagtg 13980

gtatcgttgg atgttaggga ttttttttтt tttggtaatg atggtgtttt tagatattgt 14040

taggtgattt ttgggagtaa aattgttttt ttttgagaat ttataattta agtagatttt 14100

aatattattt gatatagtta aaaaattttt agggttttgt ataagaggat tgttttattt 14160

ttagttaagt gtggagaatt ttaatatttt ttttttaagt ttaaatatga agttaaataa 14220

tttttattag tttgtgttga tagattaatt ttgtatagag gggatttata gatacgtttt 14280

atatagattg attatttttа gtatattttt ttttttgga aataatatat gagtgggagt 14340

aatagaatat ttgagagaga gtatgtagga agtaaatatt tatttgaatt atttatgtgt 14400

ttttttttтc ggttatttgt tagtagatag tttggattta tttttaaata atgattattg 14460

tttttttatt tttgtgggta aatatttagt aataagaaat ttttatttta tttagtttgt 14520

agtgttagaa aaggtaaagt tattgataat tataattgtt gaagattaaa atatttagtg 14580

agttaaatta tttgtttgta atgagaaatt tttaaaagaa ttatgtgtag tttttagttg 14640

taattataag tttaagattt tgagttaata atatttgtat agttagaaga aagtaaaaat 14700

aaatatatta tatgtaaaaa ttttttttтg tgaattttta gatttaaatt tttaagtttt 14760

tattttatgg gtttgatgtt gattaatata ttttatttat gaaatgatat ataattgatg 14820

taagtaaatt tgattgttgt ttgggtatta tttttatata atttaagtat atatgaaata 14880

tattttaaaa attatttgaa taaaatggga ataataatgt ttatataaag tgagagagga 14940

-continued

taatttttttt tatatgagtt ttgggttgaa ttttaaaatt attttgaaat atgaaatatt 15000

aatattataa atatatgatt agattaattt ttttaagagt tttaaatagt agataatttt 15060

tattttatta gaaattgtaa agatatttga aattttatat ttatgtattg tttaatttag 15120

tggttgtatt ttaattgtat taggtagata attatggaag tagttagatt aatttggaga 15180

ttttatatta tttattaatt gatgtttaag taggtagggt agaggtattt gaaaatatat 15240

atatatatat atatatatat atatatatat atatatatat atttatttttt tttttttat 15300

ttttttgat ttgtgtttta attttgaaat gtgtgtttag gtggtaaagg gttgtaaata 15360

tagtatattg tgtttgatta aaatattttt ttgtttttta ggtggagaag gattttgtat 15420

agttttttgt tagtagagag ataaagtggg tgttgggtaa tttaatgata ggtggaggtc 15480

gttttcgggg agtgttgcgg agaatttgag ataggggttt aaattataga tagtggagga 15540

gagttttgtt ttatttatgg ataggaagtt taaagaaggt atagcgaatt ataggtttat 15600

ttaggtattt tatgttagat attgaacggg aagtttagta gttttttagt tattggagaa 15660

cggttaaaaa tttagaggtt ttatttttttt aaatgagtat tttttgggtt tataaattaa 15720

ggaattttat agagtattta agttatatat ttttttttttt aggttgattt tttgtatagt 15780

gggttttaat gtggtaagat ttatttatga atattttatt ttttataatg tcgttagtta 15840

ttttttttttt ttttttttggt atatttttat tatttttatt ttttatggtt ttaatttttt 15900

taatttattt gtttagtgtt tttttgttgt ttatttggat aaaataataa taataataat 15960

aatttattta ttttttaagg tttagtttaa atgttatttt ttttgtgaag tttttttttaa 16020

ggtttaggta gaattagttt tttttttttgt gtaatattat agtatttttt ttggattgtt 16080

aatataatag tagattgtgt tggggtttat tgtatgtgtt tgtttttttag agagattatg 16140

aatttataga ggggagagat tatatttttt ttttttcgta attttaatat ttggagatgt 16200

taatggaaat taagtgattg tattatttta tgtatatttt taaagggtat taggttttaa 16260

attagatatt tgtaaaggat gaattttgtt aatttttttgg aaaattggtt tttttttgtt 16320

tgtggttatg aaagttggtt tattggttat tttttatttt aatttattta aagttttatt 16380

tgtattaaag tattattgat tttttagtgg aaaaataggt atgttcggcg tatattggta 16440

tttttaataa attgatattt aaaagataga tttttcgttg aggtttatgg tttttataat 16500

tttagttttt tttagaaata gattttgatt tttttggttg ttttttgtta agatcgattt 16560

tatgttattt tgaagaattt ttaattttta gaattataga ttttatttga gagaaagtat 16620

gttttgtatt tgtttgtgtt agtgttttaa aatgtgagga ttaaaaattt tagatgtatt 16680

tattaaaaat atacgtggtt ttttgttgag ggaaattata ttttttttttt taggtataat 16740

tgaggaagta aaaaaatttt gtttagtttt tagtggtttg tttttaggta ttttttatat 16800

aaagatgagt aagatttttt aaaaattatt aatttgttta tttaggggggg aaagtttttt 16860

tatgtttaga aataatatta aattaagatt attgttttta atagttttaa aaattgtggt 16920

tttatttttt tgtatgagtt atttttagt atagtgtagt atatgattgt attatattat 16980

gataaagtat atttgttttg tgttttagtg ggttatttta tttattagcg ttagatgatt 17040

agattagaag agagatttag tttaattttt ttattttgta gatgagatgt tgtattatgt 17100

gattttaaga ttttaagtta tttatttaga ataaaataga aaaagagaat tttatgttgg 17160

ggatatttta tgtttttaat tttatatttt attattttat ttttatttgt ttgtttttga 17220

-continued

```
tgaggagttg agaatgttgg aattagatta tttttgtgtg tatttaggtt ttgttattta  17280
ttagcgatgt gattttggtt atgtaatttt ttgagtggtt gtaagttata gttttagtat  17340
gtgtatatgg ggaataataa tagtattcgt tttatggagt tgtaaggaat aattgaattt  17400
atgtaggaaa gatatttggt ttagtgtttg gtttattata aaagtttagg ttatagtttg  17460
aattgatatt ttggaataaa atgttgagtg tatttgatat ttagagaagg aaatttatgt  17520
tgttaaagta agtttttttt tttaatttgt tatttaaaaa atataaataa gttatatttt  17580
gtataatata ttaaaataga gatgatagag gttatttttg agaggaatga ttagagggag  17640
taagagagag gttttggaat gttgattatg ttttttattt tggtttggtt gttggttatg  17700
tgaaagtgtt tatttaataa aaatttatta gtgattaatt tttgatttat gtattttatt  17760
ttattgtgta tatgttatat ttttttaaaa agttttaaaa atacggatat atttaaagta  17820
aaaagtggat ttatttattt ttatttttttg taaattagga taagttgtta tatttagatg  17880
tgtatttttt taggtatttt tttttttata tatgtgtaaa tgtaataatt atgtgaggat  17940
gtaatttatt tttatttttat atttttaatt ttaataggat tattgatttt tttaaaattt  18000
gtttttgatt tgttgaattt tgaatatttt tttatattag tatatatagt tttgggtgtt  18060
tttatttaat gtatgtagat attttataat tttattgttt tttttttggt gtatgtttat  18120
attgttttta tatttttatt attattaagt atgttgtatt gatgattttt atgttttttc  18180
gtatatttgt taaaaatatt tgtagataaa attttagatg taatattaat agataaatat  18240
gtatgtgtat tttttataga aaatattaaa atgtttttta aaaagattta tgaattttgt  18300
atttttaagg taatattttt atttttttta taaaaattag gtgtaaaaat tttaatttaa  18360
ttgaattttg taattatttg tgttttggtt aagaaaaatg gttatagtag taaatttttt  18420
gaaatttata tagtaatcgg aatattttat tatgattagg ataaataatt ttattaacga  18480
tatatattta atatatatat ataatttata taattagttt tttatttaag aatatttagt  18540
atttatttttt atgaatgtat attatattta tttatggttc gtaagtttaa atgttaggaa  18600
gaaataaatt agtaatatta tatttattat atttttttttt atttagtatt attaaatatt  18660
tattagaatt attaggagaa atggttttaa tttttatgtg attggtgaga atttatgagg  18720
aatgatatat tgttatttta gtaagagttt attttgtgtt aggtatagtt ttaagtattt  18780
tatatatatt acgatagttt tttaatatag agatgaaagg tatagatatt gtataagtaa  18840
taaatgatat tattttatta taaatgtgtt gataaatatt attatatatg ttataatatt  18900
agttgtgtta tattaatgat taatttataa atttgttatt tattataatt tgtttaatgt  18960
ggaaataaaa gttaatgtat taaaatttag aaaattgtta tttaaatgtt ggttaaagta  19020
atttgataga tatagtttta aaatgaagta tattaattag ttgattttgt tgttcgattt  19080
ttatatgtag aaaattttgt ttttagaata tttagttttt gtagtttttg gttaagattt  19140
tgattaattt ttaatggtta tttagtgttt ttaaaaagta gtttaattta gaatagttaa  19200
tgtatttttt ttgtattgaa taaatatggt tattaatttt tgtggggttt tttttttgt  19260
agaatttttt gttattttaa gggatttaat aggatttata tatataaaat tgaaaattat  19320
attattatgg ggaagttgtt ttgtgttttt tagaaggaat ttttttgtta atttaagtat  19380
tgtatgtgtg tagaatagtt aatatttttt ttttaagaaa ttttatgtat gtagttaaaa  19440
tatttttaaa ttgattaagg atttgttata tatttttaga agtatttaag agtatttta  19500
```

-continued

tggttataag agtagagtgt attagaatgt tagaaattaa tgaatacgtt agaggttttt 19560

aaaattgtgt ttaatatttt tgattttatt atataaatat gtggggtagt ttggaaaaat 19620

aaaataattt ttttttttt aacgtaggtg aggtttgtga tgataatatt ttaattggtt 19680

tgaatattaa tttgatattt ttttgttaga agtaattttt attatttagt aaatgaaatg 19740

gagaggtaat aaatatgatt gatatttatt ttttgagtaa agtattattt tatattagat 19800

tcgattagtt atttttattt tttttggaaa atgtattata taaattatta tagtattttg 19860

tttattaatt agggaagtta aaaaacgttt tataaacgtt gaattaaaat ttttattgtt 19920

gtgagggaat taaattttaa gtaatatatt ttttttatag ggtgagaaat tgaagtatgg 19980

agaaattata aggtttttt aaagttatat attgagttat ggtagatata gaaatttata 20040

attttgtttg tttgatttaa tttgaaattt tatgaaacgt aatttattta ttttttgata 20100

tgtgttttag aagtatattt ttttatttgt ttatttatgt ttttatttat ttatttatgt 20160

atgtatttat gtattattga ttatttatgt atttatttat ttatttatgt attttaaaag 20220

aatcggtgtt tttgtatgat atagttttga tttagggatt atgaatattt tgaatttata 20280

tattttagat attttttaa agaaatttat tgtatttttt ttacgattat gaaaaagaaa 20340

ttaagatatt aagaaaatta agttggattg tttaagtgtg ggttatagta gtgtttatgg 20400

gtagtatttg aagttaattt aggagtgaat ttagtttgtt taagtgtttg aataagaatt 20460

tggttgaagt tttggaggag atgtttgggg tatagagtgg ggacgaggaa aaattatagt 20520

atttttagag agtattgtaa ggattagtat ttatattttt attatttgat gtttttatat 20580

ttggtttagt taagagagag ggggtaaatt attttgtttt ttattttgtt attttttga 20640

ttttagtttt tacgttttaa attttttttt attgcgattt ttttttatt agtttaatta 20700

gtatttttc gcgttttttt tattaaattc gttttttttt attagtttgc ggagttatat 20760

tttttgatcg aattgttggg gttataagta atttgaaatg aggaaagtgt ttagtaattt 20820

tttttagttt agtattttgt aatagagatt ttagagtacg tagtgatgat atttgttttt 20880

ttttttttt atagagagtt tagtgggtaa ttttttggt ttgttttttt gttttttttt 20940

tgtagtttta agatttagga tttagatttg gtttttgttt gtatggtttt ttatttttt 21000

ttttggaaga gttttttttc gggggttttt taggtttaag agtatttgga ttttgggag 21060

atgaatttgt agattttata aagaatattt aagtattagg taaattatat ttttttgtag 21120

tttaaacgtt agtttttttt ttttttttaa ttttattgaa gaataattta gtttttaata 21180

aataagtaaa tagaatttta gggttttttt taaaggtgta tttttgttgt tttgaagatt 21240

attattatta atttttattt tagttaattg gttttttttt atatttttat ttgggtataa 21300

atgtgatatt ttggtatata ttaatgttga tttatattaa tacgttaatt tataattgag 21360

tgatttaaaa ataatgatgt attatgattt agtttatatt tgtttttttat ttaatgtatt 21420

ttttaagtaa ttatgttttt gtggttgatt gaatttttttt ataatttttt gttgggaaat 21480

aaagattttt ttttaaattg aagaagtggt tttgttaatt aggggtatcg tatttaattt 21540

gagagaaaaa gattaggaat gataggaatg atagtatatt tttaatattt aatagtttgt 21600

tatatatgtg aaagtaggat atatagtagg gaagtataac gttgagttga tttaaaatat 21660

taaatgtatt tgatatatta ttttttgaat ttttataatt ttagtttttt taaaggtttt 21720

tggattttta gaagttataa gggtagtttt tggtaaaggt ggttttattt ttaatttagt 21780

-continued

tatagagtta gtggaatgat ttttatattt aaatgttata ggggaattta ttgagaggaa 21840

aaagggtatt taaatttttt tggatgtttt aattaaaaat ttggattaat aaaatttttt 21900

taagtgtttt aaatttaagg aaagtaaata gtattttatt taattatatt tttaattagt 21960

atttattata gatataattt aaattagtaa tttggtatta gatgaattag atttattgtt 22020

atattatata atttttatgt ttattttgtt gtgtttggga tttttttttt tttataataa 22080

agaatatgat ttataggtgt tatattttga tttttgagaa attaatgtta tagaaaagtt 22140

gtttggaaag atataattgt gattttgtta ttgtttttgt tgttattatt tttttttatg 22200

gggatgggta tatatttttt gagatttatt agatttattt tgtagtttat aaattatttt 22260

tttagatgta tttaatttgt tgtttagttt gttttttgta aaaaaattta gtgaatatat 22320

ttttaaaaat atttttatgg ttttttttta ggtttgtgtg tatatttttt tcgtgtgtgg 22380

ttttttaatt ttatttttgt ttttttgtg tttgttttaa gtatatttat ttagtttttt 22440

ttatattgtt ttgtagtttt atatttttag atttttaatt tgttgttgtg gttatttttt 22500

attgtgtttt gttttttat gtagtttata atttttttt ttttttgag atggagttgt 22560

gttttgttgt ttaggttgga gtgtagtggt acgattttgg tttattgtaa tttttgtttt 22620

ttgggtttag gcgatttttt tgttttagtt tttcgaatag ttgggattat aggtgtttat 22680

tattatgttt agttaatttt tgtattttta gtagaaaagg ggtttcgtta tgttagttag 22740

gttggtttta aatttttgat tttaagtgat ttgtttgttt gagtttttta atgtgttggg 22800

attataggta tgagttatcg tatttcgttt gtagtttata atttttggtg gatttttttt 22860

ttagaatttt ttgtattttg tggagtaatt ttgtattttt ttggagttta gggattttaa 22920

atttttaggt tagtttttat tttattttat tttttgttt tgaggttttt atattataga 22980

tgtagtaaat atttagaatt tatattttgt aaggttgaag ttttgattta ttttatttaa 23040

gaaattattt ttttttattt ttttttaaa ggttttatta taggataggt tttttgttg 23100

aatttgatgg tgggtcgagt ttttttattt tttttttaat aaatgtgata aatttttag 23160

atttttttt ttgtggattg ttttttgttt ttgttttttg tttatcgtta taaaagaaga 23220

taatgataat aaaacgataa taatataaat gtaaacgtat ttttttttt taatttttt 23280

atattattaa ttttttatga attaaggtgt gatatttgaa agttatattt gttttattag 23340

ttatatttat atagtttttt tgttttattg atgtattttt tatattattt tttatattta 23400

ttttttttagg ttttttttta ttttatgtta gtttagattt ttttggtttt ttatttggag 23460

cgttatagta gtttttaagt tgttatttaa ttttatttt tgtttatttt gtttgttaga 23520

aatttgaagt agatgtgttg ttgtaatgat attatatgtg ttatttttg ttttgggggt 23580

tttaaggttt ttaaggttat taaaatattg taataattaa ttgttttaat tgtattttgt 23640

atttattttt ttgtttttgt taatttaaaa tgttttaata ttttgttaat attgaatgtt 23700

gatgtttttt tatttttttt ttttttatat gttcgtttat aattgtttat tgattatatt 23760

agttttttaa tattaaaggt attagttata attttgatgt tatatagaat gataattaaa 23820

aggtgataaa tgttaatgat ttaatcgagg agttgtttat ttttataagt ttatttgagt 23880

ttagagaatt ggtaaaagat tggtgatttt tggttattat ttaaatataa ttggttaatg 23940

tagtttttgg agattagtgg ttagtataat atttttagtt gagtagggtt aaaaataatt 24000

atatttgttt aagttgtaga tattatttta ttgtatagta aagaatgtta aaatgtttta 24060

-continued

atttagtata tgaagagtaa aatataaaaa taggtttttt tttatttta ttagtaagta 24120

aagtggtttg tatttttgaa atatattttt tgtttaatta taataaaata taatataaat 24180

gttattaaaa gttttatttt atggatgaag atagtgatat ttagagatat taattagttt 24240

ttttaagatt atatatttat ttagtagtaa aagtaagttt ttataagttg atttatttta 24300

ttttagtttt tatttggaat tattaaattt atatttttt tagtaaaatt atcgtttata 24360

tttttagggt tttaagagtt taatgttaaa tttgggagat ttatttttaa atttttttaa 24420

agtgtaatat atttttttta ggatgattat aaagttatta gatgtgtgtt aagtaatata 24480

tatagttata tatttttgat taaaaaagat ttttaggtta tatagaagag agatttatgg 24540

ttgagaaaaa ggtagattgt ttttaagaaa ggttttaaat atatttttt ttgataggtt 24600

ataggagagt tagttagatg aattattata gtgtgtataa aatgtttagt agaataattt 24660

tttagtattg gtagggaaga gtaataggag taatataaga gaaggaaagt tggtttgtag 24720

agtttgtttt agtattcgta ggggtgatga ggttgtttgg gttaagaatt tttttatatg 24780

ttttttttggg ataatttatt tagaattatt tttgtgtttt ttaggttatt taggtaatta 24840

ttattaatta gtaaatatag ataaaataga atattttgtt tggatttgtt tgttaaaaat 24900

taaatgatta taatttataa ttttaggaag atttgttatt aaatgaattt atgataaatg 24960

atttaaatga tttttataaa gtagagtttg ttgtaatttt tagtatgaga agtatgaatt 25020

ttattattat tttttaaata ttttgagtta aatggatcgt tttgtaaagt ttatgtttta 25080

tttttataga tagtttaaaa atagtttagt atttagtttg taggttggtt ttatttaagg 25140

agtttgaagt taggtaaggt gtttattatt atttatttag atttgttttt tggatatttt 25200

agttgttttt ttttttttacg gttagggagg agagttatgt gtatagttta agaatgtgta 25260

tttttatgtt tttgaatagt tggttataga attggagtta ggtgtttttt gtgttattgg 25320

ttttttttttt tggtatgttt ttcgggtggt tggagttagt tagtgtttga tttagtttta 25380

tagatttagt aatgaaattt ggagggtaga gtaggtaatt aggtatataa gaattgtaaa 25440

gtaatgttta tttggatagt tgattataat tataattttt taataatgta ataggaatat 25500

aaattgttag atttttttata ttattgtaat ataaagaaga taattgaagg tcgtgaagat 25560

agtttatgaa atgtaaggaa gaattggata attaagtaat aggaagagta gagaaatagt 25620

gagattttgg gagttataag atttgggtgt ttaaattttg ttaggatttt tttgttattt 25680

ttatttttgt ttttttttttt cggttagttt ttttttgttt ttttttatag attagttttt 25740

ttttatatgg atatgaaacg tttttaagtt ttatatttta ggggttttgt tattgaagga 25800

ggattagttt gatatttttt tagatttata ttttaaggaa gatgaattat tagatttaag 25860

taatttcgtg taattggaga agatttgtag attttattta atgggttaag ttggatatgt 25920

atttgtggga gggagatatg ttttgtgggt ataatatata tgtaagtaaa ttttaatatt 25980

gtataagata cgtttttgaa aatttatttg tagtttgaaa atttggaagt taaatagtag 26040

attaattgtt gtgagtgtta gaatttagtg taatggtatt ttttgtgatt taggtaattt 26100

tgtataggta gaaagatttt tgaaagtttt atttaggttg gaaagggttt gtgttagagg 26160

ttatgagtgg gatttttttag gtatttgttt ttttatttta aggtttgtat atggattgga 26220

ataaaagttt tggtagttgt aatttttaaa ttttttttta agggtattta gggatttaaa 26280

gtagtaggga atgtattttt ttatttttttt ttgtttttttt ttttattgta gtaagaggta 26340

-continued

agtatagttt ttagttttgt ttgtattttt ataggagatt tatgtatagg tttcgggttt 26400

tggttgtagg atagtgtgag gttgttgata gggcgagaga atggcgagaa taatggaata 26460

ggattgaggg agaaggaaga ggaatcgttt ttagaattat tatttgggac ggtggtttta 26520

gggagtaaaa aatatttttg gagtttttttt aaggggtttt ttggtagagc ggtattatcg 26580

ttagatattg ttatggttgt aggtaggatt gattaaagaa tttttgattt tttttgagga 26640

tagggtaaag ttttgaattg tttttagaga ttgtatttag atttatattt cggaattatt 26700

attttggttg tattgtgaac ggtaacgttt agggagtagg attggaggga aggagattat 26760

tacgtggttg ttgtttttgt ttaagtaggg aataatgatg gcggaacgtt tgtattggta 26820

gagaggagag agaggaggga tttggcgaat ttttggaggt attggtagga tttggtaata 26880

tattgaatgt ggagagtaaa tgggtaggga gttagtgata aagtttaggt gtttggtttt 26940

gatgattggt tatgtttgtg tcggttgtgt atagaattta gaggtttagc gtttttatgt 27000

gtagcgtcgg tttttatttc gcggtagttt ggtgtggagt tgagatgtgt tttttttatt 27060

ttatgtttac gttttttag ttagagtaaa gtatgtttgg ataatttagg ttttttttt 27120

ttttttttt ttttttttag agagagaggg ttttcgtttt gttatttaga agaagtgtag 27180

tgtcgtgatt atagtttatt gtagttttga atttttggtt ttaagtgatt agaggattag 27240

gtgtattttt ttataaattt tttaaatttt ttttagtatt ttatttttta agggatttta 27300

tagttttttt tatttggacg gcgttatggt gttttatttt atttttgaga attgttttaa 27360

aattttatat agaaatttgt tattatgtat ttaaatgaga agaaatgttg aatttgtaat 27420

tttaattttt taagataaga gtttgtagtt cgtttttagt agagttaaag aaaaagatat 27480

gatgaatggt ttatatattg aaaattgtag aagaaataat ttaatatgga atgggtgagg 27540

ttgttatagg tggtgtaaaa ataattgcgg tttttgttgt tatttttagt ggtaaaaatc 27600

gtaattgttt ttgtataaat ttaataatat tgtttgaaat ttatattttt tggtggaata 27660

gttttgagat agagcgggaa ttagattatt ggtttataaa atagtatgta gttattgtta 27720

ttttttaatt ttatattttt attatgaggt aatgtttttt ttatttgatt tggagaatgt 27780

ttttattttt tttttatata tttgtgaaaa ttaaatgtta aaggaattaa tataataatt 27840

attgttaaat tatgatgtaa aataatattg tgtttgtaat tagtttaggt agttttattt 27900

ggtttaataa tataaaatta ttaggtttat aatattttag tattttagtg tgattggtta 27960

agaaaaaaat ttacgttatt ataaaaagtt aaattaagta ataattaatt taaattttaa 28020

ggaaatataa agtaaagtat tatgttgtaa tatggaatat taatgtaaat aatttttaa 28080

tagtttatga aatagatttt gaattggtag ttgataaaga aaggttagtt ttggtttaat 28140

ttttttatgt tttaatattt tttttgaagt tttatttaat tttatttgcg ttagtaatag 28200

tattttgaga tggtggtgta tttggaaaat tattattttt tttttgataa ttagtatttt 28260

taatttaatt tttatatatg ttttttggaa atattatatt tatattttga tgacgtttta 28320

aaaataattt taatgtaata attatatatg tgtggtgagt aaattgtgtt tcgtaaaaga 28380

tatttttaaa ttttaatgtt tgtatttggg aatgtgattt tatttggaaa tagggttttt 28440

gtaggtatga ttaagttaaa atgagatttt attggattag gatgggtttt aatataatga 28500

ttgttgtatt taagaggaaa atttgtatag agatatatat atatataaaa gaatgttatg 28560

taacgttagg tacggagaat attatgtgat aatgaaggta gagattggag tgatgtattt 28620

-continued

atgagttaag gatggttagt aatttttaga agttggggag agataaagga tgtttttttа 28680

ggatttttag agtaagtata aatttgttag tattttgatt taggattttt ttttttaga 28740

attgtgagaa tagattttag ttttaagtta tttagtttgt ggtattttgt tataggagtt 28800

ttaggaaatt aatgtaatat gttatgttgt aaataaatag tagttttaat tggtataaat 28860

aataatttta agtatatttt attattaggt taggtgagta ggaaattgtt tggtagatta 28920

attattaatg ttgtattttt tgaaaagtta aaatattttt atttagtaaa taatatttgt 28980

ttaaaagttt ttatttgtat tgttttaaaa taaagtaaaa attaaaatgg agttagttat 29040

gtatttgaaa aatgtgaaaa aggaaagaaa aatttttag gaaagatttt ttttaaaatg 29100

gttatatatt tgagaaagaa ttttaaaatt taaattattt ggttgtttat tttatttttt 29160

tgtttatatt attagatata ttatgtatga ttttatatat tatgtatgtt atatttaatt 29220

aatatttatt aaatatttaa aatttgttag gttttgagtt aggtattgta gataggtaaa 29280

taagataaat attttttttt tttaaagaga ttattttggt atgaaattta atttgtagaa 29340

gaagtgaaag aggttatttg tagagtttat tatgaaaatt tatttttatt atttgttgta 29400

gttttatttg attttgtgaa attattttag tattatttgg ttttattttg tgtttttgta 29460

ttatattgga gaaaataaaa tttatttatt gtatagataa tataaaatta attaaagtat 29520

gtgaaattaa aaatgtaatt gtttattaaa tttttttag tgtttttat ttattagtat 29580

ttaaatatat ttttagttgt gtatttatgt tagtataatt aaaatgtggt gtttttattt 29640

ttttatttaa tttataatta ataatgttta tttttttatt ttttttttt gagatagagt 29700

tttattttgt tatatttagg ttggagtgta gtggtatgat tttggtatat tgtaattttc 29760

gttttttggg tttaagcgat ttttttgttt tagttttttg agtaattggg attataggcg 29820

ttcgttatta tgttcggtta atttttgta ttttagtaga gatagggttt tattatgttg 29880

gttaggttgg tttcgaattt ttgattttag gtgattcgtt tatttcggtt ttttaaagtg 29940

ttgggattat aggtatgaat tattacgttt ggtttttat tatttttata att 29993

<210> SEQ ID NO 3
<211> LENGTH: 29993
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: chemically treated genomic DNA (Homo sapiens)

<400> SEQUENCE: 3

aattataaaa atgatgaggg gttaggcgtg gtggtttatg tttgtaattt tagtattttg 60

ggaggtcgag gtgggcggat tatttgaggt taggagttcg agattagttt gattaatatg 120

gtgaaatttt gtttttatta aaatataaaa aattagtcgg gtatggtggc gggcgtttgt 180

aattttaatt atttaggagg ttgaggtagg agaatcgttt gaatttagga ggcggaggtt 240

gtagtgtgtt aagattatgt tattgtattt tagtttgggt gtgatagagt gagattttgt 300

tttaaaaaaa aaaagtggaa aagtgaatat tgttgattgt gaattagatg aaaaaataag 360

aatattatat tttagttatg ttaatatgga tgtataatta agaatgtatt tggatattga 420

tgaatgggaa atattaaaaa aaatttgata aataattata tttttggttt tatatatttt 480

aattaatttt atgttgtttg tgtaataagt aaatttttatt ttttttaata taatatagaa 540

gtataaagta aaattaaatg atgttaaaat ggttttatag gattaggtaa aattatagta 600

-continued

gatagtgaaa atgaattttt atggtagatt ttgtaaatgg ttttttttat ttttttata 660

gattaaattt tatgttaaaa taatttttt aggggaaggg aatgtttgtt ttatttattt 720

atttataata tttagtttaa ggtttagtaa attttaggta tttaataaat attgattgaa 780

tgtaatatat ataatgtatg aaattatata taatgtattt gataatgtaa ataaaaaaat 840

ggaataaata gttagatgat ttaagtttta aaatttttt ttaaatgtat agttatttta 900

ggaaaaattt tttttaaaag attttttttt ttttttttat atttttagg tatataattg 960

attttatttt aatttttgtt ttgttttagg ataatataga tagaaatttt tagataagta 1020

ttatttattg aatgggaata ttttaatttt ttagaaaata taatattgat aattagttta 1080

ttaggtaatt ttttatttat ttgatttaat aataaaatat atttaaaatt attatttata 1140

ttaattagaa ttattgttta tttataatat agtatgttgt attagttttt taggattttt 1200

ataataaagt attataaatt gggtggttta gaattgaaat ttatttttat agttttggag 1260

gaaagaagtt ttaaattaag gtgttggtag atttatgttt gttttggaga ttttagggaa 1320

gtatttttg ttttttttta gtttttgggg gttgttggtt atttttggtt tatagatgta 1380

ttattttaat ttttgttttt attgttatat gatgtttttc gtgtttaacg ttatatggta 1440

ttttttgtg tgtgtgtatg tttttgtata aatttttttt ttaagtataa tagttattgt 1500

attaggattt attttaattt agtaaaattt tattttaatt tgattatatt tgtaaagatt 1560

ttgtttttaa atgaggttat atttttaaat atagatatta ggatttaaaa gtatttttta 1620

cggaatataa tttatttatt atatatgtat aattattata ttgaaattat ttttaagacg 1680

ttattaaagt atgaatgtaa tatttttaga aagtatatgt aagaattaaa ttaaagatat 1740

tgattgttag gaaagaagtg ataatttttt agatgtatta ttattttaga gtattgttgt 1800

taacgtaagt gaaattaaat aagattttag gaaagatatt ggagtataag agaattaagt 1860

taaaattagt tttttttgt taattgttag tttagaattt attttatgaa ttgttgggga 1920

gttatttata ttaatgtttt atgttataat ataatatttt gttttatatt tttttgaaat 1980

ttagattaat tgttatttgg tttgattttt tatggtaacg tgaatttttt ttttggttaa 2040

ttatattaag gtattaaaat attatgagtt tagtgatttt atattattga attaagtaaa 2100

attatttagg ttaattgtag atatagtatt attttatatt ataatttagt aatggttgtt 2160

atgttaattt ttttaatatt tagtttttat aaatatatga gaaaaagatg agaatatttt 2220

ttaaattaag taagagaagt attattttat aataaagata taagattaag gaataatagt 2280

aattgtatgt tgttttatga gttaataatt tagttttcgt tttattttaa gattgtttta 2340

ttagaaaata tgaattttaa atagtattat taggtttgtg taaaaataat tgcggttttt 2400

gttattgaaa gtaatagtaa aaatcgtaat tatttttgta ttatttgtag taattttatt 2460

tattttatat tagattattt tttttgtagt ttttaatata tagattattt attatatttt 2520

ttttttttaat tttgttagaa gcgagttata aatttttgtt ttagaaaatt agaattataa 2580

atttaatatt ttttttttatt taaatatata atagtaagtt tttatataaa gttttaagat 2640

aatttttaga aatggaatga ggtattatgg cgtcgtttag gtgggagaga ttgtaaggtt 2700

ttttaaggaa tgagatgttg aaggaggttt gagaagtttg tagagaagta tatttaattt 2760

tttgattatt tgaggttagg agtttaaggt tgtagtgagt tatgattacg atattgtatt 2820

ttttttaggt gatagagcgg agatttttttt tttttagaga aaagaaagaa agaaagaaag 2880

-continued

aaatttaggt tgtttaggta tgttttgttt tggttaaaga aacgtgaata tggagtggag 2940

ggggtatatt ttaattttat attaggttgt cgcggaatga ggatcgacgt tgtatatgga 3000

aacgttaaat ttttggattt tgtatataat cggtataggt atagttagtt attaaagtta 3060

aatatttagg ttttgttatt gatttttgt ttatttgttt tttatattta atatattatt 3120

aagttttatt aatattttta aaagttcgtt aaatttttt ttttttttt ttttgttagt 3180

ataggcgttt cgttattatt gttttttgtt tgggtaggag taatagttac gtaatggttt 3240

ttttttttt agttttgttt tttggacgtt atcgtttata gtgtaattag agtgatggtt 3300

tcgaaatata gatttgaatg tagtttttaa aaataattta gagttttatt ttgtttttag 3360

aagaaattag aaatttttta attagtttta tttgtagtta tggtagtgtt tagcggtagt 3420

atcgttttgt taaaaggttt tttgaagggg ttttaggggt gttttttgtt ttttgggatt 3480

atcgttttaa gtagtagttt tggggacgat ttttttttt ttttttttag ttttgtttta 3540

ttgttttcgt tatttttcg ttttgttagt agttttatat tgttttgtag ttagagttcg 3600

ggatttgtat atgagttttt tgtgagggta taagtagggt tgagggttat atttgttttt 3660

tgttgtagtg aaaaggaaaa taagggaaag tgggggagta tatttttat tgttttaaat 3720

ttttagatat ttttgggaaa agatttaaaa attgtagttg ttaaaatttt tgttttagtt 3780

tatgtgtaga ttttgggatg gggaaatagg tgtttgaaga gttttattta tgatttttga 3840

tatagatttt ttttaatttg ggtggggttt ttaagaattt ttttgtttat gtagagttgt 3900

ttgggttata gaaagtgtta ttatattaaa ttttaatatt tataatagtt ggtttattgt 3960

ttggttttta gatttttaaa ttgtaagtaa atttttagaa gcgtattttg tataatatta 4020

aggtttgttt gtatgtgtat tatatttata aaatatattt ttttttata gatgtatatt 4080

tagtttaatt tattaggtag ggtttgtaag tttttttag ttgtacgaaa ttatttggat 4140

ttaatgattt attttttta ggatatgagt ttgggaaaat attaggttag ttttttttta 4200

gtggtaaaat ttttaggata taaaatttgg aaacgtttta tatttatgtg gaaaaaagtt 4260

ggtttgtagg gaaagataga agagaattga tcgagaaaga ggagtaaaaa tgaagatggt 4320

aagaaagttt tggtagaatt taagtattta gattttgtga tttttaaagt tttattgttt 4380

ttttgttttt tttgttgttt agttgtttaa ttttttttg tattttatga gttatttta 4440

cggtttttaa ttatttttt tatattgtaa tggtatagga agtttaatag tttatatttt 4500

tgttatatta ttgagaagtt atagttgtga ttaattgttt agatgggtat tgttttataa 4560

tttttgtgta tttagttatt tattttgttt tttaaatttt attattgggt ttgtgaagtt 4620

ggattaggta ttggttggtt ttaattattc gggaagtatg ttagagagga aagttaatgg 4680

tataaagaat atttagtttt aattttatgg ttaattgttt agagatatgg agatgtatat 4740

ttttggatta tgtatatgat ttttttttt ggtcgtagaa aagaaaaata gttgaggtgt 4800

ttaagaaata gatttggatg aatagtaatg agtattttgt ttgattttaa gttttttaaa 4860

tggagttaat ttataaatta gatgttgagt tatttttgag ttgtttataa agatagaata 4920

tggattttgt aaaacggttt atttaattta aaatgtttaa aaaatgataa tggggtttat 4980

atttttatg ttaaaagtta taataagttt tattttgtga gaattatttg ggttatttgt 5040

tataagttta tttgatggta agttttttg gaattataaa ttatgattat ttgatttttg 5100

ataagtaggt ttaaataagg tgttttattt tgtttgtgtt tgttaattgg tgatggttgt 5160

-continued

ttagatagtt tagaaaatat agaagtaatt ttgagtgaat tgttttaggg agatatgtga 5220

aagaattttt agtttaagta attttattat ttttacggat attaaggtag attttgtaga 5280

ttaatttttt ttttttgtg ttatttttat tgtttttttt tattagtatt gagaaattat 5340

tttgttgagt attttgtata tattgtaatg gtttatttag ttggtttttt tgtagtttgt 5400

tagaagagaa tatatttgaa gtttttttta gaagtaattt gtttttttt tagttataaa 5460

ttttttttt gtgtaatttg gaaatttttt ttgattaaga gtatatgatt atatatattg 5520

tttaatatat atttgatgat tttatgatta ttttgaggaa aatgtgttgt attttgagag 5580

aatttaagaa tagatttttt aagtttagta ttgaattttt gaaattttag aaatgtaaac 5640

gatagttttg ttaaagggaa tgtaagttta gtgattttaa ataaagattg gaataaaata 5700

gattagtttg tgaaaatttg tttttattat tggataaatg tatgattttg gaaaagttaa 5760

ttaatatttt taaatattat tgtttttatt tataaaatag ggtttttaat aatatttgta 5820

ttatattttg ttatgattaa ataaaaaata tattttaaaa atatagatta ttttgtttgt 5880

tagtgagagt ggaagaaaat ttgtttttat attttgtttt ttatatattg aattaaaata 5940

ttttaatatt ttttgttgta tagtagagta gtatttgtaa tttaaataaa tgtaattatt 6000

tttaattttg tttaattaag agtattatat taattattga tttttaggag ttatattaat 6060

taattatatt tagatagtag ttaaaagtta ttagtttttt attaattttt tgaatttagg 6120

taagtttgta aaaatgaata atttttcgat taagttatta gtatttgtta ttttttagtt 6180

gttattttgt ataatattaa agttgtgatt ggtgttttta gtgttgagag attgatataa 6240

ttaataaata attatagacg gatatgtgaa agaaaaggga ataggaaagt attagtattt 6300

agtattaata ggatgttaag atattttaag ttggtaaaag taggagaata agtataaaat 6360

ataattaaga taattggtta ttataatatt ttgataattt taaaagtttt aaaattttta 6420

aaataaagaa tggtatatgt gatattattg taataatata tttattttag atttttgata 6480

aataagatga ataagaagtg gagttaagtg gtaatttaaa ggttgttgtg acgttttaag 6540

tgagaagtta agagaattta gattgatatg gggtggaaaa aggtttggag aagtggatat 6600

gagggataat atgagagata tattaataag ataaaggaat tatatgaata taattgataa 6660

ggtaaatgtg atttttaggt attatatttt aatttatgag aaattgatga tatggaaaag 6720

ttaagaggaa aaggtacgtt tatatttgtg ttgttatcgt tttgttgtta ttatttttt 6780

ttatggcgat gggtaggaag taaaagtaag aggtaattta tagaggaagg agtttaaaaa 6840

gtttgttata tttattggag gggggataga aaaattcggt ttattattag atttagtaaa 6900

gaagtttgtt ttatgatgga gtttttggga agagaataaa agaaaataat tttttgagtg 6960

agataaatta aaattttagt tttgtaggat gtaaatttta aatgtttatt gtatttgtgg 7020

tatggaaatt ttaagataaa aaaataaaat aaaataaaga ttgatttaga ggtttgaaat 7080

ttttagattt taggggaatg taaaattgtt ttatagagta taaagggttt taagagagaa 7140

atttattaaa agttataaat tataggcgag gtgcggtggt ttatgtttgt aattttagta 7200

tattgggagg tttaggtagg tagattattt gaggttagga gtttgaggtt agtttggtta 7260

atatggcgaa attttttttt tattaaaaat ataaaagtta gttgggtatg gtggtgggta 7320

tttgtgattt tagttattcg ggaggttgag gtaggagaat cgtttgaatt taggaggtag 7380

agattgtagt aagttaagat cgtgttattg tattttagtt tgggtaatag agtataattt 7440

-continued

tattttaaaa agaaaaaaaa aattataagt tatataaaga aataaaatat agtagagagt 7500

agttataata ataaattagg aatttaagaa tgtgaaatta tagagtaatg tggaaaaaat 7560

tagataggta tatttaaaat agatataaaa gaaatagaaa taaaattgaa aagttatata 7620

cgaaaaaaat atatatataa atttggaaga gaattataaa aatgttttta aaaatatatt 7680

tattgaattt ttttataaga aataaattaa atagtaaatt agatatattt aaagaaataa 7740

tttatgaatt ataaggtaaa tttggtaaat tttaagaagt gtgtgtttat ttttataaaa 7800

gaaaataatg ataataaaaa taataataaa attataatta tatttttta aataattttt 7860

ttataatatt aatttttag gaattaaggt gtgatatttg tgagttatat tttttattat 7920

ggggagggag aagttttaaa tataataaag taaatatgag agttatataa tatggtaata 7980

aatttgattt atttaatgtt aagttattga tttgaattgt gtttatagtg aatattaatt 8040

aagaatgtaa ttaaataaga tgttgtttgt tttttttaaa tttgggatat ttgaggaaat 8100

tttattgatt tagattttta attgaggtat ttaggaaagt ttgagtattt ttttttttt 8160

taataaattt ttttgtagta tttgaatata aaaattattt tattgatttt gtaattgaat 8220

tagaagtagg gttatttta ttaaaaattg tttttatagt ttttaaaaat ttaaagattt 8280

ttaaaagggt tgaagttgta agaatttaga aaataatgta ttaaatatat ttggtgtttt 8340

gagttaattt aacgttatgt ttttttgtta tgtgttttgt ttttatatgt atggtaggtt 8400

gttagatatt aggagtatgt tgttattttt gttatttta attttttttt tttaagttaa 8460

gtacggtgtt tttggttaat aagattattt ttttaattta aaaaaaaatt tttgtttttt 8520

aatagggaat tataagggaa tttagttagt tataaaagta taattgttta ggaaatgtat 8580

taagtggaag gtagatataa attaggttat agtgtattat tatttttaaa ttatttaatt 8640

ataaattgac gtattaatat agattaatat taatgtatat taaagtatta tatttatatt 8700

taaataaaaa tgtaaaggaa aattaattag ttgaaataga aattaatgat gataattttt 8760

aaagtagtaa gaatgtattt ttgagaaaga ttttagggtt ttgtttgttt gtttgttgga 8820

gattaaatta ttttttagta aaattgaagg gggaaaaaag attaacgttt aagttatagg 8880

aagatgtggt ttgtttggta tttggatgtt ttttgtagaa tttataagtt tatttttag 8940

gggtttaaat gtttttggat ttaaaaagtt ttcgggaaag aatttttta agggaagaga 9000

tggagagtta tataagtaga aattaagttt gagttttaag ttttggggtt gtaagaaaag 9060

aataagagaa taagttaaaa aagttgttta ttaaattttt tatagaaggg gaggagggta 9120

ggtgttatta ttacgtattt tgaagttttt gttatagggt attggattga gggaagttgt 9180

tagatatttt ttttatttta aattatttgt aattttagta gttcgattaa aaaatataat 9240

ttcgtaagtt gatggaaaga aacgaatttg gtgaagaaag cgcgggaaaa tattggttaa 9300

attagtaaga aaaagatcgt aataaaaaag aatttaaagc gtgagaattg aaattaggaa 9360

gatggtagaa tgagaagtag aataatttgt ttttttttt ttgattgaat taaatgtaaa 9420

gatattaggt ggtagagatg tggatattag tttttatagt atttttaag agtgttatgg 9480

ttttttttcg tttttatttt atgttttagg tatttttttt agggttttag ttaagttttt 9540

gtttaagtat ttgaataaat tagatttatt tttgaattag ttttaggtgt tgtttataga 9600

tattgttatg gtttatattt ggataattta gtttggtttt tttaatgttt taatttttt 9660

tttatgatcg tgaaaaaaat gtaatgagtt tttttgaaga aatatttaag atatataaat 9720

-continued

ttagaatatt tatagttttt aaattaagat tgtattatat aaaaatatcg attttttaa 9780

aatgtatgaa tgagtgaatg agtgtataaa tagttaatag tgtataaatg tatgtatgaa 9840

tgaatgaatg agagtataaa taagtaaata aggaaatata tttttaaagt atatgttagg 9900

aaatggataa attacgtttt atgaagtttt agattgaatt aggtaaatag aattgtagat 9960

ttttatgttt gttatgattt agtgtatagt tttgggaaaa ttttgtaatt tttttatatt 10020

ttagtttttt attttataaa aggagtatat tgtttgaaat ttaatttttt tatagtagtg 10080

agagttttga tttaacgttt ataaaacgtt ttttagtttt tttgattaat aggtaaaata 10140

ttgtggtagt ttgtataata tatttttaa aaaggataaa ggtaattggt cgaatttgat 10200

gtaaaataat attttattta agaaataaat attagttata tttattattt ttttatttta 10260

tttgttaagt aataaaaatt atttttaata gaaaagtatt aaattaatat ttaagttagt 10320

tgaagtgtta ttattatagg ttttatttac gttgggggaa ggagagttgt tttgtttttt 10380

taggttattt tatatgttta tatggtaaag ttaggaatgt tgggtatagt tttgaaggtt 10440

tttagcgtat ttattggttt ttggtatttt aatgtatttt atttttataa ttataaaagt 10500

atttttgagt gtttttggaa atatataata aatttttaat taatttaaga gtgttttaat 10560

tgtatatatg aaatttttttg ggaggaaaat attagttatt ttatatatat atagtattta 10620

agttagtaag agagtttttt ttgaaaggta taaagtagtt tttttatagt aatataattt 10680

ttagttttat atgtgtaagt tttattgaat tttttagaat aataggaaat tttgtaggaa 10740

aaaaaatttt ataaaaattg atagttatat ttatttaatg taagaggaat atattagtta 10800

ttttgagttg gattattttt taaaggtatt agataattat tgaagattaa ttaaaatttt 10860

aattaaaaat tataagaatt gaatattttg gagatagaat tttttatata taaaaatcga 10920

ataataaaat taattgatta atatatttta ttttaaaatt gtatttatta gattatttta 10980

gttagtattt gagtagtaat tttttggatt ttgatgtatt aatttttatt tttatattaa 11040

atagattata ataagtaata gatttataaa ttagttattg atatgatata attgatattg 11100

tgatatgtat aataatattt attaatatat ttataatgga gtaatgttat ttattattta 11160

tataatgttt atatttttta tttttatgtt aaaaagttat cgtaatatat gtaaggtgtt 11220

tagaattgtg tttggtatag agtaggtttt tattagaata ataatatatt attttttata 11280

agtttttatt aattatatga gagttagaat tatttttttt aataattttg gtaaatattt 11340

gatggtatta agtaaaaaaa aatgtagtga atataatatt attgatttat tttttttaa 11400

tatttgggtt tacgaattat aagtaaatat aatatgtatt tataaaaatg aatattaaat 11460

atttttaagt gagaaattaa ttatataaat tatatgtata tattaaatat atatcgttaa 11520

tagaattatt tattttaatt atagtggagt atttcgatta ttatatgagt tttaaggaat 11580

ttattattat aattattttt tttaattaga gtataaataa ttataaggtt taattaagtt 11640

gaaatttttg tatttggttt ttgtgagaaa gatgagaata ttattttaaa gatgtaagat 11700

ttataaattt ttttaaaaag tattttggta ttttttatgg aaaatgtata tatatattta 11760

tttattaata ttgtatttag agttttattt atagatattt ttggtagatg tacgaagaga 11820

tataaaaatt attagtgtaa tatatttagt aatagtaaaa atatggaagt aatgtaaata 11880

tatattaaga ggggaatagt gaaattatga aatatttgta tatattaaat agaagtattt 11940

aaaattatat gtattgatgt ggaaaaatat ttaagattta gtagattaaa agtaagtttt 12000

-continued

aaggaaatta atgattttat tggagttaaa aatgtaaaat gaaaataaat tatattttta 12060

tatagttatt atatttatat atatatagaa aaaaaaatgt ttgaaaagat gtatatttaa 12120

gtataataat ttattttgat ttgtagagaa taagaatgag taaatttatt ttttatttta 12180

aatatattcg tatttttaaa atttttaag aaagtataat atatgtatag taaaataaaa 12240

tatataaatt aaaagttgat tattgatgaa tttttattga ataaatattt ttatatgatt 12300

agtaattaaa ttaagataaa agatataatt agtattttaa agtttttttt ttgttttttt 12360

tggttatttt ttttaaaagt aatttttatt atttttgttt taatatgtta tataaagtat 12420

gatttatttg tatttttaa gtaatagatt aaaaaaaaaa atttgtttta gtagtatgaa 12480

ttttttttt tgaatattaa atatatttag tattttattt taagatatta atttaaatta 12540

taatttgagt ttttatagtg ggttaggtat tgaattaaat gttttttttg tatgaattta 12600

attatttttt ataattttat gaggcgagta ttgttattat tttttatgta tatatgttgg 12660

aattatggtt tatagttatt tagaaaatta tatgattaaa gttatatcgt tgatgagtgg 12720

tagagtttgg atatatataa aggtagtttg attttagtat ttttaatttt ttattagaaa 12780

taaataaata aaaataaagt ggtaagatgt agagttggga gtatgaagtg tttttagtat 12840

gaagtttttt ttttttgttt tgttttgagt aaatgattta aggttttaag gttatataat 12900

ataatatttt atttgtaaaa tggaagggtt aggttagatt tttttttga tttgattatt 12960

tgacgttgat aaataaagtg atttattgaa atatagagta aatatgtttt gttatgatgt 13020

agtgtaatta tgtattgtat tatgttaaaa aatgatttat ataagaaaat aaaattataa 13080

tttttgaaat tattggaaat aataatttta atttaatgtt atttttggat ataggaagat 13140

ttttttttt aaataagtaa attggtggtt tttgaagggt tttgtttatt tttgtatgga 13200

aaatgtttaa aaataaatta ttaaaaatta aataaaattt ttttatttt ttaattgtgt 13260

ttgaaaaaaa agatgtaatt ttttttagta aaaggttacg tatattttta atagatatat 13320

ttgggatttt tggtttttat attttgaaat attgatataa ataaatatag aatatgtttt 13380

tttttaaatg aaatttataa ttttgagggt taaaaatttt ttaaaataat atgaaatcga 13440

ttttagtaga aggtaattaa agagattaag gtttgttttt ggagaaaatt agaattatga 13500

agattataaa ttttaacgag aaatttgttt tttaaatgtt agtttattga aaatattaat 13560

gtacgtcgga tatatttgtt tttttattag aaaattagta atattttggt ataaatgagg 13620

ttttaaataa gttggagtag aaaataatta ataaattagt ttttatgatt ataagtaaaa 13680

gagaattagt tttttaaaaa gttaatagga tttatttttt gtagatgttt ggtttgggat 13740

ttaatgtttt ttaaagatat gtatggaata gtgtagttat ttaattttta ttaatatttt 13800

taggtgttga ggttacggag ggaaaaagat atgatttttt ttttttgtga gtttatagtt 13860

tttttagaag atagatatat ataatgagtt ttaatataat ttgttgttat attaatagtt 13920

taaaaaaagt gttatggtat tatatagaga aaagaattaa ttttgtttga gttttaagga 13980

aggttttata gaggaggtga tatttgagtt aagttttgaa agatgagtag attattatta 14040

ttattattat tttgtttagg taggtagtaa aagagtatta ggtaagtagg ttaagggagt 14100

tggggttatg gaggataagg gtggtgagaa tatgttaggg agaaaaaagg aagtgattga 14160

cggtattata agaaataaaa tgtttataag taagttttat tatattagga tttattatat 14220

aaagaattaa tttgagaaag gagatatgtg atttgaatat tttgtggggt tttttgattt 14280

-continued

atgaatttag gaagtgttta tttggaaaaa tagaattttt gggtttttgg tcgtttttta 14340

gtaattaaag ggttattagg tttttcgttt aatatttggt ataaagtgtt tggatggatt 14400

tgtggttcgt tgtgtttttt ttggattttt tgtttatgaa tgaagtagaa ttttttttta 14460

ttatttgtga tttaaatttt tgttttaaat ttttcgtaat atttttcgag aacggttttt 14520

atttgttatt gggttgttta gtatttattt tatttttttg ttaataagga gttatgtagg 14580

gttttttttt atttaggaaa tagaaaaata ttttggttaa atatagtatg ttgtatttgt 14640

agtttttttgt tatttaagta tatattttaa aattaaagta taaattaaag gaagtgagag 14700

agagagagtg agtgtgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtt 14760

tttaagtatt tttattttgt ttatttaggt attaattagt aaatgatgtg aaatttttag 14820

gttagtttga ttgtttttat gattatttat ttaatataat taaagtataa ttattaaatt 14880

agataatgta taagtgtgga attttaagta tttttgtaat ttttaataag ataaaaatta 14940

tttattgttt agaattttta agaaaattag tttaattata tatttataat attaatgttt 15000

tatattttag aataattttg aaatttaatt taaaatttat atgggaaaag ttatttttttt 15060

ttattttatg tgaatattat tgtttttatt ttgtttaagt gatttttgaa atgtgtttta 15120

tatatgttta aattatgtaa gggtggtgtt taaataatag ttaaatttat ttatattaat 15180

tatatattat tttatagata aaatgtattg gttaatatta aatttataga atgagaattt 15240

aaaagtttgg atttgagaat ttataaaaga gagtttttat atgtaatata tttatttta 15300

ttttttttta gttgtataaa tgttattaat ttaagatttt gaatttgtgg ttataattga 15360

aagttatata taatttttt gaaaattttt tattataaat aaataattta atttattaag 15420

tattttaatt tttagtagtt gtgattatta gtaattttat ttttttaat attatagatt 15480

aaataaaata aaagtttttt attattgaat atttatttat agaaatggaa aggtaatggt 15540

tattatttaa aaataaattt aaattattta ttgataaata atcggaagaa aagatatata 15600

gatgatttaa ataaatattt atttttata tatttttttt taggtatttt gttatttta 15660

tttatatatt atttttagaa aaggaaaata tgttggagat ggttaatttg tgtgaagcgt 15720

atttgtaaat tttttttgtg taaagttaat ttattaatat agattaatga gaattgtttg 15780

attttatgtt taaatttggg aggaaggtat taggattttt tatatttgat tggaaataga 15840

ataatttttt tatgtagagt tttgggagtt ttttgattat attaaatgat attaagattt 15900

gtttaggtta tgggttttta agaagaggta attttgtttt tagagattat ttgataatgt 15960

ttggagatat tattattatt aggggaggga gggtttttgg tatttagcga tgttattaag 16020

tattttatta ggtataggat agtatttttt gaataaatta tttagtttaa aatgttaata 16080

attagagttt gaaaaatttt tatttaaata tattgaagaa tttaggtata acgttattat 16140

ttttaaaatt atatagatat atttggtagt agttttagat aagttgtaaa aatgagattt 16200

tgataattaa tattttaatt ttttttttttt ttttagtttt attttgttat atagttatta 16260

tatttgaatt taagtgataa attttgtaag tagaggtatt aattatttttt tattgttttt 16320

ttaaaggtaa gaattatagt aatatgtaaa gttatttttt aaaaagttta tattgagttt 16380

attaggttta ttgattattg aaaattatat aaatttttat aattggtatt ttacggttga 16440

aaaaaaagta attgaggaaa tttaaatatt ggttataaag tattattatt atataacgtg 16500

tttataattg tttaaagtag ttaaggaatt atatagatta aagttttaaa ataatttaga 16560

-continued

ttattgtatg cggaaatatt tattaaaaga aatattattg tataacgtaa atggttaatg 16620

ttaattgttg agtatttata gtgtttttttt ttaaggtttt aaatataatt ttagatttgt 16680

taagatgatt tatagtattt aatttatatt tggattattg gatattttat ttatggttat 16740

taatttatag ttttttaaaa aagattttta gttttaaata tttttggaat atttgggggt 16800

aatttaatga aagatattta attatagtaa taataattga tatttatatt aagtattatt 16860

ttaaatatat ttatatatat ttatttattg aatttttatt agaattttat gaggtatgtt 16920

ttgttatata tgagaaattg aagtaaaatt tgttttagta atgtgtttga tgttataaat 16980

ttattaatgt tagagttgag gtttgaattt aagtgtttgg ttttatattt ttttttttt 17040

ttatttattt atttattttt gagatggagt tttattttgt tgtttaggtt ggagtgtaat 17100

ggtgtaattt tagtttattg taatttttgt tttttgggtt taagtgattt ttttgtttta 17160

gtttttcgag tagttgggat tataggcgtt tattattacg tttagttaat ttttgtattt 17220

ttagtagaga cggggttttg ttattttggt taggttggtt tcgaattttt gattttaggt 17280

gatttattcg tttcggtttt ttaaagtgtt gggattataa gtgtgagtta tcgcgtttag 17340

tttttatatt ttttttattt atttattgtg ttataagaat tgagtttttt agattttaag 17400

tattatgaaa ttatcgtatt tttagagatt tttaggattt ttggtttatt tattagatta 17460

ttgatttttt agtggtaaga aataaatata tttttatatt tacgtatata tatatataat 17520

ttaagtttgg atttatagtt gtaattttat tgggtttata ttaaaatttt ttgaggttgt 17580

tattgaggta gttttatttt gtaatagtta ttgttttttt tattgttttt agttttaagg 17640

aggggatgtt tagtgttata atttttgttt ttatttttag tatttttatt tatttattta 17700

atgggaatat aataattatt tattgattat atgtttagta gtgtattgtg tattagcgat 17760

atagaaatga taaatatagt taatttttt agtaaatttt tagtttaaag taggatttag 17820

attaatgtaa tttattatat tttaatgtgg taagggttat gttaaatgta gaagttggat 17880

gttttgggag taataagaaa gaaacgttta atttagattt tggaggttag aggaatttat 17940

tagaggagtt aatattttgt attttaagta ttgaaaaagt agtagaaaag atatttgtag 18000

ggggagaggg gcgatgtttt ttagagaaaa agtattttac gtataaagaa atgaagtgga 18060

aatgtttaag gtgtatttga gaattgtagt tttttatata gttggaaaag agagttgttg 18120

tgagtgggga gtgagagatg agaagagtaa tttgagtaag gtttttagta tgaagggtta 18180

ttttttttgt agagaatttt ggaatttgtt ttataggtag taggtaaatt ttttaaggaa 18240

ttttaagttt aggagggatt ttataaaaat tatgaataaa tgtggttgaa tattttaggg 18300

acgtgagaga gtgttagaga tagttttaat atagtttaga atgggttttt aggagaggta 18360

ttaagatatt ataattatat ttgttatgat aatattaata cgaataagtg tgtataggat 18420

aattgtttat ttaaagttaa tttgaatatt tgaggattag atttgtgatt ttggttataa 18480

tgatttttttt tttaaatatt tttgggaatg gtatgtaaaa atataagaat ataataaaag 18540

ttttaaagtt tttaaagtta tgtagagtag aaaagaaatt tatttatttt attttaattt 18600

tgtaagtttt tagtaatatt gataaggaaa aagtatttaa taagtattta ttaagtattt 18660

ataattttgt taattatttt tatgaacgaa atttttaaaa attaaagtta ataattaagg 18720

gaaaaaatta gatttaaatt aaaatttttt atgagttaat taagaaaaat aatagtatta 18780

ttattttatt aattaaagtt aatgtaaaaa taaataaata aaaaaaaaaa taaaataaaa 18840

-continued

```
tagtagaatt gtcgtttttg ataggtttat tgaggtttgt atgtttaagt tatttattag  18900
aagtattgtt ttagattatt ggaaaatttg taaaaggaaa tttgaatgtg atttatttat  18960
aacgttatta aatttagtaa gaatgatttg agttataatc gtatatgaat atatatttgt  19020
atttaaatgt aagagaaaag aattaggtaa tgtagagatt tgttgatttt tttaagttat  19080
tattattttt aatatattga aaatattaag ttagaaatat ttaatttttt aattagaata  19140
ttcggttttt tttaaatttt ttaatattag ggagtttaaa gttagaggtg aaatgtaatg  19200
aatagattat ttcgttgggt atatgtagag gtttgtgaat tgtatatatg attttgtgta  19260
taatgagttt tttcggtatg tggtatattt atttgattag ggatagaatt ttaaattttg  19320
tattgttgtt tttgtgagta gaattaaatt taatggtata tgaatgtaaa tttttatgat  19380
ttaattgtag ttttttataa gaaatgtttt ttttatttta aaaagaaagg gggaaaggat  19440
aatagaaata gtagtaataa tttacgtgaa aatttaaagt tatgttaagt taggttattg  19500
ttggtgtttt tttttatagt ttttaaagga ggaaagatgt tttaattaat aaagttaatt  19560
tttgtaggta taagtttttt gtgttttatt tagatttgta attatatata gggaatattt  19620
tgattagttt taattttttt agtgttaaat cgaatttgaa gattggttaa tacggatatt  19680
taagttatag aagggagagt agatatattt ttttgttgat atgttatatt gggagatatt  19740
ttatcgtatt tgatagtttg ttttttagtg atgttatatg aatatgttag ggtaggtatt  19800
ttgaattaat tttttttgtt agaagatttg aatttttttg tagtagtttt tatatattga  19860
agatatttag agagaatttt agaggcggcg gttatcgtga gagggtattg ttggtgagtt  19920
atttttaggt tatttatatt ttgttttatt tagagtagtg gtatattttt ttattgggta  19980
tattattagt ttttatttaa atgaaaggat tcgtggttga gtatataagt aaataaaaaa  20040
atgaaaatta ttgttttagt ggaaaaatat tataaaattg taaaataggt tattaatagt  20100
aaaagtattt atttatagaa aattttatat atgcgataat tagataaatt gaatgtattt  20160
ttaaaatatg ggtagtttta ttataattta aatttgtttt tagaagttta ttatgtattt  20220
tttaattttt taagttttat tagttggttt aatataaatg tgtagatttg agatttaaaa  20280
ttagtattaa aataataaaa aataaaaata aagaaaatat aaagttgata atatttaatt  20340
aatttggata aggtttgaag agtaaaggaa gattttatat gttagttaaa tatttaaaag  20400
atttggttat aagtttttga taattgggaa atattataaa atatagtatt tttttaaatt  20460
tagttatttg ttgtttagtt tttatgattg tggttattta ggttatattt ttgtttattg  20520
ttatttaata cgtttttttа gatatttaat ttttttttтt tgattttaat attttgtttg  20580
gttttgtttt aaataatatt gtttatgaag ttataggttt aatataaaat ttaataaatt  20640
tttttaatat ttagttattt ttatataaaa tataagtatt attaaaataa aatgtttagg  20700
tattatttaa aaatggtggt atatgattga tggaagtaag agtttagtat acgtttaagg  20760
ataagggtgt tggttttata ttggggagtt aggattttgg ttttattgtg ttgtatgatt  20820
gggttttagt ttttttattt gtaaaatagg aataataaaa gtatttaatt tacggtatag  20880
ttaagaagat taaaattagt taatttatat aaatgtttaa aatagtgttt tgtacgaaat  20940
ttttgagtaa atgttagtta tgatgttatg atggtgatag tgattaatat tatagtaata  21000
atgatagtta aattgtaatt atttaaagat tatagtgatt atttttattt aaaaatatat  21060
tttaggatat aaatatattt aagaaaatat gtttttatat ttgatattta tattaatata  21120
```

-continued

```
tgttttggtg atataaaagt tattttatat tggtttaacg taattttatt ttgaaaatat  21180
ttatgtgata ataggaatta gattttgttt aaaaattata atttaaataa attatttgtg  21240
tagaaaaaaa aaaattaaaa aattttaata cgatagaata tataggtttt ttttttattg  21300
tttaaaagta cgttaaaata aatttattta ttgtatgtaa ataaaagttt taaaaatatg  21360
aatatattgt attaaaatat tttatattgg aaaaaaatga gaatttttgg tattttagaa  21420
tggtgttttt ttttattatt tatgtattga aattttaggg taagaaaagg gttagattaa  21480
ttttgaaagg ttttaaatat taaattatat tattgtgtta gtattttaag attaattagt  21540
atttttttaat gataaaaaag gttatagtga gatttagagg tgtatttttt gaaattgttt  21600
taaattatta tataaatgtg gttttgataa tataaaatgg tttttatttt aaattaatta  21660
gttttatttt ttttttgga ggtgtgaaag gggttagggg agtaagaata gtagtgttta  21720
tttttagtat ttttttatta ataaaaagat ggatggagaa atattaagaa tgataaattt  21780
taagcgtaaa atgatatatt attaaagtat atattaatgt atagttttat aagtaatatt  21840
gagtgatagt aatgtttata aagtttttaa atttttaaa agtaattaaa gttgtttgta  21900
tttttttga atgtgtaatt tttgtttatt tttttttatt taagtttaag ttaaatgacg  21960
ttaagtttgg ttttaaaaat gatttaatga ttatgtagtg tttttttat taaaatatat  22020
ttaattataa gatgtagtat tatatatatt gtattttatt tatttttagg gtaaatgttt  22080
gaaatagtat gttatttttt agtttagtag gaaaaattta tgagagaagt aggtatattg  22140
taatgttatt ttatttttt ggtgttgatt ttttttattt agtaattatt gtaataaaat  22200
tttttaattg tatttttgaa tataattagt ttaagatatt ttatagggag gtacgttata  22260
agagtttagt atttagtata tgtaagaata atatttattt agaaaaagaa attgttaaga  22320
ttatattttt attgaaaaaa gaagaaggtg ttttgataga ggtttgttta gttttttaat  22380
gagaggattt aaaacgtttt ttatttttga tagtatattt ttttatttat tttttaaata  22440
agtgatttga aatatttata attagatagt atttaatttt aggttttaag tgttttaata  22500
gttttatgaa atatgattag tagttgtaat atagaattat ggtgaataaa ttttttataa  22560
agaggggttt gtttttatag aatgtttagt gtttttgtat ttatttaata gaaaaaaatg  22620
ataatgagaa agtataatat atatttgtat ttttaaatta tttaatattt aattaagtag  22680
tgaaaaagat tattattttt ttttttgaaa tttgtataaa aattgtgagt ttttgtgggg  22740
taaaataagt ttgattattt gaaagttaga atgtgaattt tttaatattt taatttttta  22800
taataatagt tatcgttttt ttgttatttt attttttaa aaattttaaa aaatttaatt  22860
ttagtgtata aaatgttaaa atgaaatatt aagttatata aatatatttt tagatacgat  22920
attagttatt tagagtttgt aatagagatt gaaaatatga aattggatat tttatttttc  22980
gtggttatgt aaaatttatt tatattttaa agtataatat aaataaaaag gaaatttta  23040
gaaatggagt atttgttttt ttttttttt ttttaattta aagagtttta ttaaaagaaa  23100
aaaataaatt atagtaaaat gataagtaat ttatttgtta gttattcgtt aaaaaagaaa  23160
tttttatgta attttttttt ttagttttta gaaatttttt gaaatttagt atttattttt  23220
tttaataatt aataattatt attttttttt atataattat ttttttttat ataaatttta  23280
agattttaaa aattttaaaa tttaagttta ttatttaaaa gaagtgttaa ttgtgagtat  23340
atatatatat gtgagacgtt ttattttgga aatgagaaat ttaattttaa atatttaata  23400
```

-continued

aatattaata aaaagaaaat aattgagtaa attttatttt agttataatt gtgaattaat 23460

cggtagttat aatggttttt aaatattgaa atatattatt tttaggaaat ttataaagga 23520

aaataggttt ttgaaaatta tattgattgt agatttttat ttattatgaa agataagtat 23580

atattattaa taattagaga aaggagatat ttttaataaa tttaattatg ttgtttttaa 23640

agttgtagtt gttaaaggtt ttaatatttt tgtatagtta tttttaaata cgggtttaat 23700

tttgaatgat ttataatttt taattaggtg gaataaatta tttttacggt ttataataat 23760

aaatgtgggt gtagttatgt ttgttttcga tattgttgtt ataaaaatta gattttggta 23820

gttttgtttt atataaaata atttgttttt tgttattatt gttttttgtt ttttgggata 23880

atgttatttt taaaaatata taaatgtttt tgaatgtagt tttttaaaac gtttcgtatt 23940

acgtattaaa tatttttgg ggggattttt tttttatttt ttttagtttt tggtttttta 24000

ggtttaagtt tgttaataat attttaaaag ataaaaaaaa aaaaaaaggt attgattttg 24060

tttaaaatta aatcgtattc gggaaaggaa tttaagtata attttttttt taaggtttta 24120

aaaataaaaa taacgtaata aataaaaaaa ttatcgttaa tttttaattt tgttagttat 24180

ttacgtgtaa gtgaaatttt agaagttgtt gatggggagt tcgcggttgt taaacgtttt 24240

taagattttt atttaaattt cgtaggagtt cgtattgaaa ttgataaata ttcgagggtt 24300

tataaagtgg ttatagatga gaagaggaat aaaatgttat attttaggag gtgtatttcg 24360

ttttaataat tacgttgatt ttagggtgtt aagttaaaag gggtaaggga gaaagaaaat 24420

tgttaagtat gagtttttaa tgtgaaattt aatttgttgg ttttttttagg tattagattt 24480

tggaagtgtt tgtaatgata gttaggaaag taaattaatg gttaacggtt tattgggttt 24540

gaatattaga gttttgttat ttttttatag attaagtata taatatatat atatatatat 24600

atatatatat acgaagttgt tttacgtgga attgttttgg aattaaaata tacgttcggg 24660

cgttattttg tattttggta gaaagggtag agggatttgt attttttttt tttgttttta 24720

ggttaagatt tatgaatgat tttttaggtt cgttcgggat ggcggggaga ggcgtaggag 24780

acgcggtgag agaggatagt ttttatatta ttttttcgta tatatttta ttttaggcgt 24840

aaattcgttg tttttatagg aaggagaaag aggggtggtg gtgaagggaa atattatttt 24900

tttaagtagg gaagagatga taaaaattaa attgcgtttt ttgttttcgt tttagttttg 24960

ttatcggatt cggttcgtaa atttttttaat cgtttcgagt tgtttagaat ttcgtaaata 25020

aagggagaaa atttttttaa gtgttattaa acgaaagttc gggttgttgt gcggcgtaga 25080

ataaaataag tatatatacg tttatttta aatttcgttt tattacgtta aaaattcgag 25140

ttattttaag ttttattgtt tttttagatt tagtaggttt ggaaggagag aaaaatttgt 25200

tttatttagt taggtttttt tttttggaga agttataagt tttttatggt attcgttatt 25260

cgagttaatg aacgcgattg ttttgataag gcgtttttaa ttttatcgat tttggtaaat 25320

tgtaggagat atttagttta cgttatataa aaattattaa agtgggatcg tcgtagttga 25380

aaagttcgat tagtgtttgg agattagaac gcgagttttt aaagtatata cgtattcggt 25440

atcgggaaag tttaggcgtt tgtgattttt cgaaggattt tgggttagta ttttcgcgtt 25500

tggattattt agggggttat tagaaagtaa tatacgttat aagaaagatg tgggggagat 25560

ttcgatgttc gcgtttttgt taggtggagg cggggtgtag gtagaagtcg cgcgttggat 25620

tgttggatga atttcgttac gcgtaggttt cgcgtcgatt tcggaaggga taggtaggtt 25680

-continued

cggaagggat ttttggggtt tggggacgtt ttttagggta gagagtatcg ttgcggttcg 25740

agcggttcgg gtttttaggc gtggggtatt cggtttttaa gcgtttttcg gggacgtagg 25800

cgtttacgta tcgtttagtt gcgcgttttt agtatttagc gttcggcgtt ttcggcggag 25860

tttcgcgtat tttttcggaa agtttggggg ttttttttaat ttaggttttt tcgggagttt 25920

tggggcgggg gttggaagaa ggggttattt atttcgtcgt tcggttcgtt tcgggttttc 25980

gaagtgtttg ttttagtcgc ggtttagtgc gttcgtaatt aagtatttat tttcgttttt 26040

tttagtgtcg aagttttttt ttcgcgttgt gtttttggtg aaaataggat attttttttcg 26100

gttattttat aataaatagc gtatataagc gggggagaag cggggcggag ggagaagtcg 26160

gtttcgaggg ggaggaggaa aggagaggag ttaaaatttc ggattgcgat agggggggaaa 26220

ggagaagaaa agaaaatgag agagcgcgtt tagtcgtcgt agtcgttatt tttcggtttt 26280

tttcgtagtt ttttttttttt ttttaaggta gcgataattt tacgtggata ggatggaagt 26340

tttgcgcgga agtcgggaac ggtcggagcg tgcgtcgcgt tgtcggtagt ttcgtatttg 26400

cgtagggagg tggggcgggg gcgacgtcgt tatcggttat tgcgggttcg tgaggtcgtc 26460

gggggtttcg ggggaggtcg ttagggacgc ggggggcggc ggcgcgcggg ggatttattt 26520

cgtttttatt tgatttcggg tgattgttta ttgttatggt ttgcgatttc gcgtggggat 26580

cgtttagggt ttcggggttt tggatgtttc gggtggcgtc gtcgtaattt tcgtgttttt 26640

cgcgtcgttt ttatttcgtt tttattcggg tcgattcgag cggttgtagt ttttaggttg 26700

aggggaggga ttcgcgatcg cgtttttttt tttgggtcgg agagttattg tcgattttta 26760

tttgtttttg tggggtagtt ttcggaattg tttggaattt tttcgttatt tagttttttt 26820

gtgtaggtta ttgcgggttt tttgtcggat ttttttttat ttattttaag ggaggagata 26880

gaagggattt cgtttatttg taataacgtg aaataaaaat taatttagat tagattgggg 26940

cgtttttttt gacgggagga aatatttttt ttcgtggaga tatatgttat gaaggattaa 27000

gcggtttggg gataggttgt gttgcgaagg gtttgggttt tttttagtgt tgttgggtgt 27060

aggtataggt attcgtttta gacgtttaaa ggttaggtag taacgattta tttttaaggc 27120

ggcgttagag tttttttagg tatattgttg aaatgatatt tcgtgtttaa gttttttgcg 27180

tatgtattac gcgattatat aggatttacg attttatatt tatacgtgtg tatgttataa 27240

ttaggggata tagtaaaggt agacggaata aataatttat aaattttgta tttatttaat 27300

tatttttttt aaaaatttat tgtttatttt agcggtattt tttttgttat attgataata 27360

aatgttttaa tttattgata aattattgag tgttaattat tgtattggtt atttatggta 27420

tatgagttta tttaattttt atgataattt tacggtggtt attattaagt cggtataaaa 27480

gatgaggaaa ttaaagttta aagaattatg agtgattttt ttaaagataa atttagtggt 27540

ttttaattat aaagtttatg atataataat ttttatatta ttttaggtgt ggtttaagag 27600

attatagatt aattcgtaga aattatttttt atttaaagaa agtttagttt taatatattt 27660

attaagttac gttttgaaaa aggtttagaa aaaagatttt atttttttatt ttaggttggt 27720

tttatgtaat tgatatgagt attgtttaat ttaaaagtag tattaatttt gaatatttat 27780

ggttataatt tatttttttaa tgagtgggga attatttata atgtttaaga ggttttagaa 27840

aaggtggtgt tgtaaattta aaatgataaa ggtagtcgtt gttgttttta ttatttattg 27900

ggtgtttttt tttgtagatt tattttggag ggtgtaggta ttgttaggta taatgttttt 27960

-continued

tttttggtt tttaaggtag aaagggtttt ggtagtgggg ttggtataaa gcggatttgg 28020

agtatggttg agagtatatt ttggtttaac gaggaatgtt agttaatata tattggagag 28080

aaaaatatga atggatagat ttaattaatt ttgtagattt atttttttta ttttatatgt 28140

gagaaaatta agggtttaga atttttagaa gtttgttaat aaatggtaga tttgagtttt 28200

aagttagttt ttatttaagt ttatttttag ttattgtgat gttattatta gtattaatgg 28260

tatttagaaa aatagtaatt tttttaaata tgtaatatat tataagttaa atatgtaatt 28320

taaatgtttt gtgaattata gtgtttaaga atgttttgtg gttatagtaa atattatgta 28380

aggattaagt ataatgatga atttaatatt gtttttaaaa ggtaataata ttttgaattt 28440

tattttggta taaaatatta tgtgttagtt ttatttataa ataaaggtgg gagatgtatg 28500

atttgaataa aataaaaatt tatattttag tatattgtta atataaatat agggtgggtt 28560

tttttagtat gttttttgt atttttttaa agttagattt tttagttata ttgattttgt 28620

ttttaaggtt attttgtttt tgtatagttt tttgtatatt tagataagaa tatatgtata 28680

gaaattattt ttaaaattta ggataatata ttagttattt gattttatat tattattttt 28740

taatagttgt tttaaatata tatatattaa tatggatatt attaggtgtt agataaaacg 28800

ttaattttta gtatatattt gaaataagtt taatatagga aatagaattt tagggttata 28860

aaggtaaaaa tgtattgttt gtttaaatga ggttttattt ttagtattta tattgaatat 28920

tatttttata attatagtta tatcgtttat attattgatt taatatgtat ttttaaataa 28980

tttatttttta ttaaataagt ttatttgata aaggatattt tttatagtta ttataaatgg 29040

aaaattaata aaattcgtta tgaatagaag atgattttgt aaaggaattt atattgtaga 29100

aataaaagtt atttttatg tattatttaa aattatttta tatatagttt gagaaatatt 29160

ggtttaaaga attatatttt atatgttttt taggtttatt aagagatatt tttaagtttt 29220

tttttttat aatcggaata aaatgttgtg tttaatttat tttgaggtat tatttttaa 29280

attttgtgtt tatttaatta ttgtttttttt tgtttaaaat atatttgaag tttttgaagt 29340

tgaggcgttt ttttaaaatt tatgtattat ttattgtttt atttattatt ttatttgtgg 29400

taaaatgtaa ataatttata aatgtataat taaaaagtaa attatttttt tgaaatattg 29460

tatcggttat ttattatttt tttttagtat ttattagttt agtagtgtaa tatagtatag 29520

ataatgaaga agagagggaa agtagaatag tgggagaaac gtaaagggtt tagaaagata 29580

tatgggggaa attgaagtta gagatttgtt gttagatttt tgaagtgatt taaaaatatt 29640

agtgggtttt gtatgatatg agttaggttt ttagaatatt tgtatatgtt taatttagtt 29700

tgaaaaggga tgtgttttgg gaggagtggg gagataaaaa atataagatt ttatagggta 29760

aattagtgga aatataagat ttatttatta gtaatttttt taattaattt ttttggaatg 29820

ggatgttttt gtagttatta tttattattt gtttattttt taaacgtgta tggtattgtt 29880

ttggtattag attttgtata agttaaatta aataaagcgt tttaggagtt tataatttaa 29940

aatataattt ttgttttaat tttatttaaa agtaatttta agggaaaaat tag 29993

<210> SEQ ID NO 4
<211> LENGTH: 29993
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: chemically treated genomic DNA (Homo sapiens)

-continued

<400> SEQUENCE: 4

ttggtttttt ttttaaggtt gtttttaaat gaaattaaaa taaaaattat gttttggatt 60

atgagttttt agagtgtttt gtttaatttg atttatataa aatttaatgt taagatagtg 120

ttatgtatgt ttgaaaaata aataaatagt aaatggtaat tgtagaggta ttttatttta 180

gaggagttgg ttaagggaat tgttaataag tgaattttat atttttattg atttatttta 240

taaagtttta tatttttat ttttttattt tttttaaaat atatttttt ttagattgag 300

ttgaatatgt ataaatattt tgagagtttg atttatgtta tgtagggttt attggtgttt 360

ttgaattatt ttaagaattt ggtagtaggt ttttgatttt aatttttttt atgtgttttt 420

ttaggttttt tgtgtttttt ttattgtttt attttttttt ttttttttat tgtttgtatt 480

gtattgtatt attgggttaa tagatgttag ggaagggtaa tgagtaattg gtataatatt 540

ttaggaaagt gatttgtttt ttaattgtgt atttatgaat tgtttgtatt ttattataaa 600

taagatagta agtaaaataa taaataatat atgaatttta aaaagatgtt ttaattttag 660

agattttaag tatattttag ataagaaaag taataattaa ataagtatag aatttaaaaa 720

gtaatatttt aaagtaaatt ggatataata ttttgttttg gttatgaaaa ggggaggttt 780

gaaaatattt tttggtgagt ttgagggata tatggaatgt aatttttttaa attaatgttt 840

tttaaattgt atgtgagata attttaggtg gtatatgaga aataattttt atttttataa 900

tataggtttt tttgtaagat tatttttttat ttatggtggg ttttattagt tttttattta 960

tggtgattat aaaaaatgtt ttttattaga taaatttatt taataaaaat agattattta 1020

aagatatata ttaagttaat aatataggtg atatggttat aattataaaa gtgatattta 1080

atgtaaatgt tggaaataag gttttattta agtaaataat atatttttgt ttttataatt 1140

ttaaaatttt gttttttatg ttaaatttat tttaaatgtg tattgaaaat tgatgtttta 1200

tttgatattt agtggtgttt atgttaatat atgtatattt aagataatta ttagaaggta 1260

gtaatatgaa gttaaataat taatatatta ttttggattt taaaaataat ttttgtatat 1320

gtatttttat ttagatgtat aaaaaattat gtaggagtaa agtgattttg aaagtaaggt 1380

tagtgtaatt gaaaaatttg attttagaag ggtataaaga aatatgttaa gaaaatttat 1440

tttatattta tgttaataat atgttaaagt atgaattttt attttattta aattatatat 1500

tttttatttt tatttgtagg tgaaattagt atataatgtt ttatgttaaa atgaaattta 1560

aaatgttgtt gttttttgag gatagtatta aatttattat tgtatttaat ttttatatga 1620

tatttgttat agttatagaa tatttttaga tattatgatt tataaaatat ttaaattgta 1680

tatttggttt gtaatatatt atatatttaa aagaattatt atttttttga atattattaa 1740

tattaataat agtattatag tgattaagag tggatttaaa taaaggttgg tttgaaattt 1800

aggtttgtta tttattagta agtttttaaa aattttgagt ttttagtttt tttatatgtg 1860

aaatggagaa aataaatttg taaaattaat taaatttgtt tatttatatt ttttttttta 1920

gtgtatatta attggtattt tttgttaggt tagaatgtgt ttttaattat gttttaaatt 1980

tgttttgtgt taattttatt gttagaattt tttttatttt gagaattaga aaaggaaata 2040

ttatgtttgg taatgtttat atttttttaaa ataaatttgt aggaaagaat atttagtaag 2100

tgatgagagt agtaatgatt gtttttatta ttttaaattt ataatattat tttttttaga 2160

gttttttaag tattgtagat aatttttttat ttattaaaaa ataaattgta attataagta 2220

-continued

tttagggttg atattgtttt tgaattagat agtgtttata ttagttgtat aagattaatt 2280

taaagtagag gatgaaattt ttttttgaa ttttttttag aatgtaattt agtgaatata 2340

ttaaaattaa attttttttg aatgggagta atttttatgg attaatttgt aatttttag 2400

attatattta aggtaatgta gaggttgttg tattataggt tttgtgatta gagattattg 2460

gatttgtttt tggaaaagtt atttatgatt ttttgggttt tggttttttt attttttata 2520

ttggtttaat aatgattatt gtagagttgt tatggaagtt aaatgaattt atgtattata 2580

agtgattaat ataatgattg atatttagtg atttattaat aaattaaaat atttattatt 2640

aatatgatag agaaggtgtt gttaaaatag ataataggtt tttggaagag gtgattaaat 2700

ggatgtaaaa tttatggatt gtttattttg tttatttttg ttgtgttttt tggttgtggt 2760

atatatatgt gtgggtataa aattgtaaat tttatgtagt tgtgtagtgt atgtgtagaa 2820

ggtttagata tgaaatgtta ttttagtaat gtgtttagag aagttttgat gttgttttgg 2880

aagtaagttg ttgttgtttg atttttgggt gtttgggatg gatgtttata tttgtattta 2940

gtagtattgg aaggggttta ggtttttgt agtatagttt atttttagat tgtttagttt 3000

tttataatat atatttttat ggaaaagggt attttttttt gttagaaaaa gtgttttagt 3060

ttggtttggg ttggttttta ttttatgttg ttgtaagtag gtgaagtttt ttttgttttt 3120

ttttttgggg taagtggaaa ggagtttggt agggggtttg tagtggtttg tataggggaa 3180

ttgggtagtg agagagtttt aggtaatttt gggggttgtt ttatagaagt aggtggggat 3240

tgatagtggt tttttggttt agggaggaga gtgtggttgt gggttttttt ttttagtttg 3300

gaggttgtag ttgtttgagt tggtttgggt gggggtgggg tgggggtggt gtggagggta 3360

tggagattat ggtggtgtta tttgggatat ttagggtttt gaggttttgg gtggttttta 3420

tgtgagattg taaattatga taataggtag ttatttgagg ttaaataaaa atggagtggg 3480

ttttttgtgt gttgttgttt tttgtgtttt tggtggtttt ttttgaggtt tttggtggtt 3540

ttatgagttt gtagtagttg gtggtgatgt tgtttttgtt ttattttttt gtgtaagtgt 3600

gaggttgttg gtagtgtggt gtatgttttg gttgtttttg gtttttgtgt aaaatttttа 3660

ttttgtttat gtgaagttgt tgttgtttta gagaggggga aagagttgtg ggaaaagttg 3720

gggagtgatg attgtggtgg ttgggtgtgt ttttttattt ttttttttтt ttttttttтt 3780

tttgttgtag tttggagttt tggttttttt ttttttttтt ttttttttgg agttggtttt 3840

ttttttttgtt ttgttttttt tttgtttgtg tatgttattt gttgtggggt ggttgaaggg 3900

gatgttttgt ttttattaga ggtatagtgt gaaggggaaa ttttgatatt ggaaggaatg 3960

agaataaata tttaattatg gatgtattga attgtggttg ggatagatat tttgggaatt 4020

tgaggtggat tgggtgatga ggtgagtgat ttttttttтt aatttttgtt ttagggtttt 4080

tgggggagtt tgagttgaga gaatttttaa attttttggg aaagtgtgtg aggttttgtt 4140

ggggatgttg agtgttgggt attgaggatg tgtagttgga tggtgtgtgg gtgtttgtgt 4200

ttttgggggg tgtttggagg ttgggtgttt tatgtttgag ggtttgggtt gtttggattg 4260

tagtggtgtt ttttgtttta gaagatgttt ttaagtttta agggtttttt ttgagtttgt 4320

ttgtttttтt tggggttggt gtggagtttg tgtgtaatgg agtttattta gtagtttagt 4380

gtgtggtttt tatttgtatt ttgtttttat ttggtagagg tgtgagtatt ggggtttttt 4440

ttatattttt tttatgatgt gtattatttt ttgatgattt tttagatggt ttaggtgtga 4500

-continued

ggatgttgat ttagagtttt ttggagggtt ataggtgttt gggtttttttt ggtgttgggt 4560

gtgtgtgtat tttaaaggtt tgtgttttaa tttttaggta ttgattgggt tttttaattg 4620

tggtgatttt attttaatag tttttatgtg gtgtggattg aatgtttttt gtagtttgtt 4680

agggttggtg aaattagagg tgttttgtta gagtagttgt gtttattggt ttgagtagtg 4740

ggtgttatgg aaggtttata atttttttaa aggaagggat ttggttgggt agagtaggtt 4800

ttttttttttt tttaagtttg ttgggtttgg ggaggtagtg gaatttgaaa tggtttggat 4860

ttttagtgtg gtgaagtgag gtttggaagt agatgtgtgt gtgtttgttt tattttgtgt 4920

tgtatagtaa tttgaatttt tgtttggtag tatttgaaag agtttttttt ttttgtttgt 4980

gagattttga atagtttgga gtgattaggg aatttgtgga ttgagtttgg tggtagagtt 5040

ggggtgaaag tagagagtgt aatttaattt ttgttatttt ttttttgttt gggaggatag 5100

tgtttttttt tattattatt ttttttttttt ttttttatga agataatgga tttgtgtttg 5160

gggtgagagt gtgtgtggga gagtggtgtg gagattgttt ttttttattg tgtttttttgt 5220

gtttttttttt gttattttga gtgggtttag agagttattt atgaatttta atttgagggt 5280

aggggaggaa ggtgtaggtt tttttgtttt ttttgttaag gtgtagaata gtgtttgggt 5340

gtgtgttttg gttttagagt agttttatgt ggagtaattt tgtgtgtgtg tgtgtgtgtg 5400

tgtgtgtgtg ttgtgtattt gatttgtgag gaggtaatag gattttggtg tttaaattta 5460

gtgggttgtt ggttattagt ttgtttttttt ggttgttatt atagatattt ttaaaatttg 5520

atatttaaga gaattaatag gttaggtttt atattaaggg tttatattta atagtttttt 5580

ttttttttta ttttttttgg tttggtattt tgggattaat gtaattgttg gagtgaaata 5640

tatttttttgg aatatggtat tttgtttttt tttttatttg tggttatttt gtgaattttt 5700

gggtgtttgt tagttttagt gtgggttttt gtgggattta ggtgggagtt ttaggagtgt 5760

ttaataattg tgggtttttt attagtagtt tttgaagttt tatttatatg taggtgattg 5820

ataggattga aagttgatga tggtttttttt gtttgttgtg ttgtttttgt ttttaaaatt 5880

ttagggaagg gattgtattt gaatttttttt tttgggtatg gtttggtttt aagtagaatt 5940

agtgtttttt ttttttttttt tgtttttttaa aatattattg gtaagtttaa atttgaagaa 6000

ttaaaaatta gaggggggtgg ggagagaatt tttttaaaaa atatttgata tgtgatatgg 6060

agtgttttag gagattgtat ttaaagatat ttgtgtattt ttaaaaataa tattatttta 6120

agaaataaaa agtagtagta ataagagata gattgttttg tgtggagtaa gattgttaga 6180

atttgatttt tatggtaata atattgaaag tagatataat tatatttata tttattgtta 6240

taaattgtaa aaatagtttg ttttatttga ttaaaagttg taagttattt aaagttaaat 6300

ttgtatttag gaatgattgt ataagaatgt taaaattttt gatagttata gttttgaaag 6360

taatataatt agatttgttg aaaatgtttt tttttttttga ttattagtga tatgtatttg 6420

ttttttataa taagtaaagg tttataatta atatggtttt taaaagtttg tttttttttg 6480

taaatttttt gaaaatagta tgttttaata tttaaagatt attgtaatta ttggttggtt 6540

tataattata attaaaataa gatttattta gttgtttttt ttttattagt atttgttgaa 6600

tatttgaaat tgaatttttt atttttaaaa taaaatgttt tatatatatg tatgtattta 6660

taattaatat ttttttttaag tagtaggttt aagttttaaa atttttaaaa ttttaagatt 6720

tgtataaaaa ggagtgattg tataaaaagg aataataatt attaattgtt aagaaaaata 6780

-continued

gatgttgaat tttagagggt ttttagaagt tggagaaaaa aattgtatag aagtttttt 6840

tttgatgggt gattggtaga tgagttattt gttattttgt tataatttat ttttttttt 6900

taataaggtt ttttgggtta aaaaaaaaaa aggaaaaata agtattttat ttttaagagt 6960

ttttttttta tttatgttgt attttggggt gtaaataaat tttatatagt tatgggaaat 7020

agagtattta attttatgtt tttagttttt gttgtaagtt ttaaatgatt gatgttgtgt 7080

ttaaaaatat atttatatgg tttgatattt tattttaata ttttatgtat tggaattaga 7140

ttttttagga tttttagaaa gatagaatga tagaaagatg atgattattg ttataaaaag 7200

ttagaatgtt aggaagttta tattttaatt tttaaataat taaatttatt ttgttttata 7260

aaaatttata gtttttatat agattttaag aagaggggtg ataatttttt ttattattta 7320

gttaagtgtt aagtaattta agaatgtaag tatgtattat gtttttttat tattattttt 7380

ttttgttagg taaatgtaag aatattggat attttgtaaa aataggtttt tttttataag 7440

gagtttattt attatagttt tgtattgtag ttgttgatta tattttatgg agttgttaaa 7500

gtatttaaaa tttaaaatta ggtattgttt ggttgtaaat attttagatt atttatttaa 7560

gaaataaata gaaaagtgtg ttattaaaag taggagatgt tttgaatttt tttattgaag 7620

agttgaataa atttttatta aaatattttt ttttttttt agtgagaata taattttgat 7680

agttttttt tttaaatgga tattattttt atatgtatta aatgttaaat ttttataatg 7740

tgttttttg tagagtattt taaattaatt atatttagaa atatagttgg gggattttat 7800

tataatggtt attaggtgaa ggaaattaat attaggggaa tggggtggta ttgtagtgta 7860

tttgtttttt ttatgaattt tttttgttaa attaagaaat gatatgttgt tttaggtatt 7920

tgttttggag atgggtgaga tgtaatatgt gtaatgttgt attttatagt tagatgtgtt 7980

ttaatgaagg ggatattgta tagttattaa attatttttg gagttaaatt tggtgttatt 8040

tagtttgaat ttaagtggaa gaaaatgaat aagagttata tatttaaaag aagtataagt 8100

aattttgatt gtttttaaga ggtttgaaga ttttgtaaat attattgtta tttaatattg 8160

tttgtggagt tgtatattaa tatatgtttt ggtgatatgt tattttatgt ttgaaatttg 8220

ttatttttag tgtttttta tttatttttt tattagtaaa gagatattga aaatgaatat 8280

tattattttt attttttaa ttttttttat attttagaa aaagagatga aattgattaa 8340

tttaaaatag aaattatttt gtgttattaa aattatattt atatagtgat ttgagatagt 8400

tttagagagt gtatttttga gttttattgt aatttttttt gttattgaaa ggtgttaatt 8460

gattttaggg tattgatata atagtatagt ttgatatttg aaatttttta gagttggttt 8520

ggtttttttt ttattttgag attttagtgt atggatgatg aagaaagata ttattttaaa 8580

atattagaaa tttttatttt tttttaatat gaaatgtttt aatatagtat gtttatattt 8640

ttaaagtttt tatttatata tagtaagtaa atttatttta atgtattttt ggatagtagg 8700

agaaagattt atatgtttta ttgtgttaga attttttagt ttttttttt ttgtataggt 8760

agtttattta ggttataatt tttaggtaaa gtttgatttt tattattata tgaatatttt 8820

taaagtgaaa ttgtgttaaa ttaatgtgga atagtttttg tattattaag gtatatatta 8880

atgtagatgt taaatatgag agtatatttt tttgagtata tttatatttt aaagtgtatt 8940

tttaaataaa agtggttatt gtagttttta gataattata atttggttgt tattattatt 9000

ataatattaa ttattattat tattataata ttatagttag tatttattta ggaattttgt 9060

-continued

gtaaaatatt gttttaagta tttatatgga ttagttaatt ttaatttttt taattatgtt 9120

gtaaattagg tatttttgtt atttttattt tatagataag gaagttgaga tttagttatg 9180

tagtatagtg gagttaggat tttaattttt tagtatgagg ttagtatttt tatttttaag 9240

tgtgtgttgg gtttttgttt ttattagtta tatattatta tttttaggtg gtatttgaat 9300

attttgtttt aataatattt atattttatg taaggataat tagatattag aaaaatttgt 9360

taaattttgt attaaatttg taattttatg ggtaatattg tttgagataa gattaaataa 9420

agtattgaag ttaaagaaaa aaaattaagt atttgaagaa atgtattaag taatagtgaa 9480

taagaatatg gtttaaataa ttatagttat gaaggttgga tagtaaatga ttgaatttgg 9540

agaaatgttg tattttgtaa tgtttttaa ttattaagaa tttatgatta gatttttaa 9600

atatttaatt aatatgtgga attttttttt gttttttaag ttttatttaa attggttaaa 9660

tgttattaat tttgtatttt ttttgttttt gttttttgtt gttttaatgt tggttttgaa 9720

ttttaaattt gtatatttat gttgaattaa ttaataaggt ttgaagagtt aaagagtgta 9780

tgatggattt ttggaggtag gtttaaatta taatggagtt gtttatattt tggaaatata 9840

tttaatttat ttggttattg tatgtgtaag gttttttgta ggtaaatatt tttgttgtta 9900

ataatttgtt ttataatttt atagtatttt tttattgaag tagtggtttt tatttttta 9960

tttatttata tatttagtta tggatttttt tatttaaatg gaaattgatg atatgtttag 10020

tagagaaatg tgttattgtt ttaggtgaag taggatgtag gtagtttgag aatgatttat 10080

taatagtatt tttttatggt ggttgttgtt tttggagttt tttttaagtg tttttaatgt 10140

atggaaattg ttgtaaaaaa atttaagttt tttgataaaa ggggttaatt tagagtattt 10200

gttttaatat gtttatgtgg tattattaaa aaatagattg ttagatatgg taaaatattt 10260

tttagtgtga tatattagta gaaaggtgtg tttatttttt tttttataat ttgagtattt 10320

gtattaatta gtttttaaat ttgatttagt attgagaaaa ttaaaattga ttaaaatgtt 10380

ttttgtgtgt agttataggt ttgaatgagg tataaaggat ttgtatttgt aaaggttgat 10440

tttattaatt agaatatttt tttttttttа aagattgtaa gaagaaatat tagtagtggt 10500

ttaatttgat atgattttag atttttatgt aaattattgt tattattttt gttatttttt 10560

ttttttttt ttttaaaatg aaagggatat tttttgtgaa agattataat taaattataa 10620

aaatttatat ttatgtgtta ttaagtttaa ttttatttat aaaagtaata gtatagagtt 10680

tgaaatttta tttttaatta agtaggtgta ttatatattg ggagggttta ttatgtataa 10740

ggttatatat ataatttata gatttttgta tatatttaat ggagtgattt atttattata 10800

ttttattttt gattttgaat tttttaatgt taaaagattt gaaaagaatt gaatgttttg 10860

attaagagat tgaatatttt taatttaatg tttttagtat gttgaaagtg atgatgattt 10920

gggggaatta gtagattttt atattattta attttttttt tttatatttg aatgtaaatg 10980

tatatttatg tgtggttatg atttaagtta tttttgttaa atttaatgat gttgtaggtg 11040

aattatattt agatttttтt ttgtaggttt tttagtaatt taaaataatg tttttagtag 11100

gtaatttaag tatgtaaatt ttaataaatt tgttaagaat ggtaatttta ttgttttatt 11160

ttgttttttt ttttgtttgt ttgtttttgt attaatttta gttgataaga tgatggtatt 11220

gttatttttt ttagttgatt tatgaagaat tttaatttag gtttagtttt ttttttтaat 11280

tgttgatttt agtttttaaa ggttttgttt atgaaaatgg ttagtaaagt tgtgggtatt 11340

-continued

```
tggtaaatgt ttgttaaatg ttttttttt attagtgttg ttgaagattt gtaaaattag  11400
agtgggatgg atagattttt tttttatttt gtatggtttt gaagattttg gagtttttat  11460
tgtattttta tatttttata tattattttt aggaatattt agagagagaa ttattgtaat  11520
taaggttata ggtttaattt ttaggtattt aaattagttt taggtggata attgttttat  11580
atatatttgt ttgtattaat gttgttataa taaatatagt tataatattt tgatgttttt  11640
tttgggggtt tattttggat tgtgttgaag ttgtttttaa tatttttta tgtttttaag  11700
atatttaatt atatttgttt atgattttta tgaggttttt tttggattta aaatttttta  11760
aaaaatttgt ttgttgttta tagggtaaat tttaaaattt tttgtaggga agataatttt  11820
ttatattgag aattttgttt aggttgtttt ttttattttt tatttttat ttataataat  11880
ttttttttt agttgtatgg aaaattgtag tttttaaata tattttggat atttttattt  11940
tatttttttg tgtgtggaat gttttttttt tggaaaatat tgttttttt tttttgtaaa  12000
tatttttttt attgtttttt taatatttaa ggtgtaaaat gttagttttt ttgataagtt  12060
tttttgattt ttagagtttg agttgaatgt ttttttttg ttatttttaa agtatttagt  12120
ttttatattt aatatagttt ttgttatatt gaaatgtaat agattatatt agtttaaatt  12180
ttgttttaag ttgagaattt attggaagga ttagttgtat ttgttatttt tgtattgtta  12240
atgtatagtg tattgttgga tatatagtta gtaggtagtt gttatatttt tattgaatga  12300
atgagtagag gtattgggaa tgagagtaga gattgtgata ttgaatattt ttttttgag  12360
attgggaata gtgagaaggg tagtgattat tataaggtgg gattatttta ataataattt  12420
tagggagttt tggtataaat ttagtagaat tgtagttgtg aatttaggtt taggttatat  12480
gtatgtatat gtgaatgtag aaatgtgttt attttttatt attgggaagt tagtgatttg  12540
gtggataagt taaggatttt gaaaattttt ggagatatgg taattttata gtatttgaaa  12600
tttgagagat ttagttttta tagtatagtg agtaagtaga aagaatatgg gggttgggtg  12660
tggtggttta tatttgtaat tttagtattt tgggaggttg aggtggatgg attatttgaa  12720
gttaggagtt tgagattagt ttgattaaaa tggtaaaatt ttgtttttat taaaaatata  12780
aaaattagtt gggtgtggtg gtgggtgttt gtaattttag ttatttggga ggttgaggta  12840
ggagaattat ttgaatttag aaggtagagg ttgtagtgag ttgagattgt attattgtat  12900
tttagtttgg ataatagagt gagattttat tttaaaaata aataaataaa taaaaaggaa  12960
gaagaatatg gagttaggta tttgggttta gattttagtt ttaatattga tgagtttgta  13020
atattaaata tattattgaa atagattttg ttttagtttt ttatgtgtaa tagaatatgt  13080
tttatggggt tttgatgaga atttaatgag taaatatatg taaatatatt tagagtagtg  13140
tttgatatga gtattagttg ttattattat gattgggtat tttttattag attatttta  13200
aatgttttag agatatttag agttgaaggt tttttttggg gggttatgga ttggtgatta  13260
taaatggagt gtttagtagt ttaagtataa attagatatt gtgggttatt ttggtaaatt  13320
tgaggttgta tttggagttt taagagaaga tattataggt gtttaatagt tggtattggt  13380
tatttatgtt gtgtagtaat attttttta gtaaatattt ttgtatatag taatttagat  13440
tgttttagag ttttaatttg tgtggttttt taattatttt aagtaattat aagtatgttg  13500
tataatggta gtattttata gttaatattt aagttttttt agttgttttt tttttagttg  13560
taaggtatta attatgagaa tttgtataat ttttaatagt tagtgggttt gatgaattta  13620
```

-continued

atgtaaattt tttaaaaggt agttttatat attgttatga tttttatttt tagagagata 13680

gtaggaggta attagtattt ttatttatag aatttattat ttggatttag atataatggt 13740

tatgtggtag gatggggttg aaggaaaaag gaaagttaaa atattaattg ttaaggtttt 13800

atttttatag tttgtttgga attgttatta ggtgtatttg tatagtttta aaaatgataa 13860

tgttgtattt gaatttttta gtatatttaa ataagagttt tttaagtttt ggttattgat 13920

attttgggtt ggataattta tttaggggat attgttttgt gtttggtagg atgtttagtg 13980

gtattgttgg atgttaggga ttttttttt tttggtaatg atggtgtttt tagatattgt 14040

taggtgattt ttgggagtaa aattgttttt ttttgagaat ttataattta agtagatttt 14100

aatattattt gatatagtta aaaaattttt agggttttgt ataagaggat tgttttattt 14160

ttagttaagt gtggagaatt ttaatatttt ttttttaagt ttaaatatga agttaaataa 14220

tttttattag tttgtgttga tagattaatt ttgtatagag gggatttata gatatgtttt 14280

atatagattg attatttta gtatattttt ttttttgga aataatatat gagtgggagt 14340

aatagaatat ttgagagaga gtatgtagga agtaaatatt tatttgaatt atttatgtgt 14400

ttttttttt ggttatttgt tagtagatag tttggattta tttttaaata atgattattg 14460

tttttttatt tttgtgggta aatatttagt aataagaaat ttttatttta tttagtttgt 14520

agtgttagaa aaggtaaagt tattgataat tataattgtt gaagattaaa atatttagtg 14580

agttaaatta tttgtttgta atgagaaatt tttaaaagaa ttatgtgtag tttttagttg 14640

taattataag tttaagattt tgagttaata atatttgtat agttagaaga aagtaaaaat 14700

aaatatatta tatgtaaaaa ttttttttg tgaattttta gatttaaatt tttaagtttt 14760

tattttatgg gtttgatgtt gattaatata ttttatttat gaaatgatat ataattgatg 14820

taagtaaatt tgattgttgt ttgggtatta tttttatata atttaagtat atatgaaata 14880

tattttaaaa attatttgaa taaaatggga ataataatgt ttatataaag tgagagagga 14940

taatttttt tatatgagtt ttgggttgaa ttttaaaatt attttgaaat atgaaatatt 15000

aatattataa atatatgatt agattaattt ttttaagagt tttaaatagt agataatttt 15060

tattttatta gaaattgtaa agatatttga aattttatat ttatgtattg tttaatttag 15120

tggttgtatt ttaattgtat taggtagata attatggaag tagttagatt aatttggaga 15180

ttttatatta tttattaatt gatgtttaag taggtagggt agaggtattt gaaaatatat 15240

atatatatat atatatatat atatatatat atatatatat atttatttt ttttttttat 15300

ttttttgat ttgtgtttta attttgaaat gtgtgtttag gtggtaaagg gttgtaaata 15360

tagtatattg tgtttgatta aaatattttt ttgtttttta ggtggagaag gattttgtat 15420

agtttttgt tagtagagag ataaagtggg tgttgggtaa tttaatgata ggtggaggtt 15480

gtttttgggg agtgttgtgg agaatttgag ataggggttt aaattataga tagtggagga 15540

gagttttgtt ttatttatgg ataggaagtt taaagaaggt atagtgaatt ataggtttat 15600

ttaggtattt tatgttagat attgaatggg aagtttagta gttttttagt tattggagaa 15660

tggttaaaaa tttagaggtt ttatttttt aaatgagtat tttttgggtt tataaattaa 15720

ggaattttat agagtattta agttatatat ttttttttt aggttgattt tttgtatagt 15780

gggttttaat gtggtaagat ttatttatga atattttatt ttttataatg ttgttagtta 15840

ttttttttt ttttttggt atatttttat tatttttatt ttttatggtt ttaatttttt 15900

-continued

taatttattt gtttagtgtt tttttgttgt ttatttggat aaaataataa taataataat 15960

aatttattta ttttttaagg tttagtttaa atgttatttt ttttgtgaag tttttttaa 16020

ggtttaggta gaattagttt tttttttgt gtaatattat agtatttttt ttggattgtt 16080

aatataatag tagattgtgt tggggtttat tgtatgtgtt tgtttttag agagattatg 16140

aatttataga ggggagagat tatatttttt ttttttgta attttaatat ttggagatgt 16200

taatggaaat taagtgattg tattatttta tgtatatttt taaagggtat taggttttaa 16260

attagatatt tgtaaaggat gaattttgtt aatttttgg aaaattggtt ttttttgtt 16320

tgtggttatg aaagttggtt tattggttat tttttatttt aatttattta aagttttatt 16380

tgtattaaag tattattgat tttttagtgg aaaaataggt atgtttggtg tatattggta 16440

tttttaataa attgatattt aaaagataga ttttttgttg aggtttatgg tttttataat 16500

tttagttttt tttagaaata gattttgatt tttttggttg ttttttgtta agattgattt 16560

tatgttattt tgaagaattt ttaattttta gaattataga ttttatttga gagaaagtat 16620

gttttgtatt tgtttgtgtt agtgttttaa aatgtgagga ttaaaaattt tagatgtatt 16680

tattaaaaat atatgtggtt ttttgttgag ggaaattata ttttttttt taggtataat 16740

tgaggaagta aaaaaatttt gtttagtttt tagtggtttg tttttaggta ttttttatat 16800

aaagatgagt aagatttttt aaaaattatt aatttgttta tttaggggg aaagtttttt 16860

tatgtttaga aataatatta aattaagatt attgttttta atagttttaa aaattgtggt 16920

tttatttttt tgtatgagtt atttttagt atagtgtagt atatgattgt attatattat 16980

gataaagtat atttgttttg tgttttagtg ggttatttta tttattagtg ttagatgatt 17040

agattagaag agagatttag tttaattttt ttattttgta gatgagatgt tgtattatgt 17100

gattttaaga ttttaagtta tttatttaga ataaaataga aaaagagaat tttatgttgg 17160

ggatatttta tgtttttaat tttatatttt attattttat ttttatttgt ttgtttttga 17220

tgaggagttg agaatgttgg aattagatta tttttgtgtg tatttaggtt ttgttattta 17280

ttagtgatgt gattttggtt atgtaatttt ttgagtggtt gtaagttata gttttagtat 17340

gtgtatatgg ggaataataa tagtatttgt tttatggagt tgtaaggaat aattgaattt 17400

atgtaggaaa gatatttggt ttagtgtttg gtttattata aaagtttagg ttatagtttg 17460

aattgatatt ttggaataaa atgttgagtg tatttgatat ttagagaagg aaatttatgt 17520

tgttaaagta agtttttttt tttaatttgt tatttaaaaa atataaataa gttatatttt 17580

gtataatata ttaaaataga gatgatagag gttatttttg agaggaatga ttagagggag 17640

taagagagag gttttggaat gttgattatg ttttttattt tggtttggtt gttggttatg 17700

tgaaagtgtt tatttaataa aaatttatta gtgattaatt tttgatttat gtattttatt 17760

ttattgtgta tatgttatat ttttttaaaa agttttaaaa atatggatat atttaaagta 17820

aaaagtggat ttatttattt ttatttttttg taaattagga taagttgtta tatttagatg 17880

tgtatttttt taggtatttt ttttttata tatgtgtaaa tgtaataatt atgtgaggat 17940

gtaatttatt tttattttat atttttaatt ttaataggat tattgatttt tttaaaattt 18000

gtttttgatt tgttgaattt tgaatatttt tttatattag tatatatagt tttgggtgtt 18060

tttatttaat gtatgtagat attttataat tttattgttt tttttttggt gtatgtttat 18120

attgttttta tatttttatt attattaagt atgttgtatt gatgattttt atgttttttt 18180

-continued

gtatatttgt taaaaatatt tgtagataaa attttagatg taatattaat agataaatat 18240

gtatgtgtat tttttataga aaatattaaa atgtttttta aaaagattta tgaattttgt 18300

atttttaagg taatattttt atttttttta taaaaattag gtgtaaaaat tttaatttaa 18360

ttgaattttg taattatttg tgttttggtt aagaaaaatg gttatagtag taaatttttt 18420

gaaatttata tagtaattgg aatattttat tatgattagg ataaataatt ttattaatga 18480

tatatattta atatatatat ataatttata taattagttt tttatttaag aatatttagt 18540

atttattttt atgaatgtat attatattta tttatggttt gtaagtttaa atgttaggaa 18600

gaaataaatt agtaatatta tatttattat atttttttt atttagtatt attaaatatt 18660

tattagaatt attaggagaa atggttttaa tttttatgtg attggtgaga atttatgagg 18720

aatgatatat tgttatttta gtaagagttt attttgtgtt aggtatagtt ttaagtattt 18780

tatatatatt atgatagttt tttaatatag agatgaaagg tatagatatt gtataagtaa 18840

taaatgatat tattttatta taaatgtgtt gataaatatt attatatatg ttataatatt 18900

agttgtgtta tattaatgat taatttataa atttgttatt tattataatt tgtttaatgt 18960

ggaaataaaa gttaatgtat taaaatttag aaaattgtta tttaaatgtt ggttaaagta 19020

atttgataga tatagtttta aaatgaagta tattaattag ttgattttgt tgtttgattt 19080

ttatatgtag aaaattttgt ttttagaata tttagttttt gtagtttttg gttaagattt 19140

tgattaattt ttaatggtta tttagtgttt ttaaaaagta gtttaattta gaatagttaa 19200

tgtatttttt ttgtattgaa taaatatggt tattaatttt tgtggggttt tttttttgt 19260

agaatttttt gttattttaa gggatttaat aggatttata tatataaaat tgaaaattat 19320

attattatgg ggaagttgtt ttgtgttttt tagaaggaat ttttttgtta atttaagtat 19380

tgtatgtgtg tagaatagtt aatatttttt ttttaagaaa ttttatgtat gtagttaaaa 19440

tatttttaaa ttgattaagg atttgttata tatttttaga agtatttaag agtattttta 19500

tggttataag agtagagtgt attagaatgt tagaaattaa tgaatatgtt agaggttttt 19560

aaaattgtgt ttaatatttt tgattttatt atataaatat gtggggtagt ttggaaaaat 19620

aaaataattt ttttttttt aatgtaggtg aggtttgtga tgataatatt ttaattggtt 19680

tgaatattaa tttgatattt ttttgttaga agtaattttt attatttagt aaatgaaatg 19740

gagaggtaat aaatatgatt gatatttatt ttttgagtaa agtattattt tatattagat 19800

ttgattagtt atttttattt tttttggaaa atgtattata taaattatta tagtattttg 19860

tttattaatt agggaagtta aaaaatgttt tataaatgtt gaattaaaat ttttattgtt 19920

gtgagggaat taaattttaa gtaatatatt ttttttatag ggtgagaaat tgaagtatgg 19980

agaaattata aggttttttt aaagttatat attgagttat ggtagatata gaaatttata 20040

attttgtttg tttgatttaa tttgaaattt tatgaaatgt aatttattta ttttttgata 20100

tgtgttttag aagtatattt ttttatttgt ttatttatgt ttttatttat ttatttatgt 20160

atgtatttat gtattattga ttatttatgt atttatttat ttatttatgt attttaaaag 20220

aattggtgtt tttgtatgat atagttttga tttagggatt atgaatattt tgaatttata 20280

tattttagat attttttaa agaaatttat tgtatttttt ttatgattat gaaaaagaaa 20340

ttaagatatt aagaaaatta agttggattg tttaagtgtg ggttatagta gtgtttatgg 20400

gtagtatttg aagttaattt aggagtgaat ttagtttgtt taagtgtttg aataagaatt 20460

-continued

tggttgaagt tttggaggag atgtttgggg tatagagtgg ggatgaggaa aaattatagt 20520

atttttagag agtattgtaa ggattagtat ttatattttt attatttgat gtttttatat 20580

ttggtttagt taagagagag ggggtaaatt attttgtttt ttattttgtt attttttga 20640

ttttagtttt tatgttttaa attttttttt attgtgattt ttttttatt agtttaatta 20700

gtatttttt gtgttttttt tattaaattt gttttttttt attagtttgt ggagttatat 20760

tttttgattg aattgttggg gttataagta atttgaaatg aggaaagtgt ttagtaattt 20820

tttttagttt agtattttgt aatagagatt ttagagtatg tagtgatgat atttgttttt 20880

ttttttttt atagagagtt tagtgggtaa ttttttggt ttgttttttt gttttttttt 20940

tgtagtttta agatttagga tttagatttg gtttttgttt gtatggtttt ttatttttt 21000

ttttggaaga gttttttttt gggggttttt taggtttaag agtatttgga ttttgggag 21060

atgaatttgt agattttata aagaatattt aagtattagg taaattatat ttttttgtag 21120

tttaaatgtt agtttttttt ttttttttaa ttttattgaa gaataattta gtttttaata 21180

aataagtaaa tagaatttta gggttttttt taaaggtgta ttttgttgt tttgaagatt 21240

attattatta atttttattt tagttaattg gttttttttt atatttttat ttgggtataa 21300

atgtgatatt ttggtatata ttaatgttga tttatattaa tatgttaatt tataattgag 21360

tgatttaaaa ataatgatgt attatgattt agtttatatt tgtttttttat ttaatgtatt 21420

ttttaagtaa ttatgttttt gtggttgatt gaatttttt ataatttttt gttgggaaat 21480

aaagattttt ttttaaattg aagaagtggt tttgttaatt aggggtattg tatttaattt 21540

gagagaaaaa gattaggaat gataggaatg atagtatatt tttaatattt aatagtttgt 21600

tatatatgtg aaagtaggat atatagtagg gaagtataat gttgagttga tttaaaatat 21660

taaatgtatt tgatatatta ttttttgaat ttttataatt ttagtttttt taaaggtttt 21720

tggattttta gaagttataa gggtagtttt tggtaaaggt ggttttattt ttaatttagt 21780

tatagagtta gtggaatgat ttttatattt aaatgttata ggggaattta ttgagaggaa 21840

aaagggtatt taaatttttt tggatgtttt aattaaaaat ttggattaat aaaatttttt 21900

taagtgtttt aaatttaagg aaagtaaata gtattttatt taattatatt tttaattagt 21960

atttattata gatataattt aaattagtaa tttggtatta gatgaattag atttattgtt 22020

atattatata atttttatgt ttattttgtt gtgtttggga ttttttttt tttataataa 22080

agaatatgat ttataggtgt tatattttga tttttgagaa attaatgtta tagaaaagtt 22140

gtttggaaag atataattgt gattttgtta ttgtttttgt tgttattatt ttttttatg 22200

gggatgggta tatatttttt gagatttatt agatttattt tgtagtttat aaattatttt 22260

tttagatgta tttaatttgt tgtttagttt gttttttgta aaaaaattta gtgaatatat 22320

ttttaaaaat atttttatgg ttttttttta ggtttgtgtg tatatttttt ttgtgtgtgg 22380

ttttttaatt ttatttttgt ttttttgtg tttgttttaa gtatatttat ttagtttttt 22440

ttatattgtt ttgtagtttt atatttttag atttttaatt tgttgttgtg gttatttttt 22500

attgtgtttt gtttttttat gtagtttata attttttttt ttttttgag atggagttgt 22560

gttttgttgt ttaggttgga gtgtagtggt atgattttgg tttattgtaa ttttgttttt 22620

ttgggtttag gtgatttttt tgttttagtt ttttgaatag ttgggattat aggtgtttat 22680

tattatgttt agttaatttt tgtattttta gtagaaaagg ggttttgtta tgttagttag 22740

-continued

gttggtttta aatttttgat tttaagtgat ttgtttgttt gagtttttta atgtgttggg 22800

attataggta tgagttattg tattttgttt gtagtttata atttttggtg gatttttttt 22860

ttagaatttt ttgtattttg tggagtaatt ttgtattttt ttggagttta gggattttaa 22920

atttttaggt tagtttttat tttattttat ttttttgttt tgaggttttt atattataga 22980

tgtagtaaat atttagaatt tatattttgt aaggttgaag ttttgattta ttttatttaa 23040

gaaattattt ttttttattt ttttttttaaa ggttttatta taggataggt ttttttgttg 23100

aatttgatgg tgggttgagt ttttttattt ttttttttaat aaatgtgata aatttttttag 23160

atttttttttt ttgtggattg ttttttgttt ttgttttttg tttattgtta taaaagaaga 23220

taatgataat aaaatgataa taatataaat gtaaatgtat ttttttttttt taatttttttt 23280

atattattaa ttttttatga attaaggtgt gatatttgaa agttatattt gttttattag 23340

ttatatttat atagtttttt tgttttattg atgtattttt tatattattt tttatattta 23400

ttttttttagg ttttttttta ttttatgtta gtttagattt ttttggtttt ttatttggag 23460

tgttatagta gtttttaagt tgttatttaa ttttatttttt tgtttatttt gtttgttaga 23520

aatttgaagt agatgtgttg ttgtaatgat attatatgtg ttatttttttg ttttgggggt 23580

tttaaggttt ttaaggttat taaaatattg taataattaa ttgttttaat tgtattttgt 23640

atttatttttt ttgtttttgt taatttaaaa tgttttaata ttttgttaat attgaatgtt 23700

gatgtttttt tatttttttt ttttttatat gtttgtttat aattgtttat tgattatatt 23760

agtttttttaa tattaaaggt attagttata attttgatgt tatatagaat gataattaaa 23820

aggtgataaa tgttaatgat ttaattgagg agttgtttat ttttataagt ttatttgagt 23880

ttagagaatt ggtaaaagat tggtgatttt tggttattat ttaaatataa ttggttaatg 23940

tagtttttgg agattagtgg ttagtataat atttttagtt gagtagggtt aaaaataatt 24000

atatttgttt aagttgtaga tattatttta ttgtatagta aagaatgtta aaatgtttta 24060

atttagtata tgaagagtaa aatataaaaa taggtttttt tttatttttta ttagtaagta 24120

aagtggtttg tatttttgaa atatattttt tgtttaatta taataaaata taatataaat 24180

gttattaaaa gttttatttt atggatgaag atagtgatat ttagagatat taattagttt 24240

ttttaagatt atatatttat ttagtagtaa aagtaagttt ttataagttg atttatttta 24300

ttttagtttt tatttggaat tattaaattt atatttttttt tagtaaaatt attgtttata 24360

tttttagggt tttaagagtt taatgttaaa tttgggagat ttatttttaa atttttttaa 24420

agtgtaatat atttttttta ggatgattat aaagttatta gatgtgtgtt aagtaatata 24480

tatagttata tatttttgat taaaaaagat ttttaggtta tatagaagag agatttatgg 24540

ttgagaaaaa ggtagattgt ttttaagaaa ggttttaaat atatttttttt ttgataggtt 24600

ataggagagt tagttagatg aattattata gtgtgtataa aatgtttagt agaataattt 24660

tttagtattg gtagggaaga gtaataggag taatataaga gaaggaaagt tggtttgtag 24720

agtttgtttt agtatttgta ggggtgatga ggttgtttgg gttaagaatt tttttatatg 24780

ttttttttggg ataatttatt tagaattatt tttgtgtttt ttaggttatt taggtaatta 24840

ttattaatta gtaaatatag ataaaataga atattttgtt tggatttgtt tgttaaaaat 24900

taaatgatta taatttataa ttttaggaag atttgttatt aaatgaattt atgataaatg 24960

atttaaatga tttttataaa gtagagtttg ttgtaatttt tagtatgaga agtatgaatt 25020

-continued

ttattattat tttttaaata ttttgagtta aatggattgt tttgtaaagt ttatgtttta 25080

tttttataga tagtttaaaa atagtttagt atttagtttg taggttggtt ttatttaagg 25140

agtttgaagt taggtaaggt gtttattatt atttatttag atttgttttt tggatatttt 25200

agttgttttt tttttttatg gttagggagg agagttatgt gtatagttta agaatgtgta 25260

tttttatgtt tttgaatagt tggttataga attggagtta ggtgtttttt gtgttattgg 25320

tttttttttt tggtatgttt tttgggtggt tggagttagt tagtgtttga tttagtttta 25380

tagatttagt aatgaaattt ggagggtaga gtaggtaatt aggtatataa gaattgtaaa 25440

gtaatgttta tttggatagt tgattataat tataattttt taataatgta ataggaatat 25500

aaattgttag attttttata ttattgtaat ataaagaaga taattgaagg ttgtgaagat 25560

agtttatgaa atgtaaggaa gaattggata attaagtaat aggaagagta gagaaatagt 25620

gagattttgg gagttataag atttgggtgt ttaaattttg ttaggatttt tttgttattt 25680

ttatttttgt ttttttttt tggttagttt ttttttgttt ttttttatag attagttttt 25740

ttttatatgg atatgaaatg tttttaagtt ttatatttta ggggttttgt tattgaagga 25800

ggattagttt gatatttttt tagatttata ttttaaggaa gatgaattat tagatttaag 25860

taattttgtg taattggaga agatttgtag attttattta atgggttaag ttggatatgt 25920

atttgtggga gggagatatg ttttgtgggt ataatatata tgtaagtaaa ttttaatatt 25980

gtataagata tgtttttgaa aatttatttg tagtttgaaa atttggaagt taaatagtag 26040

attaattgtt gtgagtgtta gaatttagtg taatggtatt ttttgtgatt taggtaattt 26100

tgtataggta gaaagatttt tgaaagtttt atttaggttg gaaagggttt gtgttagagg 26160

ttatgagtgg gatttttag gtatttgttt ttttatttta aggtttgtat atggattgga 26220

ataaaagttt tggtagttgt aatttttaaa ttttttttta agggtattta gggatttaaa 26280

gtagtaggga atgtattttt ttatttttt ttgttttttt ttttattgta gtaagaggta 26340

agtatagttt ttagttttgt ttgtattttt ataggagatt tatgtatagg ttttgggttt 26400

tggttgtagg atagtgtgag gttgttgata gggtgagaga atggtgagaa taatggaata 26460

ggattgaggg agaaggaaga ggaattgttt ttagaattat tatttgggat ggtggtttta 26520

gggagtaaaa aatatttttg gagtttttt aaggggtttt ttggtagagt ggtattattg 26580

ttagatattg ttatggttgt aggtaggatt gattaaagaa tttttgattt tttttgagga 26640

tagggtaaag ttttgaattg tttttagaga ttgtatttag atttatattt tggaattatt 26700

attttggttg tattgtgaat ggtaatgttt agggagtagg attggaggga aggagattat 26760

tatgtggttg ttgtttttgt ttaagtaggg aataatgatg gtggaatgtt tgtattggta 26820

gagaggagag agaggaggga tttggtgaat ttttggaggt attggtagga tttggtaata 26880

tattgaatgt ggagagtaaa tgggtaggga gttagtgata aagtttaggt gtttggtttt 26940

gatgattggt tatgtttgtg ttggttgtgt atagaattta gaggtttagt gtttttatgt 27000

gtagtgttgg tttttatttt gtggtagttt ggtgtggagt tgagatgtgt tttttttatt 27060

ttatgtttat gtttttttag ttagagtaaa gtatgtttgg ataatttagg ttttttttt 27120

ttttttttt ttttttttag agagagaggg tttttgtttt gttatttaga agaagtgtag 27180

tgttgtgatt atagtttatt gtagttttga atttttggtt ttaagtgatt agaggattag 27240

gtgtattttt ttataaattt tttaaatttt ttttagtatt ttatttttta agggatttta 27300

-continued

tagtttttttt tatttggatg gtgttatggt gttttatttt atttttgaga attgttttaa 27360

aattttatat agaaatttgt tattatgtat ttaaatgaga agaaatgttg aatttgtaat 27420

tttaattttt taagataaga gtttgtagtt tgtttttagt agagttaaag aaaaagatat 27480

gatgaatggt ttatatattg aaaattgtag aagaaataat ttaatatgga atgggtgagg 27540

ttgttatagg tggtgtaaaa ataattgtgg tttttgttgt tatttttagt ggtaaaaatt 27600

gtaattgttt ttgtataaat ttaataatat tgtttgaaat ttatattttt tggtggaata 27660

gttttgagat agagtgggaa ttagattatt ggtttataaa atagtatgta gttattgtta 27720

ttttttaatt ttatattttt attatgaggt aatgtttttt ttatttgatt tggagaatgt 27780

ttttattttt tttttatata tttgtgaaaa ttaaatgtta aaggaattaa tataataatt 27840

attgttaaat tatgatgtaa aataatattg tgtttgtaat tagtttaggt agttttattt 27900

ggtttaataa tataaaatta ttaggtttat aatattttag tattttagtg tgattggtta 27960

agaaaaaaat ttatgttatt ataaaaagtt aaattaagta ataattaatt taaattttaa 28020

ggaaatataa agtaaagtat tatgttgtaa tatggaatat taatgtaaat aatttttaa 28080

tagtttatga aatagatttt gaattggtag ttgataaaga aaggttagtt ttggtttaat 28140

ttttttatgt tttaatattt tttttgaagt tttatttaat tttatttgtg ttagtaatag 28200

tattttgaga tggtggtgta tttggaaaat tattattttt tttttgataa ttagtatttt 28260

taatttaatt tttatatatg ttttttggaa atattatatt tatattttga tgatgtttta 28320

aaaataattt taatgtaata attatatatg tgtggtgagt aaattgtgtt ttgtaaaaga 28380

tatttttaaa ttttaatgtt tgtatttggg aatgtgattt tatttggaaa tagggttttt 28440

gtaggtatga ttaagttaaa atgagatttt attggattag gatgggtttt aatataatga 28500

ttgttgtatt taagaggaaa atttgtatag agatatatat atatataaaa gaatgttatg 28560

taatgttagg tatggagaat attatgtgat aatgaaggta gagattggag tgatgtattt 28620

atgagttaag gatggttagt aatttttaga agttggggag agataaagga tgtttttta 28680

ggatttttag agtaagtata aatttgttag tattttgatt taggattttt ttttttaga 28740

attgtgagaa tagattttag ttttaagtta tttagtttgt ggtattttgt tataggagtt 28800

ttaggaaatt aatgtaatat gttatgttgt aaataaatag tagttttaat tggtataaat 28860

aataatttta agtatatttt attattaggt taggtgagta ggaaattgtt tggtagatta 28920

attattaatg ttgtattttt tgaaaagtta aaatattttt atttagtaaa taatatttgt 28980

ttaaaagttt ttatttgtat tgttttaaaa taaagtaaaa attaaaatgg agttagttat 29040

gtatttgaaa aatgtgaaaa aggaaagaaa aatttttag gaaagatttt ttttaaaatg 29100

gttatatatt tgagaaagaa ttttaaaatt taaattattt ggttgtttat tttatttttt 29160

tgtttatatt attagatata ttatgtatga ttttatatat tatgtatgtt atatttaatt 29220

aatatttatt aaatatttaa aatttgttag gttttgagtt aggtattgta gataggtaaa 29280

taagataaat attttttttt tttaaagaga ttattttggt atgaaattta atttgtagaa 29340

gaagtgaaag aggttatttg tagagtttat tatgaaaatt tatttttatt atttgttgta 29400

gttttatttg attttgtgaa attattttag tattatttgg ttttattttg tgtttttgta 29460

ttatattgga gaaaataaaa tttatttatt gtatagataa tataaaatta attaaagtat 29520

gtgaaattaa aaatgtaatt gtttattaaa tttttttag tgtttttat ttattagtat 29580

-continued

ttaaatatat ttttagttgt gtatttatgt tagtataatt aaaatgtggt gtttttattt 29640

ttttatttaa tttataatta ataatgttta ttttttttatt ttttttttt gagatagagt 29700

tttattttgt tatatttagg ttggagtgta gtggtatgat tttggtatat tgtaattttt 29760

gttttttggg tttaagtgat ttttttgttt tagttttttg agtaattggg attataggtg 29820

tttgttatta tgtttggtta atttttgta ttttagtaga gatagggttt tattatgttg 29880

gttaggttgg ttttgaattt ttgattttag gtgatttgtt tattttggtt ttttaaagtg 29940

ttgggattat aggtatgaat tattatgttt ggtttttat tatttttata att 29993

<210> SEQ ID NO 5
<211> LENGTH: 29993
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: chemically treated genomic DNA (Homo sapiens)

<400> SEQUENCE: 5

aattataaaa atgatgaggg gttaggtgtg gtggtttatg tttgtaattt tagtattttg 60

ggaggttgag gtgggtggat tatttgaggt taggagtttg agattagttt gattaatatg 120

gtgaaatttt gtttttatta aaatataaaa aattagttgg gtatggtggt gggtgtttgt 180

aattttaatt atttaggagg ttgaggtagg agaattgttt gaatttagga ggtggaggtt 240

gtagtgtgtt aagattatgt tattgtattt tagtttgggt gtgatagagt gagattttgt 300

tttaaaaaaa aaaagtggaa aagtgaatat tgttgattgt gaattagatg aaaaaataag 360

aatattatat tttagttatg ttaatatgga tgtataatta agaatgtatt tggatattga 420

tgaatgggaa atattaaaaa aaatttgata aataattata tttttggttt tatatatttt 480

aattaatttt atgttgtttg tgtaataagt aaattttatt ttttttaata taatatagaa 540

gtataaagta aaattaaatg atgttaaaat ggttttatag gattaggtaa aattatagta 600

gatagtgaaa atgaattttt atggtagatt ttgtaaatgg ttttttttat ttttttata 660

gattaaattt tatgttaaaa taatttttt aggggaaggg aatgtttgtt ttatttattt 720

atttataata tttagtttaa ggtttagtaa attttaggta tttaataaat attgattgaa 780

tgtaatatat ataatgtatg aaattatata taatgtattt gataatgtaa ataaaaaaat 840

ggaataaata gttagatgat ttaagtttta aaatttttt ttaaatgtat agttatttta 900

ggaaaaattt tttttaaaag attttttttt ttttttttat atttttagg tatataattg 960

attttatttt aatttttgtt ttgttttagg ataatataga tagaaatttt tagataagta 1020

ttatttattg aatgggaata ttttaatttt ttagaaaata taatattgat aattagttta 1080

ttaggtaatt ttttatttat ttgatttaat aataaaatat atttaaaatt attatttata 1140

ttaattagaa ttattgttta tttataatat agtatgttgt attagttttt taggattttt 1200

ataataaagt attataaatt gggtggttta gaattgaaat ttatttttat agttttggag 1260

gaaagaagtt ttaaattaag gtgttggtag atttatgttt gttttggaga ttttagggaa 1320

gtatttttg ttttttttta gttttgggg gttgttggtt atttttggtt tatagatgta 1380

ttattttaat ttttgttttt attgttatat gatgtttttt gtgtttaatg ttatatggta 1440

ttttttttgtg tgtgtgtatg tttttgtata aatttttttt ttaagtataa tagttattgt 1500

attaggattt attttaattt agtaaaattt tattttaatt tgattatatt tgtaaagatt 1560

-continued

ttgtttttaa atgaggttat atttttaaat atagatatta ggatttaaaa gtatttttta 1620

tggaatataa tttatttatt atatatgtat aattattata ttgaaattat ttttaagatg 1680

ttattaaagt atgaatgtaa tatttttaga aagtatatgt aagaattaaa ttaaagatat 1740

tgattgttag gaaagaagtg ataatttttt agatgtatta ttattttaga gtattgttgt 1800

taatgtaagt gaaattaaat aagattttag gaaagatatt ggagtataag agaattaagt 1860

taaaattagt tttttttttgt taattgttag tttagaattt attttatgaa ttgttgggga 1920

gttatttata ttaatgtttt atgttataat ataatatttt gttttatatt ttttgaaat 1980

ttagattaat tgttatttgg tttgattttt tatggtaatg tgaatttttt ttttggttaa 2040

ttatattaag gtattaaaat attatgagtt tagtgatttt atattattga attaagtaaa 2100

attatttagg ttaattgtag atatagtatt attttatatt ataatttagt aatggttgtt 2160

atgttaattt ttttaatatt tagtttttat aaatatatga gaaaaagatg agaatatttt 2220

ttaaattaag taagagaagt attattttat aataaagata taagattaag gaataatagt 2280

aattgtatgt tgttttatga gttaataatt tagtttttgt tttattttaa gattgtttta 2340

ttagaaaata tgaattttaa atagtattat taggtttgtg taaaaataat tgtggttttt 2400

gttattgaaa gtaatagtaa aaattgtaat tatttttgta ttatttgtag taattttatt 2460

tattttatat tagattattt tttttgtagt ttttaatata tagattattt attatatttt 2520

ttttttttaat tttgttagaa gtgagttata aatttttgtt ttagaaaatt agaattataa 2580

atttaatatt tttttttatt taaatatata atagtaagtt tttatataaa gttttaagat 2640

aatttttaga aatggaatga ggtattatgg tgttgtttag gtgggagaga ttgtaaggtt 2700

ttttaaggaa tgagatgttg aaggaggttt gagaagtttg tagagaagta tatttaattt 2760

tttgattatt tgaggttagg agtttaaggt tgtagtgagt tatgattatg atattgtatt 2820

ttttttaggt gatagagtgg agatttttttt tttttagaga aaagaaagaa agaaagaaag 2880

aaatttaggt tgtttaggta tgttttgttt tggttaaaga aatgtgaata tggagtggag 2940

ggggtatatt ttaattttat attaggttgt tgtggaatga ggattgatgt tgtatatgga 3000

aatgttaaat ttttggattt tgtatataat tggtataggt atagttagtt attaaagtta 3060

aatatttagg ttttgttatt gatttttttgt ttatttgttt tttatattta atatattatt 3120

aagttttatt aatattttta aaagtttgtt aaatttttttt ttttttttttt ttttgttagt 3180

ataggtgttt tgttattatt gtttttttgtt tgggtaggag taatagttat gtaatggttt 3240

ttttttttttt agttttgttt tttggatgtt attgtttata gtgtaattag agtgatggtt 3300

ttgaaatata gatttgaatg tagtttttaa aaataattta gagttttatt ttgtttttag 3360

aagaaattag aaatttttta attagtttta tttgtagtta tggtagtgtt tagtggtagt 3420

attgttttgt taaaaggttt tttgaagggg ttttaggggt gttttttgtt ttttgggatt 3480

attgttttaa gtagtagttt tggggatgat ttttttttttt ttttttttag ttttgtttta 3540

ttgtttttgt tatttttttg ttttgttagt agttttatat tgttttgtag ttagagtttg 3600

ggatttgtat atgagttttt tgtgagggta taagtagggt tgagggttat atttgttttt 3660

tgttgtagtg aaaaggaaaa taagggaaag tgggggagta tatttttttat tgttttaaat 3720

ttttagatat ttttgggaaa agatttaaaa attgtagttg ttaaaatttt tgttttagtt 3780

tatgtgtaga ttttgggatg gggaaatagg tgtttgaaga gttttattta tgatttttga 3840

-continued

tatagatttt ttttaatttg ggtggggttt ttaagaattt ttttgtttat gtagagttgt 3900

ttgggttata gaaagtgtta ttatattaaa ttttaatatt tataatagtt ggtttattgt 3960

ttggttttta gatttttaaa ttgtaagtaa atttttagaa gtgtattttg tataatatta 4020

aggtttgttt gtatgtgtat tatatttata aaatatattt tttttttata gatgtatatt 4080

tagtttaatt tattaggtag ggtttgtaag ttttttttag ttgtatgaaa ttatttggat 4140

ttaatgattt attttttta ggatatgagt ttgggaaaat attaggttag ttttttttta 4200

gtggtaaaat ttttaggata taaaatttgg aaatgtttta tatttatgtg gaaaaaagtt 4260

ggtttgtagg gaaagataga agagaattga ttgagaaaga ggagtaaaaa tgaagatggt 4320

aagaaagttt tggtagaatt taagtattta gattttgtga tttttaaagt tttattgttt 4380

ttttgttttt tttgttgttt agttgtttaa tttttttttg tattttatga gttatttta 4440

tggtttttaa ttatttttt tatattgtaa tggtatagga agtttaatag tttatatttt 4500

tgttatatta ttgagaagtt atagttgtga ttaattgttt agatgggtat tgttttataa 4560

tttttgtgta tttagttatt tattttgttt tttaaatttt attattgggt ttgtgaagtt 4620

ggattaggta ttggttggtt ttaattattt gggaagtatg ttagagagga aagttaatgg 4680

tataaagaat atttagtttt aattttatgg ttaattgttt agagatatgg agatgtatat 4740

ttttggatta tgtatatgat ttttttttt ggttgtagaa aagaaaaata gttgaggtgt 4800

ttaagaaata gatttggatg aatagtaatg agtattttgt ttgattttaa gttttttaaa 4860

tggagttaat ttataaatta gatgttgagt tatttttgag ttgtttataa agatagaata 4920

tggattttgt aaaatggttt atttaattta aaatgtttaa aaaatgataa tggggtttat 4980

atttttatg ttaaaagtta taataagttt tattttgtga gaattatttg ggttatttgt 5040

tataagttta tttgatggta agtttttttg gaattataaa ttatgattat ttgatttttg 5100

ataagtaggt ttaaataagg tgttttattt tgtttgtgtt tgttaattgg tgatggttgt 5160

ttagatagtt tagaaaatat agaagtaatt ttgagtgaat tgttttaggg agatatgtga 5220

aagaattttt agtttaagta attttattat ttttatggat attaaggtag attttgtaga 5280

ttaatttttt tttttttgtg ttatttttat tgttttttt tattagtatt gagaaattat 5340

tttgttgagt attttgtata tattgtaatg gtttatttag ttggtttttt tgtagtttgt 5400

tagaagagaa tatatttgaa gttttttta gaagtaattt gttttttttt tagttataaa 5460

ttttttttt gtgtaatttg gaaatttttt ttgattaaga gtatatgatt atatatattg 5520

tttaatatat atttgatgat tttatgatta ttttgaggaa aatgtgttgt attttgagag 5580

aatttaagaa tagatttttt aagtttagta ttgaattttt gaaattttag aaatgtaaat 5640

gatagttttg ttaaagggaa tgtaagttta gtgattttaa ataaagattg gaataaaata 5700

gattagtttg tgaaaatttg tttttattat tggataaatg tatgattttg gaaaagttaa 5760

ttaatatttt taaatattat tgtttttatt tataaaatag ggtttttaat aatatttgta 5820

ttatattttg ttatgattaa ataaaaaata tattttaaaa atatagatta ttttgtttgt 5880

tagtgagagt ggaagaaaat ttgtttttat attttgtttt ttatatattg aattaaaata 5940

ttttaatatt ttttgttgta tagtagagta gtatttgtaa tttaaataaa tgtaattatt 6000

tttaattttg tttaattaag agtattatat taattattga tttttaggag ttatattaat 6060

taattatatt tagatagtag ttaaaagtta ttagtttttt attaattttt tgaatttagg 6120

-continued

taagtttgta aaaatgaata atttttgat taagttatta gtatttgtta ttttttagtt 6180

gttatttgt ataatattaa agttgtgatt ggtgttttta gtgttgagag attgatataa 6240

ttaataaata attatagatg gatatgtgaa agaaaaggga ataggaaagt attagtattt 6300

agtattaata ggatgttaag atattttaag ttggtaaaag taggagaata agtataaaat 6360

ataattaaga taattggtta ttataatatt ttgataattt taaaagtttt aaaattttta 6420

aaataaagaa tggtatatgt gatattattg taataatata tttattttag atttttgata 6480

aataagatga ataagaagtg gagttaagtg gtaatttaaa ggttgttgtg atgttttaag 6540

tgagaagtta agagaattta gattgatatg gggtggaaaa aggtttggag aagtggatat 6600

gagggataat atgagagata tattaataag ataaaggaat tatatgaata taattgataa 6660

ggtaaatgtg atttttaggt attatatttt aatttatgag aaattgatga tatggaaaag 6720

ttaagaggaa aaggtatgtt tatatttgtg ttgttattgt tttgttgtta ttatttttt 6780

ttatggtgat gggtaggaag taaaagtaag aggtaattta tagaggaagg agtttaaaaa 6840

gtttgttata tttattggag gggggataga aaaatttggt ttattattag atttagtaaa 6900

gaagtttgtt ttatgatgga gtttttggga agagaataaa agaaaataat tttttgagtg 6960

agataaatta aaattttagt tttgtaggat gtaaatttta aatgtttatt gtatttgtgg 7020

tatggaaatt ttaagataaa aaaataaaat aaaataaaga ttgatttaga ggtttgaaat 7080

ttttagattt taggggaatg taaaattgtt ttatagagta taaagggttt taagagagaa 7140

atttattaaa agttataaat tataggtgag gtgtggtggt ttatgtttgt aattttagta 7200

tattgggagg tttaggtagg tagattattt gaggttagga gtttgaggtt agtttggtta 7260

atatggtgaa attttttttt tattaaaaat ataaaagtta gttgggtatg gtggtgggta 7320

tttgtgattt tagttatttg ggaggttgag gtaggagaat tgtttgaatt taggaggtag 7380

agattgtagt aagttaagat tgtgttattg tattttagtt tgggtaatag agtataattt 7440

tattttaaaa agaaaaaaaa aattataagt tatataaaga aataaaatat agtagagagt 7500

agttataata ataaattagg aatttaagaa tgtgaaatta tagagtaatg tggaaaaaat 7560

tagataggta tatttaaaat agatataaaa gaaatagaaa taaaattgaa aagttatata 7620

tgaaaaaaat atatatataa atttggaaga gaattataaa aatgttttta aaaatatatt 7680

tattgaattt ttttataaga aataaattaa atagtaaatt agatatattt aaagaaataa 7740

tttatgaatt ataaggtaaa tttggtaaat tttaagaagt gtgtgtttat ttttataaaa 7800

gaaaataatg ataataaaaa taataataaa attataatta tatttttttа aataattttt 7860

ttataatatt aatttttag gaattaaggt gtgatatttg tgagttatat tttttattat 7920

ggggagggag aagttttaaa tataataaag taaatatgag agttatataa tatggtaata 7980

aatttgattt atttaatgtt aagttattga tttgaattgt gtttatagtg aatattaatt 8040

aagaatgtaa ttaaataaga tgttgtttgt ttttttaaa tttgggatat ttgaggaaat 8100

tttattgatt tagattttta attgaggtat ttaggaaagt ttgagtattt ttttttttt 8160

taataaattt ttttgtagta tttgaatata aaaattattt tattgatttt gtaattgaat 8220

tagaagtagg gttatttta ttaaaaattg tttttatagt ttttaaaaat ttaaagattt 8280

ttaaaagggt tgaagttgta agaatttaga aaataatgta ttaaatatat ttggtgtttt 8340

gagttaattt aatgttatgt ttttttgtta tgtgttttgt ttttatatgt atggtaggtt 8400

-continued

gttagatatt aggagtatgt tgttattttt gttattttta attttttttt tttaagttaa 8460

gtatggtgtt tttggttaat aagattattt ttttaattta aaaaaaaatt tttgtttttt 8520

aatagggaat tataagggaa tttagttagt tataaaagta taattgttta ggaaatgtat 8580

taagtggaag gtagatataa attaggttat agtgtattat tatttttaaa ttatttaatt 8640

ataaattgat gtattaatat agattaatat taatgtatat taaagtatta tatttatatt 8700

taaataaaaa tgtaaaggaa aattaattag ttgaaataga aattaatgat gataattttt 8760

aaagtagtaa gaatgtattt ttgagaaaga ttttagggtt ttgtttgttt gtttgttgga 8820

gattaaatta ttttttagta aaattgaagg gggaaaaaag attaatgttt aagttatagg 8880

aagatgtggt ttgtttggta tttggatgtt ttttgtagaa tttataagtt tatttttag 8940

gggtttaaat gtttttggat ttaaaaagtt tttgggaaag aatttttttta agggaagaga 9000

tggagagtta tataagtaga aattaagttt gagttttaag ttttggggtt gtaagaaaag 9060

aataagagaa taagttaaaa aagttgttta ttaaattttt tatagaaggg gaggagggta 9120

ggtgttatta ttatgtattt tgaagttttt gttatagggt attggattga gggaagttgt 9180

tagatatttt ttttatttta aattatttgt aattttagta gtttgattaa aaaatataat 9240

tttgtaagtt gatggaaaga aatgaatttg gtgaagaaag tgtgggaaaa tattggttaa 9300

attagtaaga aaaagattgt aataaaaaag aatttaaagt gtgagaattg aaattaggaa 9360

gatggtagaa tgagaagtag aataatttgt ttttttttt ttgattgaat taaatgtaaa 9420

gatattaggt ggtagagatg tggatattag tttttatagt atttttaag agtgttatgg 9480

ttttttttg tttttatttt atgttttagg tatttttttt agggttttag ttaagttttt 9540

gtttaagtat ttgaataaat tagatttatt tttgaattag ttttaggtgt tgtttataga 9600

tattgttatg gtttatattt ggataattta gtttggtttt tttaatgttt taatttttt 9660

tttatgattg tgaaaaaaat gtaatgagtt tttttgaaga aatatttaag atatataaat 9720

ttagaatatt tatagttttt aaattaagat tgtattatat aaaaatattg attttttaa 9780

aatgtatgaa tgagtgaatg agtgtataaa tagttaatag tgtataaatg tatgtatgaa 9840

tgaatgaatg agagtataaa taagtaaata aggaaatata tttttaaagt atatgttagg 9900

aaatggataa attatgtttt atgaagtttt agattgaatt aggtaaatag aattgtagat 9960

ttttatgttt gttatgattt agtgtatagt tttgggaaaa ttttgtaatt tttttatatt 10020

ttagtttttt attttataaa aggagtatat tgtttgaaat ttaatttttt tatagtagtg 10080

agagttttga tttaatgttt ataaaatgtt ttttagtttt tttgattaat aggtaaaata 10140

ttgtggtagt ttgtataata tatttttttaa aaaggataaa ggtaattggt tgaatttgat 10200

gtaaaataat attttattta agaaataaat attagttata tttattattt ttttatttta 10260

tttgttaagt aataaaaatt atttttaata gaaaagtatt aaattaatat ttaagttagt 10320

tgaagtgtta ttattatagg ttttatttat gttgggggaa ggagagttgt tttgtttttt 10380

taggttattt tatatgttta tatggtaaag ttaggaatgt tgggtatagt tttgaaggtt 10440

tttagtgtat ttattggttt ttggtatttt aatgtatttt atttttataa ttataaaagt 10500

atttttgagt gtttttggaa atatataata aatttttaat taatttaaga gtgttttaat 10560

tgtatatatg aaatttttttg ggaggaaaat attagttatt ttatatatat atagtattta 10620

agttagtaag agagtttttt ttgaaaggta taaagtagtt tttttatagt aatataattt 10680

-continued

```
ttagttttat atgtgtaagt tttattgaat tttttagaat aataggaaat tttgtaggaa  10740
aaaaaatttt ataaaaattg atagttatat ttatttaatg taagaggaat atattagtta  10800
ttttgagttg gattattttt taaaggtatt agataattat tgaagattaa ttaaaatttt  10860
aattaaaaat tataagaatt gaatattttg gagatagaat tttttatata taaaaattga  10920
ataataaaat taattgatta atatatttta ttttaaaatt gtatttatta gattatttta  10980
gttagtattt gagtagtaat tttttggatt ttgatgtatt aatttttatt tttatattaa  11040
atagattata ataagtaata gatttataaa ttagttattg atatgatata attgatattg  11100
tgatatgtat aataatattt attaatatat ttataatgga gtaatgttat ttattattta  11160
tataatgttt atatttttta tttttatgtt aaaaagttat tgtaatatat gtaaggtgtt  11220
tagaattgtg tttggtatag agtaggtttt tattagaata ataatatatt atttttttata  11280
agtttttatt aattatatga gagttagaat tatttttttt aataattttg gtaaatattt  11340
gatggtatta agtaaaaaaa aatgtagtga atataatatt attgatttat tttttttaa  11400
tatttgggtt tatgaattat aagtaaatat aatatgtatt tataaaaatg aatattaaat  11460
atttttaagt gagaaattaa ttatataaat tatatgtata tattaaatat atattgttaa  11520
tagaattatt tattttaatt atagtggagt attttgatta ttatatgagt tttaaggaat  11580
ttattattat aattattttt tttaattaga gtataaataa ttataaggtt taattaagtt  11640
gaaatttttg tatttggttt ttgtgagaaa gatgagaata ttattttaaa gatgtaagat  11700
ttataaattt ttttaaaaag tattttggta ttttttatgg aaaatgtata tatatattta  11760
tttattaata ttgtatttag agttttattt atagatattt ttggtagatg tatgaagaga  11820
tataaaaatt attagtgtaa tatatttagt aatagtaaaa atatggaagt aatgtaaata  11880
tatattaaga ggggaatagt gaaattatga aatatttgta tatattaaat agaagtattt  11940
aaaattatat gtattgatgt ggaaaaatat ttaagattta gtagattaaa agtaagtttt  12000
aaggaaatta atgattttat tggagttaaa aatgtaaaat gaaaataaat tatattttta  12060
tatagttatt atatttatat atatatagaa aaaaaaatgt ttgaaaagat gtatatttaa  12120
gtataataat ttattttgat ttgtagagaa taagaatgag taaatttatt ttttatttta  12180
aatatatttg tatttttaaa atttttttaag aaagtataat atatgtatag taaaataaaa  12240
tatataaatt aaaagttgat tattgatgaa tttttattga ataaatattt ttatatgatt  12300
agtaattaaa ttaagataaa agatataatt agtattttaa agtttttttt ttgttttttt  12360
tggttatttt ttttaaaagt aatttttatt atttttgttt taatatgtta tataaagtat  12420
gatttatttg tatttttttaa gtaatagatt aaaaaaaaaa atttgtttta gtagtatgaa  12480
ttttttttttt tgaatattaa atatatttag tattttattt taagatatta atttaaatta  12540
taatttgagt ttttatagtg ggttaggtat tgaattaaat gtttttttttg tatgaattta  12600
attatttttt ataattttat gaggtgagta ttgttattat tttttatgta tatatgttgg  12660
aattatggtt tatagttatt tagaaaatta tatgattaaa gttatattgt tgatgagtgg  12720
tagagtttgg atatatataa aggtagtttg attttagtat ttttaatttt ttattagaaa  12780
taaataaata aaaataaagt ggtaagatgt agagttggga gtatgaagtg tttttagtat  12840
gaagtttttt ttttttgttt tgttttgagt aaatgattta aggttttaag gttatataat  12900
ataatatttt atttgtaaaa tggaagggtt aggttagatt tttttttga tttgattatt  12960
```

-continued

tgatgttgat aaataaagtg atttattgaa atatagagta aatatgtttt gttatgatgt 13020

agtgtaatta tgtattgtat tatgttaaaa aatgatttat ataagaaaat aaaattataa 13080

tttttgaaat tattggaaat aataatttta atttaatgtt atttttggat ataggaagat 13140

ttttttttt aaataagtaa attggtggtt tttgaagggt tttgtttatt tttgtatgga 13200

aaatgtttaa aaataaatta ttaaaaatta aataaaattt ttttatttt ttaattgtgt 13260

ttgaaaaaaa agatgtaatt ttttttagta aaaggttatg tatattttta atagatatat 13320

ttgggatttt tggtttttat attttgaaat attgatataa ataaatatag aatatgtttt 13380

tttttaaatg aaatttataa ttttgagggt taaaaatttt ttaaaataat atgaaattga 13440

ttttagtaga aggtaattaa agagattaag gtttgttttt ggagaaaatt agaattatga 13500

agattataaa ttttaatgag aaatttgttt tttaaatgtt agtttattga aaatattaat 13560

gtatgttgga tatatttgtt tttttattag aaaattagta atattttggt ataaatgagg 13620

ttttaaataa gttggagtag aaaataatta ataaattagt ttttatgatt ataagtaaaa 13680

gagaattagt tttttaaaaa gttaatagga tttatttttt gtagatgttt ggtttgggat 13740

ttaatgtttt ttaaagatat gtatggaata gtgtagttat ttaattttta ttaatatttt 13800

taggtgttga ggttatggag ggaaaaagat atgatttttt ttttttgtga gtttatagtt 13860

tttttagaag atagatatat ataatgagtt ttaatataat ttgttgttat attaatagtt 13920

taaaaaaagt gttatggtat tatatagaga aaagaattaa ttttgtttga gttttaagga 13980

aggttttata gaggaggtga tatttgagtt aagttttgaa agatgagtag attattatta 14040

ttattattat tttgtttagg taggtagtaa aagagtatta ggtaagtagg ttaagggagt 14100

tggggttatg gaggataagg gtggtgagaa tatgttaggg agaaaaaagg aagtgattga 14160

tggtattata agaaataaaa tgtttataag taagttttat tatattagga tttattatat 14220

aaagaattaa tttgagaaag gagatatgtg atttgaatat tttgtggggt tttttgattt 14280

atgaatttag gaagtgttta tttggaaaaa tagaattttt gggtttttgg ttgtttttta 14340

gtaattaaag ggttattagg ttttttgttt aatatttggt ataaagtgtt tggatggatt 14400

tgtggtttgt tgtgtttttt ttggattttt tgtttatgaa tgaagtagaa ttttttttta 14460

ttatttgtga tttaaatttt tgttttaaat tttttgtaat atttttgag aatggttttt 14520

atttgttatt gggttgttta gtatttattt tatttttttg ttaataagga gttatgtagg 14580

gtttttttttt atttaggaaa tagaaaaata ttttggttaa atatagtatg ttgtatttgt 14640

agtttttttgt tatttaagta tatattttaa aattaaagta taaattaaag gaagtgagag 14700

agagagagtg agtgtgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtt 14760

tttaagtatt tttatttttgt ttatttaggt attaattagt aaatgatgtg aaatttttag 14820

gttagtttga ttgtttttat gattatttat ttaatataat taaagtataa ttattaaatt 14880

agataatgta taagtgtgga attttaagta tttttgtaat ttttaataag ataaaaatta 14940

tttattgttt agaattttta agaaaattag tttaattata tatttataat attaatgttt 15000

tatattttag aataattttg aaatttaatt taaaatttat atgggaaaag ttatttttttt 15060

ttattttatg tgaatattat tgtttttatt ttgtttaagt gatttttgaa atgtgtttta 15120

tatatgttta aattatgtaa gggtggtgtt taaataatag ttaaatttat ttatattaat 15180

tatatattat tttatagata aaatgtattg gttaatatta aatttataga atgagaattt 15240

-continued

aaaagtttgg atttgagaat ttataaaaga gagtttttat atgtaatata tttatttta 15300

ttttttttta gttgtataaa tgttattaat ttaagatttt gaatttgtgg ttataattga 15360

aagttatata taatttttt gaaaattttt tattataaat aaataattta atttattaag 15420

tattttaatt tttagtagtt gtgattatta gtaattttat ttttttaat attatagatt 15480

aaataaaata aaagtttttt attattgaat atttatttat agaaatggaa aggtaatggt 15540

tattatttaa aaataaattt aaattattta ttgataaata attggaagaa aagatatata 15600

gatgatttaa ataaatattt atttttata tatttttttt taggtatttt gttattttta 15660

tttatatatt atttttagaa aaggaaaata tgttggagat ggttaatttg tgtgaagtgt 15720

atttgtaaat tttttttgtg taaagttaat ttattaatat agattaatga gaattgtttg 15780

attttatgtt taaatttggg aggaaggtat taggattttt tatatttgat tggaaataga 15840

ataatttttt tatgtagagt tttgggagtt ttttgattat attaaatgat attaagattt 15900

gtttaggtta tgggttttta agaagaggta attttgtttt tagagattat ttgataatgt 15960

ttggagatat tattattatt aggggaggga gggtttttgg tatttagtga tgttattaag 16020

tattttatta ggtataggat agtatttttt gaataaatta tttagtttaa aatgttaata 16080

attagagttt gaaaaatttt tatttaaata tattgaagaa tttaggtata atgttattat 16140

ttttaaaatt atatagatat atttggtagt agttttagat aagttgtaaa aatgagattt 16200

tgataattaa tattttaatt ttttttttt ttttagtttt attttgttat atagttatta 16260

tatttgaatt taagtgataa attttgtaag tagaggtatt aattattttt tattgttttt 16320

ttaaaggtaa gaattatagt aatatgtaaa gttatttttt aaaaagttta tattgagttt 16380

attaggttta ttgattattg aaaattatat aaatttttat aattggtatt ttatggttga 16440

aaaaaaagta attgaggaaa tttaaatatt ggttataaag tattattatt atataatgtg 16500

tttataattg tttaaagtag ttaaggaatt atatagatta aagttttaaa ataatttaga 16560

ttattgtatg tggaaatatt tattaaaaga aatattattg tataatgtaa atggttaatg 16620

ttaattgttg agtatttata gtgttttttt ttaaggtttt aaatataatt ttagatttgt 16680

taagatgatt tatagtattt aatttatatt tggattattg gatattttat ttatggttat 16740

taatttatag ttttttaaaa aagattttta gttttaaata tttttggaat atttgggggt 16800

aatttaatga aagatattta attatagtaa taataattga tatttatatt aagtattatt 16860

ttaaatatat ttatatatat ttatttattg aatttttatt agaattttat gaggtatgtt 16920

ttgttatata tgagaaattg aagtaaaatt tgttttagta atgtgtttga tgttataaat 16980

ttattaatgt tagagttgag gtttgaattt aagtgtttgg ttttatattt ttttttttt 17040

ttatttattt atttattttt gagatggagt tttattttgt tgtttaggtt ggagtgtaat 17100

ggtgtaattt tagtttattg taatttttgt tttttgggtt taagtgattt ttttgtttta 17160

gtttttgag tagttgggat tataggtgtt tattattatg tttagttaat ttttgtattt 17220

ttagtagaga tggggttttg ttattttggt taggttggtt ttgaattttt gattttaggt 17280

gatttatttg ttttggtttt ttaaagtgtt gggattataa gtgtgagtta ttgtgtttag 17340

tttttatatt ttttttattt atttattgtg ttataagaat tgagtttttt agattttaag 17400

tattatgaaa ttattgtatt tttagagatt tttaggattt ttggtttatt tattagatta 17460

ttgatttttt agtggtaaga aataaatata tttttatatt tatgtatata tatatataat 17520

-continued

ttaagtttgg atttatagtt gtaattttat tgggtttata ttaaaatttt ttgaggttgt 17580

tattgaggta gttttatttt gtaatagtta ttgtttttt tattgttttt agttttaagg 17640

aggggatgtt tagtgttata atttttgttt ttatttttag tatttttatt tatttattta 17700

atgggaatat aataattatt tattgattat atgtttagta gtgtattgtg tattagtgat 17760

atagaaatga taaatatagt taatttttt agtaaatttt tagtttaaag taggatttag 17820

attaatgtaa tttattatat tttaatgtgg taagggttat gttaaatgta gaagttggat 17880

gttttgggag taataagaaa gaaatgttta atttagattt tggaggttag aggaatttat 17940

tagaggagtt aatattttgt attttaagta ttgaaaaagt agtagaaaag atatttgtag 18000

ggggagaggg gtgatgtttt ttagagaaaa agtattttat gtataaagaa atgaagtgga 18060

aatgtttaag gtgtatttga gaattgtagt tttttatata gttggaaaag agagttgttg 18120

tgagtgggga gtgagagatg agaagagtaa tttgagtaag gtttttagta tgaagggtta 18180

tttttttgt agagaatttt ggaatttgtt ttataggtag taggtaaatt ttttaaggaa 18240

ttttaagttt aggagggatt ttataaaaat tatgaataaa tgtggttgaa tattttaggg 18300

atgtgagaga gtgttagaga tagttttaat atagtttaga atgggttttt aggagaggta 18360

ttaagatatt ataattatat ttgttatgat aatattaata tgaataagtg tgtataggat 18420

aattgtttat ttaaagttaa tttgaatatt tgaggattag atttgtgatt ttggttataa 18480

tgatttttt tttaaatatt tttgggaatg gtatgtaaaa atataagaat ataataaaag 18540

ttttaaagtt tttaaagtta tgtagagtag aaaagaaatt tatttatttt attttaattt 18600

tgtaagtttt tagtaatatt gataaggaaa aagtatttaa taagtattta ttaagtattt 18660

ataattttgt taattatttt tatgaatgaa atttttaaaa attaaagtta ataattaagg 18720

gaaaaaatta gatttaaatt aaaatttttt atgagttaat taagaaaaat aatagtatta 18780

ttattttatt aattaaagtt aatgtaaaaa taaataaata aaaaaaaaaa taaaataaaa 18840

tagtagaatt gttgtttttg ataggtttat tgaggtttgt atgtttaagt tatttattag 18900

aagtattgtt ttagattatt ggaaaatttg taaaaggaaa tttgaatgtg atttatttat 18960

aatgttatta aatttagtaa gaatgatttg agttataatt gtatatgaat atatatttgt 19020

atttaaatgt aagagaaaag aattaggtaa tgtagagatt tgttgatttt tttaagttat 19080

tattattttt aatatattga aaatattaag ttagaaatat ttaatttttt aattagaata 19140

tttggttttt tttaaatttt ttaatattag ggagtttaaa gttagaggtg aaatgtaatg 19200

aatagattat tttgttgggt atatgtagag gtttgtgaat tgtatatatg attttgtgta 19260

taatgagttt ttttggtatg tggtatattt atttgattag ggatagaatt ttaaattttg 19320

tattgttgtt tttgtgagta gaattaaatt taatggtata tgaatgtaaa tttttatgat 19380

ttaattgtag ttttttataa gaaatgtttt ttttatttta aaaagaaagg gggaaaggat 19440

aatagaaata gtagtaataa tttatgtgaa aatttaaagt tatgttaagt taggttattg 19500

ttggtgtttt tttttatagt ttttaaagga ggaaagatgt tttaattaat aaagttaatt 19560

tttgtaggta taagtttttt gtgttttatt tagatttgta attatatata gggaatattt 19620

tgattagttt taatttttt agtgttaaat tgaatttgaa gattggttaa tatggatatt 19680

taagttatag aagggagagt agatatattt ttttgttgat atgttatatt gggagatatt 19740

ttattgtatt tgatagtttg ttttttagtg atgttatatg aatatgttag ggtaggtatt 19800

-continued

ttgaattaat tttttttgtt agaagatttg aatttttttg tagtagtttt tatatattga 19860

agatatttag agagaatttt agaggtggtg gttattgtga gagggtattg ttggtgagtt 19920

atttttaggt tatttatatt ttgttttatt tagagtagtg gtatattttt ttattgggta 19980

tattattagt ttttatttaa atgaaaggat ttgtggttga gtatataagt aaataaaaaa 20040

atgaaaatta ttgttttagt ggaaaaatat tataaaattg taaaataggt tattaatagt 20100

aaaagtattt atttatagaa aattttatat atgtgataat tagataaatt gaatgtattt 20160

ttaaaatatg ggtagtttta ttataattta aatttgtttt tagaagttta ttatgtattt 20220

tttaattttt taagttttat tagttggttt aatataaatg tgtagatttg agatttaaaa 20280

ttagtattaa aataataaaa aataaaaata aagaaaatat aaagttgata atatttaatt 20340

aatttggata aggtttgaag agtaaaggaa gattttatat gttagttaaa tatttaaaag 20400

atttggttat aagtttttga taattgggaa atattataaa atatagtatt tttttaaatt 20460

tagttatttg ttgtttagtt tttatgattg tggttattta ggttatattt ttgtttattg 20520

ttatttaata tgtttttttа gatatttaat ttttttttтt tgattttaat attttgtttg 20580

gttttgtttt aaataatatt gtttatgaag ttataggttt aatataaaat ttaataaatt 20640

tttttaatat ttagttattt ttatataaaa tataagtatt attaaaataa aatgtttagg 20700

tattatttaa aaatggtggt atatgattga tggaagtaag agtttagtat atgtttaagg 20760

ataagggtgt tggttttata ttggggagtt aggattttgg ttttattgtg ttgtatgatt 20820

gggttttagt ttttttattt gtaaaatagg aataataaaa gtatttaatt tatggtatag 20880

ttaagaagat taaaattagt taatttatat aaatgtttaa aatagtgttt tgtatgaaat 20940

ttttgagtaa atgttagtta tgatgttatg atggtgatag tgattaatat tatagtaata 21000

atgatagtta aattgtaatt atttaaagat tatagtgatt atttttattt aaaaatatat 21060

tttaggatat aaatatattt aagaaaatat gtttttatat ttgatattta tattaatata 21120

tgttttggtg atataaaagt tattttatat tggtttaatg taattttatt ttgaaaatat 21180

ttatgtgata ataggaatta gattttgttt aaaaattata atttaaataa attatttgtg 21240

tagaaaaaaa aaaattaaaa aattttaata tgatagaata tataggtttt ttttttattg 21300

tttaaaagta tgttaaaata aatttattta ttgtatgtaa ataaaagttt taaaaatatg 21360

aatatattgt attaaaatat tttatattgg aaaaaaatga gaatttttgg tattttagaa 21420

tggtgttttt ttttattatt tatgtattga aattttaggg taagaaaagg gttagattaa 21480

ttttgaaagg ttttaaatat taaattatat tattgtgtta gtattttaag attaattagt 21540

atttttttaat gataaaaaag gttatagtga gatttagagg tgtatttttt gaaattgttt 21600

taaattatta tataaatgtg gttttgataa tataaaatgg tttttatttt aaattaatta 21660

gttttatttt ttttttgga ggtgtgaaag gggttagggg agtaagaata gtagtgttta 21720

tttttagtat ttttttatta ataaaaagat ggatggagaa atattaagaa tgataaattt 21780

taagtgtaaa atgatatatt attaaagtat atattaatgt atagttttat aagtaatatt 21840

gagtgatagt aatgtttata aagtttttaa atttttttaaa agtaattaaa gttgtttgta 21900

tttttttttga atgtgtaatt tttgtttatt tttttttatt taagtttaag ttaaatgatg 21960

ttaagtttgg ttttaaaaat gatttaatga ttatgtagtg tttttttttat taaaatatat 22020

ttaattataa gatgtagtat tatatatatt gtattttatt tatttttagg gtaaatgttt 22080

-continued

gaaatagtat gttatttttt agtttagtag gaaaaattta tgagagaagt aggtatattg 22140

taatgttatt ttatttttt ggtgttgatt ttttttattt agtaattatt gtaataaaat 22200

tttttaattg tatttttgaa tataattagt ttaagatatt ttatagggag gtatgttata 22260

agagtttagt atttagtata tgtaagaata atatttattt agaaaaagaa attgttaaga 22320

ttatattttt attgaaaaaa gaagaaggtg ttttgataga ggtttgttta gttttttaat 22380

gagaggattt aaaatgtttt ttatttttga tagtatattt ttttatttat tttttaaata 22440

agtgatttga aatatttata attagatagt atttaatttt aggttttaag tgttttaata 22500

gttttatgaa atatgattag tagttgtaat atagaattat ggtgaataaa ttttttataa 22560

agaggggttt gtttttatag aatgtttagt gtttttgtat ttatttaata gaaaaaaatg 22620

ataatgagaa agtataatat atatttgtat ttttaaatta tttaatattt aattaagtag 22680

tgaaaaagat tattattttt ttttttgaaa tttgtataaa aattgtgagt ttttgtgggg 22740

taaaataagt ttgattattt gaaagttaga atgtgaattt tttaatattt taatttttta 22800

taataatagt tattgttttt ttgttatttt attttttaa aaattttaaa aaatttaatt 22860

ttagtgtata aaatgttaaa atgaaatatt aagttatata aatatatttt tagatatgat 22920

attagttatt tagagtttgt aatagagatt gaaaatatga aattggatat tttatttttt 22980

gtggttatgt aaaatttatt tatattttaa agtataatat aaataaaaag gaaatttta 23040

gaaatggagt atttgttttt ttttttttt ttttaattta aagagtttta ttaaaagaaa 23100

aaaataaatt atagtaaaat gataagtaat ttatttgtta gttatttgtt aaaaaagaaa 23160

tttttatgta attttttttt ttagttttta gaaatttttt gaaatttagt atttattttt 23220

tttaataatt aataattatt attttttttt atataattat ttttttttat ataaatttta 23280

agattttaaa aattttaaaa tttaagttta ttatttaaaa gaagtgttaa ttgtgagtat 23340

atatatatat gtgagatgtt ttattttgga aatgagaaat ttaattttaa atatttaata 23400

aatattaata aaaagaaaat aattgagtaa attttatttt agttataatt gtgaattaat 23460

tggtagttat aatggttttt aaatattgaa atatattatt tttaggaaat ttataaagga 23520

aaataggttt ttgaaaatta tattgattgt agatttttat ttattatgaa agataagtat 23580

atattattaa taattagaga aaggagatat ttttaataaa tttaattatg ttgtttttaa 23640

agttgtagtt gttaaaggtt ttaatatttt tgtatagtta tttttaaata tgggtttaat 23700

tttgaatgat ttataatttt taattaggtg gaataaatta tttttatggt ttataataat 23760

aaatgtgggt gtagttatgt ttgtttttga tattgttgtt ataaaaatta gattttggta 23820

gttttgtttt atataaaata atttgttttt tgttattatt gtttttttgtt ttttgggata 23880

atgttatttt taaaaatata taaatgtttt tgaatgtagt tttttaaaat gttttgtatt 23940

atgtattaaa tatttttttgg ggggatttttt tttttatttt ttttagtttt tggtttttta 24000

ggtttaagtt tgttaataat attttaaaag ataaaaaaaa aaaaaaaggt attgattttg 24060

tttaaaatta aattgtattt gggaaaggaa tttaagtata attttttttt taaggtttta 24120

aaaataaaaa taatgtaata aataaaaaaa ttattgttaa tttttaattt tgttagttat 24180

ttatgtgtaa gtgaaatttt agaagttgtt gatggggagt ttgtggttgt taaatgtttt 24240

taagattttt atttaaattt tgtaggagtt tgtattgaaa ttgataaata tttgagggtt 24300

tataaagtgg ttatagatga gaagaggaat aaaatgttat attttaggag gtgtattttg 24360

-continued

ttttaataat tatgttgatt ttagggtgtt aagttaaaag gggtaaggga gaaagaaaat 24420

tgttaagtat gagtttttaa tgtgaaattt aatttgttgg ttttttttagg tattagattt 24480

tggaagtgtt tgtaatgata gttaggaaag taaattaatg gttaatggtt tattgggttt 24540

gaatattaga gttttgttat ttttttatag attaagtata taatatatat atatatatat 24600

atatatatat atgaagttgt tttatgtgga attgttttgg aattaaaata tatgtttggg 24660

tgttattttg tattttggta gaaagggtag agggatttgt attttttttt tttgttttta 24720

ggttaagatt tatgaatgat tttttaggtt tgtttgggat ggtggggaga ggtgtaggag 24780

atgtggtgag agaggatagt ttttatatta tttttttgta tatattttta ttttaggtgt 24840

aaatttgttg tttttatagg aaggagaaag aggggtggtg gtgaagggaa atattatttt 24900

tttaagtagg gaagagatga taaaaattaa attgtgtttt ttgtttttgt tttagttttg 24960

ttattggatt tggtttgtaa atttttttaat tgttttgagt tgtttagaat tttgtaaata 25020

aagggagaaa atttttttaa gtgttattaa atgaaagttt gggttgttgt gtggtgtaga 25080

ataaaataag tatatatatg tttatttttа aattttgttt tattatgtta aaaatttgag 25140

ttattttaag ttttattgtt tttttagatt tagtaggttt ggaaggagag aaaaatttgt 25200

tttatttagt taggtttttt tttttggaga agttataagt tttttatggt atttgttatt 25260

tgagttaatg aatgtgattg ttttgataag gtgtttttaa ttttattgat tttggtaaat 25320

tgtaggagat atttagttta tgttatataa aaattattaa agtgggattg ttgtagttga 25380

aaagtttgat tagtgtttgg agattagaat gtgagttttt aaagtatata tgtatttggt 25440

attgggaaag tttaggtgtt tgtgattttt tgaaggattt tgggttagta tttttgtgtt 25500

tggattattt agggggttat tagaaagtaa tatatgttat aagaaagatg tgggggagat 25560

tttgatgttt gtgtttttgt taggtggagg tggggtgtag gtagaagttg tgtgttggat 25620

tgttggatga attttgttat gtgtaggttt tgtgttgatt ttggaaggga taggtaggtt 25680

tggaagggat ttttggggtt tggggatgtt ttttagggta gagagtattg ttgtggtttg 25740

agtggtttgg gtttttaggt gtggggtatt tggtttttaa gtgttttttg gggatgtagg 25800

tgtttatgta ttgtttagtt gtgtgttttt agtatttagt gtttggtgtt tttggtggag 25860

ttttgtgtat ttttttggaa agtttgggggg ttttttttaat ttaggttttt ttgggagttt 25920

tggggtgggg gttggaagaa ggggttattt attttgttgt ttggtttgtt ttgggttttt 25980

gaagtgtttg ttttagttgt ggtttagtgt gtttgtaatt aagtatttat ttttgttttt 26040

tttagtgttg aagttttttt tttgtgttgt gtttttggtg aaaataggat attttttttg 26100

gttattttat aataaatagt gtatataagt gggggagaag tggggtggag ggagaagttg 26160

gttttgaggg ggaggaggaa aggagaggag ttaaaatttt ggattgtgat agggggggaaa 26220

ggagaagaaa agaaaatgag agagtgtgtt tagttgttgt agttgttatt ttttggtttt 26280

ttttgtagtt ttttttttttt ttttaaggta gtgataattt tatgtggata ggatggaagt 26340

tttgtgtgga agttgggaat ggttggagtg tgtgttgtgt tgttggtagt tttgtatttg 26400

tgtagggagg tggggtgggg gtgatgttgt tattggttat tgtgggtttg tgaggttgtt 26460

gggggttttg ggggaggttg ttagggatgt ggggggtggt ggtgtgtggg ggatttattt 26520

tgtttttatt tgattttggg tgattgttta ttgttatggt ttgtgatttt gtgtggggat 26580

tgtttagggt tttggggttt tggatgtttt gggtggtgtt gttgtaattt ttgtgttttt 26640

-continued

```
tgtgttgttt ttattttgtt tttatttggg ttgatttgag tggttgtagt ttttaggttg  26700
aggggaggga tttgtgattg tgtttttttt tttgggttgg agagttattg ttgattttta  26760
tttgtttttg tggggtagtt tttggaattg tttggaattt ttttgttatt tagttttttt  26820
gtgtaggtta ttgtgggttt tttgttggat ttttttttat ttattttaag ggaggagata  26880
gaagggattt tgtttatttg taataatgtg aaataaaaat taatttagat tagattgggg  26940
tgtttttttt gatgggagga aatatttttt tttgtggaga tatatgttat gaaggattaa  27000
gtggtttggg gataggttgt gttgtgaagg gtttgggttt tttttagtgt tgttgggtgt  27060
aggtataggt atttgtttta gatgtttaaa ggttaggtag taatgattta tttttaaggt  27120
ggtgttagag tttttttagg tatattgttg aaatgatatt ttgtgtttaa gttttttgtg  27180
tatgtattat gtgattatat aggatttatg attttatatt tatatgtgtg tatgttataa  27240
ttaggggata tagtaaaggt agatggaata aataatttat aaattttgta tttatttaat  27300
tatttttttt aaaaatttat tgtttatttt agtggtattt tttttgttat attgataata  27360
aatgttttaa tttattgata aattattgag tgttaattat tgtattggtt atttatggta  27420
tatgagttta tttaattttt atgataattt tatggtggtt attattaagt tggtataaaa  27480
gatgaggaaa ttaaagttta aagaattatg agtgattttt ttaaagataa atttagtggt  27540
ttttaattat aaagtttatg atataataat ttttatatta ttttaggtgt ggtttaagag  27600
attatagatt aatttgtaga aattattttt atttaaagaa agtttagttt taatatattt  27660
attaagttat gttttgaaaa aggtttagaa aaaagatttt atttttttatt ttaggttggt  27720
tttatgtaat tgatatgagt attgtttaat ttaaaagtag tattaatttt gaatatttat  27780
ggttataatt tatttttttaa tgagtgggga attatttata atgtttaaga ggttttagaa  27840
aaggtggtgt tgtaaattta aaatgataaa ggtagttgtt gttgttttta ttatttattg  27900
ggtgtttttt tttgtagatt tattttggag ggtgtaggta ttgttaggta taatgttttt  27960
ttttttggtt tttaaggtag aaagggtttt ggtagtgggg ttggtataaa gtggatttgg  28020
agtatggttg agagtatatt ttggtttaat gaggaatgtt agttaatata tattggagag  28080
aaaaatatga atggatagat ttaattaatt ttgtagattt atttttttta ttttatatgt  28140
gagaaaatta agggtttaga atttttagaa gtttgttaat aaatggtaga tttgagtttt  28200
aagttagttt ttatttaagt ttatttttag ttattgtgat gttattatta gtattaatgg  28260
tatttagaaa aatagtaatt tttttaaata tgtaatatat tataagttaa atatgtaatt  28320
taaatgtttt gtgaattata gtgtttaaga atgttttgtg gttatagtaa atattatgta  28380
aggattaagt ataatgatga atttaatatt gtttttaaaa ggtaataata ttttgaattt  28440
tattttggta taaaatatta tgtgttagtt ttatttataa ataaaggtgg gagatgtatg  28500
atttgaataa aataaaaatt tatattttag tatattgtta atataaatat agggtgggtt  28560
tttttagtat gttttttttgt attttttttaa agttagattt tttagttata ttgattttgt  28620
ttttaaggtt attttgtttt tgtatagttt tttgtatatt tagataagaa tatatgtata  28680
gaaattattt ttaaaattta ggataatata ttagttattt gattttatat tattattttt  28740
taatagttgt tttaaatata tatatattaa tatggatatt attaggtgtt agataaaatg  28800
ttaattttta gtatatattt gaaataagtt taatatagga aatagaattt tagggttata  28860
aaggtaaaaa tgtattgttt gtttaaatga ggttttattt ttagtattta tattgaatat  28920
```

-continued

```
tatttttata attatagtta tattgtttat attattgatt taatatgtat ttttaaataa  28980

tttatttttа ttaaataagt ttatttgata aaggatattt tttatagtta ttataaatgg  29040

aaaattaata aaatttgtta tgaatagaag atgattttgt aaaggaattt atattgtaga  29100

aataaaagtt atttttatg tattatttaa aattatttta tatatagttt gagaaatatt  29160

ggtttaaaga attatatttt atatgttttt taggtttatt aagagatatt tttaagtttt  29220

ttttttttat aattggaata aaatgttgtg tttaatttat tttgaggtat tatttttaa  29280

attttgtgtt tatttaatta ttgtttttтt tgtttaaaat atatttgaag tttttgaagt  29340

tgaggtgttt ttttaaaatt tatgtattat ttattgtttt atttattatt ttatttgtgg  29400

taaaatgtaa ataatttata aatgtataat taaaaagtaa attatttttt tgaaatattg  29460

tattggttat ttattatttt tttttagtat ttattagttt agtagtgtaa tatagtatag  29520

ataatgaaga agagagggaa agtagaatag tgggagaaat gtaaagggtt tagaaagata  29580

tatgggggaa attgaagtta gagatttgtt gttagatttt tgaagtgatt taaaaatatt  29640

agtgggtttt gtatgatatg agttaggttt ttagaatatt tgtatatgtt taatttagtt  29700

tgaaaaggga tgtgttttgg gaggagtggg gagataaaaa atataagatt ttatagggta  29760

aattagtgga aatataagat ttatttatta gtaatttttt taattaattt ttttggaatg  29820

ggatgttttt gtagttatta tttattattt gtttatttтt taaatgtgta tggtattgtt  29880

ttggtattag attttgtata agttaaatta aataaagtgt tttaggagtt tataatttaa  29940

aatataattt ttgttttaat tttatttaaa agtaatttta agggaaaaat tag         29993
```

<210> SEQ ID NO 6
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PRIMER

<400> SEQUENCE: 6

```
cggagggtac ggagattacg                                              20
```

<210> SEQ ID NO 7
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PRIMER

<400> SEQUENCE: 7

```
cgacgacgcg cgaaa                                                   15
```

<210> SEQ ID NO 8
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PRIMER

<400> SEQUENCE: 8

```
tggtgatgga ggaggtttag taagt                                        25
```

<210> SEQ ID NO 9
<211> LENGTH: 25
<212> TYPE: DNA

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PRIMER

<400> SEQUENCE: 9

ggtgattgtt tattgttatg gtttg 25

<210> SEQ ID NO 10
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PRIMER

<400> SEQUENCE: 10

cccctcaacc taaaaactac aac 23

<210> SEQ ID NO 11
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PROBE

<400> SEQUENCE: 11

cgaaacccta aatatcccga ataacgccg 29

<210> SEQ ID NO 12
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PROBE

<400> SEQUENCE: 12

accaccaccc aacacacaat aacaaacaca 30

<210> SEQ ID NO 13
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PROBE

<400> SEQUENCE: 13

aaaattacga cgacgccacc cgaaa 25

<210> SEQ ID NO 14
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLOCKER

<400> SEQUENCE: 14

gttatggttt gtgattttgt gtggg 25

<210> SEQ ID NO 15
<211> LENGTH: 26

-continued

<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BLOCKER

<400> SEQUENCE: 15

aaactacaac cactcaaatc aaccca 26

1. A method of diagnosing a colon cell proliferative disorder in a subject, comprising the steps of:

(a) obtaining one or more test samples from colon tissue or serum or both of said subject; and

(b) detecting a decrease in the amount or expression of a polypeptide expressed from the EYA4 gene

2. The method of claim 1, wherein said colon cell proliferative disorders are taken from the group comprising adenocarcinomas, squamous cell cancers, carcinoid tumours, sarcomas, and lymphomas.

3. The method of claim 1 or 2, wherein said detection is by immunoassay, in particular by an ELISA.

4. The method of claim 3, wherein said immunoassay is a radioimmunoassay.

5. A method of diagnosing a colon cell proliferative disorder in a subject, comprising the steps of:

a) obtaining one or more test samples from colon tissue or serum or both of said subject; and

b) contacting said sample with an antibody immunoreactive with the EYA4 polypeptide to form an immunocomplex;

c) detecting said immunocomplex;

d) comparing the quantity of said immunocomplex to the quantity of immunocomplex formed under identical conditions with the same antibody and a control sample from one or more subjects known not to have colon cancer; wherein a decrease in quantity of said immunocomplex in the sample from said subject relative to said control sample is indicative of colon cancer.

6. The method of claim 5, wherein said immunocomplex is detected in a Western blot assay.

7. The method of claim 5, wherein said immunocomplex is detected in an ELISA.

8. The method of claim 1, wherein said detection is by expression analysis.

9. The method of claim 8, comprising detecting the presence or absence of mRNA encoding a EYA4 polypeptide in a sample from a patient, wherein a decreased concentration of said mRNA above the concentration for a healthy individual indicates the presence of colon cell proliferative disorder cells.

10. The method of claim 8, comprising the steps of:

(a) providing a polynucleotide probe which specifically hybridises or is identical to a polynucleotide consisting of SEQ ID NO: 1,

(b) incubating said sample with said polynucleotide probe under high stringency conditions to form a specific hybridisation complex between an mRNA and said probe;

(c) detecting said hybridisation complex.

11. The method according to claim 10 wherein the detecting step further comprises the steps of:

a) producing a cDNA from mRNA in the sample;

b) providing two oligonucleotides which specifically hybridise to regions flanking a segment of the cDNA;

c) performing a polymerase chain reaction on the cDNA of step a) using the oligonucleotides of step b) as primers to amplify the cDNA segment; and

d) detecting the amplified cDNA segment.

12. A method for repressing transformation in a colon cell, the method comprising contacting said cell with a EYA4 polypeptide in an amount effective to inhibit a transformed phenotype.

13. The method of claim 12, wherein said EYA4 polypeptide is introduced into said cell by the direct introduction of said EYA4 polypeptide.

14. The method of claim 12, wherein said EYA4 polypeptide is introduced into the cell through the introduction of a EYA4-encoding polynucleotide.

15. The method of claim 14, wherein said EYA4-encoding polynucleotide further comprises control sequences operatively linked to said EYA4 encoding polynucleotide.

16. The method of claim 14, wherein said EYA4-encoding polynucleotide is present in a vector.

17. Use of the vector according to claim 16 for therapy of colon cell proliferative disorders.

18. A method for preventing or treating a colon cell proliferative disorder in a subject which comprises administering to the subject a therapeutically effective amount of a compound that agonises EYA4.

19. Use of a polypeptide expressed from the EYA4 gene for detecting, differentiating or distinguishing between colon cell proliferative disorders.

20. Use of a polypeptide expressed from the EYA4 gene for therapy of colon cell proliferative disorders.

21. The method of claim 8, wherein said detection comprises methylation analysis of the gene EYA 4, its promoter and/or regulatory elements, in particular through the methylation analysis of a genomic DNA sequence according to SEQ ID NO: 1.

22. A nucleic acid comprising a sequence at least 18 bases in length of a segment of the chemically pretreated genomic DNA according to one of the sequences taken from the group comprising SEQ ID NO: 2 to SEQ ID NO: 5 and sequences complementary thereto.

23. An oligomer, in particular an oligonucleotide or peptide nucleic acid (PNA)-oligomer, said oligomer comprising in each case at least one base sequence having a length of at least 9 nucleotides which is complementary to, or hybridises under moderately stringent or stringent con-

ditions to a pretreated genomic DNA according to one of the SEQ ID NO: 2 to SEQ ID NO: 5 and sequences complementary thereto.

24. The oligomer as recited in claim 23; wherein the base sequence includes at least one CpG, TpG or CpA dinucleotide.

25. The oligomer as recited in claim 24; characterised in that the cytosine of the CpG dinucleotide is located approximately in the middle third of the oligomer.

26. A set of oligomers, comprising at least two oligomers according to any of claims 23 to 25.

27. A set of at least two oligonucleotides as recited in claims 23 to 26, which can be used as primer oligonucleotides for the amplification of DNA sequences of one of SEQ ID NO: 2 to SEQ ID NO: 5 and sequences complementary thereto.

28. A set of oligonucleotides as recited in claim 23, characterised in that at least one oligonucleotide is bound to a solid phase.

29. Use of a set of oligomer probes comprising at least ten of the oligomers according to any of claims 24 to 28 for detecting the cytosine methylation state and/or single nucleotide polymorphisms (SNPs) within one of the sequences according to SEQ ID NO: 1, and sequences complementary thereto.

30. A method for manufacturing an arrangement of different oligomers (array) fixed to a carrier material for analysing diseases associated with the methylation state of the CpG dinucleotides of one of SEQ ID NO: 1, and sequences complementary thereto wherein at least one oligomer according to any of the claims **23** through **26** and **28** is coupled to a solid phase.

31. An arrangement of different oligomers (array) obtainable according to claim 30.

32. An array of different oligonucleotide- and/or PNA-oligomer sequences as recited in claim 31, characterised in that these are arranged on a plane solid phase in the form of a rectangular or hexagonal lattice.

33. The array as recited in any of the claims **31** or **32**, characterised in that the solid phase surface is composed of silicon, glass, polystyrene, aluminium, steel, iron, copper, nickel, silver, or gold.

34. A composition of matter comprising the following:

A nucleic acid comprising a sequence at least 18 bases in length of a segment of the chemically pretreated genomic DNA according to one of the sequences taken from the group comprising SEQ ID NO: 1 to SEQ ID NO: 5 and sequences complementary thereto, and

A buffer comprising at least one of the following substances: 1 to 5 mM Magnesium Chloride, 100-500 μM dNTP, 0.5-5 units of taq polymerase, an oligomer, in particular an oligonucleotide or peptide nucleic acid (PNA)-oligomer, said oligomer comprising in each case at least one base sequence having a length of at least 9 nucleotides which is complementary to, or hybridises under moderately stringent or stringent conditions to a pretreated genomic DNA according to one of the SEQ ID NO: 2 to SEQ ID NO: 5 and sequences complementary thereto.

35. Use of the gene EYA 4, its promoter and/or regulatory elements for detecting, differentiating or distinguishing between colon cell proliferative disorders.

36. A method for detecting, differentiating or distinguishing between colon cell proliferative disorders according to claim 21, comprising:

a) obtaining, from a subject, a biological sample having subject genomic DNA;

b) treating the genomic DNA, or a fragment thereof, with one or more reagents to convert 5-position unmethylated cytosine bases to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridisation properties;

c) contacting the treated genomic DNA, or the treated fragment thereof, with an amplification enzyme and at least two primers comprising, in each case a contiguous sequence at least 18 nucleotides in length that is complementary to, or hybridises under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS: 2 to 5, and complements thereof, wherein the treated DNA or a fragment thereof is either amplified to produce one or more amplificates, or is not amplified; and

d) determining, based on the presence or absence of, or on a property of said amplificate, the methylation state of at least one CpG dinucleotide sequence of SEQ ID NO: 1, or an average, or a value reflecting an average methylation state of a plurality of CpG dinucleotide sequences of SEQ ID NO: 1.

37. A method for detecting, differentiating or distinguishing between colon cell proliferative disorders according to claim 21, comprising the following steps of

a) obtaining, from a subject, a biological sample having subject genomic DNA;

b) treating the genomic DNA, or a fragment thereof, with one or more reagents to convert 5-position unmethylated cytosine bases to uracil or to another base that is detectably dissimilar to cytosine in terms of hybridisation properties;

c) amplifying one or more fragments of the treated DNA such that only DNA originating from colon or colon cell proliferative disorder cells are amplified d) detecting the amplificates or characteristics thereof and thereby deducing on the presence or absence of a colon cell proliferative disorder.

38. The method of one of claims **36** or **37**, wherein in step a) the biological sample obtained from the subject is selected from the group consisting of histological slides, biopsies, paraffin-embedded tissue, bodily fluids, serum, plasma, stool, urine, blood, and combinations thereof.

39. The method of one of claims 36 to 38, wherein in step b) treating the genomic DNA, or the fragment thereof, comprises use of a solution selected from the group consisting of bisulfite, hydrogen sulfite, disulfite, and combinations thereof.

40. The method of one of claims 36 to 39, wherein treating in b) is subsequent to embedding the DNA in agarose.

41. The method of one of claims 36 to 40, where treating in b) comprises treating in the presence of at least one of a DNA denaturing reagent or a radical trap reagent.

42. The method of one of claims 36 to 41, wherein contacting or amplifying in step c) comprises use of at least one method selected from the group consisting of: use of a

heat-resistant DNA polymerase as the amplification enzyme; use of a polymerase lacking 5'-3' exonuclease activity; use of a polymerase chain reaction (PCR); generation of a amplificate nucleic acid molecule carrying a detectable labels; and combinations thereof.

43. The method of claim 42, wherein the detectable amplificate label is selected from the label group consisting of: fluorescent labels; radionuclides or radiolabels; amplificate mass labels detectable in a mass spectrometer; detachable amplificate fragment mass labels detectable in a mass spectrometer; amplificate, and detachable amplificate fragment mass labels having a single-positive or single-negative net charge detectable in a mass spectrometer; and combinations thereof.

44. The method of claim 43, comprising in step d) use of mass spectrometry for detecting the amplificate, or detachable amplificate fragment mass labels.

45. The method of claim 44, wherein the mass spectrometry technique is selected from the group consisting of matrix assisted laser desorption/ionisation mass spectrometry (MALDI), electron spray mass spectrometry (ESI), and combinations thereof.

46. The method according to one of claims 36 to 45 wherein in step c) of the method 6 or more different fragments are amplified.

47. The method according to one of claims 36 to 46 wherein said amplificates are between 100 and 200 base pairs in length.

48. The method according to one of claims 36 to 47 wherein said amplificates are between 100 and 350 base pairs in length.

49. The method according to one of claims 36 to 48 wherein one or more of said primers comprise sequences taken from the group according to SEQ ID NO: 11 to SEQ ID NO: 15.

50. The method according to one of claims 36 to 48 wherein one or more of said primers comprise one or more CpG, TpG or CpA dinucleotides.

51. The method of claim 50 wherein said primers comprise between two to four CpG, TpG or CpA dinucleotides.

52. The method according to one of claims **50** or **51** wherein said one or more CpG, TpG or CpA dinucleotides are located within the 3' half of the primer.

53. The method according to one of claims 50 to 52 wherein said primers comprise one or more bases which hybridise to positions that were converted in the treatment of step b).

54. The method of claim 53 wherein said bases are located within the 3' half of the primer.

55. The method according to one of claims 50 to 54 wherein said primers do not comprise more than 2 cytosine or guanine bases within the first 5 bases at the 3' end.

56. The method according to one of claims 36 to 55 wherein said amplificates obtained in step d) comprise at least one 20 base pair sequence that comprises 3 or more CpG, TpG or CpA dinucleotides.

57. The method according to one of claims 36 to 56, further comprising in step c) the use of at least one nucleic acid molecule or peptide nucleic acid molecule at least 18 base pairs in length comprising one or more CpG, TpG or CpA dinucleotides and wherein the sequence of said molecule is complementary or identical to a sequence selected from the group consisting of SEQ ID NOS: 2 to 5, and complements thereof, and wherein said nucleic acid molecule or peptide nucleic acid molecule suppresses amplification of the nucleic acid to which it is hybridised.

58. The method according to claim 57 wherein the sequence of said nucleic acid(s) or peptide nucleic acid(s) is selected from the group consisting SEQ ID NO: 6 to SEQ ID NO: 10, and sequences complementary thereto.

59. The method of claim 57, wherein amplification of DNA that was unmethylated prior to treatment of step b) is suppressed.

60. The method of one of claims 57 to 59, wherein said nucleic acid molecule or peptide nucleic acid molecule is in each case modified at the 5'-end thereof to preclude degradation by an enzyme having a 5'-3' exonuclease activity.

61. The method of one of claims 57 to 60, wherein said nucleic acid molecule or peptide nucleic acid molecule in each case lack a 3' hydroxyl group.

62. The method of one of claims 57 to 61, wherein the amplification enzyme is a polymerase lacking 5'-3' exonuclease activity.

63. The method of one of claims 57 to 62, wherein the binding site of the oligonucleotide or PNA oligomer is identical to, or overlaps with that of the primer and thereby hinders hybridisation of the primer to its binding site.

64. The method of one of claims 57 to 63, wherein the binding sites of at least two of the oligonucleotides or PNA oligomers are identical to, or overlap with those of at least two of the primers, and thereby hinder hybridisation of the primers to their binding site.

65. The method of claim 64, wherein hybridisation of at least one of the oligonucleotides or peptide nucleic acid oligomers hinders hybridisation of a forward primer, and the hybridisation of at least one of the oligonucleotides or peptide nucleic acid oligomers hinders the hybridisation of a reverse primer that binds to the elongation product of said forward primer.

66. The method of one of claims 57 to 64, wherein said oligonucleotide or peptide nucleic acid oligomer hybridises between the binding sites of the forward and reverse primers.

67. The method of one of claims **36** or **37**, wherein determining in step d), comprises hybridisation of at least one nucleic acid molecule or peptide nucleic acid (PNA) molecule in each case comprising a contiguous sequence at least 9 nucleotides in length comprising one or more CpG, TpG or CpA dinucleotides and wherein the sequence of said molecule that is complementary or identical to a sequence selected from the group consisting of SEQ ID NOS: 2 to 5, and complements thereof.

68. The method of claim 67, wherein at least one such hybridising nucleic acid molecule or peptide nucleic acid molecule is bound to a solid phase.

69. The method of claim 68, wherein a plurality of such hybridising nucleic acid molecules or peptide nucleic acid molecules are bound to a solid phase in the form of a nucleic acid or peptide nucleic acid array selected from the array group consisting of linear, hexagonal, rectangular, and combinations thereof.

70. The method of one of claims **36** or **37**, wherein determining in step d), comprises sequencing of the amplificate.

71. The method of one of claims **36** or **37**, wherein determining in step d), comprises: hybridising at least one nucleic acid molecule comprising a contiguous sequence at least 9 nucleotides in length that is complementary to, or

hybridises under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS: 2 to 5, and complements thereof; and extending at least one such hybridised nucleic acid molecule by at least one nucleotide base.

72. The method according to claim 71 wherein the sequence of said nucleic acid(s) or peptide nucleic acid(s) is selected from the group consisting SEQ ID NO: 11 to SEQ ID NO: 15, and sequences complementary thereto.

73. The method according to claim 67 wherein said oligonucleotides or PNA-oligomers are fluorescently labelled, and wherein detection thereof is by either an increase or a decrease in fluorescence or fluorescence polarisation.

74. The method according to claim 73 wherein the hybridisation of the oligonucleotides or PNA oligomers is detectable by fluorescence resonance energy transfer, and wherein the detection is by either an increase or a decrease in fluorescence.

75. The method of one of claims **36** or **37**, wherein the background DNA concentration is at between 100 to 1000 fold excess of the concentration of the DNA to be investigated.

76. A method for detecting a colon cell proliferative disorder according to claim 21, comprising:

a) obtaining, from a subject, a biological sample having subject genomic DNA;

b) extracting the genomic DNA;

c) contacting the genomic DNA, or a fragment thereof, comprising SEQ ID NO:1 or a sequence that hybridises under stringent conditions to SEQ ID NO:1, with one or more methylation-sensitive restriction enzymes, wherein the genomic DNA is either digested thereby to produce digestion fragments, or is not digested thereby; and

d) determining, based on a presence or absence of, or on property of at least one such fragment, the methylation state of at least one CpG dinucleotide sequence of SEQ ID NO: 1, or an average, or a value reflecting an average methylation state of a plurality of CpG dinucleotide sequences of SEQ ID NO: 1, whereby at least one of detecting the prostate cell proliferative disorder, or distinguishing between a transitional and a peripheral zone of origin of the prostate cell proliferative disorder is, at least in part, afforded.

77. The method of claim 76, further comprising, prior to determining in step d), amplifying of the digested or undigested genomic DNA.

78. The method of claim 77, wherein amplifying comprises use of at least one method selected from the group consisting of: use of a heat resistant DNA polymerase as an amplification enzyme; generation of a amplificate nucleic acid carrying a detectable label; and combinations thereof.

79. The method of claim 78, wherein the detectable amplificate label is selected from the label group consisting of: fluorescent labels; radionuclides or radiolabels; amplificate mass labels detectable in a mass spectrometer; detachable amplificate fragment mass labels detectable in a mass spectrometer; amplificate, and detachable amplificate fragment mass labels having a single-positive or single-negative net charge detectable in a mass spectrometer; and combinations thereof.

80. The method of claim 79, comprising use of mass spectrometry for detecting amplificate, or detachable amplificate fragment mass labels.

81. The method of claim 80, wherein the mass spectrometry is selected from the group consisting of matrix assisted laser desorption/ionisation mass spectrometry (MALDI), electron spray mass spectrometry (ESI), and combinations thereof.

82. The method of claim 76, wherein the biological sample obtained from the subject is selected from the group consisting of histological slides, biopsies, paraffin-embedded tissue, bodily fluids, urine, serum, plasma, stool, blood, and combinations thereof.

83. A kit useful for detecting, differentiating or distinguishing between colon cell proliferative disorders, comprising:

a) a bisulfite reagent; and

b) at least one nucleic acid molecule or peptide nucleic acid molecule comprising, in each case a contiguous sequence at least 9 nucleotides in length that is complementary to, or hybridises under moderately stringent or stringent conditions to a sequence selected from the group consisting of SEQ ID NOS: 1 to 5, and complements thereof.

84. The kit of claim 83, further comprising standard reagents for performing a methylation assay selected from the group consisting of MS-SNuPE, MSP, MethylLight™, HeavyMethyl™, COBRA, nucleic acid sequencing, and combinations thereof.

85. The use of a nucleic acid according to claim 22, of an oligonucleotide or PNA-oligomer according to one of the claims 23 to 28, of a kit according to claims **83** or **84**, of an array according to one of the claims 32 to 33, of a set of oligonucleotides according to one of claims 26 to 28, or a method according to claims 36 to 82, for the classification, differentiation and/or diagnosis of colon cell proliferative disorders or the predisposition to colon cell proliferative disorders.

86. The use of a nucleic acid according to claim 22, of an oligonucleotide or PNA-oligomer according to one of the claims 23 to 28, of a kit according to claims **83** or **84**, of an array according to one of the claims 32 to 33, of a set of oligonucleotides according to one of claims 26 to 28, or a method according to claims 36 to 82, for the therapy of colon cell proliferative disorders.

* * * * *

专利名称(译)	用于分析结肠直肠细胞增殖性疾病的方法和核酸		
公开(公告)号	US20050003463A1	公开(公告)日	2005-01-06
申请号	US10/482823	申请日	2003-02-13
[标]申请(专利权)人(译)	ADORJAN PETER BURGER MATTHIAS MAIER SABINE LESCHE RALF COTTRELL SUSAN DE VOS THEO		
申请(专利权)人(译)	ADORJAN PETER BURGER MATTHIAS MAIER SABINE LESCHE RALF COTTRELL SUSAN DE VOS THEO		
当前申请(专利权)人(译)	ADORJAN PETER BURGER MATTHIAS MAIER SABINE LESCHE RALF COTTRELL SUSAN DE VOS THEO		
[标]发明人	ADORJAN PETER BURGER MATTHIAS MAIER SABINE LESCHE RALF COTTRELL SUSAN DE VOS THEO		
发明人	ADORJAN, PETER BURGER, MATTHIAS MAIER, SABINE LESCHE, RALF COTTRELL, SUSAN DE VOS, THEO		
IPC分类号	G01N27/62 A61K48/00 A61P35/00 A61P43/00 C12M1/00 C12N15/09 C12Q1/02 G01N21/78 G01N33/53 G01N33/543 G01N33/574 G01N33/58 G01N37/00 C12Q1/68		
CPC分类号	C12Q1/6886 Y10T436/143333 C12Q2600/154 A61P35/00 A61P43/00		
优先权	2002004551 2002-02-27 EP		
其他公开文献	US8691503		
外部链接	Espacenet USPTO		

摘要(译)

本发明提供了通过分析基因EYA4及其启动子和调节序列来检测，区分或区分结肠细胞增殖性疾病及其治疗的方法和核酸。本发明进一步提供了可用于所述基因的细胞增殖性疾病特异性分析的新型核酸序列及其方法，测定和试剂盒。

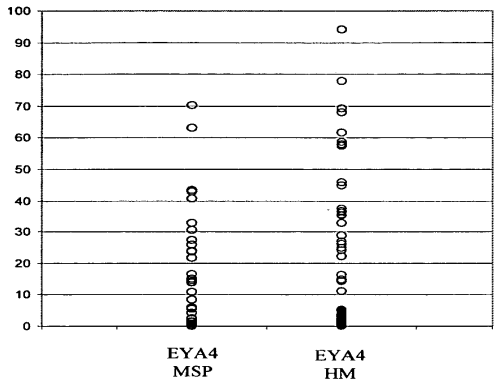



FIGURE 1