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(54) **MN GENE AND PROTEIN**

MN-GEN UND -PROTEIN

PROTEINE ET GENE MN

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WO-A-93/18152

- **PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 85, December 1988 WASHINGTON US, pages 8998-9002, M.A.FROHMAN ET AL. 'Rapid production of full-length cDNAs from rare transcripts: Amplification using a single gene-specific oligonucleotide primer'**
- **VIROLOGY, vol. 187, no. 2, April 1992 ORLANDO US, pages 620-626, S.PASTOREKOV ET AL. 'A novel quasi-viral agent, MaTu, is a two-component system'**
- **ONCOGENE, vol. 9, no. 10, October 1994 pages 2877-2888, J.PASTOREK ET AL. 'Cloning and characterization of MN, a human tumor-associated protein with a domain homologous to carbonic anhydrase and a putative helix-loop-helix DNA binding segment'**

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- **KALUZ S. ET AL: ’Transcriptional regulation of the MN/CA9 gene coding for the tumour-associated carbonic anhydrase IX’ JOURNAL OF BIOLOGICAL CHEMISTRY vol. 274, no. 46, 12 November 1999, pages 32588 - 32595**

Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

Description

[0001] The present invention is in the general area of medical genetics and in the fields of biochemical engineering and immunochemistry. More specifically, it relates to the identification of a new gene - the MN gene - a cellular gene coding for the MN protein. The inventors hereof found MN proteins to be associated with tumorigenicity. Evidence indicates that the MN protein appears to represent a potentially novel type of oncoprotein. Identification of MN antigen as well as antibodies specific therefor in patient samples provides the basis for diagnostic/prognostic assays for cancer. Specifically, the present invention relates to the MN promoter.

BACKGROUND OF THE INVENTION

[0002] A novel quasi-viral agent having rather unusual properties was detected by its capacity to complement mutants of vesicular stomatitis virus (VSV) with heat-labile surface G protein in HeLa cells (cell line derived from human cervical adenocarcinoma) , which had been cocultivated with human breast carcinoma cells. [Zavada et al., Nature New Biol., 240: 124 (1972); Zavada et al., J. Gen. Virol., 24: 327 (1974); Zavada J., Arch. Virol., 50: 1 (1976); Zavada, J., J. Gen. Virol., 63: 15-24 (1982); Zavada and Zavadova, Arch, Virol., 118: 189 (1991).] The quasi viral agent was called MaTu as it was presumably derived from a human mammary tumor.

[0003] There was significant medical interest in studying and characterizing MaTu as it appeared to be an entirely new type of molecular parasite of living cells, and possibly originated from a human tumor. Zavada et al., International Publication Number WO 93/18152 (published 1 September 1993), describes the elucidation of the biological and molecular nature of MaTu which resulted in the discovery of the MN gene and protein. MaTu was found by the inventors to be a two-component system, having an exogenous transmissible component, MX, and an endogenous cellular component, MN. The MN component was found to be a cellular gene, showing only very little homology with known DNA sequences. The MN gene was found to be present in the chromosomal DNA of all vertebrates tested, and its expression was found to be strongly correlated with tumorigenicity.

[0004] The exogenous MaTu-MX transmissible agent was identified as lymphocytic choriomeningitis virus (LCMV) which persistently infects HeLa cells. The inventors discovered that the MN expression in HeLa cells is positively regulated by cell density, and also its expression level is increased by persistent infection with LCMV.

[0005] Research results provided herein show that cells transfected with MN cDNA undergo changes indicative of malignant transformation. Further research findings indicate that the disruption of cell cycle control is one of the mechanisms by which MN may contribute to the complex process of tumor development.

[0006] Described herein is the cloning and sequencing of the MN gene and the recombinant production of MN proteins. The full-length MN cDNA sequence [SEQ. ID. NO.: 1], the amino acid sequence deduced therefrom [SEQ. ID. NO.: 2], a full-length genomic sequence for MN [SEQ. ID. NO.: 5] including a proposed promoter sequence [SEQ. ID. NO.: 27] are provided. Eleven exons [SEQ. ID. NOS. 28-38] and ten introns [SEQ. ID. NOS.: 39-48] are comprised by the MN gene. Also a 1.4 kilobase region [SEQ. ID. NO. 49] within the middle of the MN genomic sequence is described herein, which has the character of a typical CpG-rich island, and which contains multiple putative binding sites for transcription factors AP2 and Sp1.

[0007] Also described are antibodies prepared against proteins/polypeptides. MN proteins/ polypeptides can be used in serological assays to detect MN-specific antibodies. Further, MN proteins/polypeptides and/or antibodies reactive with MN antigen can be used in immunoassays to detect and/or quantitate MN antigen. Such assays may be diagnostic and/or prognostic for neoplastic/pre-neoplastic disease.

SUMMARY OF THE INVENTION

[0008] The present invention provides an isolated nucleic acid characterised in that it contains at least 16 nucleotides and has a nucleotide sequence selected from:

(a) the MN promoter nucleotide sequence as shown in accompanying Figure 6, and nucleotide sequences complementary to the said MN promoter nucleotide sequence;
and
(b) nucleotide sequences that are at least 80% homologous to the nucleotide sequences of (a) and to the complements of the said nucleotide sequences;

the said isolated nucleic acid having promoter activity and/or acting as a polymerase chain reaction primer for identifying the MN promoter.

[0009] Having indicated the scope of the present invention, it will be further described and illustrated in more general terms.

[0010] This disclosure is directed to the MN gene, fragments thereof and the related cDNA which are useful, for example, as follows: 1) to produce MN proteins/polypeptides by biochemical engineering; 2) to prepare nucleic acid probes to test for the presence of the MN gene in cells of a subject; 3) to prepare appropriate polymerase chain reaction (PCR) primers for use, for example, in PCR-based assays or to produce nucleic acid probes; 4) to identify MN proteins and polypeptides as well as homologs or near homologs thereto; 5) to identify various mRNAs transcribed from MN genes in various tissues and cell lines, preferably human; and 6) to identify mutations in MN genes. It further concerns purified and isolated DNA molecules comprising the MN gene or fragments thereof, or the related cDNA or fragments thereof.

[0011] Thus, this disclosure in one aspect concerns isolated nucleic acid sequences that encode MN proteins or polypeptides wherein the nucleotide sequences for said nucleic acids are selected from the group consisting of:

(a) SEQ. ID. NO.: 1;
(b) nucleotide sequences that hybridize under stringent conditions to SEQ. ID. NO.: 1 or to its complement;
(c) nucleotide sequences that differ from SEQ. ID. NO.: 1 or from the nucleotide sequences of (b) in codon sequence because of the degeneracy of the genetic code. Further, such nucleic acid sequences are selected from nucleotide sequences that but for the degeneracy of the genetic code would hybridize to SEQ. ID. NO.: 1 or to its complement under stringent hybridization conditions.

[0012] Further, such isolated nucleic acids that encode MN proteins or polypeptides can also include the MN nucleic acids of the genomic sequence shown in Figure 3(A-F), that is, SEQ. ID. NO.: 5, as well as sequences that hybridize to it or its complement under stringent conditions, or would hybridize to SEQ. ID. NO.: 5 or to its complement under such conditions, but for the degeneracy of the genetic code. Degenerate variants of SEQ. ID. NOS.: 1 and 5 are within the scope of the disclosure.

[0013] Further, this disclosure concerns nucleic acid probes which are fragments of the isolated nucleic acids that encode MN proteins or polypeptides as described above. Preferably said nucleic acid probes are comprised of at least 29 nucleotides, more preferably of at least 50 nucleotides, still more preferably at least 100 nucleotides, and even more preferably at least 150 nucleotides.

[0014] Still further, this disclosure is directed to isolated nucleic acids containing at least twenty-seven nucleotides selected from the group consisting of:

(a) SEQ. ID. NOS.: 1, 5 and 27-49 and that are complementary to SEQ. ID. NOS.: 1, 5 and 27-49;
(b) nucleotide sequences that hybridize under standard stringent hybridization conditions to one or more of the following nucleotide sequences: SEQ. ID. NOS.: 1, 5, and 27-49 and the respective complements of SEQ. ID. NOS.: 1, 5 and 27-49; and
(c) nucleotide sequences that differ from the nucleotide sequences of (a) and (b) in codon sequence because of the degeneracy of the genetic code. The disclosure also concerns nucleic acids that but for the degeneracy of the genetic code would hybridize to the nucleic acids of (a) and (b) under standard stringent hybridization conditions. Further this disclosure concerns nucleic acids of (b) and (c) that hybridize partially or wholly to the non-coding regions of SEQ. ID. NO.: 5 or its complement as, for example, sequences that function as nucleic acid probes to identify MN nucleic acid sequences. Conventional technology can be used to determine whether the nucleic acids of (b) and (c) or of fragments of SEQ. ID. NO.: 5 are useful to identify MN nucleic acid sequences, for example, as outlined in Benton and Davis, Science, 196: 180 (1977) and Fuscoe et al. Genomics, 5: 100 (1989). In general, such nucleic acids are preferably at least 29 nucleotides, most preferably at least 50 nucleotides and still more preferably at least 100 nucleotides. An exemplary and preferred nucleic acid probe is SEQ. ID. NO.: 55 (a 470 bp probe useful in RNase portection assays).

[0015] Test kits can comprise the nucleic acid probes which are useful diagnostically/prognostically for neoplastic and/or pre-neoplastic disease. Preferred test kits comprise means for detecting or measuring the hybridization of said probes to the MN gene or to the mRNA product of the MN gene, such as a visualizing means.

[0016] Fragments of the isolated nucleic acids can also be used as PCR primers to amplify segments of MN genes, and may be useful in identifying mutations in MN genes. Typically, said PCR primers are oligonucleotides, preferably at least 16 nucleotides, but they may be considerably longer. Exemplary primers may be from about 16 nucleotides to about 50 nucleotides, preferably from about 19 nucleotides to about 45 nucleotides.

[0017] Further, it concerns the use of such PCR primers in methods to detect mutations in an isolated MN gene and/or fragment(s) thereof. For example, such methods can comprise amplifying one or more fragment(s) of an MN gene by PCR, and determining whether any of said one or more fragments contain mutations, by, for example, comparing the size of the amplified fragments to those of similarly amplified corresponding fragments of MN genes known to be normal, by using a PCR-single-strand conformation polymorphism assay or a denaturing gradient gel electrophoretic assay.

[0018] This disclosure also concerns nucleic acids which encode MN proteins or polypeptides that are specifically bound by monoclonal antibodies designated M75 that are produced by the hybridoma VU-M75 deposited at the American Type Culture Collection (ATCC) at 10801 University Blvd., Manassas, Virginia 20110-2209 (USA) under ATCC No. HB 11128, and/or by monoclonal antibodies designated MN12 produced by the hybridoma MN 12.2.2 deposited at the ATCC under ATCC No. HB 11647.

[0019] This disclosure further concerns isolated nucleic acids containing at least sixteen nucleotides, preferably at least twenty-nine nucleotides, more preferably at least fifty nucleotides, wherein said nucleic acid is selected from the group consisting of:

(a) the MN nucleic acids contained in plasmids A4a, XE1 and XE3 which were deposited at the American Type Culture Collection (ATCC) in Manassas, Virginia in the United States of America under the respective ATCC Nos. 97199, 97200, and 97198;
(b) nucleic acids that hybridize under stringent conditions to the MN nucleic acids of (a); and
(c) nucleic acids that differ from the nucleic acids of (a) or (b) in codon sequence due to the degeneracy of the genetic code. Such isolated nucleic acids, for example, can be polymerase chain reaction (PCR) primers.

[0020] It further concerns isolated nucleic acids that code for an MN protein, MN fusion protein or MN polypeptide that is operatively linked to an expression control sequence within a vector; unicellular hosts, prokaryotic or eukaryotic, that are transformed or transfected therewith; and methods of recombinantly producing MN proteins, MN fusion proteins and MN polypeptides comprising transforming or transfecting unicellular hosts with said nucleic acid operatively linked to an expression control sequence, culturing said transformed or transfected unicellular hosts so that said MN proteins, fusion proteins or polypeptides are expressed, and extracting and isolating said MN protein fusion protein or polypeptide.

[0021] Recombinant nucleic acids that encode MN fusion proteins are claimed as consisting essentially of an MN protein or MN polypeptide and a non-MN protein or polypeptide wherein the nucleotide sequence for the portion of the nucleic acid encoding the MN protein or polypeptide is selected from the group consisting of:

(a) SEQ. ID. NO.: 1;
(b) nucleotide sequences that hybridize under stringent conditions to SEQ. ID. NO.: 1 or to its complement; and
(c) degenerate variants of SEQ. ID. NO.: 1, and of the nucleotide sequences of (b);

wherein the nucleic acid encoding said MN protein or polypeptide contains at least twenty-nine nucleotides.

[0022] Said non-MN protein or polypeptide may preferably be nonimmunogenic to humans and not typically reactive to antibodies in human body fluids. Examples of such a DNA sequence is the alpha-peptide coding region of beta-galactosidase and a sequence coding for glutathione S-transferase or a fragment thereof. However, in some instances, a non-MN protein or polypeptide that is serologically active, immunogenic and/or antigenic may be preferred as a fusion partner to a MN antigen. Further, disclosed herein are such recombinant fusion proteins/polypeptides which are substantially pure and non-naturally occurring. Exemplary fusion proteins are GEX-3X-MN, MN-Fc and MN-PA, described infra.

[0023] In HeLa and in tumorigenic HeLa x fibroblast hybrid (H/F-T) cells, MN protein is manifested as a "twin" protein p54/58N; it is glycosylated and forms disulfide-linked oligomers. As determined by electrophoresis upon reducing gels, MN proteins have molecular weights in the range of from about 40 kd to about 70 kd, preferably from about 45 kd to about 65 kd, more preferably from about 48 kd to about 58 kd, upon non-reducing gels. MN proteins in the form of oligomers have molecular weights in the range of from about 145 kd to about 160 kd, preferably from about 150 to about 155 kd, still more preferably from about 152 to about 154 kd. A predicted amino acid sequence for a preferred MN protein is shown in Figure 1(A-C) [SEQ. ID. NO. 2].

[0024] Other particular MN proteins or polypeptides are exemplified by the putative MN signal peptide shown as the first thirty-seven amino acids in Figure 1(A-C) [SEQ. ID. NO.: 6], preferred MN antigen epitopes [SEQ. ID. NOS.: 10-16], and domains of the MN protein represented in Figure 1(A-C) as amino acids 38-135 [SEQ. ID NO. : 50], 136-391 [SEQ. ID. NO.: 51], 415-434 [SEQ. ID. NO.: 52], and 435-459 [SEQ. ID. NO.: 53].

[0025] The discovery of the MN gene and protein and thus, of substantially complementary MN genes and proteins encoded thereby, led to the finding that the expression of MN proteins was associated with tumorigenicity. That finding resulted in the creation of methods that are diagnostic/ prognostic for cancer and precancerous conditions. Methods and compositions are disclosed for identifying the onset and presence of neoplastic disease by detecting and/or quantitating MN antigen in patient samples, including tissue sections and smears, cell and tissue extracts from vertebrates, preferably mammals and more preferably humans. Such MN antigen may also be found in body fluids.

[0026] MN proteins and genes are of use in research concerning the molecular mechanisms of oncogenesis, in cancer diagnostics/prognostics, and may be of use in cancer immunotherapy. The present discovery is useful for defecting a wide variety of neoplastic and/or pre-neoplastic diseases. Exemplary neoplastic diseases include carcinomas, such as mammary, bladder, ovarian, uterine, cervical, endometrial, squamous cell and adenosquamous carcinomas; and head and neck cancers; mesodermal tumors, such as neuroblastomas and retinoblastomas; sarcomas, such as osteosarcomas and Ewing's sarcoma; and melanomas. Of particular interest are head and neck cancers, gynecologic cancers including ovarian, cervical, vaginal, endometrial and vulval cancers; gastrointestinal cancer, such as, stomach, colon and esophageal cancers; urinary tract cancer, such as, bladder and kidney cancers; skin cancer; liver cancer; prostate cancer; lung cancer; and breast cancer. Of still further particular interest are gynecologic cancers; breast cancer; urinary tract cancers, especially bladder cancer; lung cancer; and liver cancer. Even further of particular interest are gynecologic cancers and breast cancer. Gynecologic cancers of particular interest are carcinomas of the uterine cervix, endometrium and ovaries; more particularly such gynecologic cancers include cervical squamous cell carcinomas, adenosquamous carcinomas, adenocarcinomas as well as gynecologic precancerous conditions, such as metaplastic cervical tissues and condylomas.

[0027] The disclosure further relates to the biochemical engineering of the MN gene, fragments thereof or related cDNA. For example, said gene or a fragment thereof or related cDNA can be inserted into a suitable expression vector, wherein it is operatively linked to an expression control sequence; host cells, preferably unicellular, can be transformed or transfected with such an expression vector; and an MN protein/polypeptide, preferably an MN protein, is expressed therein. Such a recombinant protein or polypeptide can be glycosylated or nonglycosylated, preferably glycosylated, and can be purified to substantial purity. It further concerns MN proteins/polypeptides which are synthetically or otherwise biologically prepared.

[0028] Said MN proteins/polypeptides can be used in assays to detect MN antigen in patient samples and in serological assays to test for MN-specific antibodies. MN proteins/polypeptides of this invention are serologically active, immunogenic and/or antigenic. They can further be used as immunogens to produce MN-specific antibodies, polyclonal and/or monoclonal, as well as an immune T-cell response.

[0029] The disclosure further is directed to MN-specific antibodies, which can be used diagnostically/prognostically and may be used therapeutically.

Preferred are MN-specific antibodies reactive with the epitopes represented respectively by the amino acid sequences of the MN protein shown in Figure 1(A-C) as follows: from AA 62 to AA 67 [SEQ. ID. NO.: 10]; from AA 55 to AA 60 [SEQ. ID. NO.: 11]; from AA 127 to AA 147 [SEQ. ID. NO.: 12]; from AA 36 to AA 51 [SEQ. ID. NO.: 13]; from AA 68 to AA 91 [SEQ. ID. NO.: 14]; from AA 279 to AA 291 [SEQ. ID. NO.: 15]; and from AA 435 to AA 450 [SEQ. ID. NO.: 16]. More preferred are antibodies reactive with epitopes represented by SEQ. ID. NOS.: 10, 11 and 12. still more preferred are antibodies reactive with the epitopes represented by SEQ. ID NOS: 10 and 11, as for example, respectively Mabs M75 and MN12. Most preferred are monoclonal antibodies reactive with the epitope represented by SEQ. ID. NO.: 10.

[0030] Also preferred are

antibodies prepared against recombinantly produced MN proteins as, for example, GEX-3X-MN, MN 20-19, MN-Fc and MN-PA. Also preferred are MN-specific antibodies prepared against glycosylated MN proteins, such as, MN 20-19 expressed in baculovirus infected Sf9 cells.

[0031] A hybridoma that produces a representative MN-specific antibody, the monoclonal antibody M75 (Mab M75), was deposited at the ATCC under Number HB 11128 as indicated above. The M75 antibody was used to discover and identify the MN protein and can be used to identify readily MN antigen in Western blots, in radioimmunoassays and immunohistochemically, for example, in tissue samples that are fresh, frozen, or formalin-, alcohol-, acetone- or otherwise fixed and/or paraffin-embedded and deparaffinized. Another representative MN-specific antibody, Mab MN12, is secreted by the hybridoma MN 12.2.2, which was deposited at the ATCC under the designation HB 11647.

[0032] MN-specific antibodies can be used, for example, in laboratory diagnostics, using immunofluorescence microscopy or immunohistochemical staining; as a component in immunoassays for detecting and/or quantitating MN antigen in, for example, clinical samples; as probes for immunoblotting to detect MN antigen; in immunoelectron microscopy with colloid gold beads for localization of MN proteins and/or polypeptides in cells; and in genetic engineering for cloning the MN gene or fragments thereof, or related cDNA. Such MN-specific antibodies can be used as components of diagnostic/prognostic kits, for example, for in vitro use on histological sections; such antibodies can also and used for in vivo diagnostics/prognostics, for example, such antibodies can be labeled appropriately, as with a suitable radioactive isotope, and used in vivo to locate metastases by scintigraphy. Further such antibodies may be used in vivo therapeutically to treat cancer patients with or without toxic and/or cytostatic agents attached thereto. Further, such antibodies can be used in vivo to detect the presence of neoplastic and/or pre-neoplastic disease. Still further, such antibodies can be used to affinity purify MN proteins and polypeptides.

[0033] This disclosure also concerns methods of treating neoplastic disease and/or pre-neoplastic disease comprising inhibiting the expression of MN genes by administering antisense nucleic acid sequences that are substantially complementary to mRNA transcribed from MN genes. Said antisense nucleic acid sequences are those that hybridize to such mRNA under stringent hybridization conditions. Preferred are antisense nucleic acid sequences that are substantially complementary to sequences at the 5' end of the MN cDNA sequence shown in Figure 1(A-C). Preferably said antisense nucleic acid sequences are oligonucleotides.

[0034] This disclosure also concerns vaccines comprising an immunogenic amount of one or more substantially pure MN proteins and/or polypeptides dispersed in a physiologically acceptable, nontoxic vehicle, which amount is effective to immunize a vertebrate, preferably a mammal, more preferably a human, against a neoplastic disease associated with the expression of MN proteins. Said proteins can be recombinantly, synthetically or otherwise biologically produced. A particular use of said vaccine would be to prevent recidivism and/or metastasis. For example, it could be administered to a patient who has had an MN-carrying tumor surgically removed, to prevent recurrence of the tumor.

[0035] The immunoassays can be embodied in test kits which comprise MN proteins/polypeptides and/or MN-specific antibodies. Such test kits can be in solid phase formats, but are not limited thereto, and can also be in liquid phase format, and can be based on immunohistochemical assays, ELISAs, particle assays, radiometric or fluorometric assays either unamplified or amplified, using, for example, avidin/biotin technology.

Abbreviations

[0036] The following abbreviations are used herein:

AA -	amino acid
ATCC -	American Type Culture Collection
bp -	base pairs
BLV -	bovine leukemia virus
BSA -	bovine serum albumin
BRL -	Bethesda Research Laboratories
CA -	carbonic anhydrase
CAT -	chloramphenicol acetyltransferase
Ci -	curie
cm -	centimeter
CMV -	cytomegalovirus
cpm -	counts per minute
C-terminus -	carboxyl-terminus
°C -	degrees centigrade
DEAE -	diethylaminoethyl
DMEM -	Dulbecco modified Eagle medium
EDTA -	ethylenediaminetetraacetate
EIA -	enzyme immunoassay
ELISA -	enzyme-linked immunosorbent assay
F -	fibroblasts
FCS -	fetal calf serum
FITC -	fluorescein isothiocyanate
GEX-3X-MN -	fusion protein MN glutathione S-transferase
H -	HeLa cells
HEF -	human embryo fibroblasts
HeLa K -	standard type of HeLa cells
HeLa S -	Stanbridge's mutant HeLa D98/AH.2
H/F-T -	hybrid HeLa fibroblast cells that are tumorigenic; derived from HeLa D98/AH.2
H/F-N -	hybrid HeLa fibroblast cells that are nontumorigenic; derived from HeLa D98/AH.2
HRP -	horseradish peroxidase
Inr -	initiator
IPTG -	isopropyl-beta-D-thiogalacto-pyranoside
kb -	kilobase
kbp -	kilobase pairs
kd -	kilodaltons
LCMV -	lymphocytic choriomeningitis virus
LTR -	long terminal repeat
M -	molar
mA -	milliampere
MAb -	monoclonal antibody

ME -	mercaptoethanol
MEM -	minimal essential medium
min. -	minute(s)
mg -	milligram
ml -	milliliter
mM -	millimolar
MMC -	mitomycin C
MLV -	murine leukemia virus
N -	normal concentration
NEG -	negative
ng -	nanogram
nt -	nucleotide
N-terminus -	amino-terminus
ODN -	oligodeoxynucleotide
ORF -	open reading frame
PA -	Protein A
PBS -	phosphate buffered saline
PCR -	polymerase chain reaction
PEST -	combination of one-letter abbreviations for proline, glutamic acid, serine, threonine
pI -	isoelectric point
PMA -	phorbol 12-myristate 13-acetate
POS -	positive
Py -	pyrimidine
RIA -	radioimmunoassay
RIP -	radioimmunoprecipitation
RIPA -	radioimmunoprecipitation assay
RNP -	RNase protection assay
SDRE -	serum dose response element
SDS -	sodium dodecyl sulfate
SDS-PAGE -	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SINE -	short interspersed repeated sequence
SP-RIA -	solid-phase radioimmunoassay
SSDS -	synthetic splice donor site
SSPE -	NaCl (0.18 M), sodium phosphate (0.01 M), EDTA (0.001 M)
TBE -	Tris-borate/EDTA electrophoresis buffer
TCA -	trichloroacetic acid
TC media -	tissue culture media
TMB -	tetramethylbenzidine
Tris -	tris (hydroxymethyl) aminomethane
μCi -	microcurie
μg -	microgram
μl -	microliter
μM -	micromolar
VSV -	vesicular stomatitis virus
X-MLV -	xenotropic murine leukemia virus

	Cell Lines
HeLa K	-- standard type of HeLa cells; aneuploid, epithelial-like cell line isolated from a human cervical adenocarcinoma [Gey et al., Cancer Res., 12: 264 (1952); Jones et al., Obstet. Gynecol., 38: 945-949 (1971)] obtained from Professor B. Korych, [Institute of Medical Microbiology and Immunology, Charles University; Prague, Czech Republic]

(continued)

	Cell Lines
HeLa D98/AH.2 (also HeLa S)	-- Mutant HeLa clone that is hypoxanthine guanine phosphoribosyl transferase-deficient (HGPRT$^-$) kindly provided by Eric J. Stanbridge [Department of Microbiology, College of Medicine, University of California, Irvine, CA (USA)] and reported in Stanbridge et al., Science, 215: 252-259 (15 Jan. 1982); parent of hybrid cells H/F-N and H/F-T, also obtained from E.J. Stanbridge.
NIH-3T3	-- murine fibroblast cell line reported in Aaronson, Science, 237: 178 (1987).
XC	-- cells derived from a rat rhabdomyosarcoma induced with Rous sarcoma virus-induced rat sarcoma [Svoboda, J., Natl. Cancer Center Institute Monograph No. 17, IN: "International Conference on Avian Tumor Viruses" (J.W. Beard ed.), pp. 277-298 (1964)], kindly provided by Jan Svoboda [Institute of Molecular Genetics, Czechoslovak Academy of Sciences; Prague, Czech Republic]; and
CGL1	-- H/F-N hybrid cells (HeLa D98/AH.2 derivative)
CGL2	-- H/F-N hybrid cells (HeLa D98/AH.2 derivative)
CGL3	-- H/F-T hybrid cells (HeLa D98/AH.2 derivative)
CGL4	-- H/F-T hybrid cells (HeLa D98/Ah.2 derivative)

Nucleotide and Amino Acid Sequence Symbols

[0037] The following symbols are used to represent nucleotides herein:

Base Symbol	Meaning
A	adenine
C	cytosine
G	guanine
T	thymine
U	uracil
I	inosine
M	A or C
R	A or G
W	A or T/U
S	C or G
Y	C or T/U
K	G or T/U
V	A or C or G
H	A or C or T/U
D	A or G or T/U
B	C or G or T/U
N/X	A or C or G or T/U

[0038] There are twenty main amino acids, each of which is specified by a different arrangement of three adjacent nucleotides (triplet code or codon), and which are linked together in a specific order to form a characteristic protein. A three-letter or one-letter convention is used herein to identify said amino acids, as, for example, in Figure 1(A-C) as follows:

Amino acid name	3 Ltr. Abbrev.	1 Ltr. Abbrev.
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Cysteine	Cys	C

(continued)

Amino acid name	3 Ltr. Abbrev.	1 Ltr. Abbrev.
Glutamic Acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Unknown or other		X

BRIEF DESCRIPTION OF THE FIGURES

[0039] Figure 1 (A-C) provides the nucleotide sequence for a full-length MN cDNA [SEQ. ID. NO.: 1] clone isolated as described herein. Figure 1 (A-C) also sets forth the predicted amino acid sequence [SEQ. ID. NO.: 2] encoded by the cDNA.

[0040] Figure 2 compares the results of immunizing baby rats to XC tumor cells with rat serum prepared against the fusion protein MN glutathione S-transferase (GEX-3X-MN) (the IM group) with the results of immunizing baby rats with control rat sera (the C group). Each point on the graph represents the tumor weight of a tumor from one rat. Example 2 details those experiments.

[0041] Figure 3 (A-F) provides a 10,898 bp complete genomic sequence of MN [SEQ. ID. NO.: 5]. The base count is as follows: 2654 A; 2739 C; 2645 G; and 2859 T. The 11 exons are shown in capital letters.

[0042] Figure 4 is a restriction map of the full-length MN cDNA. The open reading frame is shown as an open box. The thick lines below the restriction map illustrate the sizes and positions of two overlapping cDNA clones. The horizontal arrows indicate the positions of primers R1 [SEQ. ID. NO.: 7] and R2 [SEQ. ID. NO.: 8] used for the 5' end RACE. Relevant restriction sites are BamHI (B), EcoRV (V), EcoRI (E), PstI (Ps), PvuII (Pv).

[0043] Figure 5 is a map of the human MN gene. The numbered cross-hatched boxes represent exons. The box designated LTR denotes a region of homology to HERV-K LTR. The empty boxes are Alu-related sequences.

[0044] Figure 6 is a nucleotide sequence for the proposed promoter of the human MN gene [SEQ. ID. No.: 27]. The nucleotides are numbered from the transcription initiation site according to RNase protection assay. Potential regulatory elements are overlined. Transcription start sites are indicated by asterisks (RNase protection) and dots (RACE). The sequence of the 1st exon begins under the asterisks.

[0045] Figure 7 provides a schematic of the alignment of MN genomic clones according to their position related to the transcription initiation site. All the genomic fragments except Bd3 were isolated from a lambda FIX III genomic library derived from HeLa cells. Clone Bd3 was derived from a human fetal brain library.

[0046] Figure 8 shows the construction and cloning of a series of 5' deletion mutants of MN's putative promoter region linked to the bacterial CAT gene.

DETAILED DESCRIPTION

[0047] The MN gene is shown herein to be organized into 11 exons and 10 introns. Described herein is the cloning and sequencing of the MN cDNA and genomic sequences, and the genetic engineering of MN proteins -- such as the GEX-3X-MN, MN-PA, MN-Fc and MN 20-19 proteins. The recombinant MN proteins can be conveniently purified by affinity chromatography.

[0048] MN is manifested in HeLa cells by a twin protein, p54/58N. Immunoblots using a monoclonal antibody reactive with p54/58N (MAb M75) revealed two bands at 54 kd and 58 kd. Those two bands may correspond to one type of protein that differs by glycosylation pattern or by how it is processed. Herein, the phrase "twin protein" indicates p54/58N.

[0049] The expression of MN proteins appears to be diagnostic/prognostic for neoplastic disease. The MN twin protein, p54/58N, was found to be expressed in HeLa cells and in Stanbridge's tumorigenic (H/F-T) hybrid cells [Stanbridge et al., Somatic Cell Genet, 7: 699-712 (1981); and Stanbridge et al., Science, 215: 252-259 (1982)] but not in fibroblasts or in non-tumorigenic (H/F-N) hybrid cells [Stanbridge et al., id.]. In early studies reported in Zavada et al. WO 93/18152, supra, MN proteins were found in immunoblots prepared from human ovarian, endometrial and uterine cervical carcinomas, and in some benign neoplasias (as mammary papilloma) but not from normal ovarian, endometrial, uterine or placental tissues. Example 1 herein details further research on MN gene expression wherein MN antigen, as detected by immunohistochemical staining, was found to be prevalent in tumor cells of a number of cancers, including cervical, bladder, head and neck, and renal cell carcinomas among others. Further, the immunohistochemical staining experiments of Example 1 show that among normal tissues tested, only normal stomach tissues showed routinely and extensively the presence of MN antigen. MN antigen is further shown herein to be present sometimes in morphologically normal-appearing areas of tissue specimens exhibiting dysplasia and/or malignancy.

MN Gene--Cloning and Sequencing

[0050] Figure 1(A-C) provides the nucleotide sequence for a full-length MN cDNA clone isolated as described below [SEQ. ID. NO.: 1]. Figure 3(A-F) provides a complete MN genomic sequence [SEQ. ID. NO. : 5]. Figure 6 shows the nucleotide sequence for a proposed MN promoter [SEQ. ID. NO. : 27].

[0051] It is understood that because of the degeneracy of the genetic code, that is, that more than one codon will code for one amino acid [for example, the codons TTA, TTG, CTT, CTC, CTA and CTG each code for the amino acid leucine (leu)], that variations of the nucleotide sequences in, for example, SEQ. ID. NOS.: 1 and 5 wherein one codon is substituted for another, would produce a substantially equivalent protein or polypeptide. All such variations in the nucleotide sequences of the MN cDNA and complementary nucleic acid sequences are included within the scope of this disclosure.

[0052] It is further understood that the nucleotide sequences herein described and shown in Figures 1(A-C), 3(A-F) and 6, represent only the precise structures of the cDNA, genomic and promoter nucleotide sequences isolated and described herein. It is expected that slightly modified nucleotide sequences will be found or can be modified by techniques known in the art to code for substantially similar or homologous MN proteins and polypeptides, for example, those having similar epitopes, and such nucleotide sequences and proteins/ polypeptides are considered to be equivalents for the purpose of this disclosure. DNA or RNA having equivalent codons is considered within the scope of the disclosure , as are synthetic nucleic acid sequences that encode proteins/polypeptides homologous or substantially homologous to MN proteins/polypeptides, as well as those nucleic acid sequences that would hybridize to said exemplary sequences [SEQ. ID. NOS. 1, 5 and 27] under stringent conditions, or that, but for the degeneracy of the genetic code would hybridize to said cDNA nucleotide sequences under stringent hybridization conditions. Modifications and variations of nucleic acid sequences as indicated herein are considered to result in sequences that are substantially the same as the exemplary MN sequences and fragments thereof.

Partial cDNA clone

[0053] In Zavada et al., id., the isolation of a partial MN cDNA clone of 1397 bp in length was described. A lambda gt11 cDNA library of LMCV-infected HeLa cells was prepared and subjected to immunoscreening with Mab M75 in combination with goat anti-mouse antibodies conjugated with alkaline phosphatase. One positive clone was picked and subcloned into the NotI site of pBlusecript KS [Stratagen; La Jolla, CA (USA)] thereby creating pBluscript-MN.

[0054] Two oppositely oriented nested deletions were made using Erase-a-Base™ kit [Promega; Madison, WI (USA)] and sequenced by dideoxy method with a T7 sequencing kit [Pharmacia; Piscataway, NJ (USA)]. The sequencing showed a partial cDNA clone, the insert being 1397 bp long. The sequence comprises a large 1290 bp open reading frame and 107 bp 3' untranslated region containing a polyadenylation signal (AATAAA). However, the sequence surrounding the first ATG codon in the open reading frame (ORF) did not fit the definition of a translational start site. In addition, as followed from a comparison of the size of the MN clone with that of the corresponding mRNA in a Northern blot, the cDNA was shown to be missing about 100 bp from the 5' end of its sequence.

Full-Length cDNA Clone

[0055] Attempts to isolate a full-length clone from the original cDNA library failed. Therefore, the inventors performed a rapid amplification of cDNA ends (RACE) using MN-specific primers, R1 and R2 [SEQ. ID. NOS.: 7 and 8], derived from the 5' region of the original cDNA clone. The RACE product was inserted into pBluescript, and the entire population of recombinant plasmids was sequenced with an MN-specific primer ODN1 [SEQ. ID. NO.: 3]. In that way, a reliable sequence at the very 5' end of the MN cDNA as shown in Figure 1(A-C) [SEQ. ID. NO.: 1] was obtained.

[0056] Specifically, RACE was performed using 5' RACE System [GIBCO BRL; Gaithersburg, MD (USA)] as follows. 1 µg of mRNA (the same as above) was used as a template for the first strand cDNA synthesis which was primed by the MN-specific antisense oligonucleotide, R1 (5'-TGGGGTTCTTGAGGATCTCCAGGAG-3') [SEQ. ID. NO.: 7]. The first strand product was precipitated twice in the presence of ammonium acetate and a homopolymeric C tail was attached to its 3' end by TdT. Tailed cDNA was then amplified by PCR using a nested primer, R2 (5'-CTCTAACTTCAGGGAGCCCTCTTCTT-3') [SEQ. ID. NO.: 8] and an anchor primer that anneals to the homopolymeric tail (5'-CUACUACUACUAGGCCACGCGTCGACTAGTACGGGI IGGGIIGGGIIG-3') [SEQ. ID. NO.: 9]. The amplified product was digested with BamHI and SalI restriction enzymes and cloned into pBluescript II KS plasmid. After transformation, plasmid DNA was purified from the whole population of transformed cells and used as a template for sequencing with the MN-specific primer ODN1 [SEQ. ID. NO.: 3; a 29-mer 5' CGCCCAGTGGGTCATCTTCCCCAGAAGAG 3'].

[0057] Based upon results of the RACE analysis, the full-length MN cDNA sequence was seen to contain a single ORF starting at position 12, with an ATG codon that is in a good context (GCGCATGG) with the rule proposed for translation initiation [Kozak, J. Cell. Biol., 108: 229-241 (1989)]. [See below under Mapping of MN Gene Transcription Initiation Site for fine mapping of the 5' end of the MN gene.] The AT rich 3' untranslated region contains a polyadenylation signal (AATAAA) preceding the end of the cDNA by 10 bp. Surprisingly, the sequence from the original clone as well as from four additional clones obtained from the same cDNA library did not reveal any poly(A) tail. Moreover, just downstream of the poly(A) signal, an ATTTA motif that is thought to contribute to mRNA instability [Shaw and Kamen, Cell, 46: 659-667 (1986)] was found. That fact raised the possibility that the poly (A) tail is missing due to the specific degradation of the MN mRNA.

Genomic clones

[0058] To study MN regulation, MN genomic clones were isolated. One MN genomic clone (Bd3) was isolated from a human cosmid library prepared from fetal brain using both MN cDNA as a probe and the MN-specific primers derived from the 5' end of the cDNA ODN1 [SEQ. ID. NO.: 3, supra] and ODN2 [SEQ. ID NO.: 4; 19-mer (5' GGAATCCTCCTGCATCCGG 3')]. Sequence analysis revealed that that genomic clone covered a region upstream from a MN transcription start site and ending with the BamHI restriction site localized inside the MN cDNA. Other MN genomic clones can be similarly isolated.

[0059] In order to identify the complete genomic region of MN, the human genomic library in Lambda FIX II vector (Stratagene) was prepared from HeLa chromosomal DNA and screened by plaque hybridization using MN cDNA as described below. Several independent MN recombinant phages were identified, isolated and characterized by restriction mapping and hybridization analyses. Four overlapping recombinants covering the whole genomic region of MN were selected, digested and subcloned into pBluescript. The subclones were then subjected to'bidirectional nested deletions and sequencing. DNA sequences were compiled and analyzed by computer using the DNASIS software package.

[0060] The details of isolating genomic clones covering the complete genomic region for MN are provided below. Figure 7 provides a schematic of the alignment of MN genomic clones according to the transcription initiation site. Plasmids containing the A4a clone and the XE1 and XE3 subclones were deposited at the American Type Culture Collection (ATCC) at 10801 University Blvd., Manassas, Virginia 20110 - 2209 (USA) on June 6, 1995, respectively under ATCC Deposit Nos. 97199, 97200, and 97198.

Isolation of Genomic DNA Clones

[0061] The Sau3AI human HeLa genomic library was prepared in Lambda FIX II vector [Stratagene; La Jolla, CA (USA)] according to manufacturer's protocol. Human fetal brain cosmid library in SuperCos cosmid was from Stratagene. Recombinant phages or bacteria were plated at 1 x 10^5 plaque forming units on 22x22 cm Nunc plates or 5 x 10^4 cells on 150 mm Petri dishes, and plaques or colonies were transferred to Hybond N membranes (Amersham). Hybridization was carried out with the full-length MN cDNA labeled with [P^{32}]PdCTP by the Multiprime DNA labeling method (Amersham) at 65°C in 6 x SSC, 0.5% SDS, 10 x Denhardt's and 0.2 mg/l ml salmon sperm DNA. Filters were washed twice in 2 x SSC, 0.1% SDS at 65°C for 20 min. The dried filters were exposed to X-ray films, and positive clones were picked up. Phages and bacteria were isolated by 3-4 sequential rounds of screening.

Subcloning and DNA Sequencing

[0062] Genomic DNA fragments were subcloned into a pBluescript KS and templates for sequencing were generated by serial nested deletions using the Erase-a-Base system. Sequencing was performed by the dideoxynucleotide chain termination method using T7 sequencing kit (Pharmacia). Nucleotide sequence alignments and analyses were carried out using the DNASIS software package (Hitachi Software Engineering).

Exon-Intron Structure of Complete MN Genomic Region

[0063] The complete sequence of the overlapping clones contains 10,898 bp (SEQ. ID. NO.: 5). Figure 5 depicts the organization of the human MN gene, showing the location of all 11 exons as well as the 2 upstream and 6 intronic Alu repeat elements. All the exons are small, ranging from 27 to 191 bp, with the exception of the first exon which is 445 bp. The intron sizes range from 89 to 1400 bp.

[0064] Table 1 below lists the splice donor and acceptor sequences that conform to consensus splice sequences including the AG-GT motif [Mount, "A catalogue of splice junction sequences," Nucleic Acids Res. 10: 459-472 (1982)].

TABLE 1

Exon-Intron Structure of the Human MN Gene

Exon	Size	Genomic Position**	SEQ ID NO	5'splice donor	SEQ ID No
1	445	*3507-3951	28	AGAAG gtaagt	67
2	30	5126-5155	29	TGGAG gtgaga	68
3	171	5349-5519	30	CAGTC gtgagg	69
4	143	5651-5793	31	CCGAG gtgagc	70
5	93	5883-5975	32	TGGAG gtacca	71
6	67	7376-7442	33	GGAAG gtcagt	72
7	158	8777-8934	34	AGCAG gtgggc	73
8	145	9447-9591	35	GCCAG gtacag	74
9	27	9706-9732	36	TGCTG gtgagt	75
10	82	10350-10431	37	CACAG gtatta	76
11	191	10562-10752	38	ATAAT end	

Intron	Size	Genomic Position**	SEQ ID NO	3'splice acceptor	SEQ ID NO
1	1174	3952-5125	39	atacag GGGAT	77
2	193	5156-5348	40	ccccag GCGAC	78
3	131	5520-5650	41	acgcag TGCAA	79
4	89	5794-5882	42	tttcag ATCCA	80
5	1400	5976-7375	43	ccccag GAGGG	81
6	1334	7443-8776	44	tcacag GCTCA	82
7	512	8935-9446	45	ccctag CTCCA	83
8	114	9592-9705	46	ctccag TCCAG	84
9	617	9733-10349	47	tcgcag GTGACA	85
10	130	10432-10561	48	acacag AAGGG	86

** positions are related to nt numbering in whole genomic sequence including the 5' flanking region [Figure 3(A-F)]

* number corresponds to transcription initiation site determined below by RNase protection assay

[0065] A search for sequences related to MN gene in the EMBL Data Library did not reveal any specific homology except for 6 complete and 2 partial Alu-type repeats with homology to Alu sequences ranging from 69.8% to 91% [Jurka and Milosavljevic, "Reconstruction and analysis of human Alu genes," J. Mol. Evol. 32: 105-121 (1991)]. Below under the Characterization of the 5' Flanking Region, also a 222 bp sequence proximal to the 5' end of the genomic region is shown to be closely homologous to a region of the HERV-K LTR.

Mapping of MN Gene Transcription Initiation Site

[0066] In the earlier attempt to localize the site of transcription initiation of the MN gene by RACE (above), the obtained a major PCR fragment whose sequence placed the start site 12 bp upstream from the first codon of the ORF. That result was obtained probably due to a preferential amplification of the shortest form of mRNA. Therefore, the inventors used an RNase protection assay (RNP) for fine mapping of the 5' end of the MN gene. The probe was a uniformly labeled 470 nucleotide copy RNA (nt -205 to +265) [SEQ. ID. NO.: 55], which was hybridized to total RNA from MN-expressing HeLa and CGL3 cells and analyzed on a sequencing gel. That analysis has shown that the MN gene transcription initiates at multiple sites, the 5' end of the longest MN transcript being 30 nt longer than that previously characterized by RACE.

RNase Protection Assay

[0067] ^{32}P-labeled RNA probes were prepared with an RNA Transcription kit (Stratagene). In vitro transcription reactions were carried out using 1 μg of the linearized plasmid as a template, 50 μCi of [P^{32}P]rUTP (800 Ci/mmol), 10 U of either T3 or T7 RNA polymerase and other components of the Transcription Kit following instructions of the supplier. For mapping of the 5' end of MN mRNA, the 470 bp NcoI-BamHI fragment (NcoI filled in by Klenow enzyme) of Bd3 clone (nt - 205 to +265 related to transcription start) was subcloned to EcoRV-BamHI sites of pBluescript SK+, linearized with HindIII and labeled with T3 RNA polymerase. For the 3' end mRNA analysis, probe, that was prepared using T7 RNA polymerase on KS-dXE3-16 template (one of the nested deletion clones of MN genomic region XE3 subclone) digested with Sau3AI (which cuts exon 11 at position 10,629), was used. Approximately 3 x 10^{5} cpm of RNA probe were used per one RNase protection assay reaction.

[0068] RNase protection assays (RNP) were performed using Lysate RNase Protection Kit (USB/Amersham) according to protocols of the supplier. Briefly, cells were lysed using Lysis Solution at concentration of approximately 10^{7} cells/ml, and 45 μl of the cell homogenate were used in RNA/RNA hybridization reactions with ^{32}P-labeled RNA probes prepared as described above. Following overnight hybridizations at 42°C, homogenates were treated for 30 min at 37°C with RNase cocktail mix. Protected RNA duplexes were run on polyacrylamide/urea denaturing sequencing gels. Fixed and dried gels were exposed to X-ray film for 24 - 72 hours.

Mapping of MN Gene Transcription Termination Site

[0069] An RNase protection assay, as described above, was also used to verify***[deletion(s)]*** the 3' end of the MN cDNA. That was important with respect to our previous finding that the cDNA contains a poly(A) signal but lacks a poly (A) tail, which could be lost during the proposed degradation of MN mRNA due to the presence of an instability motif in its 3' untranslated region. RNP analysis of MN mRNA with the fragment of the genomic clone XE3 covering the region of interest corroborated our data from MN cDNA sequencing, since the 3' end of the protected fragment corresponded to the last base of MN cDNA (position 10,752 of the genomic sequence). That site also meets the requirement for the presence of a second signal in the genomic sequence that is needed for transcription termination and polyadenylation [McLauchlan et al., Nucleic Acids Res., 13: 1347 (1985)]. Motif TGTGTTAGT (nt 10,759-10,767) corresponds well to both the consensus sequence and the position of that signal within 22 bp downstream from the polyA signal (nt 10,737-10,742).

Characterization of the 5' Flanking Region

[0070] The Bd3 genomic clone isolated from human fetal brain cosmid library was found to cover a region of 3.5 kb upstream from the transcription start site of the MN gene. It contains no significant coding region. Two Alu repeats are situated at positions -2587 to -2296 [SEQ. ID. NO.: 56] and -1138 to -877 [SEQ. ID. NO.: 57] (with respect to the transcription start determined by RNP). The sequence proximal to the 5' end is strongly homologous (91.4% identity) to the U3 region of long terminal repeats of human endogenous retroviruses HERV-K [Ono, M., "Molecular cloning and long terminal repeat sequences of human endogenous retrovirus genes related to types A and B retrovirus genes," J. Virol, 58: 937-944 (1986)]. The LTR-like fragment is 222 bp long with an A-rich tail at its 3' end. Most probably, it represents part of SINE (short interspersed repeated sequence) type nonviral retroposon derived from HERV-K [Ono et al., "A novel human nonviral retroposon derived from an endogenous retrovirus," Nucleic Acids Res., 15: 8725-8373 (1987)]. There are no sequences corresponding to regulatory elements in this fragment, since the 3' part of U3, and the entire R and U5 regions of LTR are absent from the Bd3 genomic clone, and the glucocorticoid responsive element as well as the enhancer core sequences are beyond its 5' border.

[0071] However, two keratinocyte-dependent enhancers were identified in the sequence downstream from the LTR-like fragment at positions -3010 and -2814. Those elements are involved in transcriptional regulation of the E6-E7 oncogenes of human papillomaviruses and are thought to account for their tissue specificity [Cripe et al., "Transcriptional regulation of the human papillomavirus-16 E6-E7 promoter by a keratinocyte-dependent enhancer, and by viral E2 transactivator and repressor gene products: implications for cervical carcinogenesis," EMBO J., 6: 3745-3753 (1987)].

[0072] Nucleotide sequence analysis of the DNA 5' to the transcription start (from nt -507) revealed no recognizable TATA box within the expected distance from the beginning of the first exon (Figure 6). However, the presence of potential binding sites for transcription factors suggests that this region might contain a promoter for the MN gene. There are several consensus sequences for transcription factors AP1 and AP2 as well as for other regulatory elements, including a p53 binding site [Locker and Buzard, "A dictionary of transcription control sequences," J. DNA Sequencing and Mapping, 1: 3-11 (1990); Imagawa et al., "Transcription factor AP-2 mediates induction by two different signal-transduction pathways: protein kinase C and cAMP," Cell, 51: 251-260 (1987); El Deiry et al., "Human genomic DNA sequences define a consensus binding site for p53," Nat. Genet., 1: 44-49 (1992)]. Although the putative promoter region contains 59.3% C+G, it does not have additional attributes of CpG-rich islands that are typical for TATA-less promoters of housekeeping genes [Bird, "CpG-rich islands and the function of DNA methylation," Nature, 321: 209-213 (1986)]. Another class of genes lacking TATA box utilizes the initiator (Inr) element as a promoter. Many of these genes are not constitutively active, but they are rather regulated during differentiation or development. The Inr has a consensus sequence of PyPyPyCAPyPyPyPyPy [SEQ. ID. NO.: 23] and encompasses the transcription start site [Smale and Baltimore, "The 'initiator' as a transcription control element," Cell, 57: 103-113 (1989)]. There are two such consensus sequences in the MN putative promoter; however, they do not overlap the transcription start (Figure 6).

[0073] In the initial experiments, the inventors were unable to show promoter activity in human carcinoma cells HeLa and CGL3 that express MN, using the 3.5 kb Bd3 fragment and series of its deletion mutants (from nt -933 to -30) [SEQ. ID. NO.: 58] fused to chloramphenicol acetyl transferase (CAT) gene in a transient system. This might indicate that either the promoter activity of the region 5' to the MN transcription start is below the sensitivity of the CAT assay, or additional regulatory elements not present in our constructs are required for driving the expression of MN gene.

[0074] With respect to this fact, an interesting region was found in the middle of the MN gene. The region is about 1.4 kb in length [nt 4,600-6,000 of the genomic sequence; SEQ. ID. NO.: 49] and spans from the 3' part of the 1st intron to the end of the 5th exon. The region has the character of a typical CpG-rich island, with 62.8% C+G content and 82 CpG: 131 GpC dinucleotides. Moreover, there are multiple putative binding sites for transcription factors AP2 and Sp1 [Locker and Buzard, supra; Briggs et al., "Purification and biochemical characterization of the promoter-specific transcription factor Sp-1," Science, 234: 47-52 (1986)] concentrated in the center of this area. Particularly the 3rd intron of 131 bp in length contains three Sp1 and three AP2 consensus sequences. That data indicates the possible involvement of that region in the regulation of MN gene expression. However, functionality of that region, as well as other regulatory elements found in the proposed 5' MN promoter, remains to be determined.

MN Promoter Analysis

[0075] To define sequences necessary for MN gene expression, a series of 5' deletion mutants of the putative promoter region were fused to the bacterial chloramphenicol acetyltransferase (CAT) gene. [See Figure 8.] The pMN-CAT deletion constructs were transfected using a DEAE dextran method for transient expression into HeLa and CGL3 cells. Those cells were used since they naturally express MN protein, and thus, should contain all the required transcription factors.

[0076] After 48 hours, crude cell lysates were prepared and the activity of the expressed CAT was evaluated according to acetylation of [^{14}C]chloramphenicol by thin layer chromatography. However, no MN promoter CAT activity was detected in either the HeLa or the CGL3 cells in a transient system. On the other hand, reporter CAT plasmids with viral promoters (e.g. pBLV-LTR + tax transactivator, pRSV CAT and pSV2 CAT), that served as positive controls, gave strong signals on the chromatogram. [pSV2 CAT carries the SV40 origin and expresses CAT from the SV40 early promoter (P_E). pRSV CAT expresses CAT from the Rous sarcoma virus (RSV)LTR promoter (P_{LTR}).]

[0077] No detectable CAT activity was observed in additional experiments using increasing amounts of transfected plasmids (from 2 to 20 g DNA per dish) and prolonged periods of cell incubation after transcription. Increased cell density also did not improve the results (in contrast to the expectations based on density-dependent expression of native MN protein in HeLa cells). Since the inventors had found consensus sequences for transcription factors AP2 and AP1 in the putative MN promoter, they studied the effect of their inducers dexamethasone (1 m) and phorbol ester phorbol 12-myristate 13-acetate (PMA 50 ng/ml) on CAT activity. However, the MN promoter was unresponsive to those compounds.

[0078] The following provides explanations for the results: --the putative MN promoter immediately preceding the transcription initiation site is very weak, and its activity is below the sensitivity of a standard CAT assay; --additional sequences (e.g enhancers) are necessary for MN transcription.

[0079] To further shed light on the regulation of MN expression at the level of transcription, constructs, analogously prepared to the MN-CAT constructs, are prepared, wherein the MN promoter region is upstream from the neomycin phosphotransferase gene engineered for mammalian expression. Such constructs are then transfected to cells which are subjected to selection with G418. Activity of the promoter is then evaluated on the basis of the number of G418 resistant colonies that result. That method has the capacity to detect activity of a promoter that is 50 to 100 times weaker in comparison to promoters detectable by a CAT assay.

Deduced Amino Acid Sequence

[0080] The ORF of the MN cDNA shown in Figure 1(A-C) has the coding capacity for a 459 amino acid protein with a calculated molecular weight of 49.7 kd. MN protein has an estimated pI of about 4. As assessed by amino acid sequence analysis, the deduced primary structure of the MN protein can be divided into four distinct regions. The initial hydrophobic region of hydrophobic region of 37 amino acids (AA) corresponds to a signal peptide. The mature protein has an N-terminal part of 377 AA, a hydrophobic transmembrane segment of 20 AA and a C-terminal region of 25 AA. Alternatively, the MN protein can be viewed as having five domains as follows: (1) a signal peptide [amino acids (AA) 1-37; SEQ. ID. NO.: 6]; (2) a region of homology to collagen alphal chain (AA 38-135; SEQ. ID. NO.: 50); (3) a carbonic anhydrase domain (AA 136-391; SEQ. ID. NO.: 51); (4) a transmembrane region (AA 415-434; SEQ. ID. NO.: 52); and (5) an intracellular C terminus (AA 435-459; SEQ. ID. NO.: 53). [The AA numbers are keyed to Figure 1(A-C).]

[0081] More detailed insight into MN protein primary structure disclosed the presence of several consensus sequences. One potential N-glycosylation site was found at position 346 of Figure 1(A-C). That feature, together with a predicted membrane-spanning region are consistent with the results, in which MN was shown to be an N-glycosylated protein localized in the plasma membrane. MN protein sequence deduced from cDNA was also found to contain seven S/TPXX sequence elements [SEQ. ID. NOS.: 25 AND 26] (one of them is in the signal peptide) defined by Suzuki, J. Mol. Biol., 207: 61-84 (1989) as motifs frequently found in gene regulatory proteins. However, only two of them are composed of the suggested consensus amino acids.

[0082] Experiments have shown that the MN protein is able to bind zinc cations, as shown by affinity chromatography using Zn-charged chelating sepharose. MN protein immunoprecipitated from HeLa cells by Mab M75 was found to have weak catalytic activity of CA. The CA-like domain of MN has a structural predisposition to serve as a binding site for small soluble domains. Thus, MN protein could mediate some kind of signal transduction.

[0083] MN protein from LCMV-infected HeLA cells was shown by using DNA cellulose affinity chromatography to bind to immobilized double-stranded salmon sperm DNA. The binding activity required both the presence of zinc cations and the absence of a reducing agent in the binding buffer.

Sequence Similarities

[0084] Computer analysis of the MN cDNA sequence was carried out using DNASIS and PROSID (Pharmacia Software packages). GenBank, EMBL, Protein Identification Resource and SWISS-PROT databases were searched for all possible sequence similarities. In addition, a search for proteins sharing sequence similarities with MN was performed in the MIPS databank with the FastA program [Pearson and Lipman, PNAS (USA), 85: 2444 (1988)].

[0085] The MN gene was found to clearly be a novel sequence derived from the human genome. Searches for amino acid sequence similarities in protein databases revealed as the closest homology a level of sequence identity (38.9% in 256 AA or 44% in an 170 AA overlap) between the central part of the MN protein [AAs 136-391 (SEQ. ID. NO: 51)] or 221-390 [SEQ. ID. NO.: 54] of Figure 1(A-C) and carbonic anhydrases (CA). However, the overall sequence homology between the cDNA MN sequence and cDNA sequences encoding different CA isoenzymes is in a homology range of 48-50% which is considered by ones in the art to be low. Therefore, the MN cDNA sequence is not closely related to any CA cDNA sequences.

[0086] Only very closely related nt sequences having a homology of at least 80-90% would hybridize to each other under stringent conditions. A sequence comparison of the MN cDNA sequence shown in Figure 1(A-C) and a corresponding cDNA of the human carbonic anhydrase II (CA II) showed that there are no stretches of identity between the two sequences that would be long enough to allow for a segment of the CA II cDNA sequence having 50 or more nucleotides to hybridize under stringent hybridization conditions to the MN cDNA or vice versa.

[0087] Although MN deduced amino acid sequences show some homology to known carbonic anhydrases, they differ from them in several repects. Seven carbonic anhydrases are known [Dodgson et al. (eds.), The Carbonic Anhydrases, (Plenum Press; New York/London (1991)]. All of the known carbonic anhydrases are proteins of about 30 kd, smaller than the p54/58N-related products of the MN gene. Further, the carbonic anhydrases do not form oligomers as do the MN-related proteins.

[0088] The N-terminal part of the MN protein (AA 38-135; SEQ. ID. NO.: 50) shows a 27-30% identity with human collagen alpha1 chain, which is an important component of the extracellular matrix.

MN Proteins and/or Polypeptides

[0089] The phrase "MN proteins and/or polypeptides" (MN proteins/polypeptides) is herein defined to mean proteins and/or polypeptides encoded by an MN gene or fragments thereof. An exemplary and preferred MN protein has the deduced amino acid sequence shown in Figure 1(A-C). Preferred MN proteins/polypeptides are those proteins and/or polypeptides that have substantial homology with the MN protein shown in Figure 1(A-C). For example, such substantially homologous MN proteins/ polypeptides are those that are reactive with the MN-specific antibodies, preferably the Mabs M75, MN12, MN9 and MN 7 or their equivalents.

[0090] A "polypeptide" is a chain of amino acids covalently bound by peptide linkages and is herein considered to be composed of 50 or less amino acids. A "protein" is herein defined to be a polypeptide composed of more than 50 amino acids.

[0091] MN proteins exhibit several interesting features: cell membrane localization, cell density dependent expression in HeLa cells, correlation with the tumorigenic phenotype of HeLa x fibroblast somatic cell hybrids, and expression in several human carcinomas among other tissues. As demonstrated herein, for example, in Example 1, MN protein can be found directly in tumor tissue sections but not in general in counterpart normal tissues (exceptions noted infra in Example 1 as in normal stomach tissues). MN is also expressed sometimes in morphologically normal appearing areas of tissue specimens exhibiting dysplasia and/or malignancy. Taken together, these features suggest a possible involvement of MN in the regulation of cell proliferation, differentiation and/or transformation.

[0092] It can be appreciated that a protein or polypeptide produced by a neoplastic cell in vivo could be altered in sequence from that produced by a tumor cell in cell culture or by a transformed cell. Thus, MN proteins and/or polypeptides which have varying amino acid sequences including without limitation, amino acid substitutions, extensions, deletions, truncations and combinations thereof, fall within the scope of this disclosure . It can also be appreciated that a protein extant within body fluids is subject to degradative processes, such as, proteolytic processes; thus, MN proteins that are significantly truncated and MN polypeptides may be found in body fluids, such as, sera. The phrase "MN antigen" is used herein to encompass MN proteins and/or polypeptides.

[0093] It will further be appreciated that the amino acid sequence of MN proteins and polypeptides can be modified by genetic techniques. One or more amino acids can be deleted or substituted. Such amino acid changes may not cause any measurable change in the biological activity of the protein or polypeptide and result in proteins or polypeptides which are within the scope of this disclosure, as well as, MN muteins.

[0094] The MN proteins and polypeptides can be prepared in a variety of ways, for example, recombinantly, synthetically or otherwise biologically, that is, by cleaving longer proteins and polypeptides enzymatically and/or chemically. A preferred method to prepare MN proteins is by a recombinant means. Particularly preferred methods of recombinantly producing MN proteins are described below for the GEX-3X-MN, MN 20-19, MN-Fc and MN-PA proteins.

Recombinant Production of MN Proteins and Polypeptides

[0095] A representative method to prepare the MN proteins shown in Figure 1(A-C) or fragments thereof would be to insert the full-length or an appropriate fragment of MN cDNA into an appropriate expression vector as exemplified below. In Zavada et al., WO 93/18152, supra, production of a fusion protein GEX-3X-MN using the partial cDNA clone (described above) in the vector pGEX-3X (Pharmacia) is described. Nonglycosylated GEX-3X-MN (the Mn fusion protein MN glutathione S-transferase) from XL1-Blue cells. Herein described is the recombinant production of both a glycosylated MN protein expressed from insect cells and a nonglycosylated MN protein expressed from E. coli using the expression plasmid pEt-22b [Novagen Inc.; Madison, WI (USA)].

[0096] Baculovirus Expression Systems. Recombinant baculovirus express vectors have been developed for infection into several types of insect cells. For example, recombinant baculoviruses have been developed for among others: Aedes aegypti, Autographa californica, Bombyx mor, Drosphila melanogaster, Heliothis zea, Spodoptera frugiperda, and Trichoplusia ni [PCT Pub. No. WO 89/046699; Wright, Nature, 321: 718 (1986); Fraser et al., In Vitro Cell Dev. Biol., 25: 225 (1989). Methods of introducing exogenous DNA into insect hosts are well-known in the art. DNA transfection and viral infection procedures usually vary with the insect genus to be transformed. See, for example, Autographa [Carstens et al., Virology, 101: 311 (1980)]; Spodoptera [Kang, "Baculovirus Vectors for Expression of Foreign Genes," in: Advances in Virus Research, 35 (1988)]; and Heliothis (virescens) [PCT Pub. No. WO 88/02030].

[0097] A wide variety of other host-cloning vector combinations may be usefully employed in cloning the MN DNA isolated as described herein. For example, useful cloning vehicles may include chromosomal, nonchromosomal and synthetic DNA sequences such as various known bacterial plasmids such as pBR322, other E. coli plasmids and their derivatives and wider host range plasmids such as RP4, phage DNA, such as, the numerous derivatives of phage lambda, e.g., NB989 and vectors derived from combinations of plasmids and phage DNAs such as plasmids which have been modified to employ phage DNA expression control sequences.

[0098] Useful hosts may be eukaryotic or prokaryotic and include bacterial hosts such as E. coli and other bacterial strains, yeasts and other fungi, animal or plant hosts such as animal or plant cells in culture, insect cells and other hosts. Of course, not all hosts may be equally efficient. The particular selection of host-cloning vehicle combination may be made by those of skill in the art after due consideration of the principles set forth herein.

[0099] The particular site chosen for insertion of the selected DNA fragment into the cloning vehicle to form a recombinant DNA molecule is determined by a variety of factors. These include size and structure of the protein or polypeptide to be expressed, susceptibility of the desired protein or polypeptide to endoenzymatic degradation by the host cell components and contamination by its proteins, expression characteristics such as the location of start and stop codons, and other factors recognized by those of skill in the art.

[0100] The recombinant nucleic acid molecule containing the MN gene, fragment thereof, or cDNA therefrom, may be employed to transform a host so as to permit that host (transformant) to express the structural gene or fragment thereof and to produce the protein or polypeptide for which the hybrid DNA encodes. The recombinant nucleic acid molecule may also be employed to transform a host so as to permit that host on replication to produce additional recombinant nucleic acid molecules as a source of MN nucleic acid and fragments thereof. The selection of an appropriate host for either of those uses is controlled by a number of factors recognized in the art. These include, for example, compatibility with the chosen vector, toxicity of the co-products, ease of recovery of the desired protein or polypeptide, expression characteristics, biosafety and costs.

[0101] Where the host cell is a procaryote such as E. coli, competent cells which are capable of DNA uptake are prepared from cells harvested after exponential growth phase and subsequently treated by the $CaCl_2$ method by well known procedures.. Transformation can also be performed after forming a protoplast of the host cell.

[0102] Where the host used is an eukaryote, transfection methods such as the use of a calcium phosphate-precipitate, electroporation, conventional mechanical procedures such as microinjection, insertion of a plasmid encapsulated in red blood cell ghosts or in liposomes, treatment of cells with agents such as lysophosphatidyl-choline or use of virus vectors, or the like may be used.

[0103] The level of production of a protein or polypeptide is governed by three major factors: (1) the number of copies of the gene or DNA sequence encoding for it within the cell; (2) the efficiency with which those gene and sequence copies are transcribed and translated; and (3) the stability of the mRNA. Efficiencies of transcription and translation (which together comprise expression) are in turn dependent upon nucleotide sequences, normally situated ahead of the desired coding sequence. Those nucleotide sequences or expression control sequences define, inter alia, the location at which an RNA polymerase interacts to initiate transcription (the promoter sequence) and at which ribosomes bind and interact with the mRNA (the product of transcription) to initiate translation. Not all such expression control sequences function with equal efficiency. It is thus of advantage to separate the specific coding sequences for the desired protein from their adjacent nucleotide sequences and fuse them instead to known expression control sequences so as to favor higher levels of expression. This having been achieved, the newly engineered DNA fragment may be inserted into a multicopy plasmid or a bacteriophage derivative in order to increase the number of gene or sequence copies within the cell and thereby further improve the yield of expressed protein.

[0104] Several expression control sequences may be employed. These include the operator, promoter and ribosome binding and interaction sequences (including sequences such as the Shine-Dalgarno sequences) of the lactose operon of E. coli ("the lac system"), the corresponding sequences of the tryptophan synthetase system of E. coli ("the trp system"), a fusion of the trp and lac promoter ("the tac system"), the major operator and promoter regions of phage lambda (O_LP_L and O_RP_R,), and the control region of the phage fd coat protein. DNA fragments containing these sequences are excised by cleavage with restriction enzymes from the DNA isolated from transducing phages that carry the lac or trp operons, or from the DNA of phage lambda or fd. Those fragments are then manipulated in order to obtain a limited population of molecules such that the essential controlling sequences can be joined very close to, or in juxtaposition with, the initiation codon of the coding sequence.

[0105] The fusion product is then inserted into a cloning vehicle for transformation or transfection of the appropriate hosts and the level of antigen production is measured. Cells giving the most efficient expression may be thus selected. Alternatively, cloning vechicles carrying the lac, trp or lambda P_L control system attached to an initiation codon may be employed and fused to a fragment containing a sequence coding for a MN protein or polypeptide such that the gene or sequence is correctly translated from the initiation codon of the cloning vehicle.

[0106] The phrase "recombinant nucleic acid molecule" is herein defined to mean a hybrid nucleotide sequence comprising at least two nucleotide sequences, the first sequence not normally being found together in nature with the second.

[0107] The phrase "expression control sequence" is herein defined to mean a sequence of nucleotides that controls and regulates expression of structural genes when operatively linked to those genes.

[0108] The following are representative examples of genetically engineering MN proteins of this disclosure. The descriptions are exemplary.

Expression of MN 20-19 Protein

[0109] A representative, recombinantly produced MN protein is the MN 20-19 protein which, when produced in baculovirus-infected Sf9 cells [Spodoptera frugiperda cells; Clontech; Palo Alto, CA (USA)], is glycosylated. The MN 20-19 protein misses the putative signal peptide (AAs 1-37) of SEQ. ID. NO.: 6 (Figure 1 (A-C)), has a methionine (Met) at the N-terminus for expression, and a Leu-Glu-His-His-His-His-His-His [SEQ. ID NO.: 22] added to the C-terminus for purification.

[0110] In order to insert the portion of the MN coding sequence for the GEX-3X-MN fusion protein into alternate expression systems, a set of primers for PCR was designed. The primers were constructed to provide restriction sites at each end of the coding sequence, as well as in-frame start and stop codons. The sequences of the primers, indicating restriction enzyme cleavage sites and expression landmarks, are shown below.

```
Primer #20:N-terminus

                        ┌Translation start
5'GTCGCTAGCTCCATGGGTCATATGCAGAGGTTGCCCCGGATGCAG 3'
     NheI  NcoI    NdeI └MN cDNA #1      [SEQ. ID. NO. 17]


Primer #19:C-terminus

              ┌Translation stop
5'GAAGATCTCTTACTCGAGCATTCTCCAAGATCCAGCCTCTAGG 3'
    BglII    XhoI └MN cDNA                [SEQ. ID. NO. 18]
```

The SEQ. ID. NOS.: 17 and 18 primers were used to amplify the MN coding sequence present in the GEX-3X-MN vector using standard PCR techniques. The resulting PCR product (termed MN 20-19) was electrophoresed on a 0.5% agarose/1X TBE gel; the 1.3 kb band was excised; and the DNA recovered using the Gene Clean II kit according to the manufacturer's instructions [Bio101; LaJolla, CA (USA)].

[0111] MN 20-19 and plasmid pET-22b were cleaved with the restriction enzymes NdeI and XhoI, phenol-chloroform extracted, and the appropriate bands recovered by agarose gel electrophoresis as above. The isolated fragments were ethanol co-precipitated at a vector:insert ratio of 1:4. After resuspension, the fragments were ligated using T4 DNA ligase. The resulting product was used to transform competent Novablue E. coli cells [Novagen, Inc.]. Plasmid mini-preps [Magic Minipreps; Promega] from the resultant ampicillin resistant colonies were screened for the presence of the correct insert by restriction mapping. Insertion of the gene fragment into the pET-22b plasmid using the NdeI and XhoI sites added a 6-histidine tail to the protein that could be used for affinity isolation.

[0112] To prepare MN 20-19 for insertion into the baculovirus expression system, the MN 20-19 gene fragment was excised from pET-22b using the restriction endonucleases XbaI and PvuI. The baculovirus shuttle vector pBacPAK8 [Clontech] was cleaved with XbaI and PacI. The desired fragments (1.3 kb for MN 20-19 and 5.5 kb for pBacPAK8) were isolated by agarose gel electrophoresis, recovered using Gene Clean II, and co-precipitated at an insert:vector ratio of 2.4:1.

[0113] After ligation with T4 DNA ligase, the DNA was used to transform competent NM522 E. coli cells (Stratagene). Plasmid mini-preps from resultant ampicillin resistant colonies were screened for the presence of the correct insert by restriction mapping. Plasmid DNA from an appropriate colony and linearized BacPAK6 baculovirus DNA [Clontech] were used to transform Sf9 cells by standard techniques. Recombination produced BacPAK viruses carrying the MN 20-19 sequence. Those viruses were plated onto Sf9 cells and overlaid with agar.

[0114] Plaques were picked and plated onto Sf9 cells. The conditioned media and cells were collected. A small aliquot of the conditioned media was set aside for testing. The cells were extracted with PBS with 1% Triton X100.

[0115] The conditioned media and the cell extracts were dot blotted onto nitrocellulose paper. The blot was blocked with 5% non-fat dried milk in PBS. Mab M75 were used to detect the MN 20-19 protein in the dot blots. A rabbit anti-mouse Ig-HRP was used to detect bound Mab M75. The blots were developed with TMB/H_2O_2 with a membrane enhancer [KPL; Gaithersburg, MD (USA)]. Two clones producing the strongest reaction on the dot blots were selected for expansion. One was used to produce MN 20-19 protein in High Five cells [Invitrogen Corp., San Diego, CA (USA); BTI-TN-5BI-4; derived from Trichoplusia ni egg cell homogenate]. MN 20-19 protein was purified from the conditioned media from the virus infected High Five cells.

[0116] The MN 20-19 protein was purified from the conditioned media by immunoaffinity chromatography. 6.5 mg of Mab M75 was coupled to 1 g of Tresyl activated Toyopearl™ [Tosoh, Japan (#14471)]. Approximately 150 ml of the conditioned media was run through the M75-Toyopearl column. The column was washed with PBS, and the MN 20-19 protein was eluted with 1.5 M MgCl. The eluted protein was then dialyzed against PBS.

Fusion Proteins with C-Terminal Part Including Transmembrane Region Replaced by Fc or PA

[0117] MN fusion proteins in which the C terminal part including the transmembrane region is replaced by the Fc fragment of human IgG or by Protein A were constructed. Such fusion proteins are useful to identify MN binding protein (s). In such MN chimaeras, the whole N-terminal part of MN is accessible to interaction with heterologous proteins, and the C terminal tag serves for simple detection and purification of protein complexes.

Fusion Protein MN-PA (Protein A)

[0118] In a first step, the 3' end of the MN cDNA encoding the transmembrane region of the MN protein was deleted. The plasmid pFLMN (e.g. pBluescript with full length MN cDNA) was cleaved by EcoRI and blunt ended by S1 nuclease. Subsequent cleavage by *Sac*I resulted in the removal of the *Eco*RI-*Sac*I fragment. The deleted fragment was then replaced by a Protein A coding sequence that was derived from plasmid pEZZ (purchased from Pharmacia), which had been cleaved with *Rsa*I and *Sac*I. The obtained MN-PA construct was subcloned into a eukaryotic expression vector pSG5C (described in Example 3), and was then ready for transfection experiments.

Fusion Protein MN-Fc

[0119] The cloning of the fusion protein MN-Fc was rather complicated due to the use of a genomic clone containing the Fc fragment of human IgG which had a complex structure in that it contained an enhancer, a promoter, exons and introns. Moreover, the complete sequence of the clone was not available. Thus, it was necessary to ensure the correct in-phase splicing and fusion of MN to the Fc fragment by the addition of a synthetic splice donor site (SSDS) designed according to the splicing sequences of the MN gene.

[0120] The construction procedure was as follows:

1. Plasmid pMH4 (e.g. pSV2*gpt* containing a genomic clone of the human IgG Fc region) was cleaved by *BamH*I *in* order to get a 13 kb fragment encoding Fc. [In pSV2*gpt*, the E. coli xanthine-guanine phosphoribosyl transferase gene (*gpt*) is expressed using the SV40 early promoter (P_E) located in the SV40 origin, the SV40 small T intron, and the SV40 polyadenylation site.]
2. At the same time, plasmid pFLMN (with full length MN cDNA) was cleaved by *Sal*I-*EcoR*I. The released fragment was purified and ligated with a synthetic adapter *EcoR*I-*Bgl*II containing a synthetic splice donor site (SSDS).
3. Simultaneously, the plasmid pBKCMV was cleaved by *Sal*I-*BamH*I. Then advantage was taken of the fact that the *BamH*I cohesive ends (of the Fc coding fragment) are compatible with the *Bgl*II ends of the SSDS, and Fc was ligated to MN. The MN-Fc ligation product was then inserted into pBKCMV by directional cloning through the *Sal*I and *BamH*I sites.

[0121] Verification of the correct orientation and in-phase fusion of the obtained MN-Fc chimaeric clones was problematic in that the sequence of Fc was not known. Thus, functional constructs are selected on the basis of results of transient eukaryotic expression analyses.

Synthetic and Biologic Production of MN Proteins and Polypeptides

[0122] MN proteins and polypeptides may be prepared not only by recombinant means but also by synthetic and by other biologic means. Synthetic formation of the polypeptide or protein requires chemically synthesizing the desired chain of amino acids by methods well known in the art. Exemplary of other biologic means to prepare the desired polypeptide or protein is to subject to selective proteolysis a longer MN polypeptide or protein containing the desired amino acid sequence; for example, the longer polypeptide or protein can be split with chemical reagents or with enzymes.

[0123] Chemical synthesis of a peptide is conventional in the art and can be accomplished, for example, by the Merrifield solid phase synthesis technique [Merrifield, J., Am. Chem. Soc., 85: 2149-2154 (1963); Kent et al., Synthetic Peptides in Biology and Medicine, 29 f.f. eds. Alitalo et al., (Elsevier Science Publishers 1985); and Haug, J.D., "Peptide Synthesis and Protecting Group Strategy", American Biotechnology Laboratory, 5(1): 40-47 (Jan/Feb. 1987)].

[0124] Techniques of chemical peptide synthesis include using automatic peptide synthesizers employing commercially available protected amino acids, for example, Biosearch [San Rafael, CA (USA)] Models 9500 and 9600; Applied Biosystems, Inc. [Foster City, CA (USA)] Model 430; Milligen [a division of Millipore Corp.; Bedford, MA (USA)] Model 9050; and Du Pont's RAMP (Rapid Automated Multiple Peptide Synthesis) [Du Pont Compass, Wilmington, DE (USA)].

Regulation of MN Expression and MN Promoter

[0125] MN appears to be a novel regulatory protein that is directly involved in the control of cell proliferation and in cellular transformation. In HeLa cells, the expression of MN is positively regulated by cell density. Its level is increased by persistent infection with LCMV. In hybrid cells between HeLa and normal fibroblasts, MN expression correlates with tumorigenicity. The fact that MN is not present in nontumorigenic hybrid cells (CGL1), but is expressed in a tumorigenic segregant lacking chromosome 11, indicates that MN is negatively regulated by a putative suppressor in chromosome 11.

[0126] Evidence supporting the regulatory role of MN protein was found in the generation of stable transfectants of NIH 3T3 cells that constitutively express MN protein as described in Example 3. As a consequence of MN expression, the NIH 3T3 cells acquired features associated with a transformed phenotype: altered morphology, increased saturation density, proliferative advantage in serum-reduced media, enhanced DNA synthesis and capacity for anchorage-independent growth. Further, as shown in Example 4, flow cytometric analyses of asynchronous cell populations indicated that the expression of MN protein leads to accelerated progression of cells through G1 phase, reduction of cell size and the loss of capacity for growth arrest under inappropriate conditions. Also, Example 4 shows that MN expressing cells display a decreased sensitivity to the DNA damaging drug mitomycin C.

[0127] Nontumorigenic human cells, CGL1 cells, were also transfected with the full-length MN cDNA. The same pSG5C-MN construct in combination with pSV2neo plasmid as used to transfect the NIH 3T3 cells (Example 3) was used. Also the protocol was the same except that the G418 concentration was increased to 1000 μg/ml.

[0128] Out of 15 MN-positive clones (tested by SP-RIA and Western blotting), 3 were chosen for further analysis. Two MN-negative clones isolated from CGL1 cells transfected with empty plasmid were added as controls. Initial analysis indicates that the morphology and growth habits of MN-transfected CGL1 cells are not changed dramatically, but their proliferation rate and plating efficiency is increased.

[0129] MN cDNA and promoter. When the promoter region from the MN genomic clone, isolated as described above, was linked to MN cDNA and transfected into CGL1 hybrid cells, expression of MN protein was detectable immediately after selection. However, then it gradually ceased, indicating thus an action of a feedback regulator. The putative regulatory element appeared to be acting via the MN promoter, because when the full-length cDNA (not containing the promoter) was used for transfection, no similar effect was observed.

[0130] An "antisense" MN cDNA/MN promoter construct was used to transfect CGL3 cells. The effect was the opposite of that of the CGL1 cells transfected with the "sense" construct. Whereas the transfected CGL1 cells formed colonies several times larger than the control CGL1, the transfected CGL3 cells formed colonies much smaller than the control CGL3 cells.

[0131] For those experiments, the part of the promoter region that was linked to the MN cDNA through a BamHI site was derived from a NcoI - BamHI fragment of the MN genomic clone [Bd3] and represents a region a few hundred bp upstream from the transcription initiation site. After the ligation, the joint DNA was inserted into a pBK-CMV expression vector [Stratagene]. The required orientation of the inserted sequence was ensured by directional cloning and subsequently verified by restriction analysis. The tranfection procedure was the same as used in transfecting the NIH 3T3 cells (Example 3), but co-transfection with the pSV2neo plasmid was not necessary since the neo selection marker was already included in the pBK-CMV vector.

[0132] After two weeks of selection in a medium containing G418, remarkable differences between the numbers and sizes of the colonies grown were evident as noted above. Immediately following the selection and cloning, the MN-transfected CGL1 and CGL3 cells were tested by SP-RIA for expression and repression of MN, respectively. The isolated transfected CGL1 clones were MN positive (although the level was lower than obtained with the full-length cDNA), whereas MN protein was almost absent from the transfected CGL3 clones. However, in subsequent passages, the expression of MN in transfected CGL1 cells started to cease, and was then blocked perhaps evidencing a control feedback mechanism.

[0133] As a result of the very much lowered proliferation of the transfected CGL3 cells, it was difficult to expand the majority of cloned cells (according to SP-RIA, those with the lowest levels of MN), and they were lost during passaging. However, some clones overcame that problem and again expressed MN. It is possible that once those cells reached a higher quantity, that the level of endogenously produced MN mRNA increased over the amount of ectopically expressed antisense mRNA.

Transformation and Reversion

[0134] As illustrated in Examples 3 and 4, vertebrate cells transfected with MN cDNA in suitable vectors show striking morphologic transformation. Transformed cells may be very small, densely packed, slowly growing, with basophilic cytoplasm and enlarged Golgi apparatus. However, it has been found that transformed clones revert over time, for example, within 3-4 weeks, to nearly normal morphology, even though the cells may be producing MN protein at high levels. MN protein is biologically active even in yeast cells; depending upon the level of its expression, it stimulates or retards their growth and induces morphologic alterations.

[0135] Full-length MN cDNA was inserted into pGD, a MLV-derived vector, which together with standard competent MLV (murine leukemia virus), forms an infectious, transmissible complex [pGD-MN + MLV]. That complex also transforms vertebrate cells, such as, NIH 3T3 cells and mouse embryo fibroblasts BALB/c, which also revert to nearly normal morphology. Such revertants again contain MN protein and produce the [pGD-MN + MLV] artificial virus complex, which retains its transforming capacity. Thus, reversion of MN-transformed cells is apparently not due to a loss, silencing or mutation of MN cDNA, but may be the result of the activation of suppressor gene (s) .

Nucleic Acid Probes and Test Kits

[0136] Nucleic acid probes of this disclosure are those comprising sequences that are complementary or substantially complementary to the MN cDNA sequence shown in Figure 1(A-C) or to other MN gene sequences, such as, the complete genomic sequence of Figure 3(A-F) [SEQ. ID. NO.: 5] and the putative promoter sequence [SEQ. ID. NO.: 27 of Figure 6]. The phrase "substantially complementary" is defined herein to have the meaning as it is well understood in the art and, thus, used in the context of standard hybridization conditions. The stringency of hybridization conditions can be adjusted to control the precision of complementarity. Two nucleic acids are, for example, substantially complementary to each other, if they hybridize to each other under stringent hybridization conditions.

[0137] Stringent hybridization conditions are considered herein to conform to standard hybridization conditions understood in the art to be stringent. For example, it is generally understood that stringent conditions encompass relatively low salt and/or high temperature conditions, such as provided by 0.02 M to 0.15 M NaCl at temperatures of 50°C to 70°C. Less stringent conditions, such as, 0.15 M to 0.9 M salt at temperatures ranging from 20°C to 55°C can be made more stringent by adding increasing amounts of formamide, which serves to destabilize hybrid duplexes as does increased temperature.

[0138] Exemplary stringent hybridization conditions are described in Sambrook et al., Molecular Cloning: A Laboratory Manual, pages 1.91 and 9.47-9.51 (Second Edition, Cold Spring Harbor Laboratory Press; Cold Spring Harbor, NY; 1989); Maniatis et al., Molecular Cloning: A Laboratory Manual, pages 387-389 (Cold Spring Harbor Laboratory; Cold Spring Harbor, NY; 1982); Tsuchiya et al., Oral Surgery, Oral Medicine, Oral Pathology, 71(6): 721-725 (June 1991).

[0139] Preferred nucleic acid probes are fragments of the isolated nucleic acid sequences that encode MN proteins or polypeptides. Preferably those probes are composed of at least twenty-nine nucleotides, more preferably, fifty nucleotides.

[0140] Nucleic acid probes need not hybridize to a coding region of MN. For example, nucleic acid probes may hybridize partially or wholly to a non-coding region of the genomic sequence shown in Figure 3(A-F) [SEQ. ID. NO.: 5]. Conventional technology can be used to determine whether fragments of SEQ. ID. NO.: 5 or related nucleic acids are useful to identify MN nucleic acid sequences. [See, for example, Benton and Davis, supra and Fuscoe et al., supra.]

[0141] Areas of homology of the MN nt sequence to other non-MN nt sequences are indicated above. In general, nucleotide sequences that are not in the Alu or LTR-like regions, of preferably 29 bases or more, or still more preferably of 50 bases or more, can be routinely tested and screened and found to hybridize under stringent conditions to only MN nucleotide sequences. Further, not all homologies within the Alu-like MN genomic sequences are so close to Alu repeats as to give a hybridization signal under stringent hybridization conditions. The percent of homology between MN Alu-like regions and a standard Alu-J sequence are indicated as follows:

Region of Homology within MN Genomic Sequence [SEQ. ID. NO.: 5; Figure 3 (A-F)]	SEQ. ID. NOS.	% Homology to Entire Alu-J Sequence
921-1212	59	89.1%
2370-2631	60	78.6%
4587-4880	61	90.1%
6463-6738	62	85.4%
7651-7939 9020-9317	63 64	91.0% 69.8%
		% Homology to One Half of Alu-J Sequence
8301-8405	65	88.8%
10040-10122	66	73.2%.

[0142] Nucleic acid probes can be used to detect MN DNA and/or RNA, and thus can be used to test for the presence or absence of MN genes, and amplification(s), mutation(s) or genetic rearrangements of MN genes in the cells of a patient. For example, overexpression of an MN gene may be detected by Northern blotting and RNase protection analysis using probes. Gene alterations, as amplifications, translocations, inversions, and deletions among others, can be detected by using probes for in situ hybridization to chromosomes from a patient's cells, whether in metaphase spreads or interphase nuclei. Southern blotting could also be used with the probes to detect amplifications or deletions of MN genes. Restriction Fragment Length Polymorphism (RFLP) analysis using said probes is a preferred method of detecting gene alterations, mutations and deletions. Said probes can also be used to identify MN proteins and/or polypeptides as well as homologs or near homologs thereto by their hybridization to various mRNAs transcribed from MN genes in different tissues.

[0143] Probes thus can be useful diagnostically/prognostically. Said probes can be embodied in test kits, preferably with appropriate means to enable said probes when hybridized to an appropriate MN gene or MN mRNA target to be visualized. Such samples include tissue specimens including smears, body fluids and tissue and cell extracts.

PCR Assays

[0144] To detect relatively large genetic rearrangements, hybridization tests can be used. To detect relatively small genetic rearrangements, as, for example, small deletions or amplifications, or point mutations, PCR would preferably be used. [U.S. Patent Nos. 4,800,159; 4,683,195; 4,683,202; and Chapter 14 of Sambrook et al., Molecular Cloning: A Laboratory Manual, supra]

[0145] An exemplary assay would use cellular DNA from normal and cancerous cells, which DNA would be isolated and amplified employing appropriate PCR primers. The PCR products would be compared, preferably initially, on a sizing gel to detect size changes indicative of certain genetic rearrangements. If no differences in sizes are noted, further comparisons can be made, preferably using, for example, PCR-single-strand conformation polymorphism (PCR-SSCP) assay or a denaturing gradient gel electrophoretic assay. [See, for example, Hayashi, K. , "PCR-SSCP: A Simple and Sensitive Method for Detection of Mutations in the Genomic DNA," in PCR Methods and Applications, 1: 34-38 (1991); and Meyers et al., "Detection and Localization of Single Base Changes by Denaturing Gradient Gel Electrophoresis," Methods in Enzymology, 155: 501 (1987).]

Assays

[0146] Assays are disclosed to detect and/or quantitate MN antigen or MN-specific antibodies in vertebrate samples, preferably mammalian samples, more preferably human samples. Such samples include tissue specimens, body fluids, tissue extracts and cell extracts. MN antigen may be detected by immunoassay, immunohistochemical staining, immunoelectron and scanning microscopy using immunogold among other techniques.

[0147] Preferred tissue specimens to assay by immunohistochemical staining include cell smears, histological sections from biopsied tissues or organs, and imprint preparations among other tissue samples. Such tissue specimens can be variously maintained, for example, they can be fresh, frozen, or formalin-, alcohol- or acetone- or otherwise fixed and/or paraffin-embedded and deparaffinized. Biopsied tissue samples can be, for example, those samples removed by aspiration, bite, brush, cone, chorionic villus, endoscopic, excisional, incisional, needle, percutaneous punch, and surface biopsies, among other biopsy techniques.

[0148] Preferred cervical tissue specimens include cervical smears, conization specimens, histologic sections from hysterectomy specimens or other biopsied cervical tissue samples. Preferred means of obtaining cervical smears include routine swab, scraping or cytobrush techniques, among other means. More preferred are cytobrush or swab techniques. Preferably, cell smears are made on microscope slides, fixed, for example, with 55% EtOH or an alcohol based spray fixative and air-dried.

[0149] Papanicolaou-stained cervical smears (Pap smears) can be screened by the methods of this disclosure, for example, for retrospective studies. Preferably, Pap smears would be decolorized and re-stained with labeled antibodies against MN antigen. Also archival specimens, for example, matched smears and biopsy and/or tumor specimens, can be used for retrospective studies. Prospective studies can also be done with matched specimens from patients that have a higher than normal risk of exhibiting abnormal cervical cytopathology.

[0150] Preferred samples in which to assay MN antigen by, for example, Western blotting or radioimmunoassay, are tissue and/or cell extracts. However, MN antigen may be detected in body fluids, which can include among other fluids: blood, serum, plasma, semen, breast exudate, saliva, tears, sputum, mucous, urine, lymph, cytosols, ascites, pleural effusions, amniotic fluid, bladder washes, bronchioalveolar lavages and cerebrospinal fluid. It is preferred that the MN antigen be concentrated from a larger volume of body fluid before testing. Preferred body fluids to assay would depend on the type of cancer for which one was testing, but in general preferred body fluids would be breast exudate, pleural effusions and ascites.

[0151] MN-specific antibodies can be bound by serologically active MN proteins/polypeptides in samples of such body fluids as blood, plasma, serum, lymph, mucous, tears, urine, spinal fluid and saliva; however, such antibodies are found most usually in blood, plasma and serum, preferably in serum. Correlation of the results from the assays to detect and/or quantitate MN antigen and MN-specific antibodies reactive therewith, provides a preferred profile of the disease condition of a patient.

[0152] The assays are both diagnostic and/or prognostic, i.e., diagnostic/prognostic. The term "diagnostic/ prognostic" is herein defined to encompass the following processes either individually or cumulatively depending upon the clinical context: determining the presence of disease, determining the nature of a disease, distinguishing one disease from another, forecasting as to the probable outcome of a disease state, determining the prospect as to recovery from a disease as indicated by the nature and symptoms of a case, monitoring the disease status of a patient, nonitoring a patient for recurrence of disease, and/or determining the preferred therapeutic regimen for a patient. The diagnostic/ prognostic methods of this disclosure are useful, for example, for screening populations for the presence of neoplastic or pre-neoplastic disease, determining the risk of developing neoplastic disease, diagnosing the presence of neoplastic and/or pre-neoplastic disease, monitoring the disease status of patients with neoplastic disease, and/or determining the prognosis for the course of neoplastic disease. For example, it appears that the intensity of the immunostaining with MN-specific antibodies may correlate with the severity of dysplasia present in samples tested.

[0153] This discovery is useful for screening for the presence of a wide variety of neoplastic diseases as indicated above. The disclosure provides methods and compositions for evaluating the probability of the presence of malignant or pre-malignant cells, for example, in a group of cells freshly removed from a host. Such an assay can be used to detect tumors, quantitate their growth, and help in the diagnosis and prognosis of disease. The assays can also be used to detect the presence of cancer metastasis, as well as confirm the absence or removal of all tumor tissue following surgery, cancer chemotherapy and/or radiation therapy. It can further be used to monitor cancer chemotherapy and tumor reappearance.

[0154] The presence of MN antigen or antibodies can be detected and/or quantitated using a number of well-defined diagnostic assays. Those in the art can adapt any of the conventional immunoassay formats to detect and/or quantitate MN antigen and/or antibodies.

[0155] Many formats for detection of MN antigen and MN-specific antibodies are, of course available. Those can be Western blots, ELISAs, RIAs, competitive EIA or dual antibody sandwich assays, immunohistochemical staining, among other assays all commonly used in the diagnostic industry. In such immunoassays, the interpretation of the results is based on the assumption that the antibody or antibody combination will not cross-react with other proteins and protein fragments present in the sample that are unrelated to MN.

[0156] Representative of one type of ELISA test for MN antigen is a format wherein a microtiter plate is coated with antibodies made to MN proteins/polypeptides or antibodies made to whole cells expressing MN proteins, and to this is added a patient sample, for example, a tissue or cell extract. After a period of incubation permitting any antigen to bind to the antibodies, the plate is washed and another set of anti-MN antibodies which are linked to an enzyme is added, incubated to allow reaction to take place, and the plate is then rewashed. Thereafter, enzyme substrate is added to the microtiter plate and incubated for a period of time to allow the enzyme to work on the substrate, and the adsorbance of the final preparation is measured. A large change in absorbance indicates a positive result.

[0157] It is also apparent to one skilled in the art of immunoassays that MN proteins and/or polypeptides can be used to detect and/or quantitate the presence of MN antigen in the body fluids, tissues and/or cells of patients. In one such embodiment, a competition immunoassay is used, wherein the MN protein/polypeptide is labeled and a body fluid is added to compete the binding of the labeled MN protein/polypeptide to antibodies specific to MN protein/polypeptide.

[0158] In another embodiment, an immunometric assay may be used wherein a labeled antibody made to a MN protein or polypeptide is used. In such an assay, the amount of labeled antibody which complexes with the antigen-bound antibody is directly proportional to the amount of MN antigen in the sample.

[0159] A representative assay to detect MN-specific antibodies is a competition assay in which labeled MN protein/polypeptide is precipitated by antibodies in a sample, for example, in combination with monoclonal antibodies recognizing MN proteins/polypeptides. One skilled in the art could adapt any of the conventional immunoassay formats to detect and/or quantitate MN-specific antibodies. Detection of the binding of said antibodies to said MN protein/polypeptide could be by many ways known to those in the art, e.g., in humans with the use of anti-human labeled IgG.

[0160] An exemplary immuncassay method to detect and/or quantitate MN antigen in a vertebrate sample comprises the steps of:

a) incubating said vertebrate sample with one or more sets of antibodies (an antibody or antibodies) that bind to MN antigen wherein one set is labeled or otherwise detectable;
b) examining the incubated sample for the presence of immune complexes comprising MN antigen and said antibodies.

[0161] Another exemplary immunoassay method is that wherein a competition immunoassay is used to detect and/or quantitate MN antigen in a vertebrate sample and wherein said method comprises the steps of:

a) incubating a vertebrate sample with one or more sets of MN-specific antibodies and a certain amount of a labeled or otherwise detectable MN protein/polypeptide wherein said MN protein/ polypeptide competes for binding to said antibodies with MN antigen present in the sample;
b) examining the incubated sample to determine the amount of labeled/detectable MN protein/polypeptide bound to said antibodies; and
c) determining from the results of the examination in step b) whether MN antigen is present in said sample and/or the amount of MN antigen present in said sample.

[0162] Once antibodies (including biologically active antibody fragments) having suitable specificity have been prepared, a wide variety of immunological assay methods are available for determining the formation of specific antibody-antigen complexes. Numerous competitive and non-competitive protein binding assays have been described in the scientific and patent literature, and a large number of such assays are commercially available. Exemplary immunoassays which are suitable for detecting a serum antigen include those described in U.S. Patent Nos. 3,984,533; 3,996,345; 4,034,074; and 4,098,876.

[0163] Antibodies employed in assays may be labeled or unlabeled. Unlabeled antibodies may be employed in agglutination; labeled antibodies may be employed in a wide variety of assays, employing a wide variety of labels.

[0164] Suitable detection means include the use of labels such as radionuclides, enzymes, coenzymes, fluorescers, chemiluminescers, chromogens, enzyme substrates or co-factors, enzyme inhibitors, free radicals, particles, dyes and the like. Such labeled reagents may be used in a variety of well known assays, such as radioimmunoassays, enzyme immunoassays, e.g., ELISA, fluorescent immunoassays, and the like. See for example, U.S. Patent Nos. 3,766,162; 3,791,932; 3,817,837; and 4,233,402.

Immunoassay Test Kits

[0165] The above outlined assays can be embodied in test kits to detect and/or quantitate MN antigen and/or MN-specific antibodies (including biologically active antibody fragments). Kits to detect and/or quantitate MN antigen can comprise MN protein(s)/polypeptides(s) and/or MN-specific antibodies, polyclonal and/or monoclonal. Such diagnostic/prognostic test kits can comprise one or more sets of antibodies, polyclonal and/or monoclonal, for a sandwich format wherein antibodies recognize epitopes on the MN antigen, and one set is appropriately labeled or is otherwise detectable.

[0166] Test kits for an assay format wherein there is competition between a labeled (or otherwise detectable) MN protein/polypeptide and MN antigen in the sample, for binding to an antibody, can comprise the combination of the labeled protein/polypeptide and the antibody in amounts which provide for optimum sensitivity and accuracy.

[0167] Test kits for MN-specific antibodies preferably comprise labeled/detectable MN proteins(s) and/or polypeptides(s), and may comprise other components as necessary, such as, controls, buffers, diluents and detergents. Such test kits can have other appropriate formats for conventional assays.

[0168] A kit for use in an enzyme-immunoassay typically includes an enzyme-labelled reagent and a substrate for the enzyme. The enzyme can, for example, bind either an MN-specific antibody or to an antibody to such an MN-specific antibody.

Preparation of MN-Specific Antibodies

[0169] The term "antibodies" is defined herein to include not only whole antibodies but also biologically active fragments of antibodies, preferably fragments containing the antigen binding regions. Such antibodies may be prepared by conventional methodology and/or by genetic engineering. Antibody fragments may be genetically engineered, preferably from the variable regions of the light and/or heavy chains (V_H and V_L), including the hypervariable regions, and still more preferably from both the V_H and V_L regions. For example, the term "antibodies" as used herein comprehends polyclonal and monoclonal antibodies and biologically active fragments thereof including among other possibilities "univalent" antibodies [Glennie et al., Nature, 295: 712 (1982)]; Fab proteins including Fab' and $F(ab')_2$ fragments whether covalently or non-covalently aggregated; light or heavy chains alone, preferably variable heavy and light chain regions (V_H and V_L regions), and more preferably including the hypervariable regions [otherwise known as the complementarity determining regions (CDRs) of said V_H and V_L regions]; F_C proteins; "hybrid" antibodies capable of binding more than one antigen; constant-variable region chimeras; "composite" immunoglobulins with heavy and light chains of different origins; "altered" antibodies with improved specificity and other characteristics as prepared by standard recombinant techniques and also by oligonucleotide-directed mutagenesis techniques [Dalbadie-McFarland et al., PNAS (USA), 79: 6409 (1982)].

[0170] It may be preferred for therapeutic and/or imaging uses that the antibodies be biologically active antibody fragments, preferably genetically engineered fragments, more preferably genetically engineered fragments from the V_H and/or V_L regions, and still more preferably comprising the hypervariable regions thereof.

[0171] There are conventional techniques for making polyclonal and monoclonal antibodies well-known in the immunoassay art. Immunogens to prepare MN-specific antibodies include MN proteins and/or polypeptides, preferably purified, and MX-infected tumor line cells, for example, MX-infected HeLa cells, among other immunogens.

[0172] Anti-peptide antibodies are also made by conventional methods in the art as described in European Patent Publication No. 44,710 (published Jan. 27, 1982). Briefly, such anti-peptide antibodies are prepared by selecting a peptide from an MN amino acid sequence as from Figure 1(A-C), chemically synthesizing it, conjugating it to an appropriate immunogenic protein and injecting it into an appropriate animal, usually a rabbit or a mouse; then, either polyclonal or monoclonal antibodies are made, the latter by a Kohler-Milstein procedure, for example.

[0173] Besides conventional hybridoma technology, newer technologies can be used to produce antibodies. For example, the use of the PCR to clone and express antibody V-genes and phage display technology to select antibody genes encoding fragments with binding activities has resulted in the isolation of antibody fragments from repertoires of PCR amplified V-genes using immunized mice or humans. [Marks et al. , BioTechnology, 10: 779 (July 1992) for references; Chiang et al., BioTechniques, 7(4): 360 (1989); Ward et al., Nature, 341: 544 (Oct. 12, 1989); Marks et al., J. Mol. Biol., 222: 581 (1991); Clackson et al., Nature, 352: (15 August 1991) ; and Mullinax et al., PNAS (USA), 87: 8095 (Oct. 1990).]

[0174] Descriptions of preparing antibodies, which term is herein defined to include biologically active antibody fragments, by recombinant techniques can be found in U.S. Patent No . 4 , 816 , 5 67 (issued March 28, 1989); European Patent Application Publication Number (EP) 338,745 (published Oct. 25, 1989); EP 368,684 (published June 16, 1990) ; EP 239,400 (published September 30, 1987) ; WO 90/14424 (published Nov. 29, 1990) ; WO 90/14430 (published May 16, 1990) ; Huse et al., Science, 246: 1275 (Dec. 8, 1989) ; Marks et al., BioTechnology, 10: 779 (July 1992); La Sastry et al., PNAS (USA), 86: 5728 (August 1989); Chiang et al., BioTechniques, 7 (40) : 360 (1989) ; Orlandi et al., PNAS (USA), 86: 3833 (May 1989); Ward et al. Nature, 341: 544 (October 12, 1989); Marks et al., J. Mol. Biol., 222: 581 (1991) ; and Hoogenboom et al. , Nucleic Acids Res. , 19(15): 4133 (1991).

Representative Mabs

[0175] Monoclonal antibodies for use in the assays may be obtained by methods well known in the art for example, Galfre and Milstein, "Preparation of Monoclonal Antibodies: Strategies and Procedures," in Methods in Enzymology: Immunochemical Techniques, 73: 1-46 [Langone and Vanatis (eds); Academic Press (1981)]; and in the classic reference, Milstein and Kohler, Nature, 256: 495-497 (1975).]

[0176] Although representative hybridomas are formed by the fusion of murine cell lines, human/human hybridomas [Olsson et al., PNAS (USA), 77: 5429 (1980)] and human/murine hybridomas [Schlom et al., PNAS (USA), 77: 6841 (1980); Shearman et al. J. Immunol., 146: 928-935 (1991); and Gorman et al., PNAS (USA), 88: 4181-4185 (1991)] can also be prepared among other possiblities. Such humanized monoclonal antibodies would be preferred monoclonal antibodies for therapeutic and imaging uses.

[0177] Monoclonal antibodies specific for this disclosure can be prepared by immunizing appropriate mammals, preferably rodents, more preferably rabbits or mice, with an appropriate immunogen, for example, MaTu-infected HeLa cells, MN fusion proteins, or MN proteins/polypeptides attached to a carrier protein if necessary. Exemplary methods of producing antibodies of this invention are described below.

[0178] The monoclonal antibodies useful according to this disclosure to identify MN proteins/polypeptides can be labeled in any conventional manner, for example, with enzymes such as horseradish peroxidase (HRP), fluorescent compounds, or with radioactive isotopes such as, ^{125}I, among other labels. A preferred label, is ^{125}I, and a preferred method of label ing the antibodies is by using chloramine-T [Hunter, W . M . , "Radioimmunoassay", In: Handbook of Experimental Immunology, pp. 14.1-14.40 (D.W. Weir ed.; Blackwell , Oxford/London/Edinburgh/Melbourne; 1978)].

[0179] Representative mabs include Mabs M75, MN9, MN12 and MN7 described below. Monoclonal antibodies serve to identify MN proteins/polypeptides in various laboratory diagnostic tests, for example, in tumor cell cultures or in clinical samples.

Mabs Prepared Against HeLa Cells

[0180] MAb M75. Monoclonal antibody M75 (MAb M75) is produced by mouse lymphocytic hybridoma VU-M75, which was initially deposited in the Collection of Hybridomas at the Institute of Virology, Slovak Academy of Sciences (Bratislava, Slovak Republic) and was deposited under ATCC Designation HB 11128 on September 17, 1992 at the American Type Culture Collection (ATCC) in Manassas, VA (USA). The production of hybridoma VU-M75 is described in Zavada et al., WO 93/18152.

[0181] Mab M75 recognizes both the nonglycosylated GEX-3X-MN fusion protein and native MN protein as expressed in CGL3 cells equally well. Mab M75 was shown by epitope mapping to be reactive with the epitope represented by the amino acid sequence from AA 62 to AA 67 [SEQ. ID. NO.: 10] of the MN protein shown in Figure 1(A-C).

Mabs Prepared Against Fusion Protein GEX-3X-MN

[0182] Monoclonal antibodies were also prepared against the MN glutathione S-transferase fusion protein (GEX-3X-MN). BALB/C mice were immunized intraperitoneally according to standard procedures with the GEX-3X-MN fusion protein in Freund's adjuvant. Spleen cells of the mice were fused with SP/20 myeloma cells [Milstein and Kohler, supra].

[0183] Tissue culture media from the hybridomas were screened against CGL3 and CGL1 membrane extracts in an ELISA employing HRP labelled-rabbit anti-mouse. The membrane extracts were coated onto microtiter plates. Selected were antibodies reacted with the CGL3 membrane extract. Selected hybridomas were cloned twice by limiting dilution.

[0184] The mabs prepared by the just described method were characterized by Western blots of the GEX-3X-MN fusion protein, and with membrane extracts from the CGL1 and CGL3 cells. Representative of the mabs prepared are Mabs MN9, MN12 and MN7.

[0185] Mab MN9. Monoclonal antibody MN9 (Mab MN9) reacts to the same epitope as Mab M75, represented by the sequence from AA 62 to AA 67 [SEQ. ID. NO.: 10] of the Figure 1(A-C) MN protein. As Mab M75, Mab MN9 recognizes both the GEX-3X-MN fusion protein and native MN protein equally well.

[0186] Mabs corresponding to Mab MN9 can be prepared reproducibly by screening a series of mabs prepared against an MN protein/polypeptide, such as, the GEX-3X-MN fusion protein, against the peptide representing the epitope for Mabs M75 and MN9, that is, SEQ. ID. NO.: 10. Alternatively, the Novatope system [Novagen] or competition with the deposited Mab M75 could be used to select mabs comparable to Mabs M75 and MN9.

[0187] Mab MN12. Monoclonal antibody MN12 (Mab MN12) is produced by the mouse lymphocytic hybridoma MN 12.2.2 which was deposited under ATCC Designation HB 11647 on June 9, 1994 at the American Type Culture Collection (ATCC) at 10801 University Blvd, Manassas VA 20110-2209 (USA). Antibodies corresponding to Mab MN12 can also be made, analogously to the method outlined above for Mab MN9, by screening a series of antibodies prepared against an MN protein/polypeptide, against the peptide representing the epitope for Mab MN12. That peptide is AA 55 - AA 60 of Figure 1 (A-C) [SEQ. ID. NO.: 11]. The Novatope system could also be used to find antibodies specific for said epitope.

[0188] Mab MN7. Monoclonal antibody MN7 (Mab MN7) was selected from mabs prepared against nonglycosylated GEX-3X-MN as described above. It recognizes the epitope on MN represented by the amino acid sequence from AA 127 to AA 147 [SEQ. ID. NO.: 12] of the Figure 1(A-C) MN protein. Analogously to methods described above for Mabs MN9 and MN12, mabs corresponding to Mab MN7 can be prepared by selecting mabs prepared against an MN protein/polypeptide that are reactive with the peptide having SEQ. ID. NO.: 12, or by the stated alternative means.

Epitope Mapping

[0189] Epitope mapping was performed by the Novatope system, a kit for which is commercially available from Novagen, Inc. [See, for analogous example, Li et al., Nature, 363: 85-88 (6 May 1993).] In brief, the MN cDNA was cut into overlapping short fragments of approximately 60 base pairs. The fragments were expressed in E. coli, and the E. coli colonies were transferred onto nitrocellulose paper, lysed and probed with the mab of interest. The MN cDNA of clones reactive with the mab of interest was sequenced, and the epitopes of the mabs were deduced from the overlapping polypeptides found to be reactive with each mab.

Therapeutic Use of MN-Specific Antibodies

[0190] The MN-specific antibodies, monoclonal and/or polyclonal, preferably monoclonal, and as outlined above, may be used therapeutically in the treatment of neoplastic and/or pre-neoplastic disease, either alone or in combination with chemotherapeutic drugs or toxic agents, such as ricin A. Further preferred for therapeutic use would be biologically active antibody fragments as described herein. Also preferred MN-specific antibodies for such therapeutic uses would be humanized monoclonal antibodies.

[0191] The MN-specific antibodies can be administered in a therapeutically effective amount, preferably dispersed in a physiologically acceptable, nontoxic liquid vehicle.

Imaging Use of Antibodies

[0192] Further, the MN-specific antibodies when linked to an imaging agent, such as a radionuclide, can be used for imaging. Biologically active antibody fragments or humanized monoclonal antibodies, may be preferred for imaging use.

[0193] A patient's neoplastic tissue can be identified as, for example, sites of transformed stem cells, of tumors and locations of any metastases. Antibodies, appropriately labeled or linked to an imaging agent, can be injected in a physiologically acceptable carrier into a patient, and the binding of the antibodies can be detected by a method appropriate to the label or imaging agent, for example, by scintigraphy.

Antisense MN Nucleic Acid Sequences

[0194] MN genes are herein considered putative oncogenes and the encoded proteins thereby are considered to be putative oncoproteins. Antisense nucleic acid sequences substantially complementary to mRNA transcribed from MN genes, as represented by the antisense oligodeoxynucleotides ODN1 and ODN2 [SEQ. ID. NOS.: 3 and 4] can be used to reduce or prevent expression of the MN gene. [Zamecnick, P.C., "Introduction: Oligonucleotide Base Hybridization as a Modulator of Genetic Message Readout," pp. 1-6, Prospects for Antisense Nucleic Acid Therapy of Cancer and AIDS, (Wiley-Liss, Inc., New York, NY, USA; 1991); Wickstrom, E., "Antisense DNA Treatment of HL-60 Promyelocytic Leukemia Cells: Terminal Differentiation and Dependence on Target Sequence," pp. 7-24, id.; Leserman et al., "Targeting and Intracellular Delivery of Antisense Oligonucleotides Interfering with Oncogene Expression," pp. 25-34, id.; Yokoyama, K., "Transcriptional Regulation of c-myc Proto-oncogene by Antisense RNA," pp. 35-52, id.; van den Berg et al., "Antisense fos Oligodeoxyribonucleotides Suppress the Generation of Chromosomal Aberrations," pp. 63-70, id.; Mercola, D., "Antisense fos and fun RNA," pp. 83-114, id.; Inouye, Gene, 72: 25-34 (1988); Miller and Ts'o, Ann. Reports Med. Chem., 23: 295-304 (1988); Stein and Cohen, Cancer Res., 48: 2659-2668 (1988); Stevenson and Inversen, J. Gen. Virol.. 70: 2673-2682 (1989); Goodchild, "Inhibition of Gene Expression by Oligonucleotides," pp. 53-77, Oligodeoxynucleotides: Antisense Inhibitors of Gene Expression (Cohen, J.S., ed; CRC Press, Boca Raton, Florida, USA; 1989); Dervan et al., "Oligonucleotide. Recognition of Double-helical DNA by Triple-helix Formation," pp. 197-210, id.; Neckers, L.M., "Antisense Oligodeoxynucleotides as a Tool for Studying Cell Regulation: Mechanisms of Uptake and Application to the Study of Oncogene Function," pp. 211-232, id.; Leitner et al., PNAS (USA), 87: 3430-3434 (1990); Bevilacqua et al., PNAS (USA), 85: 831-835 (1988); Loke et al. Curr. Top. Microbiol. Immunol., 141: 282-288 (1988); Sarin et al., PNAS (USA), 85: 7448-7451 (1988); Agrawal et al., "Antisense Oligonucleotides: A Possible Approach for Chemotherapy and AIDS," International Union of Biochemistry Conference on Nucleic Acid Therapeutics (Jan. 13-17, 1991; Clearwater Beach, Florida, USA); Armstrong, L., Ber. Week, pp. 88-89 (March 5, 1990); and Weintraub et al., Trends, 1: 22-25 (1985).] Such antisense nucleic acid sequences, preferably oligonucleotides, by hybridizing to the MN mRNA, particularly in the vicinity of the ribosome binding site and translation initiation point, inhibits translation of the mRNA. Thus, the use of such antisense nucleic acid sequences may be considered to be a form of cancer therapy.

[0195] Preferred antisense oligonucleotides are gene-specific ODNs or oligonucleotides complementary to the 5' end of MN mRNA. Particularly preferred are the 29-mer ODN1 and 19-mer ODN2 [SEQ. ID. NOS.: 3 and 4]. Those antisense ODNs are representative of the many antisense nucleic acid sequences that can function to inhibit MN gene expression. Ones of ordinary skill in the art could determine appropriate antisense nucleic acid sequences, preferably antisense oligonucleotides, from the nucleic acid sequences of Figures 1(A-C) and 3(A-F).

[0196] Also, as described above, CGL3 cells transfected with an "antisense" MN cDNA/promoter construct formed colonies much smaller than control CGL3 cells.

Vaccines

[0197] It will be readily appreciated that MN proteins and polypeptides can be incorporated into vaccines capable of inducing protective immunity against neoplastic disease and a dampening effect upon tumorigenic activity. Efficacy of a representative MN fusion protein GEX-3X-MN as a vaccine in a rat model is shown in Example 2.

[0198] MN proteins and/or polypeptides may be synthesized or prepared recombinantly or otherwise biologically, to comprise one or more amino acid sequences corresponding to one or more epitopes of the MN proteins either in monomeric or multimeric form. Those proteins and/or polypeptides may then be incorporated into vaccines capable of inducing protective immunity. Techniques for enhancing the antigenicity of such polypeptides include incorporation into a multimeric structure, binding to a highly immunogenic protein carrier, for example, keyhole limpet hemocyanin (KLH), or diphtheria toxoid, and administration in combination with adjuvants or any other enhancers of immune response.

[0199] Preferred MN proteins/polypeptides to be used in a vaccine would be genetically engineered MN proteins. Preferred recombinant MN protein are the GEX-3X-MN, MN 20-19, MN-Fc and MN-PA proteins.

[0200] Other exemplary vaccines include vaccinia-MN (live vaccinia virus with full-length MN cDNA), and baculovirus-MN (full length MN cDNA inserted into baculovirus vector, e.g. in suspension of infected insect cells). Different vaccines may be combined and vaccination periods can be prolonged.

[0201] A preferred exemplary use of such a vaccine would be its administration to patients whose MN-carrying primary cancer had been surgically removed. The vaccine may induce active immunity in the patients and prevent recidivism or metastasis.

[0202] It will further be appreciated that anti-idiotype antibodies to antibodies to MN proteins/polypeptides are also useful as vaccines and can be similarly formulated.

[0203] An amino acid sequence corresponding to an epitope of an MN protein/polypeptide either in monomeric or multimeric form may also be obtained by'chemical synthetic means or by purification from biological sources including genetically modified microorganisms or their culture media. [See Lerner, "Synthetic Vaccines", Sci. Am. 248(2) : 66-74 (1983).] The protein/polypeptide may be combined in an amino acid sequence with other proteins/polypeptides including fragments of other proteins, as for example, when synthesized as a fusion protein, or linked to other antigenic or non-antigenic polypeptides of synthetic or biological origin. In some instances, it may be desirable to fuse a MN protein or polypeptide to an immunogenic and/or antigenic protein or polypeptide, for example, to stimulate efficacy of a MN-based vaccine.

[0204] The term "corresponding to an epitope of an MN protein/polypeptide" will be understood to include the practical possibility that, in some instances, amino acid sequence variations of a naturally occurring protein or polypeptide may be antigenic and confer protective immunity against neoplastic disease and/or anti-tumorigenic effects. Possible sequence variations include, without limitation, amino acid substitutions, extensions, deletions, truncations, interpolations and combinations thereof. Such variations fall within the contemplated scope of the disclosure provided the protein or polypeptide containing them is immunogenic and antibodies elicited by such a polypeptide or protein cross-react with naturally occurring MN proteins and polypeptides to a sufficient extent to provide protective immunity and/or anti-tumorigenic activity when administered as a vaccine.

[0205] Such vaccine compositions will be combined with a physiologically acceptable medium, including immunologically acceptable diluents and carriers as well as commonly employed adjuvants such as Freund's Complete Adjuvant, saponin, alum, and the like. Administration would be in immunologically effective amounts of the MN proteins or polypeptides, preferably in quantities providing unit doses of from 0.01 to 10.0 micrograms of immunologically active MN protein and/or polypeptide per kilogram of the recipient's body weight. Total protective doses may range from 0.1 to about 100 micrograms of antigen. Routes of administration, antigen dose, number and frequency of injections are all matters of optimization within the scope of the ordinary skill in the art.

[0206] The following examples are for purposes of illustration only.

Example 1

Immunohistochemical Staining of Tissue Specimens

[0207] To study and evaluate the tissue distribution range and expression of MN proteins, the monoclonal antibody M75 was used to stain immunohistochemically a variety of human tissue specimens. The primary antibody used in these immunohistochemical staining experiments was the M75 monoclonal antibody. A biotinylated second antibody and streptavidin-peroxidase were used to detect the M75 reactivity in sections of formalin-fixed, paraffin-embedded tissue samples. A commercially available amplification kit, specifically the DAKO LSABT™ kit [DAKO Corp., Carpinteria, CA (USA)] which provides matched, ready made blocking reagent, secondary antibody and steptavidin-horseradish peroxidase was used in these experiments.

[0208] M75 immunoreactivity was tested in multiple-tissue sections of breast, colon, cervical, lung and normal tissues. Such multiple-tissue sections were cut from paraffin blocks of tissues called "sausages" that were purchased from the City of Hope [Duarte, CA (USA)]. Combined in such a multiple-tissue section were normal, benign and malignant specimens of a given tissue; for example, about a score of tissue samples of breast cancers from different patients, a similar number of benign breast tissue samples, and normal breast tissue samples would be combined in one such multiple-breast-tissue section. The normal multiple-tissue sections contained only normal tissues from various organs, for example, liver, spleen, lung, kidney, adrenal gland, brain, prostate, pancreas, thyroid, ovary, and testis.

[0209] Also screened for MN gene expression were multiple individual specimens from cervical cancers, bladder cancers, renal cell cancers, and head and neck cancers. Such specimens were obtained from U.C. Davis Medical Center in Sacramento, CA and from Dr. Shu Y. Liao [Department of Pathology; St. Joseph Hospital; Orange, CA (USA)].

[0210] Controls used in these experiments were the cell lines CGL3 (H/F-T hybrid cells) and CGL1 (H/F-N hybrid cells) which are known to stain respectively, positively and negatively with the M75 monoclonal antibody. The M75 monoclonal antibody was diluted to a 1:5000 dilution wherein the diluent was either PBS [0.05 M phosphate buffered saline (0.15 M NaCl), pH 7.2-7.4] or PBS containing 1% protease-free BSA as a protein stabilizer.

Immunohistochemical Staining Protocol

[0211] The immunohistochemical staining protocol was followed according to the manufacturer's instructions for the DAKO LSAB™ kit. In brief, the sections were dewaxed, rehydrated and blocked to remove non-specific reactivity as well as endogenous peroxidase activity. Each section was then incubated with dilutions of the M75 monoclonal antibody. After the unbound M75 was removed by rinsing the section, the section was sequentially reacted with a biotinylated antimouse IgG antibody and streptavidin conjugated to horseradish peroxidase; a_rinsing step was included between those two reactions and after the second reaction. Following the last rinse, the antibody-enzyme complexes were detected by reaction with an insoluble chromogen (diaminobenzidine) and hydrogen peroxide. A positive result was indicated by the formation of an insoluble reddish-brown precipitate at the site of the primary antibody reaction. The sections were then rinsed, counterstained with hematoxylin, dehydrated and cover slipped. Then the sections were examined using standard light microscopy.

[0212] Interpretation. A deposit of a reddish brown precipitate over the plasma membrane was taken as evidence that the M75 antibody had bound to a MN antigen in the tissue. The known positive control (CGL3) had to be stained to validate the assay. Section thickness was taken into consideration to compare staining intensities, as thicker sections produce greater staining intensity independently of other assay parameters.

Results

[0213] Preliminary examination of cervical specimens showed that 62 of 68 squamous cell carcinoma specimens (91.2%) stained positively with M75. Additionally, 2 of 6 adenocarcinomas and 2 of 2 adenosquamous cancers of the cervix also stained positively. In early studies, 55.6% (10 of 18) of cervical dysplasias stained positively. A total of 9 specimens including both cervical dysplasias and tumors, exhibited some MN expression in normal appearing areas of the endocervical glandular epithelium, usually at the basal layer. In some specimens, whereas morphologically normal-looking areas showed expression of MN antigen, areas exhibiting dysplasia and/or malignancy did not show MN expression.

[0214] M75 positive immunoreactivity was most often localized to the plasma membrane of cells, with the most apparent stain being present at the junctions between adjacent cells. Cytoplasmic staining was also evident in some cells; however, plasma membrane staining was most often used as the main criterion of positivity.

[0215] M75 positive cells tended to be near areas showing keratin differentiation in cervical specimens. In some specimens, positive staining cells were located in the center of nests of non-staining cells. Often, there was very little, if any, obvious morphological difference between staining cells and non-staining cells. In some specimens, the positive staining cells were associated with adjacent areas of necrosis.

[0216] In most of the squamous cell carcinomas of the cervix, the M75 immunoreactivity was focal in distribution, i. e., only certain areas of the specimen stained. Although the distribution of positive reactivity within a given specimen was rather sporadic, the intensity of the reactivity was usually very strong. In most of the adenocarcinomas of the cervix, the staining pattern was more homogeneous, with the majority of the specimen staining positively.

[0217] Among the normal tissue samples, intense, positive and specific M75 immunoreactivity was observed only in normal stomach tissues, with diminishing reactivity in the small intestine, appendix and colon. No other normal tissue stained extensively positively for M75. occasionally, however, foci of intensely staining cells were observed in normal intestine samples (usually at the base of the crypts) or were sometimes seen in morphologically normal appearing areas of the epithelium of cervical specimens exhibiting dysplasia and/or malignancy. In such, normal appearing areas of cervical specimens, positive staining was seen in focal areas of the basal layer of the ectocervical epithelium or in the basal layer of endocervical glandular epithelium. In one normal specimen of human skin, cytoplasmic MN staining was observed in the basal layer. The basal layers of these epithelia are usually areas of proliferation, suggesting the MN expression may be involved in cellular growth. In a few cervical biopsied specimens, MN positivity was observed in the morphologically normal appearing stratified squamous epithelium, sometimes associated with cells undergoing koilocytic changes.

[0218] Some colon adenomas (4 of 11) and adenocarcinomas (9 of 15) were positively stained. One normal colon specimen was positive at the base of the crypts. Of 15 colon cancer specimens, 4 adenocarcinomas and 5 metastatic lesions were MN positive. Fewer malignant breast cancers (3 of 25) and ovarian cancer specimens (3 of 15) were positively stained. Of 4 head and neck cancers, 3 stained very intensely with M75.

[0219] Although normal stomach tissue was routinely positive, 4 adenocarcinomas of the stomach were MN negative. Of 3 bladder cancer specimens (1 adenocarcinoma, 1 non-papillary transitional cell carcinoma, and 1 squamous cell carcinoma), only the squamous cell carcinoma was MN positive. Approximately 40% (12 of 30) of lung cancer specimens were positive; 2 of 4 undifferentiated carcinomas; 3 of 8 adenocarcinomas; 2 of 8 oat cell carcinomas; and, 5 of 10 squamous cell carcinomas. One hundred percent (4 of 4) of the renal cell carcinomas were MN positive.

[0220] In summary, MN antigen, as detected by M75 and immunohistochemistry in the experiments described above, was shown to be prevalent in tumor cells, most notably in tissues of cervical cancers. MN antigen was also found in some cells of normal tissues, and sometimes in morphologically normal appearing areas of specimens exhibiting dysplasia and/or malignancy. However, MN is not usually extensively expressed in most normal tissues, except for stomach tissues where it is extensively expressed and in the tissues of the lower gastrointestinal tract where it is less extensively expressed. MN expression is most often localized to the cellular plasma membrane of tumor cells and may play a role in intercellular communication or cell adhesion. Representative results of experiments performed as described above are tabulated in Table 2.

TABLE 2

Immunoreactivitv of M75 in Various Tissues

TISSUE	TYPE	POS/NEG (#pos/#tested)
liver, spleen, lung, kidney, adrenal gland, brain, prostate, pancreas, thyroid, ovary, testis	normal	NEG (all)
skin	normal	POS (in basal layer) (1/1)
stomach	normal	POS
small intestine	normal	POS
colon	normal	POS
breast	normal	NEG (0/10)
cervix	normal	NEG (0/2)
breast	benign	NEG (0/17)
colon	benign	POS (4/11)
cervix	benign	POS (10/18)
breast	malignant	POS (3/25)
colon	malignant	POS (9/15)
ovarian	malignant	POS (3/15)
lung	malignant	POS (12/30)
bladder	malignant	POS (1/3)
head & neck	malignant	POS (3/4)
kidney	malignant	POS (4/4)
stomach	malignant	NEG (0/4)
cervix	malignant	POS (62/68)

[0221] The results recorded in this example indicate that the presence of MN proteins in a tissue sample from a patient may, in general, depending upon the tissue involved, be a marker signaling that a pre-neoplastic or neoplastic process is occurring. Thus, one may conclude from these results that diagnostic/prognostic methods that detect MN antigen may be particularly useful for screening patient samples for a number of cancers which can thereby be detected at a pre-neoplastic stage or at an early stage prior to obvious morphologic changes associated with dysplasia and/or malignancy being evident or being evident on a widespread basis.

Example 2

Vaccine -- Rat Model

[0222] As shown above in Example 7 of WO 93/18152 (International Publication Date: 16 September 1993), in some rat tumors, for example, the XC tumor cell line (cells from a rat rhabdomyosarcoma), a rat MN protein, related to human MN, is expressed. Thus a model was afforded to study antitumor immunity induced by experimental MN-based vaccines. The following representative experiments were performed.

[0223] Nine- to eleven-day-old Wistar rats from several families were randomized, injected intraperitoneally with 0.1 ml of either control rat sera (the C group) or with rat serum against the MN fusion protein GEX-3X-MN (the IM group). Simultaneously both groups were injected subcutaneously with 10^6 XC tumor cells.

[0224] Four weeks later, the rats were sacrificed, and their tumors weighed. The results are shown in Figure 2. Each point on the graph represents a tumor from one rat. The difference between the two groups -- C and IM -- was significant by Mann-Whitney rank test ($U = 84$, $\alpha < 0.025$). The results indicate that the IM group of baby rats developed tumors about one-half the size of the controls, and 5 of the 18 passively immunized rats developed no tumor at all, compared to 1 of 18 controls.

Example 3

Expression of Full-Length MN cDNA in NIH 3T3 Cells

[0225] The role of MN in the regulation of cell proliferation was studied by expressing the full-length cDNA in NIH 3T3 cells. That cell line was chosen since it had been used successfully to demonstrate the phenotypic effect of a number of proto-oncogenes [Weinberg, R.A., Cancer Res., 49: 3713 (1989); Hunter, T., Cell, 64: 249 (1991)]. Also, NIH 3T3 cells express no endogenous MN-related protein that is detectable by Mab M75.

[0226] The full length MN cDNA was obtained by ligation of the two cDNA clones using the unique BamHI site and subcloned from pBluescript into KpnI-SacI sites of the expression vector pSG5C. pSG5C was kindly provided by Dr. Richard Kettman [Department of Molecular Biology, Faculty of Agricultural Sciences, B-5030 Gembloux, Belgium]. pSG5C was derived from pSG5 [Stratagene] by inserting a polylinker consisting of a sequence having several neighboring sites for the following restriction enzymes: EcoRI, XhoI, KpnI, BamHI, SacI, 3 times TAG stop codon and BglII.

[0227] The recombinant pSG5C-MN plasmid was co-transfected in a 10:1 ratio (10 µg : 1 µg) with the pSV2neo plasmid [Southern and Berg, J. Mol. Appl. Genet., 1: 327 (1982)] which contains the neo gene as a selection marker. The co-transfection was carried out by calcium phosphate precipitation method [Mammalian Transfection Kit; Stratagene] into NIH 3T3 cells plated a day before at a density of 1 x 10^5 per 60 mm dish. As a control, pSV2neo was co-transfected with empty pSG5C.

[0228] Transfected cells were cultured in DMEM medium supplemented with 10% FCS and 600 µg ml^{-1} of G418 [Gibco BRL] for 14 days. The G418-resistant cells were clonally selected, expanded and analysed for expression of the transfected cDNA by Western blotting using iodinated Mab M75.

[0229] For an estimation of cell proliferation, the clonal cell lines were plated in triplicates (2 x 10^4 cells/well) in 24-well plates and cultivated in DMEM with 10% FCS and 1% FCS, respectively. The medium was changed each day, and the cell number was counted using a hemacytometer.

[0230] To determine the DNA synthesis, the cells were plated in triplicate in 96-well plate at a density of 10^4/well in DMEM with 10% FCS and allowed to attach overnight. Then the cells were labeled with ^{3}H-thymidine for 24 hours, and the incorporated radioactivity was counted.

[0231] For the anchorage-independent growth assay, cells (2 x 10^4) were suspended in a 0.3% agar in DMEM containing 10% FCS and overlaid onto 0.5% agar medium in 60 mm dish. Colonies grown in soft agar were counted two weeks after plating.

[0232] Several clonal cell lines constitutively expressing both 54 and 58 kd forms of MN protein in levels comparable to those found in LCMV-infected HeLa cells were obtained. Selected MN-positive clones and negative control cells (mock-transfected with an empty pSG5C plasmid) were subjected to further analyses directed to the characterization of their phenotype and growth behavior.

[0233] The MN-expressing NIH 3T3 cells displayed spindle-shaped morphology, and increased refractility; they were less adherent to the solid support and smaller in size. The control (mock transfected cells) had a flat morphology, similar to parental NIH 3T3 cells. In contrast to the control cells that were aligned and formed a monolayer with an ordered pattern, the cells expressing MN lost the capacity for growth arrest and grew chaotically on top of one another. Correspondingly, the MN-expressing cells were able to reach significantly higher (more than 2x) saturation densities (Table 3) and were less dependent on growth factors than the control cells.

[0234] MN transfectants also showed faster doubling times (by 15%) and enhanced DNA synthesis (by 10%), as determined by the amount of [^{3}H]-thymidine incorporated in comparison to control cells. Finally, NIH 3T3 cells expressing MN protein grew in soft agar. The diameter of colonies grown for 14 days ranged from 0.1 to 0.5 mm; however, the cloning efficiency of MN transfectants was rather low (2.4%). Although that parameter of NIH 3T3 cells seems to be less affected by MN than by conventional oncogenes, all other data are consistent with the idea that MN plays a role in cell growth control.

Table 3

Growth Properties of NIH 3T3 Cells Expressing MN Protein		
Transfected DNA	pSG5C/pSV2neo	pSG5C-MN/pSV2neo
Doubling time[a] (hours)	27.9 ± 0.5	24.1 ± 1.3
Saturation density[b] (cells x 10^4/cm^2)	4.9 ± 0.2	11.4 ± 0.4
Cloning efficiency (%)[c]	< 0.01	2.4 ± 0.2

[a] For calculation of the doubling time, the proliferation rate of exponentially growing cells was used.

[b] The saturation cell density was derived from the cell number 4 days after reaching confluency.

[c] Colonies greater than 0.1 mm in diameter were scored at day 14. Cloning efficiency was estimated as a percentage of colonies per number of cells plated, with correction for cell viability.

Example 4

Acceleration of G1 Transit and Decrease in Mitomycin C Sensitivity Caused by MN Protein

[0235] For the experiments described in this example, the stable MN transfectants of NIH 3T3 cells generated as described in Example 3 were used. Four selected MN-positive clones and four control mock-transfected clones were either used individually or in pools.

[0236] Flow cytometric analyses of asynchronous cell populations. Cells that had been grown in dense culture were plated at 1 x 10^6 cells per 60 mm dish. Four days later, the cells were collected by trypsinization, washed, resuspended in PBS, fixed by dropwise addition of 70% ethanol and stained by propidium iodine solution containing RNase. Analysis was performed by FACStar using DNA cell cycle analysis software [Becton Dickinson; Franklin Lakes, NJ (USA)].

[0237] Exponentially growing cells were plated at 5 x 10^5 cells per 60 mm dish and analysed as above 2 days later. Forward light scatter was used for the analysis of relative cell sizes. The data were evaluated using Kolmogorov-Smirnov test [Young, J. Histochem. Cytochem., 25: 935 (1977)].

[0238] The flow cytometric analyses revealed that clonal populations constitutively expressing MN protein showed a decreased percentage of cells in G1 phase and an increased percentage of cells in G2-M phases. Those differences were more striking in cell populations grown throughout three passages in high density cultures than in exponentially growing subconfluent cells. That observation supports the idea that MN protein has the capacity to perturb contact inhibition.

[0239] Also observed was a decrease in the size of MN expressing cells seen in both exponentially proliferating and high density cultures. It is possible that the MN-mediated acceleration of G1 transit is related to the above-noted shorter doubling time (by about 15%) of exponentially proliferating MN-expressing NIH 3T3 cells. Also, MN expressing cells displayed substantially higher saturation density and lower serum requirements than the control cells. Those facts suggest that MN-transfected cells had the capacity to continue to proliferate despite space limitations and diminished levels of serum growth factors, whereas the control cells were arrested in G1 phase.

[0240] Limiting conditions. The proliferation of MN-expressing and control cells was studied both in optimal and limiting conditions. Cells were plated at 2 x 10^4 per well of 24-well plate in DMEM with 10% FCS. The medium was changed at daily intervals until day 4 when confluency was reached, and the medium was no longer renewed. Viable cells were counted in a hemacytometer at appropriate times using trypan blue dye exclusion. The numbers of cells were plotted versus time wherein each plot point represents a mean value of triplicate determination.

[0241] The results showed that the proliferation of MN expressing and control cells was similar during the first phase when the medium was renewed daily, but that a big difference in the number of viable cells occurred after the medium was not renewed. More than half of the control cells were not able to withstand the unfavorable growth conditions. In contrast, the MN-expressing cells continued to proliferate even when exposed to increasing competition for nutrients and serum growth factors.

[0242] Those results were supported also by flow cytometric analysis of serum starved cells grown for two days in medium containing 1% FCS. While 83% of control cells accumulated in G0-G1 phase (S = 5%, G2-M = 12%), expression of MN protein partially reversed the delay in G1 as indicated by cell cycle distribution of MN tranfectants (G0-G1 = 65%, S = 10%, G2-M = 26%). The results of the above-described experiments suggest that MN protein might function to release the G1/S checkpoint and allow cells to proliferate under unfavorable conditions.

[0243] MMC. To test that assumption, unfavorable conditions were simulated by treating cells with the DNA damaging drug mitomycin C (MMC) and then following their proliferation and viability. The mechanism of action of MMC is thought to result from its intracellular activation and subsequent DNA alkylation and crosslinking [Yier and Szybalski, Science, 145: 55 (1964)]. Normally, cells respond to DNA damage by arrest of their cell cycle progression to repair defects and prevent acquisition of genomic instability. Large damage is accompanied by marked cytotoxicity. However, many studies [for example, Peters et al., Int. J. Cancer, 54: 450 (1993)] concern the emergence of drug resistant cells both in tumor cell populations and after the introduction of oncogenes into nontransformed cell lines.

[0244] The response of MN-transfected NIH 3T3 cells to increasing concentrations of MMC was determined by continuous [^{3}H]-thymidine labeling. Cells were plated in 96-well microtiter plate concentration of 10^4 per well and incubated overnight in DMEM with 10% FCS to attach. Then the growth medium was replaced with 100 µl of medium containing increasing concentrations of MMC from 1 µl/ml to 32 µg/ml. All the drug concentrations were tested in three replicate wells. After 5 hours of treatment, the MMC was removed, cells were washed with PBS and fresh growth medium without the drug was added. After overnight recovery, the fractions of cells that were actively participating in proliferation was determined by continuous 24-hr labeling with [^{3}H]-thymidine. The incorporation by the treated cells was compared to that of the control, untreated cells, and the proliferating fractions were considered as a percentage of the control's incorporation.

[0245] The viability of the treated cells was estimated three days later by a CellTiter 96 AQ Non-Radioactive Cell Proliferation Assay [Promega] which is based on the bioreduction of methotrexate (MTX) into a water soluble formazan that absorbs light at 490 nm. The percentage of surviving cells was derived from the values of absorbance obtained after substraction of background.

[0246] The control and MN-expressing NIH 3T3 cells showed remarkable differences in their responses to MMC. The sensitivity of the MN-transfected cells appeared considerably lower than the control's in both sections of the above-described experiments. The results suggested that the MN-transfected cells were able to override the negative growth signal mediated by MMC.

[0247] ATCC Deposits. The material listed below was deposited with the American Type Culture Collection (ATCC) at 10801 University Blvd Manassas, VA 20110-2209 (USA). The deposits were made under the provisions of the Budapest Treaty on the International Recognition of Deposited Microorganisms for the Purposes of Patent Procedure and Regulations thereunder (Budapest Treaty). Maintenance of a viable culture is assured for thirty years from the date of deposit. The hybridomas and plasmids will be made available by the ATCC under the terms of the Budapest Treaty, and subject to an agreement between the Applicants and the ATCC which assures unrestricted availability of the deposited hybridomas and plasmids to the public upon the granting of patent from the instant application. Availability of the deposited strain is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any Government in accordance with its patent laws.

Hybridoma	Deposit Date	ATCC #
VU-M75	September 17, 1992	HB 11128
MN 12.2.2	June 9, 1994	HB 11647

Plasmid	Deposit Date	ATCC #
A4a	June 6, 1995	97199
XE1	June 6, 1995	97200
XE3	June 6, 1995	97198

SEQUENCE LISTING

[0248]

(1) GENERAL INFORMATION:

(i) APPLICANT:

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(B) STREET ADDRESS: Dubravska Cesta 9
(C) CITY: Bratislava
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(F) POSTAL CODE/ZIP: 84246

(ii) TITLE OF INVENTION: MN Gene and Protein

(iii) NUMBER OF SEQUENCES: 86

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Clive FROUD
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(D) STATE: Sevenoaks, Kent
(E) COUNTRY: England
(F) ZIP: TN13 1XR

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT/US 95/07628
(B) INTERNATIONAL FILING DATE: 15-JUN-1995
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Clive FROUD
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(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: +44 1732 458 881
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(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1522 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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CCTGCTCCAG GCCTCACTGT GCAACTGCTG CTGTCACTGC TGCTTCTGAT GCCTGTCCAT     120
CCCCAGAGGT TGCCCCGGAT GCAGGAGGAT TCCCCCTTGG GAGGAGGCTC TTCTGGGGAA     180
GATGACCCAC TGGGCGAGGA GGATCTGCCC AGTGAAGAGG ATTCACCCAG AGAGGAGGAT     240
CCACCCGGAG AGGAGGATCT ACCTGGAGAG GAGGATCTAC CTGGAGAGGA GGATCTACCT     300
GAAGTTAAGC CTAAATCAGA AGAAGAGGGC TCCCTGAAGT TAGAGGATCT ACCTACTGTT     360
GAGGCTCCTG GAGATCCTCA AGAACCCCAG AATAATGCCC ACAGGGACAA AGAAGGGGAT     420
GACCAGAGTC ATTGGCGCTA TGGAGGCGAC CCGCCCTGGC CCCGGGTGTC CCCAGCCTGC     480
GCGGGCCGCT TCCAGTCCCC GGTGGATATC CGCCCCCAGC TCGCCGCCTT CTGCCCGGCC     540
CTGCGCCCCC TGGAACTCCT GGGCTTCCAG CTCCCGCCGC TCCCAGAACT GCGCCTGCGC     600
AACAATGGCC ACAGTGTGCA ACTGACCCTG CCTCCTGGGC TAGAGATGGC TCTGGGTCCC     660
GGGCGGGAGT ACCGGGCTCT GCAGCTGCAT CTGCACTGGG GGGCTGCAGG TCGTCCGGGC     720
TCGGAGCACA CTGTGGAAGG CCACCGTTTC CCTGCCGAGA TCCACGTGGT TCACCTCAGC     780
ACCGCCTTTG CCAGAGTTGA CGAGGCCTTG GGGCGCCCGG GAGGCCTGGC CGTGTTGGCC     840
GCCTTTCTGG AGGAGGGCCC GGAAGAAAAC AGTGCCTATG AGCAGTTGCT GTCTCGCTTG     900
GAAGAAATCG CTGAGGAAGG CTCAGAGACT CAGGTCCCAG GACTGGACAT ATCTGCACTC     960
CTGCCCTCTG ACTTCAGCCG CTACTTCCAA TATGAGGGGT CTCTGACTAC ACCGCCCTGT    1020
GCCCAGGGTG TCATCTGGAC TGTGTTTAAC CAGACAGTGA TGCTGAGTGC TAAGCAGCTC    1080
CACACCCTCT CTGACACCCT GTGGGGACCT GGTGACTCTC GGCTACAGCT GAACTTCCGA    1140
GCGACGCAGC CTTTGAATGG GCGAGTGATT GAGGCCTCCT TCCCTGCTGG AGTGGACAGC    1200
AGTCCTCGGG CTGCTGAGCC AGTCCAGCTG AATTCCTGCC TGGCTGCTGG TGACATCCTA    1260
GCCCTGGTTT TTGGCCTCCT TTTTGCTGTC ACCAGCGTCG CGTTCCTTGT GCAGATGAGA    1320
AGGCAGCACA GAAGGGGAAC CAAAGGGGGT GTGAGCTACC GCCCAGCAGA GGTAGCCGAG    1380
ACTGGAGCCT AGAGGCTGGA TCTTGGAGAA TGTGAGAAGC CAGCCAGAGG CATCTGAGGG    1440
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 459 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(A) First 37 amino acids represent signal peptide, and remaining amino acids represent mature protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Met Ala Pro Leu Cys Pro Ser Pro Trp Leu Pro Leu Leu Ile Pro Ala
        -35                 -30                 -25

Pro Ala Pro Gly Leu Thr Val Gln Leu Leu Leu Ser Leu Leu Leu Leu
    -20                 -15                 -10

Met Pro Val His Pro/Gln Arg Leu Pro Arg Met Gln Glu Asp Ser Pro
-5                  1               5                   10

Leu Gly Gly Gly Ser Ser Gly Glu Asp Asp Pro Leu Gly Glu Glu Asp
            15                  20                  25

Leu Pro Ser Glu Glu Asp Ser Pro Arg Glu Glu Asp Pro Pro Gly Glu
        30                  35                  40

Glu Asp Leu Pro Gly Glu Glu Asp Leu Pro Gly Glu Glu Asp Leu Pro
    45                  50                  55

Glu Val Lys Pro Lys Ser Glu Glu Glu Gly Ser Leu Lys Leu Glu Asp
60                  65                  70                  75

Leu Pro Thr Val Glu Ala Pro Gly Asp Pro Gln Glu Pro Gln Asn Asn
                80                  85                  90

Ala His Arg Asp Lys Glu Gly Asp Asp Gln Ser His Trp Arg Tyr Gly
            95                  100                 105

Gly Asp Pro Pro Trp Pro Arg Val Ser Pro Ala Cys Ala Gly Arg Phe
        110                 115                 120

Gln Ser Pro Val Asp Ile Arg Pro Gln Leu Ala Ala Phe Cys Pro Ala
    125                 130                 135

Leu Arg Pro Leu Glu Leu Leu Gly Phe Gln Leu Pro Pro Leu Pro Glu
140                 145                 150                 155

Leu Arg Leu Arg Asn Asn Gly His Ser Val Gln Leu Thr Leu Pro Pro
                160                 165                 170

Gly Leu Glu Met Ala Leu Gly Pro Gly Arg Glu Tyr Arg Ala Leu Gln
            175                 180                 185

Leu His Leu His Trp Gly Ala Ala Gly Arg Pro Gly Ser Glu His Thr
        190                 195                 200

Val Glu Gly His Arg Phe Pro Ala Glu Ile His Val Val His Leu Ser
    205                 210                 215

Thr Ala Phe Ala Arg Val Asp Glu Ala Leu Gly Arg Pro Gly Gly Leu
220                 225                 230                 235

Ala Val Leu Ala Ala Phe Leu Glu Glu Gly Pro Glu Glu Asn Ser Ala
                240                 245                 250

Tyr Glu Gln Leu Leu Ser Arg Leu Glu Glu Ile Ala Glu Glu Gly Ser
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            255                 260                 265

Glu Thr Gln Val Pro Gly Leu Asp Ile Ser Ala Leu Leu Pro Ser Asp
        270                 275                 280

Phe Ser Arg Tyr Phe Gln Tyr Glu Gly Ser Leu Thr Thr Pro Pro Cys
    285                 290                 295

Ala Gln Gly Val Ile Trp Thr Val Phe Asn Gln Thr Val Met Leu Ser
300                 305                 310                 315

Ala Lys Gln Leu His Thr Leu Ser Asp Thr Leu Trp Gly Pro Gly Asp
                320                 325                 330

Ser Arg Leu Gln Leu Asn Phe Arg Ala Thr Gln Pro Leu Asn Gly Arg
            335                 340                 345

Val Ile Glu Ala Ser Phe Pro Ala Gly Val Asp Ser Ser Pro Arg Ala
        350                 355                 360

Ala Glu Pro Val Gln Leu Asn Ser Cys Leu Ala Ala Gly Asp Ile Leu
    365                 370                 375

Ala Leu Val Phe Gly Leu Leu Phe Ala Val Thr Ser Val Ala Phe Leu
380                 385                 390                 395

Val Gln Met Arg Arg Gln His Arg Arg Gly Thr Lys Gly Gly Val Ser
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Tyr Arg Pro Ala Glu Val Ala Glu Thr Gly Ala
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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

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CGCCCAGTGG GTCATCTTCC CCAGAAGAG                                          29
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(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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GGAATCCTCC TGCATCCGG                                                        19
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(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10898 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5 :

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AAGGCAGCAT GCTCGTTAAG AGTCATCACC AATCCCTAAT CTCAAGTAAT CAGGGACACA    180
AACACTGCGG AAGGCCGCAG GGTCCTCTGC CTAGGAAAAC CAGAGACCTT TGTTCACTTG    240
TTTATCTGAC CTTCCCTCCA CTATTGTCCA TGACCCTGCC AAATCCCCCT CTGTGAGAAA    300
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AAAAAAAAAA GACTTACGAA TAGTTATTGA TAAATGAATA GCTATTGGTA AAGCCAAGTA    420
AATGATCATA TTCAAAACCA GACGGCCATC ATCACAGCTC AAGTCTACCT GATTTGATCT    480
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TTTAAACTTT ACCTCTAAGT CAGTTGGGTA GCCTTTGGCT TATTTTTGTA GCTAATTTTG    720
TAGTTAATGG ATGCACTGTG AATCTTGCTA TGATAGTTTT CCTCCACACT TTGCCACTAG    780
GGGTAGGTAG GTACTCAGTT TTCAGTAATT GCTTACCTAA GACCCTAAGC CCTATTTCTC    840
TTGTACTGGC CTTTATCTGT AATATGGGCA TATTTAATAC AATATAATTT TTGGAGTTTT    900
TTTGTTTGTT TGTTTGTTTG TTTTTTTGAG ACGGAGTCTT GCATCTGTCA TGCCCAGGCT    960
GGAGTAGCAG TGGTGCCATC TCGGCTCACT GCAAGCTCCA CCTCCCGAGT TCACGCCATT   1020
TTCCTGCCTC AGCCTCCCGA GTAGCTGGGA CTACAGGCGC CCGCCACCAT GCCCGGCTAA   1080
TTTTTTGTAT TTTTGGTAGA GACGGGGTTT CACCGTGTTA GCCAGAATGG TCTCGATCTC   1140
CTGACTTCGT GATCCACCCG CCTCGGCCTC CCAAAGTTCT GGGATTACAG GTGTGAGCCA   1200
CCGCACCTGG CCAATTTTTT GAGTCTTTTA AAGTAAAAAT ATGTCTTGTA AGCTGGTAAC   1260
TATGGTACAT TTCCTTTTAT TAATGTGGTG CTGACGGTCA TATAGGTTCT TTTGAGTTTG   1320
GCATGCATAT GCTACTTTTT GCAGTCCTTT CATTACATTT TTCTCTCTTC ATTTGAAGAG   1380
```

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CATGTTATAT CTTTTAGCTT CACTTGGCTT AAAAGGTTCT CTCATTAGCC TAACACAGTG    1440
TCATTGTTGG TACCACTTGG ATCATAAGTG GAAAAACAGT CAAGAAATTG CACAGTAATA    1500
CTTGTTTGTA AGAGGGATGA TTCAGGTGAA TCTGACACTA AGAAACTCCC CTACCTGAGG    1560
TCTGAGATTC CTCTGACATT GCTGTATATA GGCTTTTCCT TTGACAGCCT GTGACTGCGG    1620
ACTATTTTTC TTAAGCAAGA TATGCTAAAG TTTTGTGAGC CTTTTTCCAG AGAGAGGTCT    1680
CATATCTGCA TCAAGTGAGA ACATATAATG TCTGCATGTT TCCATATTTC AGGAATGTTT    1740
GCTTGTGTTT TATGCTTTTA TATAGACAGG GAAACTTGTT CCTCAGTGAC CCAAAAGAGG    1800
TGGGAATTGT TATTGGATAT CATCATTGGC CCACGCTTTC TGACCTTGGA AACAATTAAG    1860
GGTTCATAAT CTCAATTCTG TCAGAATTGG TACAAGAAAT AGCTGCTATG TTTCTTGACA    1920
TTCCACTTGG TAGGAAATAA GAATGTGAAA CTCTTCAGTT GGTGTGTGTC CCTNGTTTTT    1980
TTGCAATTTC CTTCTTACTG TGTTAAAAAA AAGTATGATC TTGCTCTGAG AGGTGAGGCA    2040
TTCTTAATCA TGATCTTTAA AGATCAATAA TATAATCCTT TCAAGGATTA TGTCTTTATT    2100
ATAATAAAGA TAATTTGTCT TTAACAGAAT CAATAATATA ATCCCTTAAA GGATTATATC    2160
TTTGCTGGGC GCAGTGGCTC ACACCTGTAA TCCCAGCACT TTGGGTGGCC AAGGTGGAAG    2220
GATCAAATTT GCCTACTTCT ATATTATCTT CTAAAGCAGA ATTCATCTCT CTTCCCTCAA    2280
TATGATGATA TTGACAGGGT TTGCCCTCAC TCACTAGATT GTGAGCTCCT GCTCAGGGCA    2340
GGTAGCGTTT TTTGTTTTTG TTTTTGTTTT TCTTTTTTGA GACAGGGTCT TGCTCTGTCA    2400
CCCAGGCCAG AGTGCAATGG TACAGTCTCA GCTCACTGCA GCCTCAACCG CCTCGGCTCA    2460
AACCATCATC CCATTTCAGC CTCCTGAGTA GCTGGGACTA CAGGCACATG CCATTACACC    2520
TGGCTAATTT TTTTGTATTT CTAGTAGAGA CAGGGTTTGG CCATGTTGCC CGGGCTGGTC    2580
TCGAACTCCT GGACTCAAGC AATCCACCCA CCTCAGCCTC CCAAAATGAG GGACCGTGTC    2640
TTATTCATTT CCATGTCCCT AGTCCATAGC CCAGTGCTGG ACCTATGGTA GTACTAAATA    2700
AATATTTGTT GAATGCAATA GTAAATAGCA TTTCAGGGAG CAAGAACTAG ATTAACAAAG    2760
GTGGTAAAAG GTTTGGAGAA AAAAATAATA GTTTAATTTG GCTAGAGTAT GAGGGAGAGT    2820
AGTAGGAGAC AAGATGGAAA GGTCTCTTGG GCAAGGTTTT GAAGGAAGTT GGAAGTCAGA    2880
AGTACACAAT GTGCATATCG TGGCAGGCAG TGGGGAGCCA ATGAAGGCTT TTGAGCAGGA    2940
GAGTAATGTG TTGAAAAATA AATATAGGTT AAACCTATCA GAGCCCCTCT GACACATACA    3000
CTTGCTTTTC ATTCAAGCTC AAGTTTGTCT CCCACATACC CATTACTTAA CTCACCCTCG    3060
GGCTCCCCTA GCAGCCTGCC CTACCTCTTT ACCTGCTTCC TGGTGGAGTC AGGGATGTAT    3120
ACATGAGCTG CTTTCCCTCT CAGCCAGAGG ACATGGGGGG CCCCAGCTCC CCTGCCTTTC    3180
CCCTTCTGTG CCTGGAGCTG GGAAGCAGGC CAGGGTTAGC TGAGGCTGGC TGGCAAGCAG    3240
CTGGGTGGTG CCAGGGAGAG CCTGCATAGT GCCAGGTGGT GCCTTGGGTT CCAAGCTAGT    3300
CCATGGCCCC GATAACCTTC TGCCTGTGCA CACACCTGCC CCTCACTCCA CCCCCATCCT    3360
AGCTTTGGTA TGGGGGAGAG GGCACAGGGC CAGACAAACC TGTGAGACTT TGGCTCCATC    3420
TCTGCAAAAG GGCGCTCTGT GAGTCAGCCT GCTCCCCTCC AGGCTTGCTC CTCCCCCACC    3480
```

Exon 3

```
CAGCTCTCGT TTCCAATGCA CGTACAGCCC GTACACACCG TGTGCTGGGA CACCCCACAG   3540
TCAGCCGCAT GGCTCCCCTG TGCCCCAGCC CCTGGCTCCC TCTGTTGATC CCGGCCCCTG   3600
CTCCAGGCCT CACTGTGCAA CTGCTGCTGT CACTGCTGCT TCTGGTGCCT GTCCATCCCC   3660
AGAGGTTGCC CCGGATGCAG GAGGATTCCC CCTTGGGAGG AGGCTCTTCT GGGGAAGATG   3720
ACCCACTGGG CGAGGAGGAT CTGCCCAGTG AAGAGGATTC ACCCAGAGAG GAGGATCCAC   3780
CCGGAGAGGA GGATCTACCT GGAGAGGAGG ATCTACCTGG AGAGGAGGAT CTACCTGAAG   3840
TTAAGCCTAA ATCAGAAGAA GAGGGCTCCC TGAAGTTAGA GGATCTACCT ACTGTTGAGG   3900
CTCCTGGAGA TCCTCAAGAA CCCCAGAATA ATGCCCACAG GGACAAAGAA GGTAAGTGGT   3960
CATCAATCTC CAAATCCAGG TTCCAGGAGG TTCATGACTC CCCTCCCATA CCCCAGCCTA   4020
GGCTCTGTTC ACTCAGGGAA GGAGGGGAGA CTGTACTCCC CACAGAAGCC CTTCCAGAGG   4080
TCCCATACCA ATATCCCCAT CCCCACTCTC GGAGGTAGAA AGGGACAGAT GTGGAGAGAA   4140
AATAAAAAGG GTGCAAAAGG AGAGAGGTGA GCTGGATGAG ATGGGAGAGA AGGGGGAGGC   4200
TGGAGAAGAG AAAGGGATGA GAACTGCAGA TGAGAGAAAA AATGTGCAGA CAGAGGAAAA   4260
AAATAGGTGG AGAAGGAGAG TCAGAGAGTT TGAGGGGAAG AGAAAAGGAA AGCTTGGGAG   4320
GTGAAGTGGG TACCAGAGAC AAGCAAGAAG AGCTGGTAGA AGTCATCTCA TCTTAGGCTA   4380
CAATGAGGAA TTGAGACCTA GGAAGAAGGG ACACAGCAGG TAGAGAAACG TGGCTTCTTG   4440
ACTCCCAAGC CAGGAATTTG GGGAAAGGGG TTGGAGACCA TACAAGGCAG AGGGATGAGT   4500
GGGGAGAAGA AAGAAGGGAG AAAGGAAAGA TGGTGTACTC ACTCATTTGG GACTCAGGAC   4560
TGAAGTGCCC ACTCACTTTT TTTTTTTTTT TTTTTGAGAC AAACTTTCAC TTTTGTTGCC   4620
CAGGCTGGAG TGCAATGGCG CGATCTCGGC TCACTGCAAC CTCCACCTCC CGGGTTCAAG   4680
TGATTCTCCT GCCTCAGCCT CTAGCCAAGT AGCTGCGATT ACAGGCATGC GCCACCACGC   4740
CCGGCTAATT TTTGTATTTT TAGTAGAGAC GGGGTTTCGC CATGTTGGTC AGGCTGGTCT   4800
CGAACTCCTG ATCTCAGGTG ATCCAACCAC CCTGGCCTCC CAAAGTGCTG GGATTATAGG   4860
CGTGAGCCAC AGCGCCTGGC CTGAAGCAGC CACTCACTTT TACAGACCCT AAGACAATGA   4920
TTGCAAGCTG GTAGGATTGC TGTTTGGCCC ACCCAGCTGC GGTGTTGAGT TTGGGTGCGG   4980
TCTCCTGTGC TTTGCACCTG GCCCGCTTAA GGCATTTGTT ACCCGTAATG CTCCTGTAAG   5040
GCATCTGCGT TTGTGACATC GTTTTGGTCG CCAGGAAGGG ATTGGGGCTC TAAGCTTGAG   5100
CGGTTCATCC TTTTCATTTA TACAGGGGAT GACCAGAGTC ATTGGCGCTA TGGAGGTGAG   5160
ACACCCACCC GCTGCACAGA CCCAATCTGG GAACCCAGCT CTGTGGATCT CCCCTACAGC   5220
CGTCCCTGAA CACTGGTCCC GGGCGTCCCA CCCGCCGCCC ACCGTCCCAC CCCCTCACCT   5280
TTTCTACCCG GGTTCCCTAA GTTCCTGACC TAGGCGTCAG ACTTCCTCAC TATACTCTCC   5340
CACCCCAGGC GACCCGCCCT GGCCCCGGGT GTCCCCAGCC TGCGCGGGCC GCTTCCAGTC   5400
CCCGGTGGAT ATCCGCCCCC AGCTCGCCGC CTTCTGCCCG GCCCTGCGCC CCCTGGAACT   5460
CCTGGGCTTC CAGCTCCCGC CGCTCCCAGA ACTGCGCCTG CGCAACAATG GCCACAGTGG   5520
TGAGGGGGTC TCCCCGCCGA GACTTGGGGA TGGGGCGGGG CGCAGGGAAG GGAACCGTCG   5580
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CGCAGTGCCT GCCCGGGGGT TGGGCTGGCC CTACCGGGCG GGGCCGGCTC ACTTGCCTCT 5640

CCCTACGCAG TGCAACTGAC CCTGCCTCCT GGGCTAGAGA TGGCTCTGGG TCCCGGGCGG 5700

GAGTACCGGG CTCTGCAGCT GCATCTGCAC TGGGGGGCTG CAGGTCGTCC GGGCTCGGAG 5760

CACACTGTGG AAGGCCACCG TTTCCCTGCC GAGGTGAGCG CGGACTGGCC GAGAAGGGGC 5820

AAAGGAGCGG GGCGGACGGG GGCCAGAGAC GTGGCCCTCT CCTACCCTCG TGTCCTTTTC 5880

AGATCCACGT GGTTCACCTC AGCACCGCCT TTGCCAGAGT TGACGAGGCC TTGGGGCGCC 5940

CGGGAGGCCT GGCCGTGTTG GCCGCCTTTC TGGAGGTACC AGATCCTGGA CACCCCCTAC 6000

TCCCCGCTTT CCCATCCCAT GCTCCTCCCG GACTCTATCG TGGAGCCAGA GACCCCATCC 6060

CAGCAAGCTC ACTCAGGCCC CTGGCTGACA AACTCATTCA CGCACTGTTT GTTCATTTAA 6120

CACCCACTGT GAACCAGGCA CCAGCCCCCA ACAAGGATTC TGAAGCTGTA GGTCCTTGCC 6180

TCTAAGGAGC CCACAGCCAG TGGGGGAGGC TGACATGACA GACACATAGG AAGGACATAG 6240

TAAAGATGGT GGTCACAGAG GAGGTGACAC TTAAAGCCTT CACTGGTAGA AAAGAAAAGG 6300

AGGTGTTCAT TGCAGAGGAA ACAGAATGTG CAAAGACTCA GAATATGGCC TATTTAGGGA 6360

ATGGCTACAT ACACCATGAT TAGAGGAGGC CCAGTAAAGG GAAGGGATGG TGAGATGCCT 6420

GCTAGGTTCA CTCACTCACT TTTATTTATT TATTTATTTT TTTGACAGTC TCTCTGTCGC 6480

CCAGGCTGGA GTGCAGTGGT GTGATCTTGG GTCACTGCAA CTTCCGCCTC CCGGGTTCAA 6540

GGGATTCTCC TGCCTCAGCT TCCTGAGTAG CTGGGGTTAC AGGTGTGTGC CACCATGCCC 6600

AGCTAATTTT TTTTTGTATT TTTAGTAGAC AGGGTTTCAC CATGTTGGTC AGGCTGGTCT 6660

CAAACTCCTG GCCTCAAGTG ATCCGCCTGA CTCAGCCTAC CAAAGTGCTG ATTACAAGTG 6720

TGAGCCACCG TGCCCAGCCA CACTCACTGA TTCTTTAATG CCAGCCACAC AGCACAAAGT 6780

TCAGAGAAAT GCCTCCATCA TAGCATGTCA ATATGTTCAT ACTCTTAGGT TCATGATGTT 6840

CTTAACATTA GGTTCATAAG CAAAATAAGA AAAAAGAATA ATAAATAAAA GAAGTGGCAT 6900

GTCAGGACCT CACCTGAAAA GCCAAACACA GAATCATGAA GGTGAATGCA GAGGTGACAC 6960

CAACACAAAG GTGTATATAT GGTTTCCTGT GGGGAGTATG TACGGAGGCA GCAGTGAGTG 7020

AGACTGCAAA CGTCAGAAGG GCACGGGTCA CTGAGAGCCT AGTATCCTAG TAAAGTGGGC 7080

TCTCTCCCTC TCTCTCCAGC TTGTCATTGA AAACCAGTCC ACCAAGCTTG TTGGTTCGCA 7140

CAGCAAGAGT ACATAGAGTT TGAAATAATA CATAGGATTT TAAGAGGGAG ACACTGTCTC 7200

TAAAAAAAAA AACAACAGCA ACAACAAAAA GCAACAACCA TTACAATTTT ATGTTCCCTC 7260

AGCATTCTCA GAGCTGAGGA ATGGGAGAGG ACTATGGGAA CCCCCTTCAT GTTCCGGCCT 7320

TCAGCCATGG CCCTGGATAC ATGCACTCAT CTGTCTTACA ATGTCATTCC CCCAGGAGGG 7380

CCCGGAAGAA AACAGTGCCT ATGAGCAGTT GCTGTCTCGC TTGGAAGAAA TCGCTGAGGA 7440

AGGTCAGTTT GTTGGTCTGG CCACTAATCT CTGTGGCCTA GTTCATAAAG AATCACCCTT 7500

TGGAGCTTCA GGTCTGAGGC TGGAGATGGG CTCCCTCCAG TGCAGGAGGG ATTGAAGCAT 7560

GAGCCAGCGC TCATCTTGAT AATAACCATG AAGCTGACAG ACACAGTTAC CCGCAAACGG 7620

CTGCCTACAG ATTGAAAACC AAGCAAAAAC CGCCGGGCAC GGTGGCTCAC GCCTGTAATC 7680

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CCAGCACTTT GGGAGGCCAA GGCAGGTGGA TCACGAGGTC AAGAGATCAA GACCATCCTG    7740
GCCAACATGG TGAAACCCCA TCTCTACTAA AAATACGAAA AAATAGCCAG GCGTGGTGGC    7800
GGGTGCCTGT AATCCCAGCT ACTCGGGAGG CTGAGGCAGG AGAATGGCAT GAACCCGGGA    7860
GGCAGAAGTT GCAGTGAGCC GAGATCGTGC CACTGCACTC CAGCCTGGGC AACAGAGCGA    7920
GACTCTTGTC TCAAAAAAAA AAAAAAAAAA GAAAACCAAG CAAAAACCAA AATGAGACAA    7980
AAAAAACAAG ACCAAAAAAT GGTGTTTGGA AATTGTCAAG GTCAAGTCTG GAGAGCTAAA    8040
CTTTTTCTGA GAACTGTTTA TCTTTAATAA GCATCAAATA TTTTAACTTT GTAAATACTT    8100
TTGTTGGAAA TCGTTCTCTT CTTAGTCACT CTTGGGTCAT TTTAAATCTC ACTTACTCTA    8160
CTAGACCTTT TAGGTTTCTG CTAGACTAGG TAGAACTCTG CCTTTGCATT TCTTGTGTCT    8220
GTTTTGTATA GTTATCAATA TTCATATTTA TTTACAAGTT ATTCAGATCA TTTTTTCTTT    8280
TCTTTTTTTT TTTTTTTTTT TTTTTTACAT CTTTAGTAGA GACAGGGTTT CACCATATTG    8340
GCCAGGCTGC TCTCAAACTC CTGACCTTGT GATCCACCAG CCTCGGCCTC CCAAAGTGCT    8400
GGGATTCATT TTTTCTTTTT AATTTGCTCT GGGCTTAAAC TTGTGGCCCA GCACTTTATG    8460
ATGGTACACA GAGTTAAGAG TGTAGACTCA GACGGTCTTT CTTCTTTCCT TCTCTTCCTT    8520
CCTCCCTTCC CTCCCACCTT CCCTTCTCTC CTTCCTTTCT TTCTTCCTCT CTTGCTTCCT    8580
CAGGCCTCTT CCAGTTGCTC CAAAGCCCTG TACTTTTTTT TGAGTTAACG TCTTATGGGA    8640
AGGGCCTGCA CTTAGTGAAG AAGTGGTCTC AGAGTTGAGT TACCTTGGCT TCTGGGAGGT    8700
GAAACTGTAT CCCTATACCC TGAAGCTTTA AGGGGGTGCA ATGTAGATGA GACCCCAACA    8760
TAGATCCTCT TCACAGGCTC AGAGACTCAG GTCCCAGGAC TGGACATATC TGCACTCCTG    8820
CCCTCTGACT TCAGCCGCTA CTTCCAATAT GAGGGGTCTC TGACTACACC GCCCTGTGCC    8880
CAGGGTGTCA TCTGGACTGT GTTTAACCAG ACAGTGATGC TGAGTGCTAA GCAGGTGGGC    8940
CTGGGGTGTG TGTGGACACA GTGGGTGCGG GGGAAAGAGG ATGTAAGATG AGATGAGAAA    9000
CAGGAGAAGA AAGAAATCAA GGCTGGGCTC TGTGGCTTAC GCCTATAATC CCACCACGTT    9060
GGGAGGCTGA GGTGGGAGAA TGGTTTGAGC CCAGGAGTTC AAGACAAGGC GGGGCAACAT    9120
AGTGTGACCC CATCTCTACC AAAAAAACCC CAACAAAACC AAAAATAGCC GGGCATGGTG    9180
GTATGCGGCC TAGTCCCAGC TACTCAAGGA GGCTGAGGTG GGAAGATCGC TTGATTCCAG    9240
GAGTTTGAGA CTGCAGTGAG CTATGATCCC ACCACTGCCT ACCATCTTTA GGATACATTT    9300
ATTTATTTAT AAAAGAAATC AAGAGGCTGG ATGGGGAATA CAGGAGCTGG AGGGTGGAGC    9360
CCTGAGGTGC TGGTTGTGAG CTGGCCTGGG ACCCTTGTTT CCTGTCATGC CATGAACCCA    9420
CCCACACTGT CCACTGACCT CCCTAGCTCC ACACCCTCTC TGACACCCTG TGGGGACCTG    9480
GTGACTCTCG GCTACAGCTG AACTTCCGAG CGACGCAGCC TTTGAATGGG CGAGTGATTG    9540
AGGCCTCCTT CCCTGCTGGA GTGGACAGCA GTCCTCGGGC TGCTGAGCCA GGTACAGCTT    9600
TGTCTGGTTT CCCCCCAGCC AGTAGTCCCT TATCCTCCCA TGTGTGTGCC AGTGTCTGTC    9660
ATTGGTGGTC ACAGCCCGCC TCTCACATCT CCTTTTTCTC TCCAGTCCAG CTGAATTCCT    9720
GCCTGGCTGC TGGTGAGTCT GCCCCTCCTC TTGGTCCTGA TGCCAGGAGA CTCCTCAGCA    9780
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CCATTCAGCC CCAGGGCTGC TCAGGACCGC CTCTGCTCCC TCTCCTTTTC TGCAGAACAG     9840
ACCCCAACCC CAATATTAGA GAGGCAGATC ATGGTGGGGA TTCCCCCATT GTCCCCAGAG     9900
GCTAATTGAT TAGAATGAAG CTTGAGAAAT CTCCCAGCAT CCCTCTCGCA AAAGAATCCC     9960
CCCCCCTTTT TTTAAAGATA GGGTCTCACT CTGTTTGCCC CAGGCTGGGG TGTTGTGGCA    10020
CGATCATAGC TCACTGCAGC CTCGAACTCC TAGGCTCAGG CAATCCTTTC ACCTTAGCTT    10080
CTCAAAGCAC TGGGACTGTA GGCATGAGCC ACTGTGCCTG GCCCCAAACG GCCCTTTTAC    10140
TTGGCTTTTA GGAAGCAAAA ACGGTGCTTA TCTTACCCCT TCTCGTGTAT CCACCCTCAT    10200
CCCTTGGCTG GCCTCTTCTG GAGACTGAGG CACTATGGGG CTGCCTGAGA ACTCGGGGCA    10260
GGGGTGGTGG AGTGCACTGA GGCAGGTGTT GAGGAACTCT GCAGACCCCT CTTCCTTCCC    10320
AAAGCAGCCC TCTCTGCTCT CCATCGCAGG TGACATCCTA GCCCTGGTTT TTGGCCTCCT    10380
TTTTGCTGTC ACCAGCGTCG CGTTCCTTGT GCAGATGAGA AGGCAGCACA GGTATTACAC    10440
TGACCCTTTC TTCAGGCACA AGCTTCCCCC ACCCTTGTGG AGTCACTTCA TGCAAAGCGC    10500
ATGCAAATGA GCTGCTCCTG GGCCAGTTTT CTGATTAGCC TTTCCTGTTG TGTACACACA    10560
GAAGGGGAAC CAAAGGGGGT GTGAGCTACC GCCCAGCAGA GGTAGCCGAG ACTGGAGCCT    10620
AGAGGCTGGA TCTTGGAGAA TGTGAGAAGC CAGCCAGAGG CATCTGAGGG GGAGCCGGTA    10680
ACTGTCCTGT CCTGCTCATT ATGCCACTTC CTTTTAACTG CCAAGAAATT TTTTAAAATA    10740
AATATTTATA ATAAAATATG TGTTAGTCAC CTTTGTTCCC CAAATCAGAA GGAGGTATTT    10800
GAATTTCCTA TTACTGTTAT TAGCACCAAT TTAGTGGTAA TGCATTTATT CTATTACAGT    10860
TCGGCCTCCT TCCACACATC ACTCCAATGT GTTGCTCC                            10898
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(A) DESCRIPTION: Signal peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Met Ala Pro Leu Cys Pro Ser Pro Trp Leu Pro Leu Leu Ile Pro Ala
1               5                   10                  15

Pro Ala Pro Gly Leu Thr Val Gln Leu Leu Leu Ser Leu Leu Leu Leu
            20                  25                  30

Met Pro Val His Pro
        35
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: primer

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
TGGGGTTCTT GAGGATCTCC AGGAG                                        25
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: primer

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
CTCTAACTTC AGGGAGCCCT CTTCTT                                       26
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: primer

(iii) HYPOTHETICAL: NO

(ix) FEATURE: N stands for inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
CUACUACUAC UAGGCCACGC GTCGACTAGT ACGGGNNGGG NNGGGNNG                     48
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
Glu Glu Asp Leu Pro Ser
1               5
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Peptide
(B) LOCATION:55..60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Gly Glu Asp Asp Pro Leu
1               5
```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
Asn Asn Ala His Arg Asp Lys Glu Gly Asp Asp Gln Ser His Trp Arg
1               5                   10                  15

Tyr Gly Gly Asp Pro
            20
```

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Peptide
(B) LOCATION:36..51

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
His Pro Gln Arg Leu Pro Arg Met Gln Glu Asp Ser Pro Leu Gly Gly
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```
Glu Glu Asp Ser Pro Arg Glu Glu Asp Pro Pro Gly Glu Glu Asp Leu
1               5                   10                  15

Pro Gly Glu Glu Asp Leu Pro Gly
            20
```

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Peptide
(B) LOCATION:279..291

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

```
Leu Glu Glu Gly Pro Glu Glu Asn Ser Ala Tyr Glu Gln
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```
Met Arg Arg Gln His Arg Arg Gly Thr Lys Gly Gly Val Ser Tyr Arg
1               5                   10                  15
```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
GTCGCTAGCT CCATGGGTCA TATGCAGAGG TTGCCCCGGA TGCAG                      45
```

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

```
GAAGATCTCT TACTCGAGCA TTCTCCAAGA TCCAGCCTCT AGG                43
```

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: AP-2 transcription factor

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

```
TCCCCCACCC                                                     10
```

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: initiator (Inr) element

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
CCACCCCCAT                                                     10
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: p53 binding site

(x) PUBLICATION INFORMATION:

(A) AUTHORS: El Deiry et al.
(B) TITLE: "Human genomic DNA sequences define a consensus binding site for p53"
(C) JOURNAL: Nature Genetics
(D) VOLUME: 1
(F) PAGES: 44-49
(G) DATE: 1992

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
AAGCTAGTCC                                                                10
```

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

```
Leu Glu His His His His His His
1               5
```

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: Initiator consensus sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```
YYYCAYYYYY                                                                10
```

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: p53 binding site

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x) PUBLICATION INFORMATION:

(A) AUTHORS: El Deiry et al.
(B) TITLE: "Human genomic DNA sequences define a consensus binding site for p53"
(C) JOURNAL: Nature Genetics
(D) VOLUME: 1
(F) PAGES: 44-49
(G) DATE: 1992

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

```
AGGCTTGCTC
```

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

```
Ser Pro Xaa Xaa
1
```

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

```
Thr Pro Xaa Xaa
1
```

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 540 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: Proposed MN promoter

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

```
CTTGCTTTTC ATTCAAGCTC AAGTTTGTCT CCCACATACC CATTACTTAA CTCACCCTCG      60
GGCTCCCCTA GCAGCCTGCC CTACCTCTTT ACCTGCTTCC TGGTGGAGTC AGGGATGTAT     120
ACATGAGCTG CTTTCCCTCT CAGCCAGAGG ACATGGGGG CCCCAGCTCC CCTGCCTTTC      180
CCCTTCTGTG CCTGGAGCTG GGAAGCAGGC CAGGGTTAGC TGAGGCTGGC TGGCAAGCAG     240
CTGGGTGGTG CCAGGGAGAG CCTGCATAGT GCCAGGTGGT GCCTTGGGTT CCAAGCTAGT     300
CCATGGCCCC GATAACCTTC TGCCTGTGCA CACACCTGCC CCTCACTCCA CCCCCATCCT     360
AGCTTTGGTA TGGGGGAGAG GGCACAGGGC CAGACAAACC TGTGAGACTT TGGCTCCATC     420


TCTGCAAAAG GGCGCTCTGT GAGTCAGCCT GCTCCCCTCC AGGCTTGCTC CTCCCCCACC     480
CAGCTCTCGT TTCCAATGCA CGTACAGCCC GTACACACCG TGTGCTGGGA CACCCCACAG     540
```

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 445 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 1st MN exon

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GCCCGTACAC ACCGTGTGCT GGGACACCCC ACAGTCAGCC GCATGGCTCC CCTGTGCCCC 60

AGCCCCTGGC TCCCTCTGTT GATCCCGGCC CCTGCTCCAG GCCTCACTGT GCAACTGCTG 120

CTGTCACTGC TGCTTCTGGT GCCTGTCCAT CCCCAGAGGT TGCCCCGGAT GCAGGAGGAT 180

TCCCCCTTGG GAGGAGGCTC TTCTGGGGAA GATGACCCAC TGGGCGAGGA GGATCTGCCC 240

AGTGAAGAGG ATTCACCCAG AGAGGAGGAT CCACCCGGAG AGGAGGATCT ACCTGGAGAG 300

GAGGATCTAC CTGGAGAGGA GGATCTACCT GAAGTTAAGC CTAAATCAGA AGAAGAGGGC 360

TCCCTGAAGT TAGAGGATCT ACCTACTGTT GAGGCTCCTG GAGATCCTCA AGAACCCCAG 420

AATAATGCCC ACAGGGACAA AGAAG 445

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 2nd MN exon

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GGGATGACCA GAGTCATTGG CGCTATGGAG 30

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 171 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 3rd MN exon

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

GCGACCCGCC CTGGCCCCGG GTGTCCCCAG CCTGCGCGGG CCGCTTCCAG TCCCCGGTGG 60

ATATCCGCCC CCAGCTCGCC GCCTTCTGCC CGGCCCTGCG CCCCCTGGAA CTCCTGGGCT 120

TCCAGCTCCC GCCGCTCCCA GAACTGCGCC TGCGCAACAA TGGCCACAGT G 171

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 143 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 4th MN exon

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

TGCAACTGAC CCTGCCTCCT GGGCTAGAGA TGGCTCTGGG TCCCGGGCGG GAGTACCGGG 60

CTCTGCAGCT GCATCTGCAC TGGGGGGCTG CAGGTCGTCC GGGCTCGGAG CACACTGTGG 120

AAGGCCACCG TTTCCCTGCC GAG 143

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 93 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 5th MN exon

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

ATCCACGTGG TTCACCTCAG CACCGCCTTT GCCAGAGTTG ACGAGGCCTT GGGGCGCCCG 60

GGAGGCCTGG CCGTGTTGGC CGCCTTTCTG GAG 93

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 67 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 6th MN exon

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

GAGGGCCCGG AAGAAAACAG TGCCTATGAG CAGTTGCTGT CTCGCTTGGA AGAAATCGCT 60

GAGGAAG 67

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 158 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 7th MN exon

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

GCTCAGAGAC TCAGGTCCCA GGACTGGACA TATCTGCACT CCTGCCCTCT GACTTCAGCC 60

GCTACTTCCA ATATGAGGGG TCTCTGACTA CACCGCCCTG TGCCCAGGGT GTCATCTGGA 120

CTGTGTTTAA CCAGACAGTG ATGCTGAGTG CTAAGCAG 158

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 145 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 8th MN exon

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CTCCACACCC TCTCTGACAC CCTGTGGGGA CCTGGTGACT CTCGGCTACA GCTGAACTTC 60

CGAGCGACGC AGCCTTTGAA TGGGCGAGTG ATTGAGGCCT CCTTCCCTGC TGGAGTGGAC 120

AGCAGTCCTC GGGCTGCTGA GCCAG 145

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 9th MN exon

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

TCCAGCTGAA TTCCTGCCTG GCTGCTG 27

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 82 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 10th MN exon

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

GTGACATCCT AGCCCTGGTT TTTGGCCTCC TTTTTGCTGT CACCAGCGTC GCGTTCCTTG 60

TGCAGATGAG AAGGCAGCAC AG 82

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 191 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 11th MN exon

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

```
AAGGGGAACC AAAGGGGGTG TGAGCTACCG CCCAGCAGAG GTAGCCGAGA CTGGAGCCTA      60

GAGGCTGGAT CTTGGAGAAT GTGAGAAGCC AGCCAGAGGC ATCTGAGGGG GAGCCGGTAA     120


CTGTCCTGTC CTGCTCATTA TGCCACTTCC TTTTAACTGC CAAGAAATTT TTTAAAATAA     180

ATATTTATAA T                                                          191
```

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1174 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 1st MN intron

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

GTAAGTGGTC ATCAATCTCC AAATCCAGGT TCCAGGAGGT TCATGACTCC CCTCCCATAC 60

CCCAGCCTAG GCTCTGTTCA CTCAGGGAAG GAGGGGAGAC TGTACTCCCC ACAGAAGCCC 120

TTCCAGAGGT CCCATACCAA TATCCCCATC CCCACTCTCG GAGGTAGAAA GGGACAGATG 180

TGGAGAGAAA ATAAAAAGGG TGCAAAAGGA GAGAGGTGAG CTGGATGAGA TGGGAGAGAA 240

GGGGGAGGCT GGAGAAGAGA AAGGGATGAG AACTGCAGAT GAGAGAAAAA ATGTGCAGAC 300

AGAGGAAAAA AATAGGTGGA GAAGGAGAGT CAGAGAGTTT GAGGGGAAGA GAAAAGGAAA 360

GCTTGGGAGG TGAAGTGGGT ACCAGAGACA AGCAAGAAGA GCTGGTAGAA GTCATCTCAT 420

CTTAGGCTAC AATGAGGAAT TGAGACCTAG GAAGAAGGGA CACAGCAGGT AGAGAAACGT 480

GGCTTCTTGA CTCCCAAGCC AGGAATTTGG GGAAAGGGGT TGGAGACCAT ACAAGGCAGA 540

GGGATGAGTG GGGAGAAGAA AGAAGGGAGA AAGGAAAGAT GGTGTACTCA CTCATTTGGG 600

ACTCAGGACT GAAGTGCCCA CTCACTTTTT TTTTTTTTTT TTTTGAGACA AACTTTCACT 660

TTTGTTGCCC AGGCTGGAGT GCAATGGCGC GATCTCGGCT CACTGCAACC TCCACCTCCC 720

GGGTTCAAGT GATTCTCCTG CCTCAGCCTC TAGCCAAGTA GCTGCGATTA CAGGCATGCG 780

CCACCACGCC CGGCTAATTT TTGTATTTTT AGTAGAGACG GGGTTTCGCC ATGTTGGTCA 840

GGCTGGTCTC GAACTCCTGA TCTCAGGTGA TCCAACCACC CTGGCCTCCC AAAGTGCTGG 900

GATTATAGGC GTGAGCCACA GCGCCTGGCC TGAAGCAGCC ACTCACTTTT ACAGACCCTA 960

AGACAATGAT TGCAAGCTGG TAGGATTGCT GTTTGGCCCA CCCAGCTGCG GTGTTGAGTT 1020

TGGGTGCGGT CTCCTGTGCT TTGCACCTGG CCCGCTTAAG GCATTTGTTA CCCGTAATGC 1080

TCCTGTAAGG CATCTGCGTT TGTGACATCG TTTTGGTCGC CAGGAAGGGA TTGGGGCTCT 1140

AAGCTTGAGC GGTTCATCCT TTTCATTTAT ACAG 1174

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 193 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 2nd MN intron

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

GTGAGACACC CACCCGCTGC ACAGACCCAA TCTGGGAACC CAGCTCTGTG GATCTCCCCT 60

ACAGCCGTCC CTGAACACTG GTCCCGGGCG TCCCACCCGC CGCCCACCGT CCCACCCCCT 120

CACCTTTTCT ACCCGGGTTC CCTAAGTTCC TGACCTAGGC GTCAGACTTC CTCACTATAC 180

TCTCCCACCC CAG 193

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 131 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 3rd MN intron

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

GTGAGGGGGT CTCCCCGCCG AGACTTGGGG ATGGGGCGGG GCGCAGGGAA GGGAACCGTC 60

GCGCAGTGCC TGCCCGGGGG TTGGGCTGGC CCTACCGGGC GGGGCCGGCT CACTTGCCTC 120

TCCCTACGCA G 131

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 89 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) 4th MN intron

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

GTGAGCGCGG ACTGGCCGAG AAGGGGCAAA GGAGCGGGGC GGACGGGGGC CAGAGACGTG 60

GCCCTCTCCT ACCCTCGTGT CCTTTTCAG 89

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1400 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 5th MN intron

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

```
GTACCAGATC CTGGACACCC CCTACTCCCC GCTTTCCCAT CCCATGCTCC TCCCGGACTC      60
TATCGTGGAG CCAGAGACCC CATCCCAGCA AGCTCACTCA GGCCCCTGGC TGACAAACTC     120
ATTCACGCAC TGTTTGTTCA TTTAACACCC ACTGTGAACC AGGCACCAGC CCCCAACAAG     180
GATTCTGAAG CTGTAGGTCC TTGCCTCTAA GGAGCCCACA GCCAGTGGGG GAGGCTGACA     240
TGACAGACAC ATAGGAAGGA CATAGTAAAG ATGGTGGTCA CAGAGGAGGT GACACTTAAA     300
GCCTTCACTG GTAGAAAAGA AAAGGAGGTG TTCATTGCAG AGGAAACAGA ATGTGCAAAG     360
ACTCAGAATA TGGCCTATTT AGGGAATGGC TACATACACC ATGATTAGAG GAGGCCCAGT     420
AAAGGGAAGG GATGGTGAGA TGCCTGCTAG GTTCACTCAC TCACTTTTAT TTATTTATTT     480
ATTTTTTTGA CAGTCTCTCT GTCGCCCAGG CTGGAGTGCA GTGGTGTGAT CTTGGGTCAC     540
TGCAACTTCC GCCTCCCGGG TTCAAGGGAT TCTCCTGCCT CAGCTTCCTG AGTAGCTGGG     600
GTTACAGGTG TGTGCCACCA TGCCCAGCTA ATTTTTTTTT GTATTTTTAG TAGACAGGGT     660
TTCACCATGT TGGTCAGGCT GGTCTCAAAC TCCTGGCCTC AAGTGATCCG CCTGACTCAG     720
CCTACCAAAG TGCTGATTAC AAGTGTGAGC CACCGTGCCC AGCCACACTC ACTGATTCTT     780
TAATGCCAGC CACACAGCAC AAAGTTCAGA GAAATGCCTC CATCATAGCA TGTCAATATG     840
TTCATACTCT TAGGTTCATG ATGTTCTTAA CATTAGGTTC ATAAGCAAAA TAAGAAAAAA     900
GAATAATAAA TAAAAGAAGT GGCATGTCAG GACCTCACCT GAAAAGCCAA ACACAGAATC     960
ATGAAGGTGA ATGCAGAGGT GACACCAACA CAAAGGTGTA TATATGGTTT CCTGTGGGGA    1020
GTATGTACGG AGGCAGCAGT GAGTGAGACT GCAAACGTCA GAAGGGCACG GGTCACTGAG    1080
AGCCTAGTAT CCTAGTAAAG TGGGCTCTCT CCCTCTCTCT CCAGCTTGTC ATTGAAAACC    1140
AGTCCACCAA GCTTGTTGGT TCGCACAGCA AGAGTACATA GAGTTTGAAA TAATACATAG    1200
GATTTTAAGA GGGAGACACT GTCTCTAAAA AAAAAAACAA CAGCAACAAC AAAAAGCAAC    1260
AACCATTACA ATTTTATGTT CCCTCAGCAT TCTCAGAGCT GAGGAATGGG AGAGGACTAT    1320
GGGAACCCCC TTCATGTTCC GGCCTTCAGC CATGGCCCTG GATACATGCA CTCATCTGTC    1380
TTACAATGTC ATTCCCCCAG                                                1400
```

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1334 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 6th MN intron

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

```
GTCAGTTTGT TGGTCTGGCC ACTAATCTCT GTGGCCTAGT TCATAAAGAA TCACCCTTTG     60
GAGCTTCAGG TCTGAGGCTG GAGATGGGCT CCCTCCAGTG CAGGAGGGAT TGAAGCATGA    120
GCCAGCGCTC ATCTTGATAA TAACCATGAA GCTGACAGAC ACAGTTACCC GCAAACGGCT    180
GCCTACAGAT TGAAAACCAA GCAAAAACCG CCGGGCACGG TGGCTCACGC CTGTAATCCC    240
AGCACTTTGG GAGGCCAAGG CAGGTGGATC ACGAGGTCAA GAGATCAAGA CCATCCTGGC    300
CAACATGGTG AAACCCCATC TCTACTAAAA ATACGAAAAA ATAGCCAGGC GTGGTGGCGG    360
GTGCCTGTAA TCCCAGCTAC TCGGGAGGCT GAGGCAGGAG AATGGCATGA ACCCGGGAGG    420
CAGAAGTTGC AGTGAGCCGA GATCGTGCCA CTGCACTCCA GCCTGGGCAA CAGAGCGAGA    480
CTCTTGTCTC AAAAAAAAAA AAAAAAAAGA AAACCAAGCA AAAACCAAAA TGAGACAAAA    540
AAAACAAGAC CAAAAAATGG TGTTTGGAAA TTGTCAAGGT CAAGTCTGGA GAGCTAAACT    600
TTTTCTGAGA ACTGTTTATC TTTAATAAGC ATCAAATATT TTAACTTTGT AAATACTTTT    660
GTTGGAAATC GTTCTCTTCT TAGTCACTCT TGGGTCATTT TAAATCTCAC TTACTCTACT    720
AGACCTTTTA GGTTTCTGCT AGACTAGGTA GAACTCTGCC TTTGCATTTC TTGTGTCTGT    780
TTTGTATAGT TATCAATATT CATATTTATT TACAAGTTAT TCAGATCATT TTTTCTTTTC    840
TTTTTTTTTT TTTTTTTTTT TTTTACATCT TTAGTAGAGA CAGGGTTTCA CCATATTGGC    900
CAGGCTGCTC TCAAACTCCT GACCTTGTGA TCCACCAGCC TCGGCCTCCC AAAGTGCTGG    960
GATTCATTTT TTCTTTTTAA TTTGCTCTGG GCTTAAACTT GTGGCCCAGC ACTTTATGAT   1020
GGTACACAGA GTTAAGAGTG TAGACTCAGA CGGTCTTTCT TCTTTCCTTC TCTTCCTTCC   1080
TCCCTTCCCT CCCACCTTCC CTTCTCTCCT TCCTTTCTTT CTTCCTCTCT TGCTTCCTCA   1140
GGCCTCTTCC AGTTGCTCCA AAGCCCTGTA CTTTTTTTTG AGTTAACGTC TTATGGGAAG   1200
GGCCTGCACT TAGTGAAGAA GTGGTCTCAG AGTTGAGTTA CCTTGGCTTC TGGGAGGTGA   1260
AACTGTATCC CTATACCCTG AAGCTTTAAG GGGGTGCAAT GTAGATGAGA CCCCAACATA   1320
GATCCTCTTC ACAG                                                     1334
```

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 512 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 7th MN intron

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

```
GTGGGCCTGG GGTGTGTGTG GACACAGTGG GTGCGGGGGA AAGAGGATGT AAGATGAGAT       60
GAGAAACAGG AGAAGAAAGA AATCAAGGCT GGGCTCTGTG GCTTACGCCT ATAATCCCAC      120
CACGTTGGGA GGCTGAGGTG GGAGAATGGT TTGAGCCCAG GAGTTCAAGA CAAGGCGGGG      180
CAACATAGTG TGACCCCATC TCTACCAAAA AAACCCCAAC AAAACCAAAA ATAGCCGGGC      240
ATGGTGGTAT GCGGCCTAGT CCCAGCTACT CAAGGAGGCT GAGGTGGGAA GATCGCTTGA      300
TTCCAGGAGT TTGAGACTGC AGTGAGCTAT GATCCCACCA CTGCCTACCA TCTTTAGGAT      360
ACATTTATTT ATTTATAAAA GAAATCAAGA GGCTGGATGG GGAATACAGG AGCTGGAGGG      420
TGGAGCCCTG AGGTGCTGGT TGTGAGCTGG CCTGGGACCC TTGTTTCCTG TCATGCCATG      480
AACCCACCCA CACTGTCCAC TGACCTCCCT AG                                    512
```

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 114 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 8th MN intron

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

```
GTACAGCTTT GTCTGGTTTC CCCCCAGCCA GTAGTCCCTT ATCCTCCCAT GTGTGTGCCA       60
GTGTCTGTCA TTGGTGGTCA CAGCCCGCCT CTCACATCTC CTTTTTCTCT CCAG            114
```

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 617 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 9th MN intron

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

```
GTGAGTCTGC CCCTCCTCTT GGTCCTGATG CCAGGAGACT CCTCAGCACC ATTCAGCCCC    60
AGGGCTGCTC AGGACCGCCT CTGCTCCCTC TCCTTTTCTG CAGAACAGAC CCCAACCCCA   120
ATATTAGAGA GGCAGATCAT GGTGGGGATT CCCCCATTGT CCCCAGAGGC TAATTGATTA   180
GAATGAAGCT TGAGAAATCT CCCAGCATCC CTCTCGCAAA AGAATCCCCC CCCCTTTTTT   240
TAAAGATAGG GTCTCACTCT GTTTGCCCCA GGCTGGGGTG TTGTGGCACG ATCATAGCTC   300
ACTGCAGCCT CGAACTCCTA GGCTCAGGCA ATCCTTTCAC CTTAGCTTCT CAAAGCACTG   360
GGACTGTAGG CATGAGCCAC TGTGCCTGGC CCCAAACGGC CCTTTTACTT GGCTTTTAGG   420
AAGCAAAAAC GGTGCTTATC TTACCCCTTC TCGTGTATCC ACCCTCATCC CTTGGCTGGC   480
CTCTTCTGGA GACTGAGGCA CTATGGGGCT GCCTGAGAAC TCGGGGCAGG GGTGGTGGAG   540
TGCACTGAGG CAGGTGTTGA GGAACTCTGC AGACCCCTCT TCCTTCCCAA AGCAGCCCTC   600
TCTGCTCTCC ATCGCAG                                                  617
```

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 130 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 10th MN intron

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

```
GTATTACACT GACCCTTTCT TCAGGCACAA GCTTCCCCCA CCCTTGTGGA GTCACTTCAT    60
GCAAAGCGCA TGCAAATGAG CTGCTCCTGG GCCAGTTTTC TGATTAGCCT TTCCTGTTGT   120
GTACACACAG                                                          130
```

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1401 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: Spans 3' part of 1st intron to beyond end of 5th exon

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

```
CAAACTTTCA CTTTTGTTGC CCAGGCTGGA GTGCAATGGC GCGATCTCGG CTCACTGCAA    60
CCTCCACCTC CCGGGTTCAA GTGATTCTCC TGCCTCAGCC TCTAGCCAAG TAGCTGCGAT   120
TACAGGCATG CGCCACCACG CCCGGCTAAT TTTTGTATTT TTAGTAGAGA CGGGGTTTCG   180
CCATGTTGGT CAGGCTGGTC TCGAACTCCT GATCTCAGGT GATCCAACCA CCCTGGCCTC   240
CCAAAGTGCT GGGATTATAG GCGTGAGCCA CAGCGCCTGG CCTGAAGCAG CCACTCACTT   300
TTACAGACCC TAAGACAATG ATTGCAAGCT GGTAGGATTG CTGTTTGGCC CACCCAGCTG   360
CGGTGTTGAG TTTGGGTGCG GTCTCCTGTG CTTTGCACCT GGCCCGCTTA AGGCATTTGT   420
TACCCGTAAT GCTCCTGTAA GGCATCTGCG TTTGTGACAT CGTTTTGGTC GCCAGGAAGG   480
GATTGGGGCT CTAAGCTTGA GCGGTTCATC CTTTTCATTT ATACAGGGGA TGACCAGAGT   540
CATTGGCGCT ATGGAGGTGA GACACCCACC CGCTGCACAG ACCCAATCTG GGAACCCAGC   600
TCTGTGGATC TCCCCTACAG CCGTCCCTGA ACACTGGTCC CGGGCGTCCC ACCCGCCGCC   660
CACCGTCCCA CCCCCTCACC TTTTCTACCC GGGTTCCCTA AGTTCCTGAC CTAGGCGTCA   720
GACTTCCTCA CTATACTCTC CCACCCCAGG CGACCCGCCC TGGCCCCGGG TGTCCCCAGC   780
CTGCGCGGGC CGCTTCCAGT CCCCGGTGGA TATCCGCCCC CAGCTCGCCG CCTTCTGCCC   840
GGCCCTGCGC CCCCTGGAAC TCCTGGGCTT CCAGCTCCCG CCGCTCCCAG AACTGCGCCT   900
GCGCAACAAT GGCCACAGTG GTGAGGGGGT CTCCCCGCCG AGACTTGGGG ATGGGGCGGG   960
GCGCAGGGAA GGGAACCGTC GCGCAGTGCC TGCCCGGGGG TTGGGCTGGC CCTACCGGGC  1020
GGGGCCGGCT CACTTGCCTC TCCCTACGCA GTGCAACTGA CCCTGCCTCC TGGGCTAGAG  1080
ATGGCTCTGG GTCCCGGGCG GGAGTACCGG GCTCTGCAGC TGCATCTGCA CTGGGGGGCT  1140
GCAGGTCGTC CGGGCTCGGA GCACACTGTG GAAGGCCACC GTTTCCCTGC CGAGGTGAGC  1200
GCGGACTGGC CGAGAAGGGG CAAAGGAGCG GGGCGGACGG GGGCCAGAGA CGTGGCCCTC  1260
TCCTACCCTC GTGTCCTTTT CAGATCCACG TGGTTCACCT CAGCACCGCC TTTGCCAGAG  1320
TTGACGAGGC CTTGGGGCGC CCGGGAGGCC TGGCCGTGTT GGCCGCCTTT CTGGAGGTAC  1380
CAGATCCTGG ACACCCCCTA C                                            1401
```

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 98 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: Region of homology to collagen alpha 1 chain

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

```
Gln Arg Leu Pro Arg Met Gln Glu Asp Ser Pro Leu Gly Gly Gly Ser
1               5                   10                  15

Ser Gly Glu Asp Asp Pro Leu Gly Glu Glu Asp Leu Pro Ser Glu Glu
            20                  25                  30

Asp Ser Pro Arg Glu Glu Asp Pro Pro Gly Glu Glu Asp Leu Pro Gly
        35                  40                  45

Glu Glu Asp Leu Pro Gly Glu Glu Asp Leu Pro Glu Val Lys Pro Lys
    50                  55                  60

Ser Glu Glu Glu Gly Ser Leu Lys Leu Glu Asp Leu Pro Thr Val Glu
65                  70                  75                  80

Ala Pro Gly Asp Pro Gln Glu Pro Gln Asn Asn Ala His Arg Asp Lys
                85                  90                  95

Glu Gly
```

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 256 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: carbonic anhydrase domain

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

```
Asp Asp Gln Ser His Trp Arg Tyr Gly Gly Asp Pro Pro Trp Pro Arg
1               5                   10                  15

Val Ser Pro Ala Cys Ala Gly Arg Phe Gln Ser Pro Val Asp Ile Arg
            20                  25                  30

Pro Gln Leu Ala Ala Phe Cys Pro Ala Leu Arg Pro Leu Glu Leu Leu
        35                  40                  45

Gly Phe Gln Leu Pro Pro Leu Pro Glu Leu Arg Leu Arg Asn Asn Gly
    50                  55                  60

His Ser Val Gln Leu Thr Leu Pro Pro Gly Leu Glu Met Ala Leu Gly
65                  70                  75                  80

Pro Gly Arg Glu Tyr Arg Ala Leu Gln Leu His Leu His Trp Gly Ala
                85                  90                  95

Ala Gly Arg Pro Gly Ser Glu His Thr Val Glu Gly His Arg Phe Pro
            100                 105                 110

Ala Glu Ile His Val Val His Leu Ser Thr Ala Phe Ala Arg Val Asp
        115                 120                 125

Glu Ala Leu Gly Arg Pro Gly Gly Leu Ala Val Leu Ala Ala Phe Leu
    130                 135                 140

Glu Glu Gly Pro Glu Glu Asn Ser Ala Tyr Glu Gln Leu Leu Ser Arg
145                 150                 155                 160

Leu Glu Glu Ile Ala Glu Glu Gly Ser Glu Thr Gln Val Pro Gly Leu
                165                 170                 175

Asp Ile Ser Ala Leu Leu Pro Ser Asp Phe Ser Arg Tyr Phe Gln Tyr
            180                 185                 190


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

Val Phe Asn Gln Thr Val Met Leu Ser Ala Lys Gln Leu His Thr Leu
    210                 215                 220

Ser Asp Thr Leu Trp Gly Pro Gly Asp Ser Arg Leu Gln Leu Asn Phe
225                 230                 235                 240

Arg Ala Thr Gln Pro Leu Asn Gly Arg Val Ile Glu Ala Ser Phe Pro
                245                 250                 255
```

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(A) DESCRIPTION: transmembrane region

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

```
Ile Leu Ala Leu Val Phe Gly Leu Leu Phe Ala Val Thr Ser Val Ala
1               5                   10                  15

Phe Leu Val Gln
            20
```

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(A) DESCRIPTION: intracellular C-terminus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

```
Met Arg Arg Gln His Arg Arg Gly Thr Lys Gly Gly Val Ser Tyr Arg
1               5                   10                  15

Pro Ala Glu Val Ala Glu Thr Gly Ala
            20                  25
```

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 170 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

```
Arg Ala Leu Gln Leu His Leu His Trp Gly Ala Ala Gly Arg Pro Gly
1               5                   10                  15

Ser Glu His Thr Val Glu Gly His Arg Phe Pro Ala Glu Ile His Val
```

```
            20                  25                  30

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

Pro Gly Gly Leu Ala Val Leu Ala Ala Phe Leu Glu Glu Gly Pro Glu
    50                  55                  60

Glu Asn Ser Ala Tyr Glu Gln Leu Leu Ser Arg Leu Glu Glu Ile Ala
65                  70                  75                  80

Glu Glu Gly Ser Glu Thr Gln Val Pro Gly Leu Asp Ile Ser Ala Leu
                85                  90                  95

Leu Pro Ser Asp Phe Ser Arg Tyr Phe Gln Tyr Glu Gly Ser Leu Thr
            100                 105                 110

Thr Pro Pro Cys Ala Gln Gly Val Ile Trp Thr Val Phe Asn Gln Thr
        115                 120                 125

Val Met Leu Ser Ala Lys Gln Leu His Thr Leu Ser Asp Thr Leu Trp
    130                 135                 140

Gly Pro Gly Asp Ser Arg Leu Gln Leu Asn Phe Arg Ala Thr Gln Pro
145                 150                 155                 160

Leu Asn Gly Arg Val Ile Glu Ala Ser Phe
                165                 170
```

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 470 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

```
CAUGGCCCCG AUAACCUUCU GCCUGUGCAC ACACCUGCCC CUCACUCCAC CCCCAUCCUA       60
GCUUUGGUAU GGGGGAGAGG GCACAGGGCC AGACAAACCU GUGAGACUUU GGCUCCAUCU      120
CUGCAAAAGG GCGCUCUGUG AGUCAGCCUG CUCCCCUCCA GGCUUGCUCC UCCCCCACCC      180
AGCUCUCGUU UCCAAUGCAC GUACAGCCCG UACACACCGU GUGCUGGGAC ACCCCACAGU      240
CAGCCGCAUG GCUCCCCUGU GCCCCAGCCC CUGGCUCCCU CUGUUGAUCC CGGCCCCUGC      300
UCCAGGCCUC ACUGUGCAAC UGCUGCUGUC ACUGCUGCUU CUGGUGCCUG UCCAUCCCCA      360
GAGGUUGCCC CGGAUGCAGG AGGAUUCCCC CUUGGGAGGA GGCUCUUCUG GGGAAGAUGA      420
CCCACUGGGC GAGGAGGAUC UGCCCAGUGA AGAGGAUUCA CCCAGAGAGG                 470
```

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 292 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: Alu repeat within MN genomic region

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

```
GTTTTTTTGA GACGGAGTCT TGCATCTGTC ATGCCCAGGC TGGAGTAGCA GTGGTGCCAT       60

CTCGGCTCAC TGCAAGCTCC ACCTCCCGAG TTCACGCCAT TTTCCTGCCT CAGCCTCCCG      120

AGTAGCTGGG ACTACAGGCG CCCGCCACCA TGCCCGGCTA ATTTTTTGTA TTTTTGGTAG      180

AGACGGGGTT TCACCGTGTT AGCCAGAATG GTCTCGATCT CCTGACTTCG TGATCCACCC      240

GCCTCGGCCT CCCAAAGTTC TGGGATTACA GGTGTGAGCC ACCGCACCTG GC              292
```

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 262 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: Alu repeat within MN genomic region

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

```
TTTCTTTTTT GAGACAGGGT CTTGCTCTGT CACCCAGGCC AGAGTGCAAT GGTACAGTCT       60

CAGCTCACTG CAGCCTCAAC CGCCTCGGCT CAAACCATCA TCCCATTTCA GCCTCCTGAG      120

TAGCTGGGAC TACAGGCACA TGCCATTACA CCTGGCTAAT TTTTTTGTAT TTCTAGTAGA      180

GACAGGGTTT GGCCATGTTG CCCGGGCTGG TCTCGAACTC CTGGACTCAA GCAATCCACC      240

CACCTCAGCC TCCCAAAATG AG                                               262
```

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 904 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

```
GCTGGTCTCG AACTCCTGGA CTCAAGCAAT CCACCCACCT CAGCCTCCCA AAATGAGGGA      60

CCGTGTCTTA TTCATTTCCA TGTCCCTAGT CCATAGCCCA GTGCTGGACC TATGGTAGTA     120
CTAAATAAAT ATTTGTTGAA TGCAATAGTA AATAGCATTT CAGGGAGCAA GAACTAGATT     180
AACAAAGGTG GTAAAAGGTT TGGAGAAAAA AATAATAGTT TAATTTGGCT AGAGTATGAG     240
GGAGAGTAGT AGGAGACAAG ATGGAAAGGT CTCTTGGGCA AGGTTTTGAA GGAAGTTGGA     300
AGTCAGAAGT ACACAATGTG CATATCGTGG CAGGCAGTGG GGAGCCAATG AAGGCTTTTG     360
AGCAGGAGAG TAATGTGTTG AAAAATAAAT ATAGGTTAAA CCTATCAGAG CCCCTCTGAC     420
ACATACACTT GCTTTTCATT CAAGCTCAAG TTTGTCTCCC ACATACCCAT TACTTAACTC     480
ACCCTCGGGC TCCCCTAGCA GCCTGCCCTA CCTCTTTACC TGCTTCCTGG TGGAGTCAGG     540
GATGTATACA TGAGCTGCTT TCCCTCTCAG CCAGAGGACA TGGGGGGCCC CAGCTCCCCT     600
GCCTTTCCCC TTCTGTGCCT GGAGCTGGGA AGCAGGCCAG GGTTAGCTGA GGCTGGCTGG     660
CAAGCAGCTG GGTGGTGCCA GGGAGAGCCT GCATAGTGCC AGGTGGTGCC TTGGGTTCCA     720
AGCTAGTCCA TGGCCCCGAT AACCTTCTGC CTGTGCACAC ACCTGCCCCT CACTCCACCC     780
CCATCCTAGC TTTGGTATGG GGGAGAGGGC ACAGGGCCAG ACAAACCTGT GAGACTTTGG     840
CTCCATCTCT GCAAAAGGGC GCTCTGTGAG TCAGCCTGCT CCCCTCCAGG CTTGCTCCTC     900
CCCC                                                                  904
```

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 292 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

```
TTTTTTTGAG ACGGAGTCTT GCATCTGTCA TGCCCAGGCT GGAGTAGCAG TGGTGCCATC       60
TCGGCTCACT GCAAGCTCCA CCTCCCGAGT TCACGCCATT TTCCTGCCTC AGCCTCCCGA      120
GTAGCTGGGA CTACAGGCGC CCGCCACCAT GCCCGGCTAA TTTTTTGTAT TTTTGGTAGA      180
GACGGGGTTT CACCGTGTTA GCCAGAATGG TCTCGATCTC CTGACTTCGT GATCCACCCG      240
CCTCGGCCTC CCAAAGTTCT GGGATTACAG GTGTGAGCCA CCGCACCTGG CC              292
```

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 262 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

```
TTCTTTTTTG AGACAGGGTC TTGCTCTGTC ACCCAGGCCA GAGTGCAATG GTACAGTCTC       60
AGCTCACTGC AGCCTCAACC GCCTCGGCTC AAACCATCAT CCCATTTCAG CCTCCTGAGT      120
AGCTGGGACT ACAGGCACAT GCCATTACAC CTGGCTAATT TTTTTGTATT TCTAGTAGAG      180
ACAGGGTTTG GCCATGTTGC CCGGGCTGGT CTCGAACTCC TGGACTCAAG CAATCCACCC      240
ACCTCAGCCT CCCAAAATGA GG                                               262
```

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 294 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

```
TTTTTTTTTG AGACAAACTT TCACTTTTGT TGCCCAGGCT GGAGTGCAAT GGCGCGATCT      60
CGGCTCACTG CAACCTCCAC CTCCCGGGTT CAAGTGATTC TCCTGCCTCA GCCTCTAGCC     120
AAGTAGCTGC GATTACAGGC ATGCGCCACC ACGCCCGGCT AATTTTTGTA TTTTTAGTAG     180
AGACGGGGTT TCGCCATGTT GGTCAGGCTG GTCTCGAACT CCTGATCTCA GGTGATCCAA     240
CCACCCTGGC CTCCCAAAGT GCTGGGATTA TAGGCGTGAG CCACAGCGCC TGGC           294
```

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 276 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

```
TGACAGTCTC TCTGTCGCCC AGGCTGGAGT GCAGTGGTGT GATCTTGGGT CACTGCAACT      60
TCCGCCTCCC GGGTTCAAGG GATTCTCCTG CCTCAGCTTC CTGAGTAGCT GGGGTTACAG     120
GTGTGTGCCA CCATGCCCAG CTAATTTTTT TTTGTATTTT TAGTAGACAG GGTTTCACCA     180
TGTTGGTCAG GCTGGTCTCA AACTCCTGGC CTCAAGTGAT CCGCCTGACT CAGCCTACCA     240
AAGTGCTGAT TACAAGTGTG AGCCACCGTG CCCAGC                               276
```

(2) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 289 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

CGCCGGGCAC GGTGGCTCAC GCCTGTAATC CCAGCACTTT GGGAGGCCAA GGCAGGTGGA 60

TCACGAGGTC AAGAGATCAA GACCATCCTG GCCAACATGG TGAAACCCCA TCTCTACTAA 120

AAATACGAAA AAATAGCCAG GCGTGGTGGC GGGTGCCTGT AATCCCAGCT ACTCGGGAGG 180

CTGAGGCAGG AGAATGGCAT GAACCCGGGA GGCAGAAGTT GCAGTGAGCC GAGATCGTGC 240

CACTGCACTC CAGCCTGGGC AACAGAGCGA GACTCTTGTC TCAAAAAAA 289

(2) INFORMATION FOR SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 298 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

AGGCTGGGCT CTGTGGCTTA CGCCTATAAT CCCACCACGT TGGGAGGCTG AGGTGGGAGA 60

ATGGTTTGAG CCCAGGAGTT CAAGACAAGG CGGGGCAACA TAGTGTGACC CCATCTCTAC 120

CAAAAAAACC CCAACAAAAC CAAAAATAGC CGGGCATGGT GGTATGCGGC CTAGTCCCAG 180

CTACTCAAGG AGGCTGAGGT GGGAAGATCG CTTGATTCCA GGAGTTTGAG ACTGCAGTGA 240

GCTATGATCC CACCACTGCC TACCATCTTT AGGATACATT TATTTATTTA TAAAAGAA 298

(2) INFORMATION FOR SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 105 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

TTTTTTACAT CTTTAGTAGA GACAGGGTTT CACCATATTG GCCAGGCTGC TCTCAAACTC 60

CTGACCTTGT GATCCACCAG CCTCGGCCTC CCAAAGTGCT GGGAT 105

(2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 83 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

```
CCTCGAACTC CTAGGCTCAG GCAATCCTTT CACCTTAGCT TCTCAAAGCA CTGGGACTGT       60

AGGCATGAGC CACTGTGCCT GGC                                               83
```

(2) INFORMATION FOR SEQ ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 5' donor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

```
AGAAGGTAAG T                                                            11
```

(2) INFORMATION FOR SEQ ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 5' donor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

```
TGGAGGTGAG A                                                            11
```

(2) INFORMATION FOR SEQ ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 5' donor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

```
CAGTCGTGAG G                                                      11
```

(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 5' donor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

```
CCGAGGTGAG C                                                      11
```

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 5' donor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

```
TGGAGGTACC A                                                      11
```

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 5' donor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

```
GGAAGGTCAG T                                                          11
```

(2) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 5' donor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

```
AGCAGGTGGG C                                                          11
```

(2) INFORMATION FOR SEQ ID NO: 74:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 5' donor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

```
GCCAGGTACA G                                                          11
```

(2) INFORMATION FOR SEQ ID NO: 75:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 5' donor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

```
TGCTGGTGAG T                                                              11
```

(2) INFORMATION FOR SEQ ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 5' donor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

```
CACAG GTATTA                                                              11
```

(2) INFORMATION FOR SEQ ID NO: 77:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 3' acceptor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

```
ATACAGGGGA T                                                              11
```

(2) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 3' acceptor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

```
CCCCAGGCGA C                                                        11
```

(2) INFORMATION FOR SEQ ID NO: 79:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 3' acceptor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:

```
ACGCAGTGCA A                                                        11
```

(2) INFORMATION FOR SEQ ID NO: 80:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 3' acceptor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:

```
TTTCAGATCC A                                                        11
```

(2) INFORMATION FOR SEQ ID NO: 81:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 3' acceptor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:

```
CCCCAGGAGG G                                                        11
```

(2) INFORMATION FOR SEQ ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 3' acceptor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:

```
TCACAGGCTC A                                                      11
```

(2) INFORMATION FOR SEQ ID NO: 83:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 3' acceptor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:

```
CCCTAGCTCC A                                                      11
```

(2) INFORMATION FOR SEQ ID NO: 84:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 3' acceptor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:

```
CTCCAGTCCA G                                                      11
```

(2) INFORMATION FOR SEQ ID NO: 85:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 3' acceptor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 85:

```
TCGCAGGTGA CA                                                          12
```

(2) INFORMATION FOR SEQ ID NO: 86:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(A) DESCRIPTION: 3' acceptor consensus splice sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 86:

```
ACACAGAAGG G                                                           11
```

Claims

1. An isolated nucleic acid **characterised in that** it contains at least 16 nucleotides and has a nucleotide sequence selected from:

   (a) the MN promoter nucleotide sequence as shown in accompanying Figure 6, and nucleotide sequences complementary to the said MN promoter nucleotide sequence;
   and
   (b) nucleotide sequences that are at least 80% homologous to the nucleotide sequences of (a) and to the complements of the said nucleotide sequences;

   the said isolated nucleic acid having promoter activity and/or acting as a polymerase chain reaction primer for identifying the MN promoter.

2. An isolated nucleic acid as claimed in claim 1 wherein the nucleotide sequences of (b) are at least 90% homologous to the nucleotide sequences of (a).

3. An isolated nucleic acid as claimed in claim 1 wherein the said nucleotide sequence is selected from:

   (a) the MN promoter sequence as shown in accompanying Figure 6 and the nucleotide sequence complementary thereto;
   and
   (b) nucleotide sequences that are at least 80% homologous to the nucleotide sequences of (a).

4. An isolated nucleic acid as claimed in any of claims 1 to 3 wherein it is from 16 to 50 nucleotides in length, preferably from 19 to 45 nucleotides in length, and functions as a polymerase chain reaction primer for MN nucleic acid sequences.

5. An isolated nucleic acid as claimed in any of claims 1 to 3 wherein it is at least 27 nucleotides in length, or is at least 29 nucleotides in length, or is at least 50 nucleotides in length, or is at least 100 nucleotides in length, or is at least 150 nucleotides in length.

6. A method of detecting mutations in an MN promoter **characterised in that** it comprises:

amplifying a fragment of the said MN promoter by the polymerase chain reaction using as a primer an isolated nucleic acid as claimed in claim 4;
and
determining whether the said fragment contains one or more mutations.

7. A vector **characterised in that** it contains an isolated nucleic acid as claimed in any of claims 1 to 5.

Patentansprüche

1. Isolierte Nukleinsäure, **dadurch gekennzeichnet, dass** sie wenigstens 16 Nukleotide enthält und eine Nukleotisequenz aufweist, ausgewählt aus:

a) der MN Promotornukleotidsequenz gemäß der beiliegenden Figur 6, und Nukleotidsequenzen, die zur MN Promotornukleotidsequenz komplementär sind; und
b) Nukleotidsequenzen, die zu wenigstens 80% mit den Nukleotidsequenzen von a) und den Komplementären dieser Nukleotidsequenzen homolog sind;

wobei die isolierte Nukleotinsäure eine Promotoraktivität besitzt und/oder als Primer für eine Polymerasekettenreaktion zur Identifizierung des MN Promotors dient.

2. Isolierte Nukleinsäure nach Anspruch 1, wobei die Nukleotidsequenzen von b) zu mindestens 90% homolog sind zu den Nukleotidsequenzen von a).

3. Isolierte Nukleinsäure nach Anspruch 1, wobei die Nukleotidsequenz ausgewählt wird aus:

a) der MN Promotorsequenz gemäß der beiliegenden Sequenz 6 und der hierzu komplementären Nukleotidsequenz; und
b) Nukleotidsequenzen, die zu mindestens 80% zu den Nukleotidsequenzen von a) homolog sind.

4. Isolierte Nukleinsäure nach einem der Ansprüche 1 bis 3, wobei sie eine Länge von 16 bis 50 Nukleotiden, bevorzugt eine Länge von 19 bis 45 Nukleotiden, besitzt, und als Primer für eine Polymerasekettenreaktion für MN Nukleinsäuresequenzen dient.

5. Isolierte Nukleinsäure nach einem der Ansprüche 1 bis 3, wobei sie eine Länge von mindestens 27 Nukleotiden besitzt oder eine Länge von mindestens 29 Nukleotiden, oder eine Länge von 50 Nukleotiden, oder eine Länge von mindestens 100 Nukleotiden, oder eine Länge von mindestens 150 Nukleotiden.

6. Verfahren zum Nachweis von Mutationen in einem MN Promotor, **dadurch gekennzeichnet, dass** es umfasst:

Amplifizieren eines Fragments des MN-Promotors durch die Polymerasekettenreaktion unter Verwendung eines Primers einer isolierten Nukleinsäure nach Anspruch 4; und
Bestimmen, ob das Fragment ein oder mehrere Mutationen enthält.

7. Vektor, **dadurch gekennzeichnet, dass** er eine isolierte Nukleinsäure nach einem der Ansprüche 1 bis 5 enthält.

Revendications

1. Un acide nucléique isolé **caractérisé en ce qu'**il contient au moins 16 nucléotides et a une séquence nucléotidique choisie parmi :

   (a) la séquence nucléotidique du promoteur MN telle que représentée sur la Figure 6 annexée, et les séquences nucléotidiques complémentaires de ladite séquence nucléotidique du promoteur MN ;

   et

   (b) des séquences nucléotidiques qui sont homologues au moins à 80 % aux séquences nucléotidiques de (a) et aux compléments desdites séquences nucléotidiques ;

   ledit acide nucléique isolé ayant une activité de promoteur et/ou agissant comme amorce d'amplification en chaîne par polymérase pour identifier le promoteur MN.

2. Un acide nucléique isolé tel que revendiqué dans la revendication 1, dans lequel les séquences nucléotidiques de (b) sont homologues au moins à 90 % aux séquences nucléotidiques de (a).

3. Un acide nucléique isolé tel que revendiqué dans la revendication 1, dans lequel ladite séquence nucléotidique est choisie parmi :

   (a) la séquence du promoteur MN telle que représentée sur la Figure 6 annexée et la séquence nucléotidique qui en est complémentaire ;
   et
   (b) des séquences nucléotidiques qui sont homologues au moins à 80 % aux séquences nucléotidiques de (a).

4. Un acide nucléique isolé tel que revendiqué dans l'une quelconque des revendications 1 à 3, qui est d'une longueur de 16 à 50 nucléotides, de préférence d'une longueur de 19 à 45 nucléotides, et agit comme amorce d'amplification en chaîne par polymérase pour des séquences d'acide nucléique MN.

5. Un acide nucléique isolé tel que revendiqué dans l'une quelconque des revendications 1 à 3, qui est d'une longueur d'au moins 27 nucléotides, ou est d'une longueur d'au moins 29 nucléotides, ou est d'une longueur d'au moins 50 nucléotides, ou est d'une longueur d'au moins 100 nucléotides ou est d'une longueur d'au moins 150 nucléotides.

6. Un procédé pour détecter des mutations dans un promoteur MN, **caractérisé en ce qu'**il comprend les étapes consistant à :

   amplifier un fragment dudit promoteur MN par l'amplification en chaîne par polymérase en utilisant comme amorce un acide nucléique isolé tel que revendiqué dans la revendication 4 ;
   et
   déterminer si ledit fragment contient une ou plusieurs mutations.

7. Un vecteur **caractérisé en ce qu'**il contient un acide nucléique isolé tel que revendiqué dans l'une quelconque des revendications 1 à 5.

```
  1                      M   A   P   L   C   P   S   P   W   L   P   L    12
  1  ACA GTC AGC CGC ATG GCT CCC CTG TGC CCC AGC CCC TGG CTC CCT CTG    48

 13   L   I   P   A   P   A   P   G   L   T   V   Q   L   L   L   S    28
 49  TTG ATC CCG GCC CCT GCT CCA GGC CTC ACT GTG CAA CTG CTG CTG TCA    96

 29   L   L   L   L   M   P   V   H   P   Q   R   L   P   R   M   Q    44
 97  CTG CTG CTT CTG ATG CCT GTC CAT CCC CAG AGG TTG CCC CGG ATG CAG   144

 45   E   D   S   P   L   G   G   G   S   S   G   E   D   D   P   L    60
145  GAG GAT TCC CCC TTG GGA GGA GGC TCT TCT GGG GAA GAT GAC CCA CTG   192

 61   G   E   E   D   L   P   S   E   E   D   S   P   R   E   E   D    76
193  GGC GAG GAG GAT CTG CCC AGT GAA GAG GAT TCA CCC AGA GAG GAG GAT   240

 77   P   P   G   E   E   D   L   P   G   E   E   D   L   P   G   E    92
241  CCA CCC GGA GAG GAG GAT CTA CCT GGA GAG GAG GAT CTA CCT GGA GAG   288

 93   E   D   L   P   E   V   K   P   K   S   E   E   E   G   S   L   108
289  GAG GAT CTA CCT GAA GTT AAG CCT AAA TCA GAA GAA GAG GGC TCC CTG   336

109   K   L   E   D   L   P   T   V   E   A   P   G   D   P   Q   E   124
337  AAG TTA GAG GAT CTA CCT ACT GTT GAG GCT CCT GGA GAT CCT CAA GAA   384

125   P   Q   N   N   A   H   R   D   K   E   G   D   D   Q   S   H   140
385  CCC CAG AAT AAT GCC CAC AGG GAC AAA GAA GGG GAT GAC CAG AGT CAT   432

141   W   R   Y   G   G   D   P   P   W   P   R   V   S   P   A   C   156
433  TGG CGC TAT GGA GGC GAC CCG CCC TGG CCC CGG GTG TCC CCA GCC TGC   480

157   A   G   R   F   Q   S   P   V   D   I   R   P   Q   L   A   A   172
481  GCG GGC CGC TTC CAG TCC CCG GTG GAT ATC CGC CCC CAG CTC GCC GCC   528
```

FIG._1A

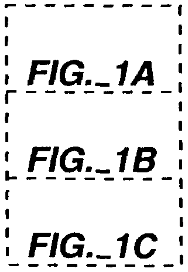
```
 173  F   C   P   A   L   R   P   L   E   L   L   G   F   Q   L   P    188
 529 TTC TGC CCG GCC CTG CGC CCC CTG GAA CTC CTG GGC TTC CAG CTC CCG    576

 189  P   L   P   E   L   R   L   R   N   N   G   H   S   V   Q   L    204
 577 CCG CTC CCA GAA CTG CGC CTG CGC AAC AAT GGC CAC AGT GTG CAA CTG    624

 205  T   L   P   P   G   L   E   M   A   L   G   P   G   R   E   Y    220
 625 ACC CTG CCT CCT GGG CTA GAG ATG GCT CTG GGT CCC GGG CGG GAG TAC    672

 221  R   A   L   Q   L   H   L   H   W   G   A   A   G   R   P   G    236
 673 CGG GCT CTG CAG CTG CAT CTG CAC TGG GGG GCT GCA GGT CGT CCG GGC    720

 237  S   E   H   T   V   E   G   H   R   F   P   A   E   I   H   V    252
 721 TCG GAG CAC ACT GTG GAA GGC CAC CGT TTC CCT GCC GAG ATC CAC GTG    768

 253  V   H   L   S   T   A   F   A   R   V   D   E   A   L   G   R    268
 769 GTT CAC CTC AGC ACC GCC TTT GCC AGA GTT GAC GAG GCC TTG GGG CGC    816

 269  P   G   G   L   A   V   L   A   A   F   L   E   E   G   P   E    284
 817 CCG GGA GGC CTG GCC GTG TTG GCC GCC TTT CTG GAG GAG GGC CCG GAA    864

 285  E   N   S   A   Y   E   Q   L   L   S   R   L   E   E   I   A    300
 865 GAA AAC AGT GCC TAT GAG CAG TTG CTG TCT CGC TTG GAA GAA ATC GCT    912

 301  E   E   G   S   E   T   Q   V   P   G   L   D   I   S   A   L    316
 913 GAG GAA GGC TCA GAG ACT CAG GTC CCA GGA CTG GAC ATA TCT GCA CTC    960

 317  L   P   S   D   F   S   R   Y   F   Q   Y   E   G   S   L   T    332
 961 CTG CCC TCT GAC TTC AGC CGC TAC TTC CAA TAT GAG GGG TCT CTG ACT   1008

 333  T   P   P   C   A   Q   G   V   I   W   T   V   F   N   Q   T    348
1009 ACA CCG CCC TGT GCC CAG GGT GTC ATC TGG ACT GTG TTT AAC CAG ACA   1056
```

FIG._1B

```
 349  V   M   L   S   A   K   Q   L   H   T   L   S   D   T   L   W    364
1057 GTG ATG CTG AGT GCT AAG CAG CTC CAC ACC CTC TCT GAC ACC CTG TGG  1104

 365  G   P   G   D   S   R   L   Q   L   N   F   R   A   T   Q   P    380
1105 GGA CCT GGT GAC TCT CGG CTA CAG CTG AAC TTC CGA GCG ACG CAG CCT  1152

 381  L   N   G   R   V   I   E   A   S   F   P   A   G   V   D   S    396
1153 TTG AAT GGG CGA GTG ATT GAG GCC TCC TTC CCT GCT GGA GTG GAC AGC  1200

 397  S   P   R   A   A   E   P   V   Q   L   N   S   C   L   A   A    412
1201 AGT CCT CGG GCT GCT GAG CCA GTC CAG CTG AAT TCC TGC CTG GCT GCT  1248

 413  G   D   I   L   A   L   V   F   G   L   L   F   A   V   T   S    428
1249 GGT GAC ATC CTA GCC CTG GTT TTT GGC CTC CTT TTT GCT GTC ACC AGC  1296

 429  V   A   F   L   V   Q   M   R   R   Q   H   R   R   G   T   K    444
1297 GTC GCG TTC CTT GTG CAG ATG AGA AGG CAG CAC AGA AGG GGA ACC AAA  1344

 445  G   G   V   S   Y   R   P   A   E   V   A   E   T   G   A   *    460
1345 GGG GGT GTG AGC TAC CGC CCA GCA GAG GTA GCC GAG ACT GGA GCC TAG  1392

1393 AGG CTG GAT CTT GGA GAA TGT GAG AAG CCA GCC AGA GGC ATC TGA GGG  1440

1441 GGA GCC GGT AAC TGT CCT GTC CTG CTC ATT ATG CCA CTT CCT TTT AAC  1488

1489 TGC CAA GAA ATT TTT TAA AAT AAA TAT TTA TAA T                    1522
```

FIG._1C

FIG._1



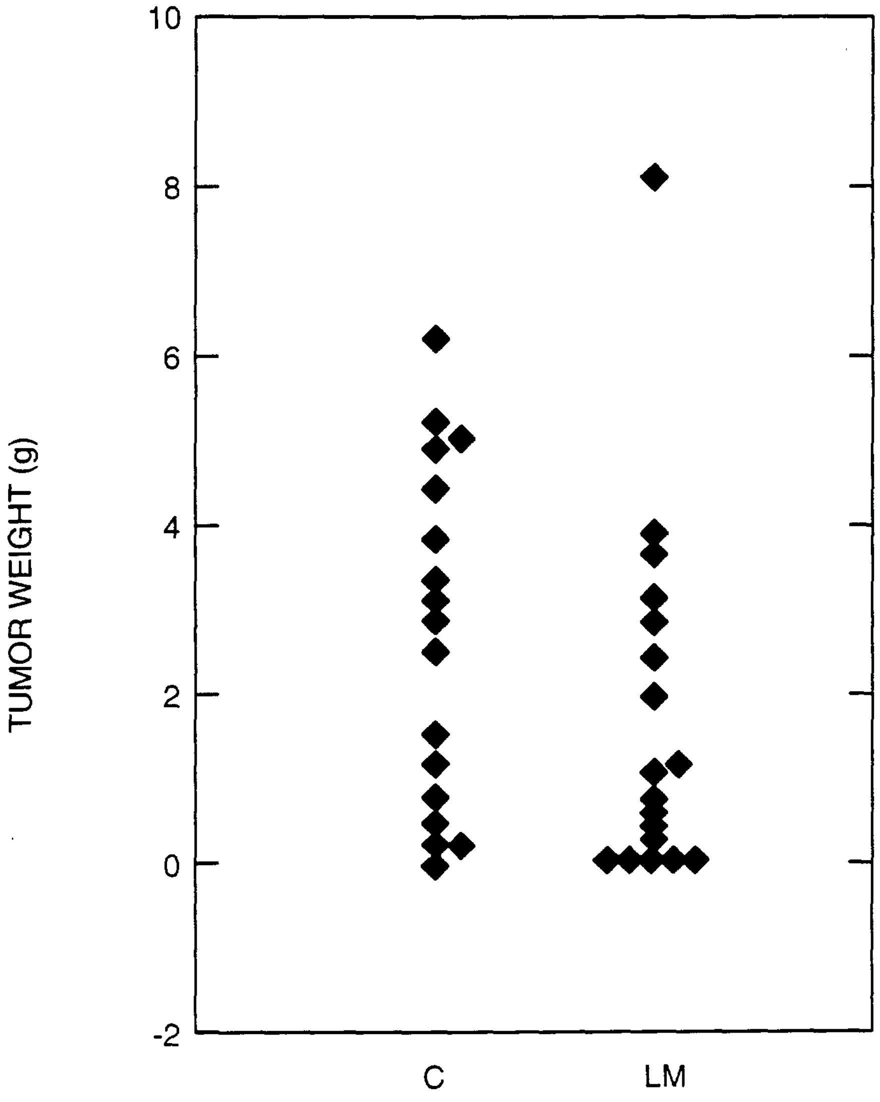


FIG._2

```
   1 ggatcctgtt gactcgtgac cttaccccca accctgtgct ctctgaaaca tgagctgtgt
  61 ccactcaggg ttaaatggat taagggcggt gcaagatgtg ctttgttaaa cagatgcttg
 121 aaggcagcat gctcgttaag agtcatcacc aatccctaat ctcaagtaat cagggacaca
 181 aacactgcgg aaggccgcag ggtcctctgc ctaggaaaac cagagacctt tgttcacttg
 241 tttatctgac cttccctcca ctattgtcca tgaccctgcc aaatccccct ctgtgagaaa
 301 cacccaagaa ttatcaataa aaaaataaat ttaaaaaaaa aatacaaaaa aaaaaaaaaa
 361 aaaaaaaaaa gacttacgaa tagttattga taaatgaata gctattggta aagccaagta
 421 aatgatcata ttcaaaacca gacggccatc atcacagctc aagtctacct gatttgatct
 481 ctttatcatt gtcattcttt ggattcacta gattagtcat catcctcaaa attctccccc
 541 aagttctaat tacgttccaa acatttaggg gttacatgaa gcttgaacct actaccttct
 601 ttgcttttga gccatgagtt gtaggaatga tgagtttaca ccttacatgc tggggattaa
 661 tttaaacttt acctctaagt cagttgggta gcctttggct tatttttgta gctaattttg
 721 tagttaatgg atgcactgtg aatcttgcta tgatagtttt cctccacact ttgccactag
 781 gggtaggtag gtactcagtt ttcagtaatt gcttacctaa gaccctaagc cctatttctc
 841 ttgtactggc ctttatctgt aatatgggca tatttaatac aatataattt ttggagtttt
 901 tttgtttgtt tgtttgtttg tttttttgag acggagtctt gcatctgtca tgcccaggct
 961 ggagtagcag tggtgccatc tcggctcact gcaagctcca cctcccgagt tcacgccatt
1021 ttcctgcctc agcctcccga gtagctggga ctacaggcgc ccgccaccat gcccggctaa
1081 tttttgtat ttttggtaga gacggggttt caccgtgtta gccagaatgg tctcgatctc
1141 ctgacttcgt gatccacccg cctcggcctc ccaaagttct gggattacag gtgtgagcca
1201 ccgcacctgg ccaatttttt gagtctttta aagtaaaaat atgtcttgta agctggtaac
1261 tatggtacat ttccttttat taatgtggtg ctgacggtca tataggttct tttgagtttg
1321 gcatgcatat gctacttttt gcagtccttt cattacattt ttctctcttc atttgaagag
1381 catgttatat cttttagctt cacttggctt aaaaggttct ctcattagcc taacacagtg
1441 tcattgttgg taccacttgg atcataagtg gaaaaacagt caagaaattg cacagtaata
1501 cttgtttgta agagggatga ttcaggtgaa tctgacacta agaaactccc ctacctgagg
1561 tctgagattc ctctgacatt gctgtatata ggcttttcct ttgacagcct gtgactgcgg
1621 actatttttc ttaagcaaga tatgctaaag ttttgtgagc ctttttccag agagaggtct
1681 catatctgca tcaagtgaga acatataatg tctgcatgtt tccatatttc aggaatgttt
1741 gcttgtgttt tatgctttta tatagacagg gaaacttgtt cctcagtgac ccaaaagagg
1801 tgggaattgt tattggatat catcattggc ccacgctttc tgaccttgga aacaattaag
1861 ggttcataat ctcaattctg tcagaattgg tacaagaaat agctgctatg tttcttgaca
1921 ttccacttgg taggaaataa gaatgtgaaa ctcttcagtt ggtgtgtgtc cct?gttttt
```

FIG._3A

```
1981 ttgcaatttc cttcttactg tgttaaaaaa aagtatgatc ttgctctgag aggtgaggca
2041 ttcttaatca tgatctttaa agatcaataa tataatcctt tcaaggatta tgtctttatt
2101 ataataaaga taatttgtct ttaacagaat caataatata atcccttaaa ggattatatc
2161 tttgctgggc gcagtggctc acacctgtaa tcccagcact ttgggtggcc aaggtggaag
2221 gatcaaattt gcctacttct atattatctt ctaaagcaga attcatctct cttccctcaa
2281 tatgatgata ttgacagggt ttgccctcac tcactagatt gtgagctcct gctcagggca
2341 ggtagcgttt tttgtttttg tttttgtttt tctttttga gacagggtct tgctctgtca
2401 cccaggccag agtgcaatgg tacagtctca gctcactgca gcctcaaccg cctcggctca
2461 aaccatcatc ccatttcagc ctcctgagta gctgggacta caggcacatg ccattacacc
2521 tggctaattt ttttgtattt ctagtagaga cagggtttgg ccatgttgcc cgggctggtc
2581 tcgaactcct ggactcaagc aatccaccca cctcagcctc ccaaaatgag ggaccgtgtc
2641 ttattcattt ccatgtccct agtccatagc ccagtgctgg acctatggta gtactaaata
2701 aatatttgtt gaatgcaata gtaaatagca tttcagggag caagaactag attaacaaag
2761 gtggtaaaag gtttggagaa aaaaataata gtttaatttg gctagagtat gagggagagt
2821 agtaggagac aagatggaaa ggtctcttgg gcaaggtttt gaaggaagtt ggaagtcaga
2881 agtacacaat gtgcatatcg tggcaggcag tggggagcca atgaaggctt ttgagcagga
2941 gagtaatgtg ttgaaaaata aatataggtt aaacctatca gagcccctct gacacataca
3001 cttgcttttc attcaagctc aagtttgtct cccacatacc cattacttaa ctcaccctcg
3061 ggctccccta gcagcctgcc ctacctcttt acctgcttcc tggtggagtc agggatgtat
3121 acatgagctg ctttccctct cagccagagg acatgggggg ccccagctcc cctgcctttc
3181 cccttctgtg cctggagctg ggaagcaggc cagggttagc tgaggctggc tggcaagcag
3241 ctgggtggtg ccagggagag cctgcatagt gccaggtggt gccttgggtt ccaagctagt
3301 ccatggcccc gataaccttc tgcctgtgca cacacctgcc cctcactcca cccccatcct
3361 agctttggta tgggggagag ggcacagggc cagacaaacc tgtgagactt tggctccatc
3421 tctgcaaaag ggcgctctgt gagtcagcct gctcccctcc aggcttgctc ctcccccacc
3481 cagctctcgt ttccaatgca cgtacagccc gtacacaccg tgtgctggga cacccccACAG
3541 TCAGCCGCAT GGCTCCCCTG TGCCCCAGCC CCTGGCTCCC TCTGTTGATC CCGGCCCCTG
3601 CTCCAGGCCT CACTGTGCAA CTGCTGCTGT CACTGCTGCT TCTGGTGCCT GTCCATCCCC
3661 AGAGGTTGCC CCGGATGCAG GAGGATTCCC CCTTGGGAGG AGGCTCTTCT GGGGAAGATG
3721 ACCCACTGGG CGAGGAGGAT CTGCCCAGTG AAGAGGATTC ACCCAGAGAG GAGGATCCAC
3781 CCGGAGAGGA GGATCTACCT GGAGAGGAGG ATCTACCTGG AGAGGAGGAT CTACCTGAAG
3841 TTAAGCCTAA ATCAGAAGAA GAGGGCTCCC TGAAGTTAGA GGATCTACCT ACTGTTGAGG
3901 CTCCTGGAGA TCCTCAAGAA CCCCAGAATA ATGCCCACAG GGACAAAGAA Ggtaagtggt
```

FIG._3B

```
3961 catcaatctc caaatccagg ttccaggagg ttcatgactc ccctcccata ccccagccta
4021 ggctctgttc actcagggaa ggaggggaga ctgtactccc cacagaagcc cttccagagg
4081 tcccatacca atatccccat ccccactctc ggaggtagaa agggacagat gtggagagaa
4141 aataaaaagg gtgcaaaagg agagaggtga gctggatgag atgggagaga aggggaggc
4201 tggagaagag aaagggatga gaactgcaga tgagagaaaa aatgtgcaga cagaggaaaa
4261 aaataggtgg agaaggagag tcagagagtt tgaggggaag agaaaaggaa agcttgggag
4321 gtgaagtggg taccagagac aagcaagaag agctggtaga agtcatctca tcttaggcta
4381 caatgaggaa ttgagaccta ggaagaaggg acacagcagg tagagaaacg tggcttcttg
4441 actcccaagc caggaatttg gggaaagggg ttggagacca tacaaggcag agggatgagt
4501 ggggagaaga aagaagggag aaaggaaaga tggtgtactc actcatttgg gactcaggac
4561 tgaagtgccc actcactttt tttttttttt tttttgagac aaactttcac ttttgttgcc
4621 caggctggag tgcaatggcg cgatctcggc tcactgcaac ctccacctcc cgggttcaag
4681 tgattctcct gcctcagcct ctagccaagt agctgcgatt acaggcatgc gccaccacgc
4741 ccggctaatt tttgtatttt tagtagagac ggggtttcgc catgttggtc aggctggtct
4801 cgaactcctg atctcaggtg atccaaccac cctggcctcc caaagtgctg ggattatagg
4861 cgtgagccac agcgcctggc ctgaagcagc cactcacttt tacagaccct aagacaatga
4921 ttgcaagctg gtaggattgc tgtttggccc acccagctgc ggtgttgagt ttgggtgcgg
4981 tctcctgtgc tttgcacctg gcccgcttaa ggcatttgtt acccgtaatg ctcctgtaag
5041 gcatctgcgt ttgtgacatc gttttggtcg ccaggaaggg attggggctc taagcttgag
5101 cggttcatcc ttttcattta tacagGGGAT GACCAGAGTC ATTGGCGCTA TGGAGgtgag
5161 acacccaccc gctgcacaga cccaatctgg gaacccagct ctgtggatct cccctacagc
5221 cgtccctgaa cactggtccc gggcgtccca cccgccgccc accgtcccac cccctcacct
5281 tttctacccg ggttccctaa gttcctgacc taggcgtcag acttcctcac tatactctcc
5341 caccccagGC GACCCGCCCT GGCCCCGGGT GTCCCCAGCC TGCGCGGGCC GCTTCCAGTC
5401 CCCGGTGGAT ATCCGCCCCC AGCTCGCCGC CTTCTGCCCG GCCCTGCGCC CCCTGGAACT
5461 CCTGGGCTTC CAGCTCCCGC CGCTCCCAGA ACTGCGCCTG CGCAACAATG GCCACAGTGg
5521 tgagggggtc tccccgccga gacttgggga tggggcgggg cgcagggaag ggaaccgtcg
5581 cgcagtgcct gcccgggggt tgggctggcc ctaccgggcg gggccggctc acttgcctct
5641 ccctacgcag TGCAACTGAC CCTGCCTCCT GGGCTAGAGA TGGCTCTGGG TCCCGGGCGG
5701 GAGTACCGGG CTCTGCAGCT GCATCTGCAC TGGGGGGCTG CAGGTCGTCC GGGCTCGGAG
5761 CACACTGTGG AAGGCCACCG TTTCCCTGCC GAGgtgagcg cggactggcc gagaaggggc
5821 aaaggagcgg ggcggacggg ggccagagac gtggccctct cctaccctcg tgtcctttc
5881 agATCCACGT GGTTCACCTC AGCACCGCCT TTGCCAGAGT TGACGAGGCC TTGGGCGCC
```

FIG._3C

```
5941 CGGGAGGCCT GGCCGTGTTG GCCGCCTTTC TGGAGgtacc agatcctgga caccccctac
6001 tccccgcttt cccatcccat gctcctcccg gactctatcg tggagccaga gaccccatcc
6061 cagcaagctc actcaggccc ctggctgaca aactcattca cgcactgttt gttcatttaa
6121 cacccactgt gaaccaggca ccagccccca acaaggattc tgaagctgta ggtccttgcc
6181 tctaaggagc ccacagccag tgggggaggc tgacatgaca gacacatagg aaggacatag
6241 taaagatggt ggtcacagag gaggtgacac ttaaagcctt cactggtaga aaagaaaagg
6301 aggtgttcat tgcagaggaa acagaatgtg caaagactca gaatatggcc tatttaggga
6361 atggctacat acaccatgat tagaggaggc ccagtaaagg gaagggatgg tgagatgcct
6421 gctaggttca ctcactcact tttatttatt tatttatttt tttgacagtc tctctgtcgc
6481 ccaggctgga gtgcagtggt gtgatcttgg gtcactgcaa cttccgcctc ccgggttcaa
6541 gggattctcc tgcctcagct tcctgagtag ctggggttac aggtgtgtgc caccatgccc
6601 agctaatttt tttttgtatt tttagtagac agggtttcac catgttggtc aggctggtct
6661 caaactcctg gcctcaagtg atccgcctga ctcagcctac caaagtgctg attacaagtg
6721 tgagccaccg tgcccagcca cactcactga ttctttaatg ccagccacac agcacaaagt
6781 tcagagaaat gcctccatca tagcatgtca atatgttcat actcttaggt tcatgatgtt
6841 cttaacatta ggttcataag caaaataaga aaaaagaata ataaataaaa gaagtggcat
6901 gtcaggacct cacctgaaaa gccaaacaca gaatcatgaa ggtgaatgca gaggtgacac
6961 caacacaaag gtgtatatat ggtttcctgt ggggagtatg tacggaggca gcagtgagtg
7021 agactgcaaa cgtcagaagg gcacgggtca ctgagagcct agtatcctag taaagtgggc
7081 tctctccctc tctctccagc ttgtcattga aaaccagtcc accaagcttg ttggttcgca
7141 cagcaagagt acatagagtt tgaaataata cataggattt taagagggag acactgtctc
7201 taaaaaaaaa aacaacagca acaacaaaaa gcaacaacca ttacaatttt atgttccctc
7261 agcattctca gagctgagga atgggagagg actatgggaa cccccttcat gttccggcct
7321 tcagccatgg ccctggatac atgcactcat ctgtcttaca atgtcattcc cccagGAGGG
7381 CCCGGAAGAA AACAGTGCCT ATGAGCAGTT GCTGTCTCGC TTGGAAGAAA TCGCTGAGGA
7441 AGgtcagttt gttggtctgg ccactaatct ctgtggccta gttcataaag aatcaccctt
7501 tggagcttca ggtctgaggc tggagatggg ctccctccag tgcaggaggg attgaagcat
7561 gagccagcgc tcatcttgat aataaccatg aagctgacag acacagttac ccgcaaacgg
7621 ctgcctacag attgaaaacc aagcaaaaac cgccgggcac ggtggctcac gcctgtaatc
7681 ccagcacttt gggaggccaa ggcaggtgga tcacgaggtc aagagatcaa gaccatcctg
7741 gccaacatgg tgaaacccca tctctactaa aaatacgaaa aaatagccag gcgtggtggc
7801 gggtgcctgt aatcccagct actcgggagg ctgaggcagg agaatggcat gaacccggga
7861 ggcagaagtt gcagtgagcc gagatcgtgc cactgcactc cagcctgggc aacagagcga
```

FIG._3D

```
7921 gactcttgtc tcaaaaaaaa aaaaaaaaaa gaaaaccaag caaaaaccaa aatgagacaa
7981 aaaaaacaag accaaaaaat ggtgtttgga aattgtcaag gtcaagtctg gagagctaaa
8041 ctttttctga gaactgttta tctttaataa gcatcaaata ttttaacttt gtaaatactt
8101 ttgttggaaa tcgttctctt cttagtcact cttgggtcat tttaaatctc acttactcta
8161 ctagaccttt taggtttctg ctagactagg tagaactctg cctttgcatt tcttgtgtct
8221 gttttgtata gttatcaata ttcatattta tttacaagtt attcagatca ttttttcttt
8281 tctttttttt tttttttttt ttttttacat ctttagtaga gacagggttt caccatattg
8341 gccaggctgc tctcaaactc ctgaccttgt gatccaccag cctcggcctc ccaaagtgct
8401 gggattcatt ttttcttttt aatttgctct gggcttaaac ttgtggccca gcactttatg
8461 atggtacaca gagttaagag tgtagactca gacggtcttt cttctttcct tctcttcctt
8521 cctcccttcc ctcccacctt cccttctctc cttcctttct ttcttcctct cttgcttcct
8581 caggcctctt ccagttgctc caaagccctg tacttttttt tgagttaacg tcttatggga
8641 agggcctgca cttagtgaag aagtggtctc agagttgagt taccttggct tctgggaggt
8701 gaaactgtat ccctataccc tgaagcttta agggggtgca atgtagatga gaccccaaca
8761 tagatcctct tcacagGCTC AGAGACTCAG GTCCCAGGAC TGGACATATC TGCACTCCTG
8821 CCCTCTGACT TCAGCCGCTA CTTCCAATAT GAGGGGTCTC TGACTACACC GCCCTGTGCC
8881 CAGGGTGTCA TCTGGACTGT GTTTAACCAG ACAGTGATGC TGAGTGCTAA GCAGgtgggc
8941 ctggggtgtg tgtggacaca gtgggtgcgg gggaaagagg atgtaagatg agatgagaaa
9001 caggagaaga aagaaatcaa ggctgggctc tgtggcttac gcctataatc ccaccacgtt
9061 gggaggctga ggtgggagaa tggtttgagc ccaggagttc aagacaaggc ggggcaacat
9121 agtgtgaccc catctctacc aaaaaaaccc caacaaaacc aaaaatagcc gggcatggtg
9181 gtatgcggcc tagtcccagc tactcaagga ggctgaggtg ggaagatcgc ttgattccag
9241 gagtttgaga ctgcagtgag ctatgatccc accactgcct accatcttta ggatacattt
9301 atttatttat aaaagaaatc aagaggctgg atggggaata caggagctgg agggtggagc
9361 cctgaggtgc tggttgtgag ctggcctggg acccttgttt cctgtcatgc catgaaccca
9421 cccacactgt ccactgacct ccctagCTCC ACACCCTCTC TGACACCCTG TGGGGACCTG
9481 GTGACTCTCG GCTACAGCTG AACTTCCGAG CGACGCAGCC TTTGAATGGG CGAGTGATTG
9541 AGGCCTCCTT CCCTGCTGGA GTGGACAGCA GTCCTCGGGC TGCTGAGCCA Ggtacagctt
9601 tgtctggttt ccccccagcc agtagtccct tatcctccca tgtgtgtgcc agtgtctgtc
9661 attggtggtc acagcccgcc tctcacatct cctttttctc tccagTCCAG CTGAATTCCT
9721 GCCTGGCTGC TGgtgagtct gcccctcctc ttggtcctga tgccaggaga ctcctcagca
9781 ccattcagcc ccagggctgc tcaggaccgc ctctgctccc tctccttttc tgcagaacag
9841 accccaaccc caatattaga gaggcagatc atggtgggga ttcccccatt gtccccagag
```

FIG._3E

```
 9901 gctaattgat tagaatgaag cttgagaaat ctcccagcat ccctctcgca aaagaatccc
 9961 cccccctttt tttaaagata gggtctcact ctgtttgccc caggctgggg tgttgtggca
10021 cgatcatagc tcactgcagc ctcgaactcc taggctcagg caatcctttc accttagctt
10081 ctcaaagcac tgggactgta ggcatgagcc actgtgcctg gccccaaacg gcccttttac
10141 ttggctttta ggaagcaaaa acggtgctta tcttacccct tctcgtgtat ccaccctcat
10201 cccttggctg gcctcttctg gagactgagg cactatgggg ctgcctgaga actcggggca
10261 ggggtggtgg agtgcactga ggcaggtgtt gaggaactct gcagacccct cttccttccc
10321 aaagcagccc tctctgctct ccatcgcagG TGACATCCTA GCCCTGGTTT TTGGCCTCCT
10381 TTTTGCTGTC ACCAGCGTCG CGTTCCTTGT GCAGATGAGA AGGCAGCACA Ggtattacac
10441 tgaccctttc ttcaggcaca agcttccccc acccttgtgg agtcacttca tgcaaagcgc
10501 atgcaaatga gctgctcctg ggccagtttt ctgattagcc tttcctgttg tgtacacaca
10561 gAAGGGGAAC CAAAGGGGGT GTGAGCTACC GCCCAGCAGA GGTAGCCGAG ACTGGAGCCT
10621 AGAGGCTGGA TCTTGGAGAA TGTGAGAAGC CAGCCAGAGG CATCTGAGGG GGAGCCGGTA
10681 ACTGTCCTGT CCTGCTCATT ATGCCACTTC CTTTTAACTG CCAAGAAATT TTTTAAAATA
10741 AATATTTATA ATaaaatatg tgttagtcac ctttgttccc caaatcagaa ggaggtattt
10801 gaatttccta ttactgttat tagcaccaat ttagtggtaa tgcatttatt ctattacagt
10861 tcggcctcct tccacacatc actccaatgt gttgctcc
```

FIG._3F

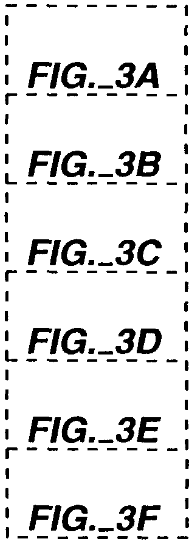


FIG._3

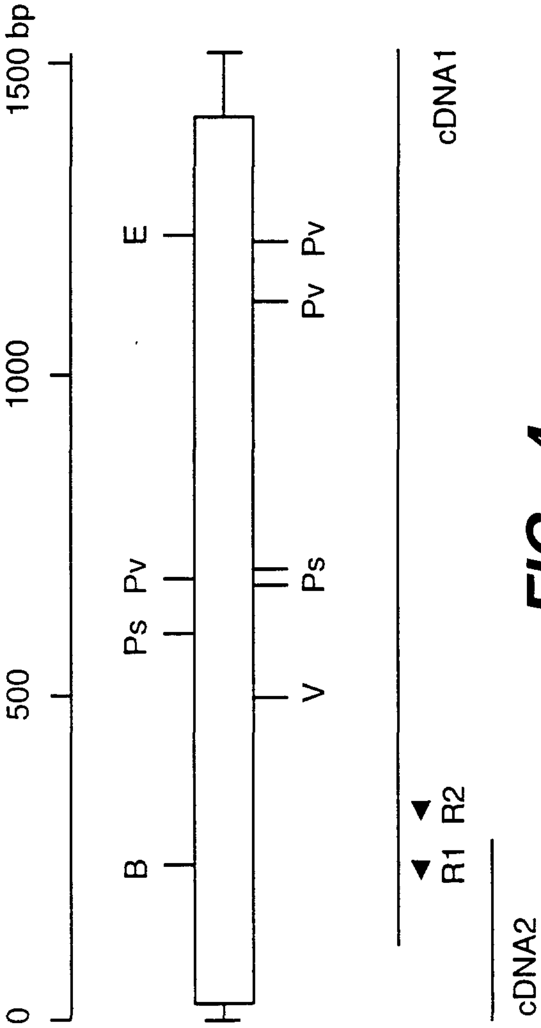

0
500
1000
1500 bp
B
Ps
Pv
E
V
Ps
Pv
Pv
R1
R2
cDNA1
cDNA2

FIG._4

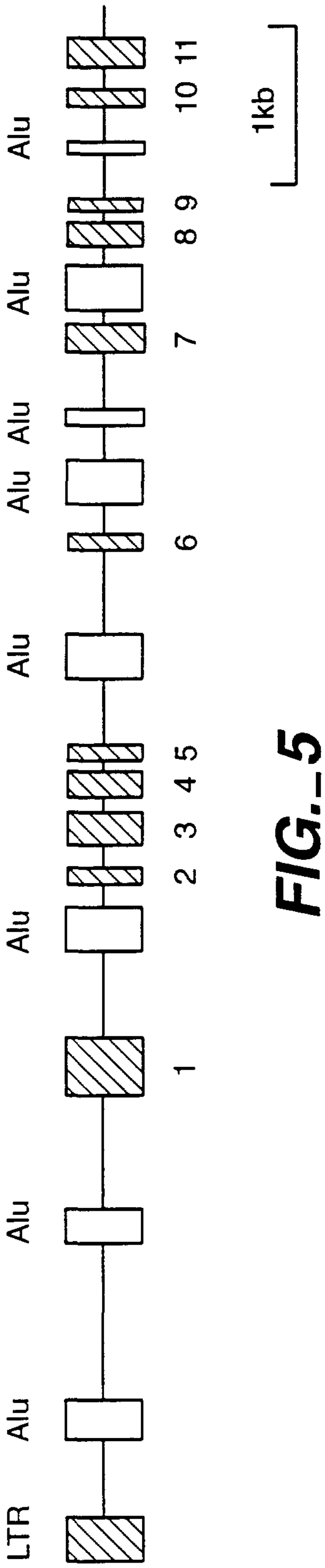

LTR
Alu
Alu
1
Alu
2 3 4 5
Alu
6
Alu
Alu
Alu
7
Alu
8 9
Alu
10 11
1kb

FIG._5

```
-506  CTTGCTTTTC ATTCAAGCTC AAGTTTGTCT CCCACATACC CATTACTTAA CTCACCCTCG

-446  GGCTCCCCTA GCAGCCTGCC CTACCTCTTT ACCTGCTTCC TGGTGGAGTC AGGGATGTAT
       AP2            AP2

-386  ACATGAGCTG CTTTCCCTCT CAGCCAGAGG ACATGGGGGG CCCCAGCTCC CCTGCCTTTC

-326  CCCTTCTGTG CCTGGAGCTG GGAAGCAGGC CAGGGTTAGC TGAGGCTGGC TGGCAAGCAG

-266  CTGGGTGGTG CCAGGGAGAG CCTGCATAGT GCCAGGTGGT GCCTTGGGTT CCAAGCTAGT
                                                                     p53

-206  CCATGGCCCC GATAACCTTC TGCCTGTGCA CACACCTGCC CCTCACTCCA CCCCCATCCT
                                                             Inr

-146  AGCTTTGGTA TGGGGGAGAG GGCACAGGGC CAGACAAACC TGTGAGACTT TGGCTCCATC
                                                                    Inr

 -86  TCTGCAAAAG GGCGCTCTGT GAGTCAGCCT GCTCCCCTCC AGGCTTGCTC CTCCCCCACC
                                 AP1                     p53         AP2
                                      ***                                  ...
 -26  CAGCTCTCGT TTCCAATGCA CGTACAGCCC GTACACACCG TGTGCTGGGA CACCCCACAG
```

FIG._6

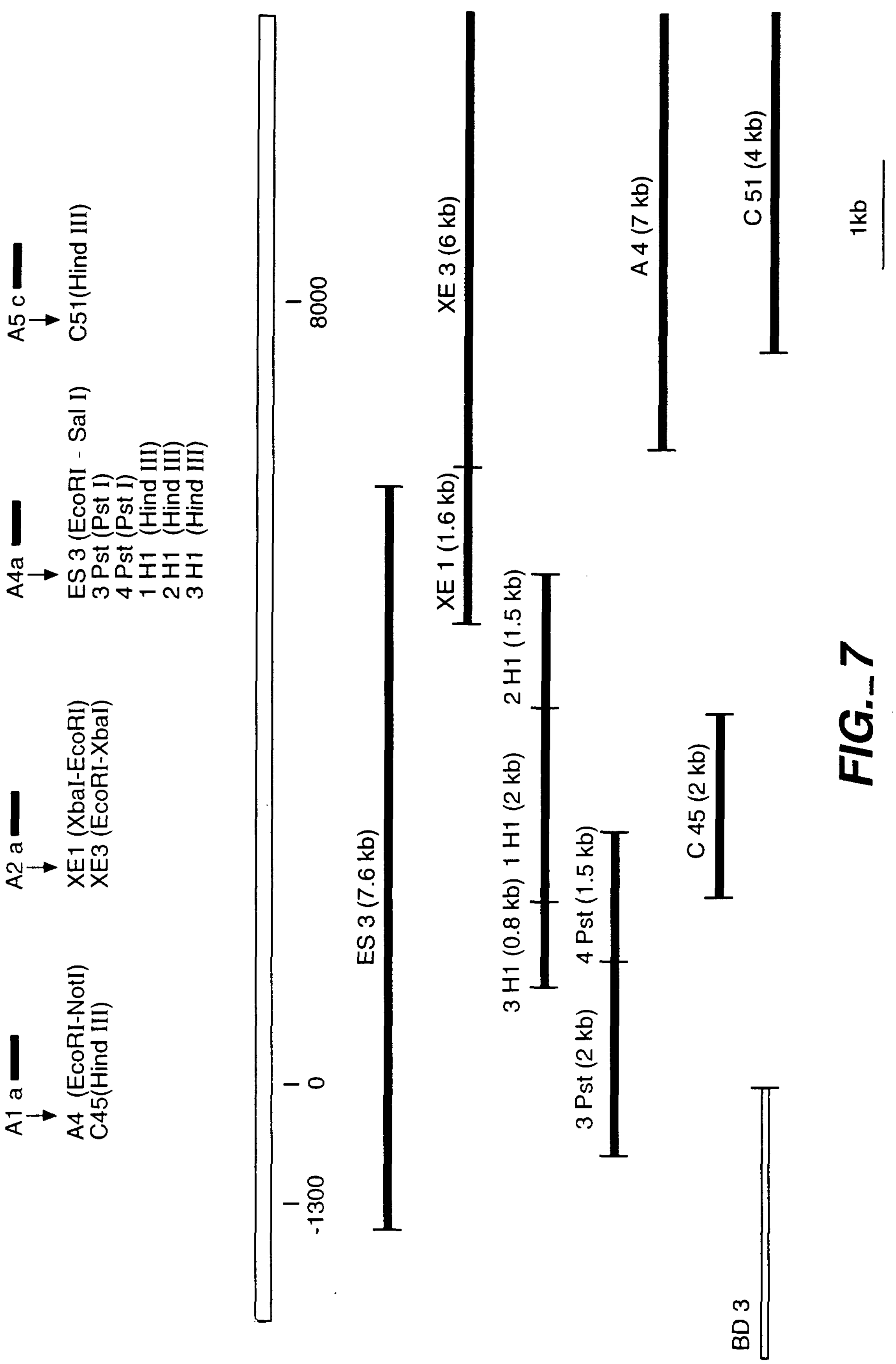

A1 a
A4 (EcoRI-NotI)
C45(Hind III)
A2 a
XE1 (XbaI-EcoRI)
XE3 (EcoRI-XbaI)
A4a
ES 3 (EcoRI - Sal I)
3 Pst (Pst I)
4 Pst (Pst I)
1 H1 (Hind III)
2 H1 (Hind III)
3 H1 (Hind III)
A5 c
C51(Hind III)
-1300
0
8000
ES 3 (7.6 kb)
XE 1 (1.6 kb)
XE 3 (6 kb)
3 H1 (0.8 kb)
1 H1 (2 kb)
2 H1 (1.5 kb)
3 Pst (2 kb)
4 Pst (1.5 kb)
C 45 (2 kb)
A 4 (7 kb)
C 51 (4 kb)
BD 3
1kb

FIG._7

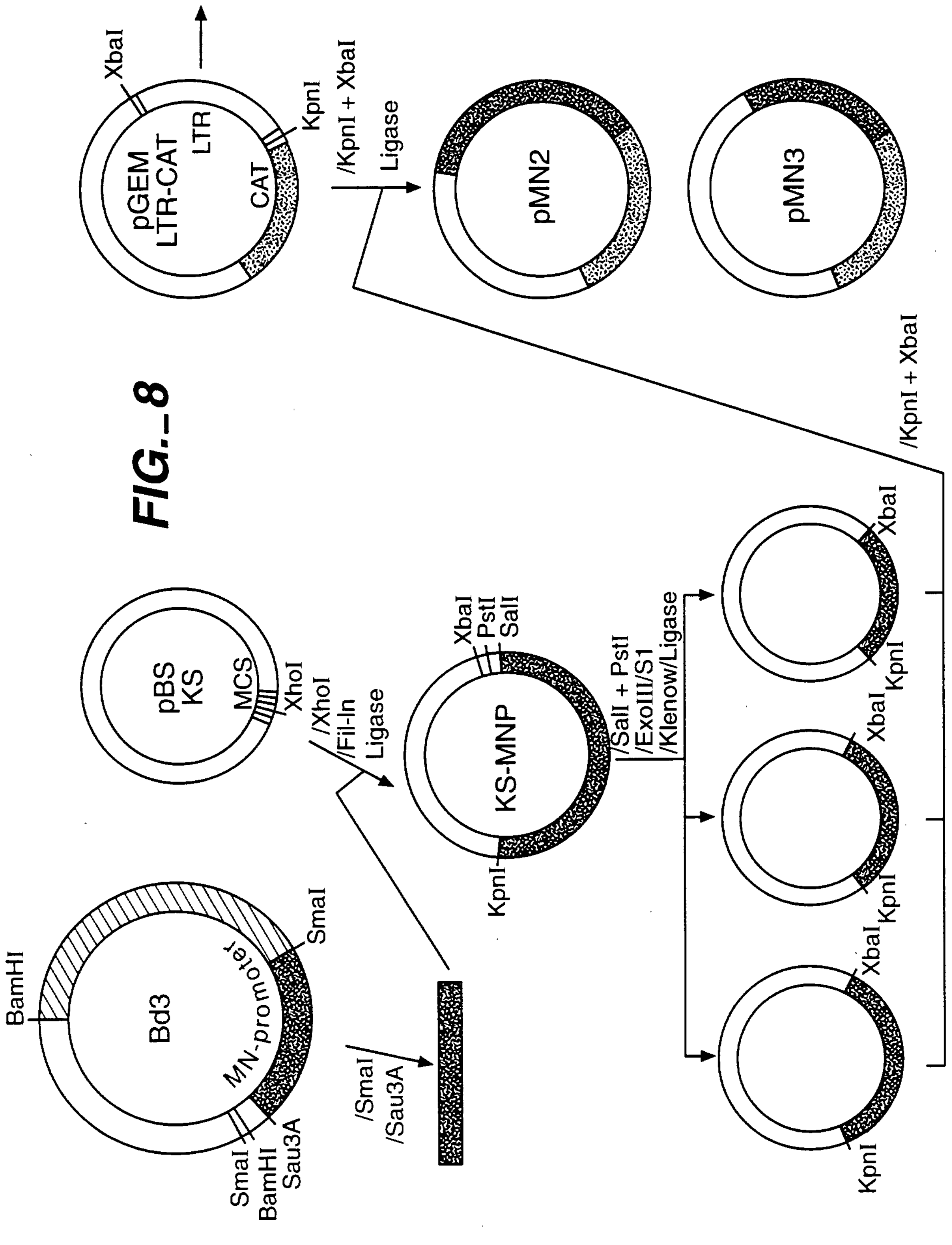


FIG._8

BamHI
Bd3
MN-promoter
SmaI
SmaI
BamHI
Sau3A
/SmaI
/Sau3A
pBS
KS
MCS
XhoI
/XhoI
/Fil-In
Ligase
KS-MNP
XbaI
PstI
SalI
KpnI
/SalI + PstI
/ExoIII/S1
/Klenow/Ligase
KpnI
XbaI
KpnI
XbaI
KpnI
XbaI
/KpnI + XbaI
pGEM
LTR-CAT
LTR
CAT
XbaI
KpnI
/KpnI + XbaI
Ligase
pMN2
pMN3