



(86) Date de dépôt PCT/PCT Filing Date: 2001/12/13
(87) Date publication PCT/PCT Publication Date: 2002/08/01
(85) Entrée phase nationale/National Entry: 2003/06/17
(86) N° demande PCT/PCT Application No.: US 2001/048917
(87) N° publication PCT/PCT Publication No.: 2002/059311
(30) Priorité/Priority: 2000/12/18 (09/740,235) US

(51) Cl.Int.⁷/Int.Cl.⁷ C12N 15/12, C12Q 1/68, C07K 14/47
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(54) Titre : **MARQUEUR DU CANCER DU COLON**
(54) Title: **COLON CANCER MARKER**

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          9          18          27          36          45          54
5'  GCC TGC CGA GTT CCG AGC GAC CGA TGG AGA TGG CGG CTG CGG CTG AGT GAC GGA
          63          72          81          90          99          108
    CGG TGG AGG CCC AGA GCC CGG GCC TGA AGG GGG GGA CAA ACC TGG GTG CCC GCA
          117          126          135          144          153          162
    GGA GCC CGG CAG GGT GTC TTA CAA GTA TCA AGA ACT TAC TAT ATG TGG TTG AAT
          171          180          189          198          207          216
    AAA CAA TCA AGG TAA AGA GCA TCA AGT AAA AAC TTC TGC TTG TTG ATA AGT ACT
          225          234          243          252          261          270
    TCA GAC ATT CCC CCA GTG GCT GAA GTG GCA TAT GAA TTA TGA AGT TGG ATC ATT
          279          288          297          306          315          324
    TGG AAT GAA TGT AAG AGA ATT GCC AAG GGC TCC TCC TAC TCC AGA GAG GAA ACC
          333          342          351          360          369          378
    TCA TCC AGG GCC ATG AAG CCA CTT CCT CAC CAT CTG TGT GCT GCT TAA GCT AAT
          387          396          405          414          423          432
    GCT GCG GGA ACC ATG GTT CCT TGG GAG GAA TCA AGC TGA CTC TTG GCA TGA GAT
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(57) **Abrégé/Abstract:**

The invention provides a cDNA which encodes a colon cancer marker. It also provides for the use of the cDNA, fragments, complements, and variants thereof and the encoded protein, portions thereof and antibodies thereto for diagnosis and treatment of colon disorders, particularly colon cancer and polyps. The invention additionally provides expression vectors and host cells for the production of the protein and a transgenic model system.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
1 August 2002 (01.08.2002)

PCT

(10) International Publication Number
WO 02/059311 A3

(51) International Patent Classification⁷: **C12N 15/12**,
C07K 14/47, C12Q 1/68

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(21) International Application Number: PCT/US01/48917

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(22) International Filing Date:

13 December 2001 (13.12.2001)

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK,
SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA,
ZW.

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/740,235 18 December 2000 (18.12.2000) US

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(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG).

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Published:

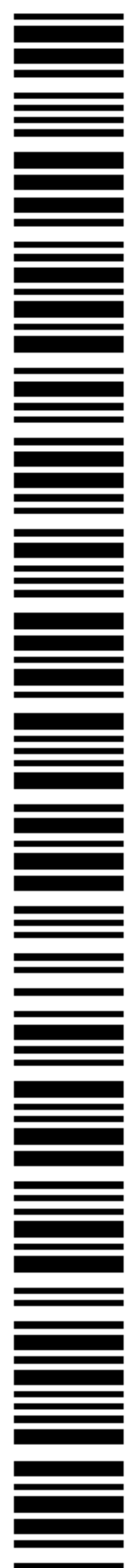
— with international search report

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(54) Title: COLON CANCER MARKER

5'	GCC	TGC	CGA	GTT	CCG	AGC	GAC	CGA	TGG	AGA	TGG	CGG	CTG	CGG	CTG	AGT	GAC	GGA
	63		72		81		90		99		108							
	CGG	TGG	AGG	CCC	AGA	GCC	CGG	GCC	TGA	AGG	GGG	GGA	CAA	ACC	TGG	GTG	CCC	GCA
	117		126		135		144		153		162							
	GGA	GCC	CGG	CAG	GGT	GTC	TTA	CAA	GTA	TCA	AGA	ACT	TAC	TAT	ATG	TGG	TTG	AAT
	171		180		189		198		207		216							
	AAA	CAA	TCA	AGG	TAA	AGA	GCA	TCA	AGT	AAA	AAC	TTC	TGC	TTG	TTG	ATA	AGT	ACT
	225		234		243		252		261		270							
	TCA	GAC	ATT	CCC	CCA	GTG	GCT	GAA	GTG	GCA	TAT	GAA	TTA	TGA	AGT	TGG	ATC	ATT
	279		288		297		306		315		324							
	TGG	AAT	GAA	TGT	AAG	AGA	ATT	GCC	AAG	GGC	TCC	TCC	TAC	TCC	AGA	GAG	GAA	ACC
	333		342		351		360		369		378							
	TCA	TCC	AGG	GCC	ATG	AAG	CCA	CTT	CCT	CAC	CAT	CTG	TGT	GCT	GCT	TAA	GCT	AAT
	387		396		405		414		423		432							
	GCT	GCG	GGA	ACC	ATG	GTT	CCT	TGG	GAG	GAA	TCA	AGC	TGA	CTC	TTG	GCA	TGA	GAT

(57) Abstract: The invention provides a cDNA which encodes a colon cancer marker. It also provides for the use of the cDNA, fragments, complements, and variants thereof and the encoded protein, portions thereof and antibodies thereto for diagnosis and treatment of colon disorders, particularly colon cancer and polyps. The invention additionally provides expression vectors and host cells for the production of the protein and a transgenic model system.



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COLON CANCER MARKER

TECHNICAL FIELD

This invention relates to a mammalian cDNA which encodes a colon cancer marker and to
5 the use of the cDNA and the encoded protein in the diagnosis and treatment of colon disorders,
particularly colon cancer and polyps.

BACKGROUND OF THE INVENTION

Phylogenetic relationships among organisms have been demonstrated many times, and
studies from a diversity of prokaryotic and eukaryotic organisms suggest a more or less gradual
10 evolution of molecules, biochemical and physiological mechanisms, and metabolic pathways.
Despite different evolutionary pressures, the proteins of nematode, fly, rat, and man have common
chemical and structural features and generally perform the same cellular function. Comparisons
of the nucleic acid and protein sequences from organisms where structure and/or function are
known accelerate the investigation of human sequences and allow the development of model
15 systems for testing diagnostic and therapeutic agents for human conditions, diseases, and
disorders.

Colorectal cancer is the fourth most common cancer and the second most common cause
of cancer death in the United States with approximately 130,000 new cases and 55,000 deaths per
year. Colon and rectal cancers share many environmental risk factors and both are found in
20 individuals with specific genetic syndromes (Potter (1999) J Natl Cancer Institute 91:916-932).
Colon cancer is the only cancer that occurs with approximately equal frequency in men and
women, and the five-year survival rate following diagnosis of colon cancer is around 55% in the
United States (Ries et al. (1990) National Institutes of Health, DHHS Publ. No. (NIH)90-2789).

Colon cancer is causally related to both genes and the environment. Several molecular
25 pathways have been linked to the development of colon cancer, and the expression of key genes in
any of these pathways may be affected by inherited or acquired mutation or by hypermethylation.
There is a particular need to identify genes for which changes in expression may provide an early
indicator of colon cancer or a predisposition for the development of colon cancer.

For example, it is well known that abnormal patterns of DNA methylation occur
30 consistently in human tumors and include, simultaneously, widespread genomic hypomethylation
and localized areas of increased methylation. In colon cancer in particular, it has been found that
these changes occur early in tumor progression such as in premalignant polyps that precede colon
cancer. Indeed, DNA methyltransferase, the enzyme that performs DNA methylation, is
significantly increased in histologically normal mucosa from patients with colon cancer or in the
35 benign polyps that precede cancer. This increase continues during the progression of colonic
neoplasms (El-Deiry et al. (1991) Proc Natl Acad Sci USA 88:3470-3474). Increased DNA

methylation occurs in G+C rich areas of genomic DNA termed “CpG islands” that are important for maintenance of an “open” transcriptional conformation around genes, and hypermethylation of these regions results in a “closed” conformation that silences gene transcription. It has been suggested that the silencing or downregulation of differentiation genes by such abnormal

5 methylation of CpG islands may prevent differentiation in immortalized cells (Antequera *et al.* (1990) *Cell* 62:503-514).

Familial adenomatous polyposis (FAP) is a rare autosomal dominant syndrome that precedes colon cancer and is caused by an inherited mutation in the adenomatous polyposis coli (APC) gene. FAP is characterized by the early development of multiple colorectal adenomas that

10 progress to cancer at a mean age of 44 years. The APC gene is a part of the APC- β -catenin-Tcf (T-cell factor) pathway. Impairment of this pathway results in the loss of orderly replication, adhesion, and migration of colonic epithelial cells that results in the growth of polyps. A series of other genetic changes follow activation of the APC- β -catenin-Tcf pathway and accompanies the transition from normal colonic mucosa to metastatic carcinoma. These changes include mutation

15 of the K-Ras proto-oncogene, changes in methylation patterns, and mutation or loss of the tumor suppressor genes p53 and Smad4/ DPC4. While the inheritance of a mutated APC gene is a rare event, the loss or mutation of APC and the consequent effects on the APC- β -catenin-Tcf pathway is believed to be central to the majority of colon cancers in the general population.

Hereditary nonpolyposis colorectal cancer (HNPCC) is another inherited autosomal

20 dominant syndrome with a less well defined phenotype than FAP. HNPCC, which accounts for about 2% of colorectal cancer cases, is distinguished by the tendency to early onset of cancer and the development of other cancers, particularly those involving the endometrium, urinary tract, stomach, and biliary system. HNPCC results from the mutation of one or more genes in the DNA mis-match repair (MMR) pathway. Mutations in two human MMR genes, MSH2 and MLH1, are

25 found in a large majority of HNPCC families identified to date. The DNA MMR pathway identifies and repairs errors that result from the activity of DNA polymerase during replication. Furthermore, loss of MMR activity contributes to cancer progression through accumulation of other gene mutations and deletions, such as loss of the BAX gene which controls apoptosis, and the TGF β receptor II gene which controls cell growth. Because of the potential for irreparable

30 damage to DNA in an individual with a DNA MMR defect, progression to carcinoma is more rapid than usual.

Although ulcerative colitis is a minor contributor to colon cancer, affected individuals have about a 20-fold increase in risk for developing cancer. Progression is associated with mutations in the p53 gene which may occur early, appearing even in histologically normal tissue.

35 The progression of the disease from ulcerative colitis to dysplasia/carcinoma without an

intermediate polyp state suggests a high degree of mutagenic activity resulting from the exposure of proliferating cells in the colonic mucosa to the colonic contents.

Almost all colon cancers arise from cells in which the estrogen receptor (ER) gene has been silenced. The silencing of ER gene transcription is age related and linked to
5 hypermethylation of the ER gene (Issa *et al.* (1994) *Nature Genetics* 7:536-540). Introduction and expression of an exogenous ER coding sequence into cultured colon carcinoma cells results in marked suppression of growth. Inhibition of cancer cell invasion depends on the function of the hormone binding domain and the N-terminal zinc finger region of the ER (Platet *et al.* (2000) *Mol Endocrinol* 14:999-1009). Activation of the ER by hormone binding induces transcription of
10 specific target genes and may be linked to the reduction in cancer invasiveness. In the absence of hormone, protein-protein interactions with the zinc finger region may also contribute to the inhibition of cancer cell migration. The connection between loss of the ER protein in colonic epithelial cells and the consequent development of cancer has not been established.

The FYVE-finger proteins play roles in cellular processes such as receptor signaling,
15 vesicular trafficking, and actin-regulated membrane rearrangements (Stenmark and Aasland (1999) *J Cell Science* 112:4175-4183). The FYVE domain is a type of zinc finger that typically contains eight conserved cysteines which bind two Zn^{2+} cations, has conserved glycine and arginine residues, and a basic motif with the consensus sequence R(R/K)HHCR. The FYVE domain binds to phosphoinositides found in specific membranes. The presence of other domains
20 in FYVE-finger proteins may mediate protein-protein interactions with other molecules at the membrane and may be involved in the recruitment of signaling molecules such as small GTPases to membranes at particular cellular locations. In mice with a null mutation in the FYVE finger protein Hrs, embryos showed defects in ventral folding morphogenesis and died *in utero* (Komada and Soriano (1999) *Genes Dev* 13:1475-1485). The embryos developed with their ventral region
25 outside of the yolk sac, had two independent bilateral heart tubes, and no foregut. Mutations in genes that control cellular differentiation and proliferation in the intestine may be linked with colon diseases (Dove *et al.* (1998) *Phil Trans R Soc Lond B* 353:915-923).

Clearly there are a number of genetic alterations associated with colon cancer and with the development and progression of the disease. Particularly, downregulation of expression or
30 deletion of genes potentially provide early indicators of cancer development, and may also be used to monitor disease progression or provide possible therapeutic targets. The specific genes affected in a given case of colon cancer depend on the molecular progression of the disease. Identification of additional genes associated with colon cancer and the precancerous state would provide more reliable diagnostic patterns associated with the development and progression of the disease.

35 The discovery of a mammalian cDNA encoding a colon cancer marker satisfies a need in

the art by providing compositions which are useful in the diagnosis and treatment of colon disorders, particularly colon cancer and polyps.

SUMMARY OF THE INVENTION

The invention is based on the discovery of a mammalian cDNA which encodes a colon cancer marker (CCM), which is useful in the diagnosis and treatment of colon disorders, particularly colon cancer and polyps.

The invention provides an isolated mammalian cDNA or a fragment thereof encoding a mammalian protein or a portion thereof selected from the group consisting of an amino acid sequence of SEQ ID NO:1, a variant having at least 85% identity to the amino acid sequence of SEQ ID NO:1, an antigenic epitope of SEQ ID NO:1, and a biologically active portion of SEQ ID NO:1. The invention also provides an isolated mammalian cDNA or the complement thereof selected from the group consisting of a nucleic acid sequence of SEQ ID NO:2, a fragment of SEQ ID NO:2 selected from SEQ ID NOs:3-22, and a variant having at least 87% identity to the nucleic acid sequence of SEQ ID NO:2 selected from SEQ ID NOs:23-34. The invention additionally provides a composition, a substrate, and a probe comprising the cDNA, or the complement of the cDNA, encoding CCM. The invention further provides a vector containing the cDNA, a host cell containing the vector and a method for using the cDNA to make CCM. In one aspect, the invention provides a substrate containing at least one of the cDNAs. In a second aspect, the invention provides a probe comprising a cDNA which can be used in methods of detection, screening, and purification. In a further aspect, the probe is a single stranded complementary RNA or DNA molecule.

The invention provides a method for using a cDNA to detect the differential expression of a nucleic acid in a sample comprising hybridizing a probe to the nucleic acids, thereby forming hybridization complexes and comparing hybridization complex formation with a standard, wherein the comparison indicates the differential expression of the cDNA in the sample. In one aspect, the method of detection further comprises amplifying the nucleic acids of the sample prior to hybridization. In another aspect, the method showing differential expression of the cDNA is used to diagnose colon disorders, particularly colon cancer and polyps. In another aspect, the cDNA or a fragment or a complement thereof may comprise an element on an array.

The invention additionally provides a method for using a cDNA or a fragment or a complement thereof to screen a library or plurality of molecules or compounds to identify at least one ligand which specifically binds the cDNA, the method comprising combining the cDNA with the molecules or compounds under conditions allowing specific binding, and detecting specific binding to the cDNA, thereby identifying a ligand which specifically binds the cDNA. In one aspect, the molecules or compounds are selected from aptamers, DNA molecules, RNA

molecules, peptide nucleic acids, artificial chromosome constructions, peptides, transcription factors, repressors, and regulatory molecules.

The invention provides a purified mammalian protein or a portion thereof selected from the group consisting of an amino acid sequence of SEQ ID NO:1, a variant having 85% identity to the amino acid sequence of SEQ ID NO:1, an antigenic epitope of SEQ ID NO:1, and a
5 biologically active portion of SEQ ID NO:1. The invention also provides a composition comprising the purified protein or a portion thereof in conjunction with a pharmaceutical carrier. The invention further provides a method of using the CCM to treat a subject with colon disorders, particularly colon cancer and polyps comprising administering to a patient in need of such
10 treatment the composition containing the purified protein. The invention still further provides a method for using a protein to screen a library or a plurality of molecules or compounds to identify at least one ligand, the method comprising combining the protein with the molecules or compounds under conditions to allow specific binding and detecting specific binding, thereby identifying a ligand which specifically binds the protein. In one aspect, the molecules or
15 compounds are selected from DNA molecules, RNA molecules, peptide nucleic acids, peptides, proteins, mimetics, agonists, antagonists, antibodies, immunoglobulins, inhibitors, and drugs. In another aspect, the ligand is used to treat a subject with colon disorders, particularly colon cancer and polyps.

The invention provides a method of using a mammalian protein to screen a subject sample
20 for antibodies which specifically bind the protein comprising isolating antibodies from the subject sample, contacting the isolated antibodies with the protein under conditions that allow specific binding, dissociating the antibody from the bound-protein, and comparing the quantity of antibody with known standards, wherein the presence or quantity of antibody is diagnostic of colon disorders, particularly colon cancer and polyps.

25 The invention also provides a method of using a mammalian protein to prepare and purify antibodies comprising immunizing a animal with the protein under conditions to elicit an antibody response, isolating animal antibodies, attaching the protein to a substrate, contacting the substrate with isolated antibodies under conditions to allow specific binding to the protein, dissociating the antibodies from the protein, thereby obtaining purified antibodies.

30 The invention provides a purified antibody which binds specifically to a protein which is expressed in colon disorders, particularly colon cancer and polyps. The invention also provides a method of using an antibody to diagnose colon disorders, particularly colon cancer and polyps comprising combining the antibody comparing the quantity of bound antibody to known standards, thereby establishing the presence of colon disorders, particularly colon cancer and
35 polyps. The invention further provides a method of using an antibody to treat colon disorders,

particularly colon cancer and polyps comprising administering to a patient in need of such treatment a pharmaceutical composition comprising the purified antibody.

The invention provides a method for inserting a marker gene into the genomic DNA of a mammal to disrupt the expression of the endogenous polynucleotide. The invention also provides
 5 a method for using a cDNA to produce a mammalian model system, the method comprising constructing a vector containing the cDNA selected from SEQ ID NOs:2-34, transforming the vector into an embryonic stem cell, selecting a transformed embryonic stem, microinjecting the transformed embryonic stem cell into a mammalian blastocyst, thereby forming a chimeric blastocyst, transferring the chimeric blastocyst into a pseudopregnant dam, wherein the dam gives
 10 birth to a chimeric offspring containing the cDNA in its germ line, and breeding the chimeric mammal to produce a homozygous, mammalian model system.

BRIEF DESCRIPTION OF THE FIGURES AND TABLE

Figures 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, 1J, 1K, 1L, 1M, 1N, 1O, 1P, 1Q, 1R, 1S, 1T, 1U and 1V show the mammalian CCM (SEQ ID NO:1) encoded by the cDNA (SEQ ID NO:2).
 15 The translation was produced using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA).

Table 1 shows the differential expression of CCM in colon cancer and colon polyps relative to normal colon tissue as determined by microarray analysis. Column 1 lists the mean differential expression (DE) values presented as log₂ DE (diseased tissue/microscopically normal
 20 tissue) for tissue samples from patients with colon cancer and colon polyps. Column 2 lists the percentage covariance (CV%) in differential expression values. Column 3 lists the tissue and patient donor (Dn) for microscopically normal samples labeled with fluorescent green dye Cy3. Column 4 lists the tissue and patient donor (Dn) for diseased samples labeled with fluorescent red dye Cy5.

25

DESCRIPTION OF THE INVENTION

It is understood that this invention is not limited to the particular machines, materials and methods described. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments and is not intended to limit the scope of the present
 30 invention which will be limited only by the appended claims. As used herein, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. For example, a reference to "a host cell" includes a plurality of such host cells known to those skilled in the art.

Unless defined otherwise, all technical and scientific terms used herein have the same
 35 meanings as commonly understood by one of ordinary skill in the art to which this invention

belongs. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

5 Definitions

"CCM" refers to a substantially purified protein obtained from any mammalian species, including bovine, canine, murine, ovine, porcine, rodent, simian, and preferably the human species, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

10 "Array" refers to an ordered arrangement of at least two cDNAs on a substrate. At least one of the cDNAs represents a control or standard sequence, and the other, a cDNA of diagnostic interest. The arrangement of from about two to about 40,000 cDNAs on the substrate assures that the size and signal intensity of each labeled hybridization complex formed between a cDNA and a sample nucleic acid is individually distinguishable.

15 The "complement" of a cDNA of the Sequence Listing refers to a nucleic acid molecule which is completely complementary over its full length and which will hybridize to the cDNA or an mRNA under conditions of high stringency.

"cDNA" refers to an isolated polynucleotide, nucleic acid molecule, or any fragment or complement thereof. It may have originated recombinantly or synthetically, be double-stranded or single-stranded, represent coding and/or noncoding 5' and 3' sequence.

20 The phrase "cDNA encoding a protein" refers to a nucleic acid sequence that closely aligns with sequences which encode conserved regions, motifs or domains that were identified by employing analyses well known in the art. These analyses include BLAST (Basic Local Alignment Search Tool; Altschul (1993) J Mol Evol 36: 290-300; Altschul et al. (1990) J Mol Biol 215:403-410) which provides identity within the conserved region.

25 "Derivative" refers to a cDNA or a protein that has been subjected to a chemical modification. Derivatization of a cDNA can involve substitution of a nontraditional base such as queosine or of an analog such as hypoxanthine. These substitutions are well known in the art. Derivatization of a protein involves the replacement of a hydrogen by an acetyl, acyl, alkyl, amino, formyl, or morpholino group. Derivative molecules retain the biological activities of the naturally occurring molecules but may confer advantages such as longer lifespan or enhanced activity.

"Differential expression" refers to an increased, upregulated or present, or decreased, downregulated or absent, gene expression as detected by the absence, presence, or at least two-fold changes in the amount of transcribed messenger RNA or translated protein in a sample.

35 "Disorder" refers to conditions, diseases or syndromes in which the cDNAs and CCM are

differentially expressed such as colon disorders, particularly colon cancer and polyps.

"Fragment" refers to a chain of consecutive nucleotides from about 200 to about 700 base pairs in length. Fragments may be used in PCR or hybridization technologies to identify related nucleic acid molecules and in binding assays to screen for a ligand. Nucleic acids and their
5 ligands identified in this manner are useful as therapeutics to regulate replication, transcription or translation.

A "hybridization complex" is formed between a cDNA and a nucleic acid of a sample when the purines of one molecule hydrogen bond with the pyrimidines of the complementary molecule, e.g., 5'-A-G-T-C-3' base pairs with 3'-T-C-A-G-5'. The degree of complementarity and
10 the use of nucleotide analogs affect the efficiency and stringency of hybridization reactions.

"Ligand" refers to any agent, molecule, or compound which will bind specifically to a complementary site on a cDNA molecule or polynucleotide, or to an epitope or a protein. Such ligands stabilize or modulate the activity of polynucleotides or proteins and may be composed of inorganic or organic substances including nucleic acids, proteins, carbohydrates, fats, and lipids.

15 "Oligonucleotide" refers a single stranded molecule from about 18 to about 60 nucleotides in length which may be used in hybridization or amplification technologies or in regulation of replication, transcription or translation. Substantially equivalent terms are amplimer, primer, and oligomer.

"Portion" refers to any part of a protein used for any purpose; but especially, to an epitope
20 for the screening of ligands or for the production of antibodies.

"Post-translational modification" of a protein can involve lipidation, glycosylation, phosphorylation, acetylation, racemization, proteolytic cleavage, and the like. These processes may occur synthetically or biochemically. Biochemical modifications will vary by cellular location, cell type, pH, enzymatic milieu, and the like.

25 "Probe" refers to a cDNA that hybridizes to at least one nucleic acid in a sample. Where targets are single stranded, probes are complementary single strands. Probes can be labeled with reporter molecules for use in hybridization reactions including Southern, northern, in situ, dot blot, array, and like technologies or in screening assays.

"Protein" refers to a polypeptide or any portion thereof. A "portion" of a protein refers to
30 that length of amino acid sequence which would retain at least one biological activity, a domain identified by PFAM or PRINTS analysis or an antigenic epitope of the protein identified using Kyte-Doolittle algorithms of the PROTEAN program (DNASTAR, Madison WI). An "oligopeptide" is an amino acid sequence from about five residues to about 15 residues that is used as part of a fusion protein to produce an antibody.

35 "Purified" refers to any molecule or compound that is separated from its natural

environment and is from about 60% free to about 90% free from other components with which it is naturally associated.

"Sample" is used in its broadest sense as containing nucleic acids, proteins, antibodies, and the like. A sample may comprise a bodily fluid; the soluble fraction of a cell preparation, or
5 an aliquot of media in which cells were grown; a chromosome, an organelle, or membrane isolated or extracted from a cell; genomic DNA, RNA, or cDNA in solution or bound to a substrate; a cell; a tissue; a tissue print; a fingerprint, buccal cells, skin, or hair; and the like.

"Specific binding" refers to a special and precise interaction between two molecules which is dependent upon their structure, particularly their molecular side groups. For example,
10 the intercalation of a regulatory protein into the major groove of a DNA molecule, the hydrogen bonding along the backbone between two single stranded nucleic acids, or the binding between an epitope of a protein and an agonist, antagonist, or antibody.

"Similarity" as applied to sequences, refers to the quantification (usually percentage) of nucleotide or residue matches between at least two sequences aligned using a standardized
15 algorithm such as Smith-Waterman alignment (Smith and Waterman (1981) J Mol Biol 147:195-197) or BLAST2 (Altschul *et al.* (1997) Nucleic Acids Res 25:3389-3402). BLAST2 may be used in a standardized and reproducible way to insert gaps in one of the sequences in order to optimize alignment and to achieve a more meaningful comparison between them.

"Substrate" refers to any rigid or semi-rigid support to which cDNAs or proteins are
20 bound and includes membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, capillaries or other tubing, plates, polymers, and microparticles with a variety of surface forms including wells, trenches, pins, channels and pores.

"Variant" refers to molecules that are recognized variations of a cDNA or a protein encoded by the cDNA. Splice variants may be determined by BLAST score, wherein the score is
25 at least 100, and most preferably at least 400. Allelic variants have a high percent identity to the cDNAs and may differ by about three bases per hundred bases. "Single nucleotide polymorphism" (SNP) refers to a change in a single base as a result of a substitution, insertion or deletion. The change may be conservative (purine for purine) or non-conservative (purine to pyrimidine) and may or may not result in a change in an encoded amino acid or its secondary,
30 tertiary, or quaternary structure.

THE INVENTION

The invention is based on the discovery of a mammalian cDNA which encodes a colon cancer marker and on the use of the cDNA, or fragments thereof, and protein, or portions thereof, directly or as compositions in the characterization, diagnosis, and treatment of colon disorders.

35 Nucleic acids encoding CCM of the present invention were first identified in Incyte Clone

5098390 from the mammary epithelial cell cDNA library (EPIMNON05) using a computer search for amino acid sequence alignments. A consensus sequence, SEQ ID NO:2, was derived from the following overlapping and/or extended nucleic acid sequences (SEQ ID NOs:3-22): Incyte Clones 5098390F6 (EPIMNON05), 8022266J1, 7394066H1, 6309461H1 (NERDTDN03), 7436032H1, 5 7676140H2, 6923049H1 (PLACFER06), 8216794J1, 6995895H1 (BRAQTDR02), 6889439H1 (BRAITDR03), 70405975D1 (SG0000195), 8117068H1, 747646H1 (BRAITUT01), 7984383H1, 8073132J1, 6744627H1 (BRAFN0T02), 6749637H1 (BRAXN0T03), 5073165F8 (COLCTUT03), 6441214H1 (BRAEN0T02), 2436362H1 (BRAVUNT02), and GenBank EST g921283 (SEQ ID NO:35), and genomic sequence GNFL.g8572864_000002_002.edit (SEQ ID 10 NO:36). Table 1 shows the differential expression of CCM in colon cancer and colon polyps relative to normal colon tissue as determined by microarray analysis. **Differential expression (DE) was considered significant if observed to be at least 2.5-fold in at least one patient and at least 2-fold in a majority of patients.** CCM shows reduced expression in tissues from patients with colon cancer relative to microscopically normal tissue from the same donors 15 (Dn3753, Dn3649, and Dn3647). CCM shows reduced expression in tissue from a patient with colon adenoma relative to microscopically normal tissue from the same donor (Dn3583). CCM also shows reduced expression in tissue from a patient with colon polyps relative to microscopically normal tissue from the same donor (Dn3753). Therefore, the cDNA is useful in diagnostic assays for colon disorders, particularly colon cancer and polyps. A fragment thereof 20 the cDNA from about nucleotide 1 to about nucleotide 50 is also useful in diagnostic assays.

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1. CCM is 784 amino acids in length and has one potential N-glycosylation site at N465; one potential cyclic AMP- or cyclic GMP-dependent protein kinase phosphorylation site at S230; ten potential casein kinase II phosphorylation sites at S3, S31, S38, 25 T132, T137, S239, T484, S635, S691, and S726; and nine potential protein kinase C phosphorylation sites at S141, S171, S226, S333, S407, S429, T501, S508, and S654. MOTIFS analysis indicates that the region of CCM from C16 through H37 is similar to a C2H2 type zinc finger motif. BLOCKS analysis indicates that the region of CCM from C182 through M191 is similar to a C3HC4 zinc finger (RING finger) motif. Pfam analysis indicates that the region of 30 CCM from V152 through K261 is similar to a FYVE-finger domain (E-value = 3.8×10^{-15}). In particular, CCM has the eight conserved cysteine residues at C163, C166, C179, C182, C187, C190, C252, and C255; the RRHHCR consensus sequence at residues R175 through R179; and the conserved glycine at G183 typical of FYVE-finger proteins. Useful antigenic epitopes extend from N156 to M186, N488 to L525, and A680 to A730, and biologically active portions of CCM 35 extend from C16 through H37 and from V152 through K261. An antibody which specifically

binds CCM is useful in an assay to diagnose colon disorders, particularly colon cancer and polyps.

The invention also encompasses CCM variants. A preferred CCM variant is one which has at least about 80%, or at least about 90%, or even at least about 95% amino acid sequence identity to the CCM amino acid sequence, and which contains at least one functional or structural
5 characteristic of CCM.

Mammalian variants of the cDNA encoding CCM were identified using BLAST2 with default parameters and the ZOOSEQ databases (Incyte Genomics). These preferred variants have from about 87% to about 95% identity to human CCM as shown in the table below. The first column shows the SEQ ID for the human cDNA (SEQ ID_H); the second column, the SEQ ID for
10 the variant cDNAs (SEQ ID_{var}); the third column, the clone number for the variant cDNAs (Clone_{var}); the fourth column, the library name; the fifth column, the alignment of the variant cDNA to the human cDNA (includes the alignment of different regions of the variant cDNA with different regions of the human cDNA in some cases); and the sixth column, the percent identity to the human cDNA.

	SEQ ID _H	SEQ ID _{var}	Clone _{var}	Library Name	Nt _H Alignment	Identity
	2	23	702073518T2	RAKITXT09	949-1543	93%
	2	24	702549617T1	RACONON05	994-1544	92%
	2	25	702448734T1	RASPNON05	1093-1544	93%
20	2	26	702081830H1	RABRTXT01	958-1376	94%
	2	27	702053775T1	RAKITXT08	1151-1442	91%
	2	28	700883909H1	RAVANOT01	721-975	90%
	2	29	700883983H1	RAVANOT01	721-970	90%
	2	30	701092129H1	RALUNOT02	1765-1967	88%
25	2	31	702137890H2	RABRFET07	397-613	87%
	2	32	701741076T1	MNBCNON01	6021-6451	95%
	2	33	702769948H2	CNLINOT01	1377-1833	88%
	2	34	702245053H1	CNLUNOT01	3494-3542	93%

30 These cDNAs are particularly useful for producing transgenic cell lines or organisms which model human disorders and upon which potential therapeutic treatments for such disorders may be tested.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of cDNA encoding CCM, some bearing minimal similarity to the
35 cDNAs of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of cDNA that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide encoding naturally occurring CCM, and all such variations are to be considered as being specifically disclosed.

40 The cDNA and fragments thereof (SEQ ID NOs:2-34) may be used in hybridization, amplification, and screening technologies to identify and distinguish among SEQ ID NO:2 and

related molecules in a sample. The mammalian cDNAs may be used to produce transgenic cell lines or organisms which are model systems for human colon disorders, particularly colon cancer and polyps and upon which the toxicity and efficacy of potential therapeutic treatments may be tested. Toxicology studies, clinical trials, and subject/patient treatment profiles may be performed and monitored using the cDNAs, proteins, antibodies and molecules and compounds identified using the cDNAs and proteins of the present invention.

Characterization and Use of the Invention

cDNA libraries

In a particular embodiment disclosed herein, mRNA was isolated from mammalian cells and tissues using methods which are well known to those skilled in the art and used to prepare the cDNA libraries. The Incyte clones listed above were isolated from mammalian cDNA libraries. Three library preparations representative of the invention are described in the EXAMPLES below. The consensus sequences were chemically and/or electronically assembled from fragments including Incyte clones and extension and/or shotgun sequences using computer programs such as PHRAP (P Green, University of Washington, Seattle WA), and AUTOASSEMBLER application (Applied Biosystems, Foster City CA). Clones, extension and/or shotgun sequences are electronically assembled into clusters and/or master clusters.

Sequencing

Methods for sequencing nucleic acids are well known in the art and may be used to practice any of the embodiments of the invention. These methods employ enzymes such as the Klenow fragment of DNA polymerase I, SEQUENASE, Taq DNA polymerase and thermostable T7 DNA polymerase (Amersham Pharmacia Biotech (APB), Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 system (Hamilton, Reno NV) and the DNA ENGINE thermal cycler (MJ Research, Watertown MA). Machines commonly used for sequencing include the ABI PRISM 3700, 377 or 373 DNA sequencing systems (Applied Biosystems), the MEGABACE 1000 DNA sequencing system (APB), and the like. The sequences may be analyzed using a variety of algorithms well known in the art and described in Ausubel *et al.* (1997; Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7) and in Meyers (1995; Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853).

Shotgun sequencing may also be used to complete the sequence of a particular cloned insert of interest. Shotgun strategy involves randomly breaking the original insert into segments of various sizes and cloning these fragments into vectors. The fragments are sequenced and

reassembled using overlapping ends until the entire sequence of the original insert is known. Shotgun sequencing methods are well known in the art and use thermostable DNA polymerases, heat-labile DNA polymerases, and primers chosen from representative regions flanking the cDNAs of interest. Incomplete assembled sequences are inspected for identity using various
5 algorithms or programs such as CONSED (Gordon (1998) Genome Res 8:195-202) which are well known in the art. Contaminating sequences including vector or chimeric sequences or deleted sequences can be removed or restored, respectively, organizing the incomplete assembled sequences into finished sequences.

Extension of a Nucleic Acid Sequence

10 The sequences of the invention may be extended using various PCR-based methods known in the art. For example, the XL-PCR kit (Applied Biosystems), nested primers, and commercially available cDNA or genomic DNA libraries may be used to extend the nucleic acid sequence. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO primer analysis software (Molecular Biology Insights, Cascade CO) to
15 be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to a target molecule at temperatures from about 55C to about 68C. When extending a sequence to recover regulatory elements, it is preferable to use genomic, rather than cDNA libraries.

Hybridization

The cDNA and fragments thereof can be used in hybridization technologies for various
20 purposes. A probe may be designed or derived from unique regions such as the 5' regulatory region or from a nonconserved region (i.e., 5' or 3' of the nucleotides encoding the conserved catalytic domain of the protein) and used in protocols to identify naturally occurring molecules encoding the CCM, allelic variants, or related molecules. The probe may be DNA or RNA, may be single stranded and should have at least 50% sequence identity to any of the nucleic acid
25 sequences, SEQ ID NOs:2-34. Hybridization probes may be produced using oligolabeling, nick translation, end-labeling, or PCR amplification in the presence of a reporter molecule. A vector containing the cDNA or a fragment thereof may be used to produce an mRNA probe in vitro by addition of an RNA polymerase and labeled nucleotides. These procedures may be conducted using commercially available kits such as those provided by APB.

30 The stringency of hybridization is determined by G+C content of the probe, salt concentration, and temperature. In particular, stringency can be increased by reducing the concentration of salt or raising the hybridization temperature. In solutions used for some membrane based hybridizations, addition of an organic solvent such as formamide allows the reaction to occur at a lower temperature. Hybridization can be performed at low stringency with
35 buffers, such as 5xSSC with 1% sodium dodecyl sulfate (SDS) at 60C, which permits the

formation of a hybridization complex between nucleic acid sequences that contain some mismatches. Subsequent washes are performed at higher stringency with buffers such as 0.2xSSC with 0.1% SDS at either 45C (medium stringency) or 68C (high stringency). At high stringency, hybridization complexes will remain stable only where the nucleic acids are completely
5 complementary. In some membrane-based hybridizations, preferably 35% or most preferably 50%, formamide can be added to the hybridization solution to reduce the temperature at which hybridization is performed, and background signals can be reduced by the use of other detergents such as Sarkosyl or TRITON X-100 (Sigma-Aldrich, St. Louis MO) and a blocking agent such as denatured salmon sperm DNA. Selection of components and conditions for hybridization are well
10 known to those skilled in the art and are reviewed in Ausubel (supra) and Sambrook et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY.

Arrays may be prepared and analyzed using methods known in the art. Oligonucleotides may be used as either probes or targets in an array. The array can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants,
15 mutations, and single nucleotide polymorphisms. Such information may be used to determine gene function; to understand the genetic basis of a condition, disease, or disorder; to diagnose a condition, disease, or disorder; and to develop and monitor the activities of therapeutic agents. (See, e.g., Brennan et al. (1995) USPN 5,474,796; Schena et al. (1996) Proc Natl Acad Sci 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon et al. (1995) PCT application WO95/35505; Heller et al. (1997) Proc Natl Acad Sci 94:2150-2155; and
20 Heller et al. (1997) USPN 5,605,662.)

Hybridization probes are also useful in mapping the naturally occurring genomic sequence. The probes may be hybridized to: 1) a particular chromosome, 2) a specific region of a chromosome, or 3) an artificial chromosome construction such as human artificial chromosome
25 (HAC), yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), bacterial P1 construction, or single chromosome cDNA libraries.

Expression

Any one of a multitude of cDNAs encoding CCM may be cloned into a vector and used to express the protein, or portions thereof, in host cells. The nucleic acid sequence can be
30 engineered by such methods as DNA shuffling (USPN 5,830,721) and site-directed mutagenesis to create new restriction sites, alter glycosylation patterns, change codon preference to increase expression in a particular host, produce splice variants, extend half-life, and the like. The expression vector may contain transcriptional and translational control elements (promoters, enhancers, specific initiation signals, and polyadenylated 3' sequence) from various sources which
35 have been selected for their efficiency in a particular host. The vector, cDNA, and regulatory

elements are combined using in vitro recombinant DNA techniques, synthetic techniques, and/or in vivo genetic recombination techniques well known in the art and described in Sambrook (supra, ch. 4, 8, 16 and 17).

A variety of host systems may be transformed with an expression vector. These include, but are not limited to, bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems transformed with baculovirus expression vectors; plant cell systems transformed with expression vectors containing viral and/or bacterial elements, or animal cell systems (Ausubel supra, unit 16). For example, an adenovirus transcription/translation complex may be utilized in mammalian cells. After sequences are ligated into the E1 or E3 region of the viral genome, the infective virus is used to transform and express the protein in host cells. The Rous sarcoma virus enhancer or SV40 or EBV-based vectors may also be used for high-level protein expression.

Routine cloning, subcloning, and propagation of nucleic acid sequences can be achieved using the multifunctional PBLUESCRIPT vector (Stratagene, La Jolla CA) or PSFORT1 plasmid (Life Technologies). Introduction of a nucleic acid sequence into the multiple cloning site of these vectors disrupts the lacZ gene and allows colorimetric screening for transformed bacteria. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence.

For long term production of recombinant proteins, the vector can be stably transformed into cell lines along with a selectable or visible marker gene on the same or on a separate vector. After transformation, cells are allowed to grow for about 1 to 2 days in enriched media and then are transferred to selective media. Selectable markers, antimetabolite, antibiotic, or herbicide resistance genes, confer resistance to the relevant selective agent and allow growth and recovery of cells which successfully express the introduced sequences. Resistant clones identified either by survival on selective media or by the expression of visible markers, such as anthocyanins, green fluorescent protein (GFP), β glucuronidase, luciferase and the like, may be propagated using culture techniques. Visible markers are also used to quantify the amount of protein expressed by the introduced genes. Verification that the host cell contains the desired cDNA is based on DNA-DNA or DNA-RNA hybridizations or PCR amplification techniques.

The host cell may be chosen for its ability to modify a recombinant protein in a desired fashion. Such modifications include acetylation, carboxylation, glycosylation, phosphorylation, lipidation, acylation and the like. Post-translational processing which cleaves a "prepro" form may also be used to specify protein targeting, folding, and/or activity. Different host cells available from the ATCC (Manassas VA) which have specific cellular machinery and characteristic mechanisms for post-translational activities may be chosen to ensure the correct

modification and processing of the recombinant protein.

Recovery of Proteins from Cell Culture

Heterologous moieties engineered into a vector for ease of purification include glutathione S-transferase (GST), 6xHis, FLAG, MYC, and the like. GST and 6-His are purified
5 using commercially available affinity matrices such as immobilized glutathione and metal-chelate resins, respectively. FLAG and MYC are purified using commercially available monoclonal and polyclonal antibodies. For ease of separation following purification, a sequence encoding a proteolytic cleavage site may be part of the vector located between the protein and the heterologous moiety. Methods for recombinant protein expression and purification are discussed
10 in Ausubel (supra, unit 16) and are commercially available.

Chemical Synthesis of Peptides

Proteins or portions thereof may be produced not only by recombinant methods, but also by using chemical methods well known in the art. Solid phase peptide synthesis may be carried out in a batchwise or continuous flow process which sequentially adds α -amino- and side chain-
15 protected amino acid residues to an insoluble polymeric support via a linker group. A linker group such as methylamine-derivatized polyethylene glycol is attached to poly(styrene-co-divinylbenzene) to form the support resin. The amino acid residues are N- α -protected by acid labile Boc (t-butyloxycarbonyl) or base-labile Fmoc (9-fluorenylmethoxycarbonyl). The carboxyl group of the protected amino acid is coupled to the amine of the linker group to anchor the residue
20 to the solid phase support resin. Trifluoroacetic acid or piperidine are used to remove the protecting group in the case of Boc or Fmoc, respectively. Each additional amino acid is added to the anchored residue using a coupling agent or pre-activated amino acid derivative, and the resin is washed. The full length peptide is synthesized by sequential deprotection, coupling of derivitized amino acids, and washing with dichloromethane and/or N, N-dimethylformamide. The
25 peptide is cleaved between the peptide carboxy terminus and the linker group to yield a peptide acid or amide. (Novabiochem 1997/98 Catalog and Peptide Synthesis Handbook, San Diego CA pp. S1-S20). Automated synthesis may also be carried out on machines such as the ABI 431A peptide synthesizer (Applied Biosystems). A protein or portion thereof may be substantially purified by preparative high performance liquid chromatography and its composition confirmed by
30 amino acid analysis or by sequencing (Creighton (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY).

Preparation and Screening of Antibodies

Various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with CCM or any portion thereof. Adjuvants such as Freund's, mineral gels, and
35 surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil

emulsions, keyhole limpet hemacyanin (KLH), and dinitrophenol may be used to increase immunological response. The oligopeptide, peptide, or portion of protein used to induce antibodies should consist of at least about five amino acids, more preferably ten amino acids, which are identical to a portion of the natural protein. Oligopeptides may be fused with proteins
5 such as KLH in order to produce antibodies to the chimeric molecule.

Monoclonal antibodies may be prepared using any technique which provides for the production of antibodies by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler *et al.* (1975) *Nature* 256:495-497; Kozbor *et al.* (1985) *J. Immunol*
10 *Methods* 81:31-42; Cote *et al.* (1983) *Proc Natl Acad Sci* 80:2026-2030; and Cole *et al.* (1984) *Mol Cell Biol* 62:109-120.)

Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce epitope specific single chain antibodies. Antibody fragments which contain specific binding sites for epitopes of the protein may also be
15 generated. For example, such fragments include, but are not limited to, F(ab')₂ fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse *et al.* (1989) *Science* 246:1275-1281.)

20 The CCM or a portion thereof may be used in screening assays of phagemid or B-lymphocyte immunoglobulin libraries to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoassays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between the protein and
25 its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes is preferred, but a competitive binding assay may also be employed (Pound (1998) *Immunochemical Protocols*, Humana Press, Totowa NJ).

Labeling of Molecules for Assay

A wide variety of reporter molecules and conjugation techniques are known by those
30 skilled in the art and may be used in various nucleic acid, amino acid, and antibody assays. Synthesis of labeled molecules may be achieved using commercially available kits (Promega, Madison WI) for incorporation of a labeled nucleotide such as ³²P-dCTP (APB), Cy3-dCTP or Cy5-dCTP (Operon Technologies, Alameda CA), or amino acid such as ³⁵S-methionine (APB). Nucleotides and amino acids may be directly labeled with a variety of substances including
35 fluorescent, chemiluminescent, or chromogenic agents, and the like, by chemical conjugation to

amines, thiols and other groups present in the molecules using reagents such as BIODIPY or FITC (Molecular Probes, Eugene OR).

DIAGNOSTICS

The cDNAs, fragments, oligonucleotides, complementary RNA and DNA molecules, and
5 PNAs and may be used to detect and quantify differential gene expression, absence/presence vs. excess, expression of mRNAs or to monitor mRNA levels during therapeutic intervention. Similarly antibodies which specifically bind CCM may be used to quantitate the protein. Disorders associated with differential expression include colon disorders, particularly colon cancer and polyps. The diagnostic assay may use hybridization or amplification technology to
10 compare gene expression in a biological sample from a patient to standard samples in order to detect differential gene expression. Qualitative or quantitative methods for this comparison are well known in the art.

For example, the cDNA or probe may be labeled by standard methods and added to a biological sample from a patient under conditions for the formation of hybridization complexes.
15 After an incubation period, the sample is washed and the amount of label (or signal) associated with hybridization complexes, is quantified and compared with a standard value. If complex formation in the patient sample is significantly altered (higher or lower) in comparison to either a normal or disease standard, then differential expression indicates the presence of a disorder.

In order to provide standards for establishing differential expression, normal and disease
20 expression profiles are established. This is accomplished by combining a sample taken from normal subjects, either animal or human, with a cDNA under conditions for hybridization to occur. Standard hybridization complexes may be quantified by comparing the values obtained using normal subjects with values from an experiment in which a known amount of a substantially purified sequence is used. Standard values obtained in this manner may be compared with values
25 obtained from samples from patients who were diagnosed with a particular condition, disease, or disorder. Deviation from standard values toward those associated with a particular disorder is used to diagnose that disorder.

Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies and in clinical trial or to monitor the treatment of an individual patient.
30 Once the presence of a condition is established and a treatment protocol is initiated, diagnostic assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in a normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

35 Immunological Methods

Detection and quantification of a protein using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies
5 reactive to two non-interfering epitopes is preferred, but a competitive binding assay may be employed. (See, e.g., Coligan *et al.* (1997) *Current Protocols in Immunology*, Wiley-Interscience, New York NY; and Pound, *supra*).

THERAPEUTICS

Regions of CCM (SEQ ID NO:1) show chemical and structural similarity to a FYVE-
10 finger domain-containing protein. In addition, differential expression of CCM is highly associated with colon disorders, particularly colon cancer and polyps, as shown in Table 1. CCM clearly plays a role in colon disorders, particularly colon cancer and polyps.

In the treatment of conditions associated with increased expression of CCM, it is desirable to decrease expression or protein activity. In one embodiment, the an inhibitor, antagonist or
15 antibody of the protein may be administered to a subject to treat a condition associated with increased expression or activity. In another embodiment, a pharmaceutical composition comprising an inhibitor, antagonist or antibody in conjunction with a pharmaceutical carrier may be administered to a subject to treat a condition associated with the increased expression or activity of the endogenous protein. In an additional embodiment, a vector expressing the
20 complement of the cDNA or fragments thereof may be administered to a subject to treat the disorder.

In the treatment of conditions associated with decreased expression of CCM, it is desirable to increase expression or protein activity. In one embodiment, the protein, an agonist or enhancer may be administered to a subject to treat a condition associated with decreased
25 expression or activity. In another embodiment, a pharmaceutical composition comprising the protein, an agonist or enhancer in conjunction with a pharmaceutical carrier may be administered to a subject to treat a condition associated with the decreased expression or activity of the endogenous protein. In an additional embodiment, a vector expressing cDNA may be administered to a subject to treat the disorder.

30 Any of the cDNAs, complementary molecules, or fragments thereof, proteins or portions thereof, vectors delivering these nucleic acid molecules or expressing the proteins, and their ligands may be administered in combination with other therapeutic agents. Selection of the agents for use in combination therapy may be made by one of ordinary skill in the art according to conventional pharmaceutical principles. A combination of therapeutic agents may act
35 synergistically to affect treatment of a particular disorder at a lower dosage of each agent.

Modification of Gene Expression Using Nucleic Acids

Gene expression may be modified by designing complementary or antisense molecules (DNA, RNA, or PNA) to the control, 5', 3', or other regulatory regions of the gene encoding CCM. Oligonucleotides designed with reference to the transcription initiation site are preferred.

- 5 Similarly, inhibition can be achieved using triple helix base-pairing which inhibits the binding of polymerases, transcription factors, or regulatory molecules (Gee *et al.* In: Huber and Carr (1994) Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177). A complementary molecule may also be designed to block translation by preventing binding between ribosomes and mRNA. In one alternative, a library or plurality of cDNAs or fragments thereof
- 10 may be screened to identify those which specifically bind a regulatory, nontranslated sequence.

- Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA followed by endonucleolytic cleavage at sites such as GUA, GUU, and GUC. Once such sites are identified, an oligonucleotide with the same
- 15 sequence may be evaluated for secondary structural features which would render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing their hybridization with complementary oligonucleotides using ribonuclease protection assays.

- Complementary nucleic acids and ribozymes of the invention may be prepared via recombinant expression, *in vitro* or *in vivo*, or using solid phase phosphoramidite chemical
- 20 synthesis. In addition, RNA molecules may be modified to increase intracellular stability and half-life by addition of flanking sequences at the 5' and/or 3' ends of the molecule or by the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. Modification is inherent in the production of PNAs and can be extended to other nucleic acid molecules. Either the inclusion of nontraditional bases such as inosine, queosine, and
- 25 wybutosine, and or the modification of adenine, cytidine, guanine, thymine, and uridine with acetyl-, methyl-, thio- groups renders the molecule less available to endogenous endonucleases.

Screening and Purification Assays

- The cDNA encoding CCM may be used to screen a library of molecules or compounds for specific binding affinity. The libraries may be aptamers, DNA molecules, RNA molecules, PNAs,
- 30 peptides, proteins such as transcription factors, enhancers, repressors, and other ligands which regulate the activity, replication, transcription, or translation of the cDNA in the biological system. The assay involves combining the cDNA or a fragment thereof with the library of molecules under conditions allowing specific binding, and detecting specific binding to identify at least one molecule which specifically binds the single stranded or, if appropriate, double stranded
- 35 molecule.

In one embodiment, the cDNA of the invention may be incubated with a plurality of purified molecules or compounds and binding activity determined by methods well known in the art, e.g., a gel-retardation assay (USPN 6,010,849) or a reticulocyte lysate transcriptional assay. In another embodiment, the cDNA may be incubated with nuclear extracts from biopsied and/or cultured cells and tissues. Specific binding between the cDNA and a molecule or compound in the nuclear extract is initially determined by gel shift assay and may be later confirmed by recovering and raising antibodies against that molecule or compound. When these antibodies are added into the assay, they cause a supershift in the gel-retardation assay.

In another embodiment, the cDNA may be used to purify a molecule or compound using affinity chromatography methods well known in the art. In one embodiment, the cDNA is chemically reacted with cyanogen bromide groups on a polymeric resin or gel. Then a sample is passed over and reacts with or binds to the cDNA. The molecule or compound which is bound to the cDNA may be released from the cDNA by increasing the salt concentration of the flow-through medium and collected.

In a further embodiment, the protein or a portion thereof may be used to purify a ligand from a sample. A method for using a protein or a portion thereof to purify a ligand would involve combining the protein or a portion thereof with a sample under conditions to allow specific binding, detecting specific binding between the protein and ligand, recovering the bound protein, and using an appropriate chaotropic agent to separate the protein from the purified ligand.

In a preferred embodiment, CCM or a portion thereof may be used to screen a plurality of molecules or compounds in any of a variety of screening assays. The portion of the protein employed in such screening may be free in solution, affixed to an abiotic or biotic substrate (e.g. borne on a cell surface), or located intracellularly. For example, in one method, viable or fixed prokaryotic host cells that are stably transformed with recombinant nucleic acids that have expressed and positioned a peptide on their cell surface can be used in screening assays. The cells are screened against a plurality or libraries of ligands and the specificity of binding or formation of complexes between the expressed protein and the ligand may be measured. Specific binding between the protein and molecule may be measured. Depending on the kind of library being screened, the assay may be used to identify DNA molecules, RNA molecules, peptide nucleic acids, peptides, proteins, mimetics, agonists, antagonists, antibodies, immunoglobulins, inhibitors, and drugs or any other ligand, which specifically binds the protein.

In one aspect, this invention contemplates a method for high throughput screening using very small assay volumes and very small amounts of test compound as described in USPN 5,876,946, incorporated herein by reference. This method is used to screen large numbers of molecules and compounds via specific binding. In another aspect, this invention also

contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding the protein specifically compete with a test compound capable of binding to the protein or oligopeptide or portion thereof. Molecules or compounds identified by screening may be used in a mammalian model system to evaluate their toxicity, diagnostic, or therapeutic potential.

Pharmacology

Pharmaceutical compositions are those substances wherein the active ingredients are contained in an effective amount to achieve a desired and intended purpose. The determination of an effective dose is well within the capability of those skilled in the art. For any compound, the therapeutically effective dose may be estimated initially either in cell culture assays or in animal models. The animal model is also used to achieve a desirable concentration range and route of administration. Such information may then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of protein or inhibitor which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity of such agents may be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED₅₀ (the dose therapeutically effective in 50% of the population) and LD₅₀ (the dose lethal to 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it may be expressed as the ratio, LD₅₀/ED₅₀. Pharmaceutical compositions which exhibit large therapeutic indexes are preferred. The data obtained from cell culture assays and animal studies are used in formulating a range of dosage for human use.

Model Systems

Animal models may be used as bioassays where they exhibit a phenotypic response similar to that of humans and where exposure conditions are relevant to human exposures. Mammals are the most common models, and most infectious agent, cancer, drug, and toxicity studies are performed on rodents such as rats or mice because of low cost, availability, lifespan, reproductive potential, and abundant reference literature. Inbred and outbred rodent strains provide a convenient model for investigation of the physiological consequences of under- or over-expression of genes of interest and for the development of methods for diagnosis and treatment of diseases. A mammal inbred to over-express a particular gene (for example, secreted in milk) may also serve as a convenient source of the protein expressed by that gene.

Toxicology

Toxicology is the study of the effects of agents on living systems. The majority of toxicity studies are performed on rats or mice. Observation of qualitative and quantitative changes in physiology, behavior, homeostatic processes, and lethality in the rats or mice are used

to generate a toxicity profile and to assess potential consequences on human health following exposure to the agent.

Genetic toxicology identifies and analyzes the effect of an agent on the rate of endogenous, spontaneous, and induced genetic mutations. Genotoxic agents usually have common chemical or physical properties that facilitate interaction with nucleic acids and are most harmful when chromosomal aberrations are transmitted to progeny. Toxicological studies may identify agents that increase the frequency of structural or functional abnormalities in the tissues of the progeny if administered to either parent before conception, to the mother during pregnancy, or to the developing organism. Mice and rats are most frequently used in these tests because their short reproductive cycle allows the production of the numbers of organisms needed to satisfy statistical requirements.

Acute toxicity tests are based on a single administration of an agent to the subject to determine the symptomology or lethality of the agent. Three experiments are conducted: 1) an initial dose-range-finding experiment, 2) an experiment to narrow the range of effective doses, and 3) a final experiment for establishing the dose-response curve.

Subchronic toxicity tests are based on the repeated administration of an agent. Rat and dog are commonly used in these studies to provide data from species in different families. With the exception of carcinogenesis, there is considerable evidence that daily administration of an agent at high-dose concentrations for periods of three to four months will reveal most forms of toxicity in adult animals.

Chronic toxicity tests, with a duration of a year or more, are used to demonstrate either the absence of toxicity or the carcinogenic potential of an agent. When studies are conducted on rats, a minimum of three test groups plus one control group are used, and animals are examined and monitored at the outset and at intervals throughout the experiment.

Transgenic Animal Models

Transgenic rodents that over-express or under-express a gene of interest may be inbred and used to model human diseases or to test therapeutic or toxic agents. (See, e.g., USPN 5,175,383 and USPN 5,767,337.) In some cases, the introduced gene may be activated at a specific time in a specific tissue type during fetal or postnatal development. Expression of the transgene is monitored by analysis of phenotype, of tissue-specific mRNA expression, or of serum and tissue protein levels in transgenic animals before, during, and after challenge with experimental drug therapies.

Embryonic Stem Cells

Embryonic (ES) stem cells isolated from rodent embryos retain the potential to form embryonic tissues. When ES cells are placed inside a carrier embryo, they resume normal

development and contribute to tissues of the live-born animal. ES cells are the preferred cells used in the creation of experimental knockout and knockin rodent strains. Mouse ES cells, such as the mouse 129/SvJ cell line, are derived from the early mouse embryo and are grown under culture conditions well known in the art. Vectors used to produce a transgenic strain contain a disease gene candidate and a marker gen, the latter serves to identify the presence of the introduced disease gene. The vector is transformed into ES cells by methods well known in the art, and transformed ES cells are identified and microinjected into mouse cell blastocysts such as those from the C57BL/6 mouse strain. The blastocysts are surgically transferred to pseudopregnant dams, and the resulting chimeric progeny are genotyped and bred to produce heterozygous or homozygous strains.

ES cells derived from human blastocysts may be manipulated in vitro to differentiate into at least eight separate cell lineages. These lineages are used to study the differentiation of various cell types and tissues in vitro, and they include endoderm, mesoderm, and ectodermal cell types which differentiate into, for example, neural cells, hematopoietic lineages, and cardiomyocytes.

15 Knockout Analysis

In gene knockout analysis, a region of a mammalian gene is enzymatically modified to include a non-mammalian gene such as the neomycin phosphotransferase gene (neo; Capecchi (1989) Science 244:1288-1292). The modified gene is transformed into cultured ES cells and integrates into the endogenous genome by homologous recombination. The inserted sequence disrupts transcription and translation of the endogenous gene. Transformed cells are injected into rodent blastulae, and the blastulae are implanted into pseudopregnant dams. Transgenic progeny are crossbred to obtain homozygous inbred lines which lack a functional copy of the mammalian gene. In one example, the mammalian gene is a human gene.

Knockin Analysis

ES cells can be used to create knockin humanized animals (pigs) or transgenic animal models (mice or rats) of human diseases. With knockin technology, a region of a human gene is injected into animal ES cells, and the human sequence integrates into the animal cell genome. Transformed cells are injected into blastulae and the blastulae are implanted as described above. Transgenic progeny or inbred lines are studied and treated with potential pharmaceutical agents to obtain information on treatment of the analogous human condition. These methods have been used to model several human diseases.

Non-Human Primate Model

The field of animal testing deals with data and methodology from basic sciences such as physiology, genetics, chemistry, pharmacology and statistics. These data are paramount in evaluating the effects of therapeutic agents on non-human primates as they can be related to

human health. Monkeys are used as human surrogates in vaccine and drug evaluations, and their responses are relevant to human exposures under similar conditions. Cynomolgus and Rhesus monkeys (Macaca fascicularis and Macaca mulatta, respectively) and Common Marmosets (Callithrix jacchus) are the most common non-human primates (NHPs) used in these investigations. Since great cost is associated with developing and maintaining a colony of NHPs, early research and toxicological studies are usually carried out in rodent models. In studies using behavioral measures such as drug addiction, NHPs are the first choice test animal. In addition, NHPs and individual humans exhibit differential sensitivities to many drugs and toxins and can be classified as a range of phenotypes from “extensive metabolizers” to “poor metabolizers” of these agents.

In additional embodiments, the cDNAs which encode the protein may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of cDNAs that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

EXAMPLES

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention. For purposes of example, preparation of the human brain posterior parietal cortex (BRAENOT02) and normalized mammary epithelial cell (EPIMNON05) libraries will be described.

I cDNA Library Construction

Brain Posterior Parietal Cortex Library

The BRAENOT02 cDNA library was constructed from brain posterior parietal cortex tissue removed from the brain of a 35-year-old Caucasian male. The frozen tissue was homogenized and lysed in TRIZOL reagent (0.8g tissue/12 ml; Life Technologies) using a POLYTRON homogenizer (Brinkmann Instruments, Westbury NJ). After brief incubation on ice, chloroform was added (1:5 v/v), and the mixture was centrifuged to separate the phases. The upper aqueous phase was removed to a fresh tube, and isopropanol was added to precipitate RNA. The RNA was resuspended in RNase-free water and treated with DNase. The RNA was re-extracted with acid phenol-chloroform and reprecipitated with sodium acetate and ethanol. Poly(A+) RNA was isolated using the OLIGOTEX kit (Qiagen, Chatsworth CA).

The mRNA was handled according to the recommended protocols in the SUPERScript plasmid system (Life Technologies) which contains a NotI primer-adaptor designed to prime the first strand cDNA synthesis at the poly(A) tail of mRNAs. Double stranded cDNA was blunted, ligated to EcoRI adaptors and digested with NotI (New England Biolabs, Beverly MA). The cDNAs were fractionated on a SEPHAROSE CL4B column (APB), and those cDNAs exceeding

400 bp were ligated into pINCY plasmid (Incyte Genomics). The plasmid pINCY was subsequently transformed into DH5 α competent cells (Life Technologies).

Normalized Mammary Epithelial Cell Library

For purposes of example, the normalization of the mammary epithelial cell library (EPIMNON05) is described. About 3.28×10^6 independent clones of the EPIMNOT01 plasmid library in *E. coli* strain DH12S competent cells (Life Technologies) were grown in liquid culture under carbenicillin (25 mg/l) and methicillin (1 mg/ml) selection following transformation by electroporation. To reduce the number of excess cDNA copies according to their abundance levels in the library, the cDNA library was normalized in a single round according to the procedure of Soares *et al.* (1994, Proc Natl Acad Sci 91:9228-9232), with the following modifications. The primer to template ratio in the primer extension reaction was increased from 2:1 to 10:1. The dNTP concentration in the reaction was reduced to 150 μ M for each dNTP to allow the generation of longer (400 to 1000 nt) primer extension products. The reannealing hybridization was extended from 13 to 48 hr. The single stranded DNA circles of the normalized library were purified by hydroxyapatite chromatography and converted to partially double-stranded by random priming, ligated into pINCY plasmid and electroporated into DH12S competent cells (Life Technologies).

II Construction of pINCY Plasmid

The plasmid was constructed by digesting the pSPORT1 plasmid (Life Technologies) with EcoRI restriction enzyme (New England Biolabs, Beverly MA) and filling the overhanging ends using Klenow enzyme (New England Biolabs) and 2'-deoxynucleotide 5'-triphosphates (dNTPs). The plasmid was self-ligated and transformed into the bacterial host, *E. coli* strain JM109.

An intermediate plasmid produced by the bacteria (pSPORT 1- Δ RI) showed no digestion with EcoRI and was digested with Hind III (New England Biolabs) and the overhanging ends were again filled in with Klenow and dNTPs. A linker sequence was phosphorylated, ligated onto the 5' blunt end, digested with EcoRI, and self-ligated. Following transformation into JM109 host cells, plasmids were isolated and tested for preferential digestibility with EcoRI, but not with Hind III. A single colony that met this criteria was designated pINCY plasmid.

After testing the plasmid for its ability to incorporate cDNAs from a library prepared using NotI and EcoRI restriction enzymes, several clones were sequenced; and a single clone containing an insert of approximately 0.8 kb was selected from which to prepare a large quantity of the plasmid. After digestion with NotI and EcoRI, the plasmid was isolated on an agarose gel and purified using a QIAQUICK column (Qiagen) for use in library construction.

III Isolation and Sequencing of cDNA Clones

Plasmid DNA was released from the cells and purified using either the MINIPREP kit (Edge Biosystems, Gaithersburg MD) or the REAL PREP 96 plasmid kit (Qiagen). This kit consists of a 96-well block with reagents for 960 purifications. The recommended protocol was employed except for the following changes: 1) the bacteria were cultured in 1 ml of sterile
5 TERRIFIC BROTH (BD Biosciences, Sparks MD) with carbenicillin at 25 mg/l and glycerol at 0.4%; 2) after inoculation, the cells were cultured for 19 hours and then lysed with 0.3 ml of lysis buffer; and 3) following isopropanol precipitation, the plasmid DNA pellet was resuspended in 0.1 ml of distilled water. After the last step in the protocol, samples were transferred to a 96-well block for storage at 4C.

10 The cDNAs were prepared for sequencing using the MICROLAB 2200 system (Hamilton) in combination with the DNA ENGINE thermal cyclers (MJ Research). The cDNAs were sequenced by the method of Sanger and Coulson (1975; J Mol Biol 94:441-448) using an ABI PRISM 377 sequencing system (Applied Biosystems) or the MEGABACE 1000 DNA sequencing system (APB). Most of the isolates were sequenced according to standard ABI protocols and kits
15 (Applied Biosystems) with solution volumes of 0.25x-1.0x concentrations. In the alternative, cDNAs were sequenced using solutions and dyes from APB.

IV Extension of cDNA Sequences

The cDNAs were extended using the cDNA clone and oligonucleotide primers. One primer was synthesized to initiate 5' extension of the known fragment, and the other, to initiate 3'
20 extension of the known fragment. The initial primers were designed using OLIGO primer analysis software (Molecular Biology Insights), to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68C to about 72C. Any stretch of nucleotides that would result in hairpin structures and primer-primer dimerizations was avoided.

25 Selected cDNA libraries were used as templates to extend the sequence. If more than one extension was necessary, additional or nested sets of primers were designed. Preferred libraries have been size-selected to include larger cDNAs and random primed to contain more sequences with 5' or upstream regions of genes. Genomic libraries are used to obtain regulatory elements, especially extension into the 5' promoter binding region.

30 High fidelity amplification was obtained by PCR using methods such as that taught in USPN 5,932,451. PCR was performed in 96-well plates using the DNA ENGINE thermal cycler (MJ Research). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing Mg^{2+} , $(NH_4)_2SO_4$, and β -mercaptoethanol, Taq DNA polymerase (APB), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the
35 following parameters for primer pair PCI A and PCI B (Incyte Genomics): Step 1: 94C, three min;

Step 2: 94C, 15 sec; Step 3: 60C, one min; Step 4: 68C, two min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68C, five min; Step 7: storage at 4C. In the alternative, the parameters for primer pair T7 and SK+ (Stratagene) were as follows: Step 1: 94C, three min; Step 2: 94C, 15 sec; Step 3: 57C, one min; Step 4: 68C, two min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68C, five min; Step 7: storage at 4C.

The concentration of DNA in each well was determined by dispensing 100 μ l PICOGREEN quantitation reagent (0.25% reagent in 1x TE, v/v; Molecular Probes) and 0.5 μ l of undiluted PCR product into each well of an opaque fluorimeter plate (Corning, Acton MA) and allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5 μ l to 10 μ l aliquot of the reaction mixture was analyzed by electrophoresis on a 1% agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended clones were desalted, concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC18 vector (APB). For shotgun sequences, the digested nucleotide sequences were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and the agar was digested with AGARACE enzyme (Promega). Extended clones were religated using T4 DNA ligase (New England Biolabs) into pUC18 vector (APB), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into *E. coli* competent cells. Transformed cells were selected on antibiotic-containing media, and individual colonies were picked and cultured overnight at 37C in 384-well plates in LB/2x carbenicillin liquid media.

The cells were lysed, and DNA was amplified using primers, Taq DNA polymerase (APB) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94C, three min; Step 2: 94C, 15 sec; Step 3: 60C, one min; Step 4: 72C, two min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72C, five min; Step 7: storage at 4C. DNA was quantified using PICOGREEN quantitative reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the conditions described above. Samples were diluted with 20% dimethylsulfoxide (DMSO; 1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT cycle sequencing kit (APB) or the ABI PRISM BIGDYE terminator cycle sequencing kit (Applied Biosystems).

V Homology Searching of cDNA Clones and Their Deduced Proteins

The cDNAs of the Sequence Listing or their deduced amino acid sequences were used to query databases such as GenBank, SwissProt, BLOCKS, and the like. These databases that contain previously identified and annotated sequences or domains were searched using BLAST or

BLAST 2 (Altschul et al. supra; Altschul, supra) to produce alignments and to determine which sequences were exact matches or homologs. The alignments were to sequences of prokaryotic (bacterial) or eukaryotic (animal, fungal, or plant) origin. Alternatively, algorithms such as the one described in Smith and Smith (1992, Protein Engineering 5:35-51) could have been used to
5 deal with primary sequence patterns and secondary structure gap penalties. All of the sequences disclosed in this application have lengths of at least 49 nucleotides, and no more than 12% uncalled bases (where N is recorded rather than A, C, G, or T).

As detailed in Karlin (supra), BLAST matches between a query sequence and a database sequence were evaluated statistically and only reported when they satisfied the threshold of 10^{-25}
10 for nucleotides and 10^{-14} for peptides. Homology was also evaluated by product score calculated as follows: the % nucleotide or amino acid identity [between the query and reference sequences] in BLAST is multiplied by the % maximum possible BLAST score [based on the lengths of query and reference sequences] and then divided by 100. In comparison with hybridization procedures used in the laboratory, the electronic stringency for an exact match was set at 70, and the
15 conservative lower limit for an exact match was set at approximately 40 (with 1-2% error due to uncalled bases).

The BLAST software suite, freely available sequence comparison algorithms (NCBI, Bethesda MD; <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>), includes various sequence analysis programs including "blastn" that is used to align nucleic acid molecules and BLAST 2 that is used
20 for direct pairwise comparison of either nucleic or amino acid molecules. BLAST programs are commonly used with gap and other parameters set to default settings, e.g.: Matrix: BLOSUM62; Reward for match: 1; Penalty for mismatch: -2; Open Gap: 5 and Extension Gap: 2 penalties; Gap x drop-off: 50; Expect: 10; Word Size: 11; and Filter: on. Identity is measured over the entire length of a sequence or some smaller portion thereof. Brenner et al. (1998; Proc Natl Acad Sci
25 95:6073-6078, incorporated herein by reference) analyzed the BLAST for its ability to identify structural homologs by sequence identity and found 30% identity is a reliable threshold for sequence alignments of at least 150 residues and 40%, for alignments of at least 70 residues.

The cDNAs of this application were compared with assembled consensus sequences or templates found in the LIFESEQ GOLD database. Component sequences from cDNA, extension,
30 full length, and shotgun sequencing projects were subjected to PHRED analysis and assigned a quality score. All sequences with an acceptable quality score were subjected to various pre-processing and editing pathways to remove low quality 3' ends, vector and linker sequences, polyA tails, Alu repeats, mitochondrial and ribosomal sequences, and bacterial contamination sequences. Edited sequences had to be at least 50 bp in length, and low-information sequences
35 and repetitive elements such as dinucleotide repeats, Alu repeats, and the like, were replaced by

“Ns” or masked.

Edited sequences were subjected to assembly procedures in which the sequences were assigned to gene bins. Each sequence could only belong to one bin, and sequences in each bin were assembled to produce a template. Newly sequenced components were added to existing bins using BLAST and CROSSMATCH. To be added to a bin, the component sequences had to have a BLAST quality score greater than or equal to 150 and an alignment of at least 82% local identity. The sequences in each bin were assembled using PHRAP. Bins with several overlapping component sequences were assembled using DEEP PHRAP. The orientation of each template was determined based on the number and orientation of its component sequences.

Bins were compared to one another and those having local similarity of at least 82% were combined and reassembled. Bins having templates with less than 95% local identity were split. Templates were subjected to analysis by STITCHER/EXON MAPPER algorithms that analyze the probabilities of the presence of splice variants, alternatively spliced exons, splice junctions, differential expression of alternative spliced genes across tissue types or disease states, and the like. Assembly procedures were repeated periodically, and templates were annotated using BLAST against GenBank databases such as GBpri. An exact match was defined as having from 95% local identity over 200 base pairs through 100% local identity over 100 base pairs and a homolog match as having an E-value (or probability score) of $\leq 1 \times 10^{-8}$. The templates were also subjected to frameshift FASTx against GENPEPT, and homolog match was defined as having an E-value of $\leq 1 \times 10^{-8}$. Template analysis and assembly was described in USSN 09/276,534, filed March 25, 1999.

Following assembly, templates were subjected to BLAST, motif, and other functional analyses and categorized in protein hierarchies using methods described in USSN 08/812,290 and USSN 08/811,758, both filed March 6, 1997; in USSN 08/947,845, filed October 9, 1997; and in USSN 09/034,807, filed March 4, 1998. Then templates were analyzed by translating each template in all three forward reading frames and searching each translation against the PFAM database of hidden Markov model-based protein families and domains using the HMMER software package (Washington University School of Medicine, St. Louis MO; <http://pfam.wustl.edu/>). The cDNA was further analyzed using MACDNASIS PRO software (Hitachi Software Engineering), and LASERGENE software (DNASTAR) and queried against public databases such as the GenBank rodent, mammalian, vertebrate, prokaryote, and eukaryote databases, SwissProt, BLOCKS, PRINTS, PFAM, and Prosite.

VI Chromosome Mapping

Radiation hybrid and genetic mapping data available from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for Genome Research (WIGR),

and Généthon are used to determine if any of the cDNAs presented in the Sequence Listing have been mapped. Any of the fragments of the cDNA encoding CCM that have been mapped result in the assignment of all related regulatory and coding sequences mapping to the same location. The genetic map locations are described as ranges, or intervals, of human chromosomes. The map position of an interval, in cM (which is roughly equivalent to 1 megabase of human DNA), is measured relative to the terminus of the chromosomal p-arm.

VII Hybridization Technologies and Analyses

Immobilization of cDNAs on a Substrate

The cDNAs are applied to a substrate by one of the following methods. A mixture of cDNAs is fractionated by gel electrophoresis and transferred to a nylon membrane by capillary transfer. Alternatively, the cDNAs are individually ligated to a vector and inserted into bacterial host cells to form a library. The cDNAs are then arranged on a substrate by one of the following methods. In the first method, bacterial cells containing individual clones are robotically picked and arranged on a nylon membrane. The membrane is placed on LB agar containing selective agent (carbenicillin, kanamycin, ampicillin, or chloramphenicol depending on the vector used) and incubated at 37C for 16 hr. The membrane is removed from the agar and consecutively placed colony side up in 10% SDS, denaturing solution (1.5 M NaCl, 0.5 M NaOH), neutralizing solution (1.5 M NaCl, 1 M Tris, pH 8.0), and twice in 2xSSC for 10 min each. The membrane is then UV irradiated in a STRATALINKER UV-crosslinker (Stratagene).

In the second method, cDNAs are amplified from bacterial vectors by thirty cycles of PCR using primers complementary to vector sequences flanking the insert. PCR amplification increases a starting concentration of 1-2 ng nucleic acid to a final quantity greater than 5 μ g. Amplified nucleic acids from about 400 bp to about 5000 bp in length are purified using SEPHACRYL-400 beads (APB). Purified nucleic acids are arranged on a nylon membrane manually or using a dot/slot blotting manifold and suction device and are immobilized by denaturation, neutralization, and UV irradiation as described above. Purified nucleic acids are robotically arranged and immobilized on polymer-coated glass slides using the procedure described in USPN 5,807,522. Polymer-coated slides are prepared by cleaning glass microscope slides (Corning, Acton MA) by ultrasound in 0.1% SDS and acetone, etching in 4% hydrofluoric acid (VWR Scientific Products, West Chester PA), coating with 0.05% aminopropyl silane (Sigma Aldrich) in 95% ethanol, and curing in a 110C oven. The slides are washed extensively with distilled water between and after treatments. The nucleic acids are arranged on the slide and then immobilized by exposing the array to UV irradiation using a STRATALINKER UV-crosslinker (Stratagene). Arrays are then washed at room temperature in 0.2% SDS and rinsed three times in distilled water. Non-specific binding sites are blocked by incubation of arrays in 0.2% casein in

phosphate buffered saline (PBS; Tropix, Bedford MA) for 30 min at 60C; then the arrays are washed in 0.2% SDS and rinsed in distilled water as before.

Probe Preparation for Membrane Hybridization

Hybridization probes derived from the cDNAs of the Sequence Listing are employed for screening cDNAs, mRNAs, or genomic DNA in membrane-based hybridizations. Probes are prepared by diluting the cDNAs to a concentration of 40-50 ng in 45 μ l TE buffer, denaturing by heating to 100C for five min, and briefly centrifuging. The denatured cDNA is then added to a REDIPRIME tube (APB), gently mixed until blue color is evenly distributed, and briefly centrifuged. Five μ l of [³²P]dCTP is added to the tube, and the contents are incubated at 37C for 10 min. The labeling reaction is stopped by adding 5 μ l of 0.2M EDTA, and probe is purified from unincorporated nucleotides using a PROBEQUANT G-50 microcolumn (APB). The purified probe is heated to 100C for five min, snap cooled for two min on ice, and used in membrane-based hybridizations as described below.

Probe Preparation for Polymer Coated Slide Hybridization

Hybridization probes derived from mRNA isolated from samples are employed for screening cDNAs of the Sequence Listing in array-based hybridizations. Probe is prepared using the GEMbright kit (Incyte Genomics) by diluting mRNA to a concentration of 200 ng in 9 μ l TE buffer and adding 5 μ l 5x buffer, 1 μ l 0.1 M DTT, 3 μ l Cy3 or Cy5 labeling mix, 1 μ l RNase inhibitor, 1 μ l reverse transcriptase, and 5 μ l 1x yeast control mRNAs. Yeast control mRNAs are synthesized by in vitro transcription from noncoding yeast genomic DNA (W. Lei, unpublished). As quantitative controls, one set of control mRNAs at 0.002 ng, 0.02 ng, 0.2 ng, and 2 ng are diluted into reverse transcription reaction mixture at ratios of 1:100,000, 1:10,000, 1:1000, and 1:100 (w/w) to sample mRNA respectively. To examine mRNA differential expression patterns, a second set of control mRNAs are diluted into reverse transcription reaction mixture at ratios of 1:3, 3:1, 1:10, 10:1, 1:25, and 25:1 (w/w). The reaction mixture is mixed and incubated at 37C for two hr. The reaction mixture is then incubated for 20 min at 85C, and probes are purified using two successive CHROMA SPIN+TE 30 columns (Clontech, Palo Alto CA). Purified probe is ethanol precipitated by diluting probe to 90 μ l in DEPC-treated water, adding 2 μ l 1mg/ml glycogen, 60 μ l 5 M sodium acetate, and 300 μ l 100% ethanol. The probe is centrifuged for 20 min at 20,800xg, and the pellet is resuspended in 12 μ l resuspension buffer, heated to 65C for five min, and mixed thoroughly. The probe is heated and mixed as before and then stored on ice. Probe is used in high density array-based hybridizations as described below.

Membrane-based Hybridization

Membranes are pre-hybridized in hybridization solution containing 1% Sarkosyl and 1x high phosphate buffer (0.5 M NaCl, 0.1 M Na₂HPO₄, 5 mM EDTA, pH 7) at 55C for two hr. The

probe, diluted in 15 ml fresh hybridization solution, is then added to the membrane. The membrane is hybridized with the probe at 55C for 16 hr. Following hybridization, the membrane is washed for 15 min at 25C in 1mM Tris (pH 8.0), 1% Sarkosyl, and four times for 15 min each at 25C in 1mM Tris (pH 8.0). To detect hybridization complexes, XOMAT-AR film (Eastman
5 Kodak, Rochester NY) is exposed to the membrane overnight at -70C, developed, and examined visually.

Polymer Coated Slide-based Hybridization

Probe is heated to 65C for five min, centrifuged five min at 9400 rpm in a 5415C microcentrifuge (Eppendorf Scientific, Westbury NY), and then 18 μ l is aliquoted onto the array
10 surface and covered with a coverslip. The arrays are transferred to a waterproof chamber having a cavity just slightly larger than a microscope slide. The chamber is kept at 100% humidity internally by the addition of 140 μ l of 5xSSC in a corner of the chamber. The chamber containing the arrays is incubated for about 6.5 hr at 60C. The arrays are washed for 10 min at 45C in 1xSSC, 0.1% SDS, and three times for 10 min each at 45C in 0.1xSSC, and dried.

15 Hybridization reactions are performed in absolute or differential hybridization formats. In the absolute hybridization format, probe from one sample is hybridized to array elements, and signals are detected after hybridization complexes form. Signal strength correlates with probe mRNA levels in the sample. In the differential hybridization format, differential expression of a set of genes in two biological samples is analyzed. Probes from the two samples are prepared and
20 labeled with different labeling moieties. A mixture of the two labeled probes is hybridized to the array elements, and signals are examined under conditions in which the emissions from the two different labels are individually detectable. Elements on the array that are hybridized to substantially equal numbers of probes derived from both biological samples give a distinct combined fluorescence (Shalon WO95/35505).

25 Hybridization complexes are detected with a microscope equipped with an Innova 70 mixed gas 10 W laser (Coherent, Santa Clara CA) capable of generating spectral lines at 488 nm for excitation of Cy3 and at 632 nm for excitation of Cy5. The excitation laser light is focused on the array using a 20X microscope objective (Nikon, Melville NY). The slide containing the array is placed on a computer-controlled X-Y stage on the microscope and raster-scanned past the
30 objective with a resolution of 20 micrometers. In the differential hybridization format, the two fluorophores are sequentially excited by the laser. Emitted light is split, based on wavelength, into two photomultiplier tube detectors (PMT R1477, Hamamatsu Photonics Systems, Bridgewater NJ) corresponding to the two fluorophores. Appropriate filters positioned between the array and the photomultiplier tubes are used to filter the signals. The emission maxima of the fluorophores used
35 are 565 nm for Cy3 and 650 nm for Cy5. The sensitivity of the scans is calibrated using the signal

intensity generated by the yeast control mRNAs added to the probe mix. A specific location on the array contains a complementary DNA sequence, allowing the intensity of the signal at that location to be correlated with a weight ratio of hybridizing species of 1:100,000.

The output of the photomultiplier tube is digitized using a 12-bit RTI-835H analog-to-digital (A/D) conversion board (Analog Devices, Norwood MA) installed in an IBM-compatible PC computer. The digitized data are displayed as an image where the signal intensity is mapped using a linear 20-color transformation to a pseudocolor scale ranging from blue (low signal) to red (high signal). The data is also analyzed quantitatively. Where two different fluorophores are excited and measured simultaneously, the data are first corrected for optical crosstalk (due to overlapping emission spectra) between the fluorophores using the emission spectrum for each fluorophore. A grid is superimposed over the fluorescence signal image such that the signal from each spot is centered in each element of the grid. The fluorescence signal within each element is then integrated to obtain a numerical value corresponding to the average intensity of the signal. The software used for signal analysis is the GEMTOOLS program (Incyte Genomics).

VIII Electronic Analysis

BLAST was used to search for identical or related molecules in the GenBank or LIFESEQ databases (Incyte Genomics). The product score for human, rat, monkey, and dog sequences was calculated as follows: the BLAST score is multiplied by the % nucleotide identity and the product is divided by (5 times the length of the shorter of the two sequences), such that a 100% alignment over the length of the shorter sequence gives a product score of 100. The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and with a product score of at least 70, the match will be exact. Similar or related molecules are usually identified by selecting those which show product scores between 8 and 40.

Electronic northern analysis is performed at a product score of 70. All sequences and cDNA libraries in the LIFESEQ database are categorized by system, organ/tissue and cell type. The categories include cardiovascular system, connective tissue, digestive system, embryonic structures, endocrine system, exocrine glands, female and male genitalia, germ cells, hemic/immune system, liver, musculoskeletal system, nervous system, pancreas, respiratory system, sense organs, skin, stomatognathic system, unclassified/mixed, and the urinary tract. For each category, the number of libraries in which the sequence is expressed are counted and shown over the total number of libraries in that category. In a non-normalized library, expression levels of two or more are significant.

IX Complementary Molecules

Molecules complementary to the cDNA, from about 5 (PNA) to about 5000 bp

(complement of a cDNA insert), are used to detect or inhibit gene expression. These molecules are selected using OLIGO primer analysis software (Molecular Biology Insights). Detection is described in Example VII. To inhibit transcription by preventing promoter binding, the complementary molecule is designed to bind to the most unique 5' sequence and includes

5 nucleotides of the 5' UTR upstream of the initiation codon of the open reading frame. Complementary molecules include genomic sequences (such as enhancers or introns) and are used in "triple helix" base pairing to compromise the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. To inhibit translation, a complementary molecule is designed to prevent ribosomal binding to the mRNA encoding the
10 protein.

Complementary molecules are placed in expression vectors and used to transform a cell line to test efficacy; into an organ, tumor, synovial cavity, or the vascular system for transient or short term therapy; or into a stem cell, zygote, or other reproducing lineage for long term or stable gene therapy. Transient expression lasts for a month or more with a non-replicating vector and for
15 three months or more if appropriate elements for inducing vector replication are used in the transformation/expression system.

Stable transformation of appropriate dividing cells with a vector encoding the complementary molecule produces a transgenic cell line, tissue, or organism (USPN 4,736,866). Those cells that assimilate and replicate sufficient quantities of the vector to allow stable
20 integration also produce enough complementary molecules to compromise or entirely eliminate activity of the cDNA encoding the protein.

X Selection of Sequences, Microarray Preparation and Use

Incyte clones represent template sequences derived from the LIFESEQ GOLD assembled human sequence database (Incyte Genomics). In cases where more than one clone was available
25 for a particular template, the 5'-most clone in the template was used on the microarray. The HUMAN GENOME GEM series 1-3 microarrays (Incyte Genomics) contain 28,626 array elements which represent 10,068 annotated clusters and 18,558 unannotated clusters. For the UNIGEM series microarrays (Incyte Genomics), Incyte clones were mapped to non-redundant Unigene clusters (Unigene database (build 46), NCBI; Shuler (1997) J Mol Med 75:694-698), and
30 the 5' clone with the strongest BLAST alignment (at least 90% identity and 100 bp overlap) was chosen, verified, and used in the construction of the microarray. The UNIGEM V microarray (Incyte Genomics) contains 7075 array elements which represent 4610 annotated genes and 2,184 unannotated clusters.

To construct microarrays, cDNAs were amplified from bacterial cells using primers
35 complementary to vector sequences flanking the cDNA insert. Thirty cycles of PCR increased the

initial quantity of cDNAs from 1-2 ng to a final quantity of greater than 5 μ g. Amplified cDNAs were then purified using SEPHACRYL-400 columns (APB). Purified cDNAs were immobilized on polymer-coated glass slides. Glass microscope slides (Corning, Corning NY) were cleaned by ultrasound in 0.1% SDS and acetone, with extensive distilled water washes between and after
5 treatments. Glass slides were etched in 4% hydrofluoric acid (VWR Scientific Products, West Chester PA), washed thoroughly in distilled water, and coated with 0.05% aminopropyl silane (Sigma Aldrich) in 95% ethanol. Coated slides were cured in a 110°C oven. cDNAs were applied to the coated glass substrate using a procedure described in USPN 5,807,522. One microliter of the cDNA at an average concentration of 100 ng/ μ l was loaded into the open capillary printing
10 element by a high-speed robotic apparatus which then deposited about 5 nl of cDNA per slide.

Microarrays were UV-crosslinked using a STRATALINKER UV-crosslinker (Stratagene), and then washed at room temperature once in 0.2% SDS and three times in distilled water. Non-specific binding sites were blocked by incubation of microarrays in 0.2% casein in phosphate buffered saline (Tropix, Bedford MA) for 30 minutes at 60°C followed by washes in
15 0.2% SDS and distilled water as before.

XI Preparation of Samples

Tissue Samples

Matched normal colon and cancerous colon or colon polyp tissue samples were provided by the Huntsman Cancer Institute, (Salt Lake City, UT). Donor 3311 is an 85 year-old male
20 diagnosed with colon cancer. Donor 3753 is an individual, sex unknown, with normal colon tissue. Donor 3757 is an individual, sex unknown, diagnosed with colon cancer. Donor 3756 is an individual, sex unknown, diagnosed with colon cancer. Donor 3755 is an individual, sex unknown, diagnosed with a colon polyp. Donor 3754 is an individual, sex unknown, diagnosed with a colon polyp. Donor 3583 is a 59 year-old male diagnosed with a right colectomy,
25 tubulovillous colon polyp, hyperplastic colon polyp, and colon adenoma. Donor 3649 is an 86 year-old individual, sex unknown, diagnosed with an invasive, well-differentiated adenocarcinoma. Donor 3647 is an 83 year-old individual, sex unknown, diagnosed with an invasive, moderately well-differentiated adenocarcinoma with metastases to the lymph nodes. Comparisons were done with matched normal and tumor or polyp tissue from the same donor.
30 Donor 3983 is a 23 year-old individual, sex unknown, diagnosed with a polyp from adenomatous polyposis coli and with moderately differentiated adenocarcinoma that had metastasized to the lymph nodes.

XII Expression of CCM

Expression and purification of the protein are achieved using either a mammalian cell
35 expression system or an insect cell expression system. The pUB6/V5-His vector system

(Invitrogen, Carlsbad CA) is used to express CCM in CHO cells. The vector contains the selectable bsd gene, multiple cloning sites, the promoter/enhancer sequence from the human ubiquitin C gene, a C-terminal V5 epitope for antibody detection with anti-V5 antibodies, and a C-terminal polyhistidine (6xHis) sequence for rapid purification on PROBOND resin (Invitrogen).

5 Transformed cells are selected on media containing blasticidin.

Spodoptera frugiperda (Sf9) insect cells are infected with recombinant Autographica californica nuclear polyhedrosis virus (baculovirus). The polyhedrin gene is replaced with the cDNA by homologous recombination and the polyhedrin promoter drives cDNA transcription. The protein is synthesized as a fusion protein with 6xhis which enables purification as described
10 above. Purified protein is used in the following activity and to make antibodies

XIII Production of Antibodies

CCM is purified using polyacrylamide gel electrophoresis and used to immunize mice or rabbits. Antibodies are produced using the protocols below. Alternatively, the amino acid sequence of CCM is analyzed using LASERGENE software (DNASTAR) to determine regions of
15 high antigenicity. An antigenic epitope, usually found near the C-terminus or in a hydrophilic region is selected, synthesized, and used to raise antibodies. Typically, epitopes of about 15 residues in length are produced using an ABI 431A peptide synthesizer (Applied Biosystems) using Fmoc-chemistry and coupled to KLH (Sigma-Aldrich) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester to increase antigenicity.

20 Rabbits are immunized with the epitope-KLH complex in complete Freund's adjuvant. Immunizations are repeated at intervals thereafter in incomplete Freund's adjuvant. After a minimum of seven weeks for mouse or twelve weeks for rabbit, antisera are drawn and tested for antipeptide activity. Testing involves binding the peptide to plastic, blocking with 1% bovine serum albumin, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-
25 rabbit IgG. Methods well known in the art are used to determine antibody titer and the amount of complex formation.

XIV Purification of Naturally Occurring Protein Using Specific Antibodies

Naturally occurring or recombinant protein is purified by immunoaffinity chromatography using antibodies which specifically bind the protein. An immunoaffinity column is constructed by
30 covalently coupling the antibody to CNBr-activated SEPHAROSE resin (APB). Media containing the protein is passed over the immunoaffinity column, and the column is washed using high ionic strength buffers in the presence of detergent to allow preferential absorbance of the protein. After coupling, the protein is eluted from the column using a buffer of pH 2-3 or a high concentration of urea or thiocyanate ion to disrupt antibody/protein binding, and the protein is collected.

35 XV Screening Molecules for Specific Binding with the cDNA or Protein

The cDNA, or fragments thereof, or the protein, or portions thereof, are labeled with ^{32}P -dCTP, Cy3-dCTP, or Cy5-dCTP (APB), or with BIODIPY or FITC (Molecular Probes, Eugene OR), respectively. Libraries of candidate molecules or compounds previously arranged on a substrate are incubated in the presence of labeled cDNA or protein. After incubation under
5 conditions for either a nucleic acid or amino acid sequence, the substrate is washed, and any position on the substrate retaining label, which indicates specific binding or complex formation, is assayed, and the ligand is identified. Data obtained using different concentrations of the nucleic acid or protein are used to calculate affinity between the labeled nucleic acid or protein and the bound molecule.

10 XVI Two-Hybrid Screen

A yeast two-hybrid system, MATCHMAKER LexA Two-Hybrid system (Clontech Laboratories, Palo Alto CA), is used to screen for peptides that bind the protein of the invention. A cDNA encoding the protein is inserted into the multiple cloning site of a pLexA vector, ligated, and transformed into *E. coli*. cDNA, prepared from mRNA, is inserted into the multiple cloning
15 site of a pB42AD vector, ligated, and transformed into *E. coli* to construct a cDNA library. The pLexA plasmid and pB42AD-cDNA library constructs are isolated from *E. coli* and used in a 2:1 ratio to co-transform competent yeast EGY48[p8op-lacZ] cells using a polyethylene glycol/lithium acetate protocol. Transformed yeast cells are plated on synthetic dropout (SD) media lacking histidine (-His), tryptophan (-Trp), and uracil (-Ura), and incubated at 30C until the colonies have
20 grown up and are counted. The colonies are pooled in a minimal volume of 1x TE (pH 7.5), replated on SD/-His/-Leu/-Trp/-Ura media supplemented with 2% galactose (Gal), 1% raffinose (Raf), and 80 mg/ml 5-bromo-4-chloro-3-indolyl β -d-galactopyranoside (X-Gal), and subsequently examined for growth of blue colonies. Interaction between expressed protein and cDNA fusion proteins activates expression of a LEU2 reporter gene in EGY48 and produces colony growth on
25 media lacking leucine (-Leu). Interaction also activates expression of β -galactosidase from the p8op-lacZ reporter construct that produces blue color in colonies grown on X-Gal.

Positive interactions between expressed protein and cDNA fusion proteins are verified by isolating individual positive colonies and growing them in SD/-Trp/-Ura liquid medium for 1 to 2 days at 30C. A sample of the culture is plated on SD/-Trp/-Ura media and incubated at 30C until
30 colonies appear. The sample is replica-plated on SD/-Trp/-Ura and SD/-His/-Trp/-Ura plates. Colonies that grow on SD containing histidine but not on media lacking histidine have lost the pLexA plasmid. Histidine-requiring colonies are grown on SD/Gal/Raf/X-Gal/-Trp/-Ura, and white colonies are isolated and propagated. The pB42AD-cDNA plasmid, which contains a cDNA encoding a protein that physically interacts with the protein, is isolated from the yeast cells
35 and characterized.

XVII CCM Assay

The localization of CCM in the intestine is detected by fluorescence microscopy as described by Boll et al. (1993; J Biol Chem 268:12901-12911). Sections of intestinal tissue are fixed with 2.5% paraformaldehyde and 0.1% glutaraldehyde and incubated with antibodies against
5 CCM. Subcellular distributions of CCM are visualized by incubation with biotinylated goat anti-guinea pig IgG (Kirkegaard and Perry Laboratories, Gaithersburg MD) followed by streptavidin complexed with the fluorescent dye Texas Red (APB).

All patents and publications mentioned in the specification are incorporated by reference
10 herein. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred
embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying
15 out the invention that are obvious to those skilled in the field of molecular biology or related fields are intended to be within the scope of the following claims.

mean log2 DE (Cy5/Cy3)	CV %	Cy3	Cy5
-1.92	4.03	Human, Colon Pool, Nrml, Dn3753	Human, Colon Tumor, Cancer, Dn3311
-1.87	5.44	Human, Colon Pool, Nrml, Dn3753	Human, Colon Tumor, Cancer, Dn3757
-1.65	0	Human, Colon Pool, Nrml, Dn3753	Human, Colon Tumor, Cancer, Dn3756
-1.61	18.65	Human, Colon Pool, Nrml, Dn3753	Human, Colon, Polyp, Dn3755
-1.41	5.18	Human, Colon Pool, Nrml, Dn3753	Human, Colon, Polyp, Dn3754
-2.29	6.8	Human, Colon Pool, Nrml, mw/AdenoCA, Dn3649	Human, Colon Tumor, AdenoCA, Dn3649
-1.04	11.76	Human, Colon Pool, Nrml, mw/Cancer, Dn3647	Human, Colon Tumor, Cancer, Dn3647
-0.84	1.41	Human, Colon Pool, Nrml, mw/Adenoma, Dn3583	Human, Colon Tumor, Adenoma, Dn3583
0.18	5.23	Human, Colon, Mucosa, mw/AdenoCA, Dn398	Human, Colon, Polyp, mw/AdenoCA, Dn3983

TABLE 1

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 KRASNOW, Randi E.
 WARREN, Bridget
 BAUGHN, Mariah R.

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<213> Homo sapiens

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<213> Homo sapiens

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<212> DNA

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<223> Incyte ID No: 6309461H1

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<221> unsure

<222> 20

<223> a, t, c, g, or other

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<211> 611

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<213> Homo sapiens

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<211> 556

<212> DNA

<213> Homo sapiens

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<223> Incyte ID No: 6923049H1

<400> 9

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<211> 915

<212> DNA

<213> Homo sapiens

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<211> 552

<212> DNA

<213> Homo sapiens

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<223> Incyte ID No: 6995895H1

<220>

<221> unsure

<222> 539, 542

<223> a, t, c, g, or other

<400> 11

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<211> 539

<212> DNA

<213> Homo sapiens

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 <213> Homo sapiens

<220>
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 <223> Incyte ID No: 70405975D1

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 ggccgcctct cccctgctct gcacttcctg tctgtacaat agaaggggga ggtgctgcta 240
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 aatatttcac cagggtctat tttctcagtt taagttcttt tttgtctctt tcaggaagtt 360
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<210> 14
 <211> 698
 <212> DNA
 <213> Homo sapiens

<220>
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 <223> Incyte ID No: 8117068H1

<400> 14
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 tttcacagg ctctattttt tcagtttaag ttctttttttg tctctttcag gaagttaagc 180
 tcccagtgca gggatcaat gtgaatctgg tcctgagctt tttagaaaat aagagtgggtg 240
 gccgggcgcg ctggctcacg cctgtaatcg caacactttg ggaggccgag gcaggcagat 300
 cacaagggtca ggagatcgag accaccctgg ctaacacagt gagatcccgt ctctgctgaa 360
 aatacaaaaa attagccagg cgtgtgtggt ggggtgcctgt ggtcccagct actttggagg 420
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 cactgtcact ccagcctggg cagtgtgagt gagactccgt ctctaaaaaa aaataagagt 540
 ggttaacttt ttggttgata ttagtattat ttgtgagaag agtttccac cttcttctga 600
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<210> 15
 <211> 172
 <212> DNA
 <213> Homo sapiens

<220>
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 <223> Incyte ID No: 747646H1

<220>
 <221> unsure
 <222> 171
 <223> a, t, c, g, or other

<400> 15
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 ctgatctggg tctgttttaa aggccgggtc tctaatttag gaatcgggtga nt 172

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<210> 16
 <211> 696
 <212> DNA
 <213> Homo sapiens

<220>
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 <223> Incyte ID No: 7984383H1

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 taaaataatt tccaaggcta tttgatcagc cttcttcctt tttggttcat tgtgtcctga 180
 cttggggcac tgatgagatt ttttattttt tttgagacgg agtttcactt tgttgcccag 240
 gctggagtgt agtggcatga tctcgggtca ctgcaacctc cacctccctg gttcaagtga 300
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 aatctctgta tttttggtag aggtagagtt tcaccatttt ggccaggctg gtttcgaact 420
 cctggcctca agtgatctgc caccttggcc tcccaaagtg ctgggaatac aggcgtgagc 480
 cactgtgccc gccctgatga gatattttat taccaatggt agtattgaga aactgaaatg 540
 tttgaagaag cacaaccagg atcgtgctgg tagcaccaca gtacttaaac tggttggtca 600
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<210> 17
 <211> 711
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 8073132J1

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 taccaggcaa catcttagat gcctgggttg caacttctca aaacaaccca taacaaccag 240
 cagggttggg aactggctga cattcattaa cacactcata tggctgtttg tgaacagatc 300
 ccaaattgcaa agcgtccttc acacacttct acccactccc catctccaca cccctataca 360
 agatcttttc attcaaccaa aaggataaga aatgtgctac agaaatgtga acatcaacac 420
 aaatacagat tattttttca tcaagagagt taaaacattc ccattatcaa gaggggtctt 480
 caccagaatg taacaaaatg aatttcagct ttcacaccag taaccagtga aagtatggcc 540
 tagagctagt tacacggcat aaagtgacta gtgggtcagtt attgttcatt cagttcagat 600
 gagtaactgc agaaactcta cccagagggt gagcacatat gggctatcat gatgtcggat 660
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<210> 18
 <211> 528
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 6744627H1

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 atttgggatc tgtacacaaa cagccatatg agtgtgttaa tgaatgtcag ccagttacca 180
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 ttctcagatg gccagtaggc tcctccctgc ttaggggtctc atttctctga aaagaggatg 360
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gtgagaacat ttctctttta attagctagt gatttttgatt aagactaatt cactaaacat 480
 aaccttccct caaatcacct caggtagcaa tctgtacgta actaaaag 528

<210> 19
 <211> 683
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 6749637H1

<400> 19
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 attatttttc ttgtgagaac atttctcttt taattagcta gtgattttga ttaagactaa 180
 ttcactaaac atacccttcc ctcaaatcac ctcaggtagc aatctgtaag taactaaaag 240
 cattgaaaaa cacacaagaa acattttttaa aaactatttt taaaggcctg gccgggtgca 300
 gtggctcact cctgtaatcc cagcactttg ggaggccgag acgggtggat cacctgaggt 360
 caggaatttg agactagcat ggccaacatg gtgaaacccc gtgtctacta aaaatacaaa 420
 aattagctgg gcgtggtggc agacgcctgt aatcccagct actcaggag gctgaggcac 480
 gagaatcgct tgaacctggg aggcggaggt tgcagtgagc caagattgcg ccactgcact 540
 ccaacctggg tgacaagagc aagactccgt ctcacaaaaa acaaacaaaa ctgactgatt 600
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 cttctatgaa ttcttccatt taa 683

<210> 20
 <211> 339
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 5073165F8

<400> 20
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 ctctacccat cagctgtacc atgtcgcaga attcatgcag gcacaaagtt ggagttacag 180
 agatggggtg acagcaggca aactttggcc tatgtattat aaccacaact tcaagttctt 240
 acctcatgtg aatattcacc ctttcttttag tcttccaagg caaacagccc cgtctcatca 300
 ccagatgagc aaggctctga tatggcatag cagatctcc 339

<210> 21
 <211> 494
 <212> DNA
 <213> Homo sapiens

<220>
 <221> misc_feature
 <223> Incyte ID No: 6441214H1

<400> 21
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 ctcatgtgaa tattcacctt ttcttttagtc ttccaaggca aacagccccg tctcatcacc 180
 agatgagcaa ggtcttgata tggcatagca gatctcccta gacacagatc atgagaaaag 240
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 tttgtattta ccctgtctaa tacactaagg atacttactc attgtacttg cagctcaata 360
 tgtctttgct gttcagatac taaaatgtac ctctgagtca ttgtgagctg tgtggtaggt 420
 tggacattgg catagttggt gatgggactc aaaatgaaaa ggtggtctct ttaccagggtc 480
 acagactggt gcag 494

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<210> 22
 <211> 226
 <212> DNA
 <213> Homo sapiens

<220>
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 <223> Incyte ID No: 2436362H1

<220>
 <221> unsure
 <222> 222
 <223> a, t, c, g, or other

<400> 22
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 ctggaagaaa ctgaggctca gagattgagg aaccttgtga ctgccatctg tgccagtcac 120
 cacctctgca tgaccacatt tccctccatt agcactacca gcatgcctgt gaggtagtta 180
 ctcagctggt catctgaccc cagacgtaga aaagttaaga gngatt 226

<210> 23
 <211> 606
 <212> DNA
 <213> Rattus norvegicus

<220>
 <221> misc_feature
 <223> Incyte ID No: 702073518T2

<400> 23
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 gtgactggct ggggctggtg tgggtgctca gggagtcctt gctggcactg gtgagcttat 480
 ttgccaaagg caggccaatg agctccatac acttcttgca catgatagac ccacagaggg 540
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 atctgg 606

<210> 24
 <211> 556
 <212> DNA
 <213> Rattus norvegicus

<220>
 <221> misc_feature
 <223> Incyte ID No: 702549617T1

<400> 24
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 ccactcgaag gtcgttggcg tgctccagac tgtaggtcgt ctcccagtc atttaatgat 180
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 ttgagcagcg tgtccttgca gtgcgtgcag cagcggatgc gctcatcatc cttctcgtcc 360
 aggactgagc tgacactgct catgctactg atgctgcctc ggccgggagcc atggacactg 420
 ttaggtgact ggctggggct ggtgtgggtg ctcagggagt ccttgctggc actggtgagc 480
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 aggcggcagt ggtgac 556

<210> 25
 <211> 452
 <212> DNA
 <213> Rattus norvegicus

<220>
 <221> misc_feature
 <223> Incyte ID No: 702448734T1

<400> 25
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 actcgaaggt cgttggcgtg ctccagactg taggtcgtct cccagcatt taatgatgct 180
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 agcagcgtgt ccttgcagtg cgtgcagcag cggatgcgct catcatcctt ctctgtccagg 360
 actgagctga cactgctcat gctactgatg ctgcctcggc gggagccatg gacactgtta 420
 ggtgactggc tggggctggg gtgggtgctc ag 452

<210> 26
 <211> 567
 <212> DNA
 <213> Rattus norvegicus

<220>
 <221> misc_feature
 <223> Incyte ID No: 702081830H1

<400> 26
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 ccggatgctg aacttattcc cacagtc 567

<210> 27
 <211> 383
 <212> DNA
 <213> Rattus norvegicus

<220>
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 <223> Incyte ID No: 702053775T1

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<210> 28
 <211> 255
 <212> DNA
 <213> Rattus norvegicus

<220>
 <221> misc_feature
 <223> Incyte ID No: 700883909H1

<400> 28
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 atcagggttg aaagcttac cgcatttgac agaacaata ctgagacctc aaagattaga 180
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 tgtgggaata agttc 255

<210> 29
 <211> 250
 <212> DNA
 <213> Rattus norvegicus

<220>
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 <223> Incyte ID No: 700883983H1

<400> 29
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 atcagggttg aaagcttac cgcatttgac agaacaata ctgagacctc aaagattaga 180
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<210> 30
 <211> 257
 <212> DNA
 <213> Rattus norvegicus

<220>
 <221> misc_feature
 <223> Incyte ID No: 701092129H1

<400> 30
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<210> 31
 <211> 473
 <212> DNA
 <213> Rattus norvegicus

<220>
 <221> misc_feature
 <223> Incyte ID No: 702137890H2

<400> 31
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 ggcttcctgt gccctctgtg cctcaaggac cttcagtctt tctatcaact tcagtcacat 420
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<210> 32

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<211> 433
 <212> DNA
 <213> Macaca fascicularis

<220>
 <221> misc_feature
 <223> Incyte ID No: 701741076T1

<400> 32
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 tgatctgtgt ctaggagat ctgctatgcc gtatcacgac cttgctcatt tggatgatgag 360
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 tgaagtgtg gtt 433

<210> 33
 <211> 645
 <212> DNA
 <213> Canis familiaris

<220>
 <221> misc_feature
 <223> Incyte ID No: 702769948H2

<220>
 <221> unsure
 <222> 562
 <223> a, t, c, g, or other

<400> 33
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 cttacaaagg gagacacagc tnattttctc caagtagtat atttcacact cctgtataca 600
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<210> 34
 <211> 542
 <212> DNA
 <213> Canis familiaris

<220>
 <221> misc_feature
 <223> Incyte ID No: 702245053H1

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tggtaatcag gaagcttttg tggaacactg ccatgggagt ggaagcttcc ttgagtcaga 540
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<210> 35
<211> 281
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: g921283

<400> 35
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acagatcatg agaaaagatg gaagagactt agggattcag gcatcagatg aagttggctt 180
ttccctttta tgccttggtt gtattttacc tgtctaatac actaaggata cttactcatt 240
gtacttgcag ctcaatatgt ctttgctgtt cagataactaa a 281

<210> 36
<211> 6719
<212> DNA
<213> Homo sapiens

<220>
<221> misc_feature
<223> Incyte ID No: GNFL.g8572864_000002_002.edit

<400> 36
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gaagtgcaga aagtgtatga gctgatagac gctttaagta agaagatctt aaccttgggc 180
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tgactctggc	aggggaaagg	gagaatcatc	tttgtaatca	gagccttctc	cataataaa	6719

What is claimed is:

1. An isolated cDNA encoding a protein having the amino acid sequence of SEQ ID NO:1.
2. An isolated cDNA selected from:
 - a) a nucleic acid sequence of SEQ ID NO:2 or the complement thereof;
 - 5 b) a fragment of SEQ ID NO:2 selected from SEQ ID NOs:3-22 or the complement thereof;
 and
 - c) a variant of SEQ ID NO:2 selected from SEQ ID NOs:23-34.
3. A composition comprising the cDNA or the complement of the cDNA of claim 1.
4. A vector comprising the cDNA of claim 1.
- 10 5. A host cell comprising the vector of claim 4.
6. A method for using a cDNA to produce a protein, the method comprising:
 - a) culturing the host cell of claim 5 under conditions for protein expression; and
 - b) recovering the protein from the host cell culture.
7. A method for using a cDNA to detect expression of a nucleic acid in a sample comprising:
 - 15 a) hybridizing the composition of claim 3 to nucleic acids of the sample, thereby forming hybridization complexes; and
 - b) comparing hybridization complex formation with a standard, wherein the comparison indicates expression of the cDNA in the sample.
8. The method of claim 7 further comprising amplifying the nucleic acids of the sample prior to
 - 20 hybridization.
9. The method of claim 7 wherein the composition is attached to a substrate.
10. The method of claim 7 wherein the cDNA is differentially expressed when compared with the standard and is diagnostic of a colon cancer or colon polyps.
11. A method of using a cDNA to screen a plurality of molecules or compounds, the method
 - 25 comprising:
 - a) combining the cDNA of claim 1 with a plurality of molecules or compounds under conditions to allow specific binding; and
 - b) detecting specific binding, thereby identifying a molecule or compound which specifically binds the cDNA.
- 30 12. The method of claim 11 wherein the molecules or compounds are selected from DNA molecules, RNA molecules, peptide nucleic acids, artificial chromosome constructions, peptides, transcription factors, repressors, and regulatory molecules.
13. A purified protein or a portion thereof selected from:
 - a) an amino acid sequence of SEQ ID NO:1;
 - 35 b) an antigenic epitope of SEQ ID NO:1; and

- c) a biologically active portion of SEQ ID NO:1.
14. A composition comprising the protein of claim 13.
15. A method for using a protein to screen a plurality of molecules or compounds to identify at least one ligand, the method comprising:
- 5 a) combining the protein of claim 13 with the molecules or compounds under conditions to allow specific binding; and
- b) detecting specific binding, thereby identifying a ligand which specifically binds the protein.
16. The method of claim 15 wherein the molecules or compounds are selected from DNA
- 10 molecules, RNA molecules, peptide nucleic acids, peptides, proteins, mimetics, agonists, antagonists, antibodies, immunoglobulins, inhibitors, and drugs.
17. A method of using a protein to prepare and purify antibodies comprising:
- a) immunizing a animal with the protein of claim 13 under conditions to elicit an antibody response;
- 15 b) isolating animal antibodies;
- c) attaching the protein to a substrate;
- d) contacting the substrate with isolated antibodies under conditions to allow specific binding to the protein;
- e) dissociating the antibodies from the protein, thereby obtaining purified antibodies.
- 20 18. An antibody produced by the method of claim 17.
19. An isolated protein comprising SEQ ID NO:1.
20. A purified antibody which binds specifically to the protein of claim 19.
21. A method for preparing and purifying a polyclonal antibody with the specificity of the antibody of claim 20 comprising:
- 25 a) immunizing a animal with a protein of SEQ ID NO:1 under conditions to elicit an antibody response;
- b) isolating animal antibodies;
- c) attaching the protein to a substrate;
- d) contacting the substrate with isolated antibodies under conditions to allow specific binding
- 30 to the protein;
- e) dissociating the antibodies from the protein, thereby obtaining purified polyclonal antibodies.
22. A polyclonal antibody produced by the method of claim 21.
23. A method for preparing a monoclonal antibody with the specificity of the antibody of claim 20
- 35 comprising:

- a) immunizing a animal with a protein of SEQ ID NO:1 under conditions to elicit an antibody response;
- b) isolating antibody-producing cells from the animal;
- c) fusing the antibody-producing cells with immortalized cells in culture to form monoclonal
- 5 antibody producing hybridoma cells;
- d) culturing the hybridoma cells; and
- e) isolating monoclonal antibodies from culture.
24. A monoclonal antibody produced by the method of claim 23.
25. A method for using an antibody to immunopurify a protein comprising:
- 10 a) attaching an antibody of claim 20 to a substrate,
- b) exposing the antibody to a sample containing protein under conditions to allow antibody:protein complexes to form,
- c) dissociating the protein from the complex, and
- d) collecting the purified protein.
- 15 26. A method for using an antibody to detect expression of a protein in a sample, the method comprising:
- a) combining the antibody of claim 20 with a sample under conditions which allow the formation of antibody:protein complexes; and
- b) detecting complex formation, wherein complex formation indicates expression of the
- 20 protein in the sample.
27. The method of claim 2 wherein complex formation is compared with standards and is diagnostic of a colon disorder, particularly colon cancer, or colon polyps.
28. A composition comprising an antibody of claim 20 and a labeling moiety.
29. A composition comprising an antibody of claim 20 and a pharmaceutical agent.

5'	9	GCC	TGC	CGA	GTT	CCG	AGC	18	GAC	CGA	TGG	AGA	36	CTG	CGG	CTG	AGT	45	GAC	54	GGA
	63	TGG	AGG	CCC	AGA	GCC	CGG	72	CGG	GCC	TGA	AGG	90	GGA	CAA	ACC	TGG	99	GTG	108	GCA
	117	GCC	CGG	CAG	GGT	GTC	TTA	126	TTA	CAA	GTA	TCA	144	ACT	TAC	TAT	ATG	153	TGG	162	AAT
	171	TCA	TCA	AGG	TAA	AGA	GCA	180	GCA	TCA	AGT	AAA	198	TTC	TGC	TTG	TTG	207	ATA	216	ACT
	225	GAC	ATT	CCC	CCA	GTG	GCT	234	GTG	GAA	GTG	GCA	252	GAA	TTA	TGA	AGT	261	TGG	270	ATT
	279	AAT	GAA	TGT	AAG	AGA	ATT	288	AGA	ATT	GCC	AAG	306	TCC	TAC	TCC	AGA	315	GAG	324	ACC
	333	TCC	AGG	GCC	ATG	AAG	CCA	342	AAG	CCA	CCT	CAC	360	CTG	TGT	GCT	GCT	369	TAA	378	AAT
	387	GCG	GGA	ACC	ATG	GTT	CCT	396	GTT	CCT	TGG	GAG	414	AGC	TGA	CTC	TTG	423	GCA	432	GAT

FIGURE 1A

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441	TCC	TGC	CTT	CCT	AGG	GTT	GAG	AGC	GGC	ACT	GCC	ATG	GCT	TCT	CTG	GAC	GAC	CCA	486
												M	A	S	L	D	D	P	
495	GGG	GAA	GTG	AGG	GAG	GGC	TTC	CTC	TGC	CCT	CTG	TGC	CTG	AAG	GAT	CTG	CAG	TCT	540
	G	E	V	R	E	G	F	L	C	P	L	C	L	K	D	L	Q	S	
549	TTC	TAT	CAG	CTT	CAC	TCA	CAT	TAC	GAG	GAA	GAA	CAC	TCA	GGG	GAA	GAC	CGT	GAT	594
	F	Y	Q	L	H	S	H	Y	E	E	E	H	S	G	E	D	R	D	
603	GTC	AAA	GGG	CAA	ATT	AAA	AGT	CTT	GTC	CAG	AAG	GCT	AAA	AAA	GCA	AAG	GAC	AGG	648
	V	K	G	Q	I	K	S	L	V	Q	K	A	K	K	A	K	D	R	
657	TTG	TTG	AAA	CGA	GAA	GGG	GAT	GAT	CGA	GCA	GAG	TCA	GGG	ACC	CAA	GGA	TAT	GAG	702
	L	L	K	R	E	G	D	D	R	A	E	S	G	T	Q	G	Y	E	

FIGURE 1B

711	720	729	738	747	756
TCT TTC AGC TAT GGA GGG GTT GAT CCT TAC ATG TGG GAA CCC CAG GAG CTT GGT					
---	---	---	---	---	---
S F S Y G G V D P Y M W E P Q E L G					
765	774	783	792	801	810
GCT GTG AGA AGC CAT CTT TCC GAC TTC AAA AAA CAC CGA GCT GCT AGA ATT GAC					
---	---	---	---	---	---
A V R S H L S D F K K H R A A R I D					
819	828	837	846	855	864
CAC TAT GTT GTG GAA GTC AAT AAA CTA ATA ATC AGG TTA GAG AAG CTC ACT GCA					
---	---	---	---	---	---
H Y V V E V N K L I I R L E K L T A					
873	882	891	900	909	918
TTT GAC AGA ACA AAT ACT GAG TCT GCA AAG ATT CGA GCA ATA GAA AAG TCT GTG					
---	---	---	---	---	---
F D R T N T E S A K I R A I E K S V					
927	936	945	954	963	972
GTG CCT TGG GTC AAC GAC CAG GAT GTC CCT TTC TGT CCA GAC TGT GGG AAT AAG					
---	---	---	---	---	---
V P W V N D Q D V P F C P D C G N K					

FIGURE 1C

981	990	999	1008	1017	1026
TTC AGC ATC CGG AAC CGC CGC CAC CAC TGC CGC CTC TGC GGG TCT ATT ATG TGC					
---	---	---	---	---	---
F S I R N R R H H C R L C G S I M C					
1035	1044	1053	1062	1071	1080
AAG AAG TGT ATG GAG CTC ATC AGC CTT CCC TTG GCA AAC AAG CTC ACC AGT GCC					
---	---	---	---	---	---
K K C M E L I S L P L A N K L T S A					
1089	1098	1107	1116	1125	1134
AGC AAG GAG TCC CTG AGC ACC CAC ACC AGC CCG AGC CAG TCA CCC AAC AGT GTC					
---	---	---	---	---	---
S K E S L S T H T S P S Q S P N S V					
1143	1152	1161	1170	1179	1188
CAT GGC TCC CGC CGA GGC AGC ATC AGC AGC ATG AGC AGT GTC AGC TCG GTC CTG					
---	---	---	---	---	---
H G S R R G S I S S M S S V S S V L					
1197	1206	1215	1224	1233	1242
GAT GAG AAG GAC GAT GAC CGG ATC CGC TGC TGT ACA CAC TGC AAG GAC ACG CTG					
---	---	---	---	---	---
D E K D D D R I R C C T H C K D T L					

FIGURE 1D

1251	1260	1269	1278	1287	1296
CTC AAG AGA GAG CAG CAG ATT GAT GAG AAG GAG CAC ACA CCT GAC ATC GTG AAG					
---	---	---	---	---	---
L K R E Q Q I D E K E H T P D I V K					
1305	1314	1323	1332	1341	1350
CTC TAC GAG AAA TTA CGA CTT TGC ATG GAG AAA GTT GAC CAG AAA GCT CCA GAA					
---	---	---	---	---	---
L Y E K L R L C M E K V D Q K A P E					
1359	1368	1377	1386	1395	1404
TAC ATC AGG ATG GCA GCA TCA TTA AAT GCT GGG GAG ACA ACC TAC AGT CTG GAA					
---	---	---	---	---	---
Y I R M A A S L N A G E T T Y S L E					
1413	1422	1431	1440	1449	1458
CAT GCC AGT GAC CTT CGA GTG GAA GTG CAG AAA GTG TAT GAG CTG ATA GAC GCT					
---	---	---	---	---	---
H A S D L R V E V Q K V Y E L I D A					
1467	1476	1485	1494	1503	1512
TTA AGT AAG AAG ATC TTA ACC TTG GGC TTG AAC CAG GAC CCT CCA CCA CAT CCA					
---	---	---	---	---	---
L S K K I L T L G L L N Q D P P P H P					

FIGURE 1E

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1521	1530	1539	1548	1557	1566
AGC AAT TTG CGG CTG CAG AGA ATG ATC AGA TAC TCA GCT ACA CTT TTT GTG CAG					
---	---	---	---	---	---
S N L R L Q R M I R Y S A T L F V Q					
1575	1584	1593	1602	1611	1620
GAA AAG TTG CTT GGT TTG ATG TCA CTG CCA ACC AAA GAA CAG TTT GAG GAA CTG					
---	---	---	---	---	---
E K L L G L M S L P T K E F E L					
1629	1638	1647	1656	1665	1674
AAA AAG AAA AGG AAG GAG GAA ATG GAG AGG AAG AGG GCC GTG GAG AGA CAA GCT					
---	---	---	---	---	---
K K K R K E E M E R K R A V E R Q A					
1683	1692	1701	1710	1719	1728
GCC CTG GAG TCC CAG CGA AGG CTT GAG GAA AGG CAG AGT AGT GGC CTG GCT TCT CGA					
---	---	---	---	---	---
A L E S Q R R L E E R Q S S A S R					
1737	1746	1755	1764	1773	1782
GCG GCC AAC GGG GAG GTG GCA TCT CTC CGC AGG GGC CCT GCC CCC TTG AGA AAG					
---	---	---	---	---	---
A A N G G E V A S L R R R G P A P L R K					

FIGURE 1F

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1791	1800	1809	1818	1827	1836
GCT GAG GGC TGG CTC CCA CTG TCA GGA GGT CAG GGG CAG AGT GAG GAC TCA GAC					
---	---	---	---	---	---
A E G W L P L S G G Q G Q S E D S D					
---	---	---	---	---	---
1845	1854	1863	1872	1881	1890
CCG CTC CTC CAG CAG ATC CAC AAC ATC ACA TCA TTC ATC AGG CAG GCC AAG GCC					
---	---	---	---	---	---
P L L Q Q I H N I T S F I R Q A K A					
---	---	---	---	---	---
1899	1908	1917	1926	1935	1944
GCG GGC CGC ATG GAT GAA GTG CGC ACT CTG CAG GAG AAC CTG CGG CAG CTG CAG					
---	---	---	---	---	---
A G R M D E V R T L Q E N L R Q L Q					
---	---	---	---	---	---
1953	1962	1971	1980	1989	1998
GAC GAG TAT GAC CAG CAG CAG ACA GAG AAG GCC ATC GAG CTG TCC CGG AGG CAG					
---	---	---	---	---	---
D E Y D Q Q Q T E K A I E L S R R Q					
---	---	---	---	---	---
2007	2016	2025	2034	2043	2052
GCT GAG GAG GAC CTG CAG CAG CGG GAA CAG CTG CAG ATG TTG CGT GAA CGG GAG					
---	---	---	---	---	---
A E E E D L Q Q R E E Q Q L L Q M L R E R E					
---	---	---	---	---	---

FIGURE 1G

2061	2070	2079	2088	2097	2106
TTG GAA CGA GAA AGG GAG CAG TTT CGG GTG GCA TCC CTG CAC ACA CGG ACT CGG					
---	---	---	---	---	---
L E R E R E Q F R V A S L H T R T R					
---	---	---	---	---	---
2115	2124	2133	2142	2151	2160
TCC CTG GAC TTC AGA GAA ATC GGC CCT TTT CAG CTG GAG CCC AGC AGA GAG CCT					
---	---	---	---	---	---
S L D F R E I G P F Q L E P S R E P					
---	---	---	---	---	---
2169	2178	2187	2196	2205	2214
CGC ACC CAC CTT GCT TAT GCT TTG GAT CTA GGC TCT TCC CCA GTT CCA AGC AGC					
---	---	---	---	---	---
R T H L A Y A L D L G S S P V P S S					
---	---	---	---	---	---
2223	2232	2241	2250	2259	2268
ACA GCT CCC AAG ACC CCT TCA CTT AGC TCA ACT CAA CCC ACC AGA GTG TGG TCT					
---	---	---	---	---	---
T A P K T P S L S S T Q P T R V W S					
---	---	---	---	---	---
2277	2286	2295	2304	2313	2322
GGG CCC CCA GCC GTT GGC CAG GAG CGC TTA CCC CAG AGC AGC ATG CCA CAG CAA					
---	---	---	---	---	---
G P P A V G Q Q E R L P Q S S M P Q Q					
---	---	---	---	---	---

FIGURE 1H

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2331	2340	2349	2358	2367	2376
CAT GAG GGG CCC TCC TTA AAC CCC TTT GAT GAG GAA GAC CTC TCC AGC CCC ATG					
---	---	---	---	---	---
H E G P S L N P F D E E D L S S P M					
---	---	---	---	---	---
2385	2394	2403	2412	2421	2430
GAA GAG GCC ACT ACT GGT CCT GCT GCA GGG GTT TCC TTA GAC CCT TCA GCC					
---	---	---	---	---	---
E E A T T G P P A A G V S L D P S A					
---	---	---	---	---	---
2439	2448	2457	2466	2475	2484
CGC ATC CTG AAA GAG TAC AAT CCT TTC GAG GAA GAG GAC GAG GAG GAG GAA GCA					
---	---	---	---	---	---
R I L K E Y N P F E E D E E E E A					
---	---	---	---	---	---
2493	2502	2511	2520	2529	2538
GTG GCA GGG AAT CCA TTC ATT CAG CCA GAC AGC CCA GCT CCT AAC CCC TTC AGT					
---	---	---	---	---	---
V A G N P F I Q P D S P A P N P F S					
---	---	---	---	---	---
2547	2556	2565	2574	2583	2592
GAG GAA GAC GAA CAT CCC CAG CAG AGG CTC TCA AGC CCT CTG GTT CCT GGT AAC					
---	---	---	---	---	---
E E D E H P Q Q R L S S P L V P G N					
---	---	---	---	---	---

FIGURE 11

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2601	2610	2619	2628	2637	2646
CCC TTT GAG GAA CCC ACC TGT ATC AAC CCC TTT GAG ATG GAC AGT GAC AGT GGG					
---	---	---	---	---	---
P F E E P T C I N P F E M D S D S G					
2655	2664	2673	2682	2691	2700
CCA GAG GCT GAG GAG CCC ATA GAG GAA GAG CTC CTC CTG CAG CAG ATC GAT AAC					
---	---	---	---	---	---
P E A E E P I E E E L L L Q Q I D N					
2709	2718	2727	2736	2745	2754
ATC AAG GCA TAC ATC TTT GAT GCC AAG CAG TGC GGC CGC CTG GAT GAG GTA GAG					
---	---	---	---	---	---
I K A Y I F D A K Q C G R L D E V E					
2763	2772	2781	2790	2799	2808
GTG CTG ACA GAG AAT CTG CGG GAG CTG AAG CAC ACC CTG GCC AAG CAG AAG GGG					
---	---	---	---	---	---
V L T E N L R E L K H T L A K Q K G					
2817	2826	2835	2844	2853	2862
GGC ACT GAC TGA CCA GCA GTG GAG AGG GCA CCT TTG GGC CCA GGC GTC TGG CAG					
---	---	---	---	---	---
G T D					

FIGURE 1J

2871	GAG CCA GTG GAG CAG GAC	2880	AGA GGG CAG GCA GGA TGG	2898	ATG GGG AAG GTG	2907	GCA GGG
2925	TGA GAA CTC AGA TGC ACA CAG GTG AGG GGC AGG AAT	2943	CTG CTG TTT TGT	2952	GTT GCG	2961	
2979	CAC TTT GAG GTA TTT CCA CTA CAG TTG AAT AAT AAA ATA GAA ACT	2997	AGA ACA GGG	3006	3015	3024	
3033	AGA ATC AGC ATT CAG TTG CTG CTT TTC CTG TTT ATT ATT ACT ATC	3051	3060	3069	3078		
3087	CGG AGG TTT ACC CCT TTT GAA GGG ACT TTA CAT TTT TAC TAC CGA GAT ATA ACT	3096	3105	3114	3123	3132	
3141	AAA TGC AGC TCT GTT GGG CCC AGG GCA GAA ATG GCT GCT GTG TAC CTC TTG GGT	3150	3159	3168	3177	3186	
3195	CCA TTT GCT ACT GCC TAG TCT TGG TTC CTT ATG CAG TAT TAT AGG GCA GCC TTT	3204	3213	3222	3231	3240	
3249	TTA GAG CCC TTC CTT TAG CCA AGA CAG AGA AGA TAG ATT CCA CTG AGC TCT ATT	3258	3267	3276	3285	3294	

FIGURE 1K

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3303 3312 3321 3330 3339 3348
CTG CTC TGA CAG AAG TCC ATC CCT AGT AGG CTG TGA GTT CCA TTT CAC CTG GGG

3357 3366 3375 3384 3393 3402
CCG CCT CTC CCC TGC TCT GCA CTT CCT GTC TGT ACA ATA GAA GGG GGA GGT GCT

3411 3420 3429 3438 3447 3456
GCT ATG AAG GGG AGA GTT TAG ACC CAG GAG AGC CCA GCA CCT CTC TTT AAG GTG

3465 3474 3483 3492 3501 3510
GGG TGA TGG GAA TAT TTC ACC AGG GTC TAT TTT CTC AGT TTA AGT TCT TTT TTG

3519 3528 3537 3546 3555 3564
TCT CTT TCA GGA AGT TAA GCT CCC AGT GCA GGG TAT CAA TGT GAA TCT GGT CCT

3573 3582 3591 3600 3609 3618
GAG CTT TTT AGA AAA TAA GAG TGG TGG CCG GGC GCG CTG GCT CAC GCC TGT AAT

3627 3636 3645 3654 3663 3672
CGC AAC ACT TTG GGA GGC CGA GGC AGG CAG ATC ACA AGG TCA GGA GAT CGA GAC

3681 3690 3699 3708 3717 3726
CAC CCT GGC TAA CAC AGT GAA ATC CCG TCT CTA CTA AAA ATA CAA AAA ATT AGC

FIGURE 1L

3735	3744	3753	3762	3771	3780
CAG GCG TGG TGG TGG GTG CCT GTG GTC CCG GCT ACT TTG GAG GCT GAG GCA GGA					
3789	3798	3807	3816	3825	3834
GAA TGC TGT GAA CCC GGG AGG AGG CAG AGC TTG CAG TGG GCC GAG ATC GCA CCA CTG					
3843	3852	3861	3870	3879	3888
CAC TCC AGC CTG GGC AGT GAG AGT GAG ACT CCG TCT CAA AAA ATA AGA GTG					
3897	3906	3915	3924	3933	3942
GTT AAC TTT TTG GTT GAT ATT AGT ATT ATT TGT GAG AAG AGT TTC CCC ACC CTT					
3951	3960	3969	3978	3987	3996
CTT CTG ATA GAA CAA TTG TCT TCT GTC ACT GGA GAA ATC TCC CTC CAG AGC TTT GGC					
4005	4014	4023	4032	4041	4050
AAA GTT ACT CTG ATC TGG GTC TGT TTA AAA GGC CGG GTC TCT AAT TTA GGA ATC					
4059	4068	4077	4086	4095	4104
GGT GAT TTG GAA GCT TTT GCA GAA CAT CAC CAG AAG AAG GGA AGC TTC CCA GAG					
4113	4122	4131	4140	4149	4158
TCA GAA GCT AAA TTA AAA TAA TTT CCA AGG CTA TTT GAT CAG CCT TCT TCC TTT					

FIGURE 1M

4167	4176	4185	4194	4203	4212
TTG GTT CAT TGT GTC CTG ACT TGG GGC ACT GAT GAG ATT TTT TAT TTT TGA					
4221	4230	4239	4248	4257	4266
GAC GGA GTT TCA CTT TGT TGC CCA GGC TGG AGT GTA GTG GCA TGA TCT CGG CTC					
4275	4284	4293	4302	4311	4320
ACT GCA ACC TCC ACC TCC CTG CTG CAA GTG ATA TTT GTG CCT CAG CCC CCT GAA					
4329	4338	4347	4356	4365	4374
TAG CTG GGA TTA CAG GCG TGG GCC ACC ACG CCT GGT TAA TCT CTG TAT TTT TGG					
4383	4392	4401	4410	4419	4428
TAG AGG TAG AGT TTC ACC ATT TTG GCC AGG CTG GTT TCG AAC TCC TGG CCT CAA					
4437	4446	4455	4464	4473	4482
GTG ATC TGC CAC CTT GGC CTC CCA AAG TGC TGG GAA TAC AGG CGT GAG CCA CTG					
4491	4500	4509	4518	4527	4536
TGC CCG CCC TGA TGA GAT ATT TTA TTA CCA ATG TTA GTA TTG AGA AAC TGA AAT					
4545	4554	4563	4572	4581	4590
GTT TGA AGA AGC ACA ACC CAG GAT CGT GCT GGT AGC ACC ACA GTA CTT AAA CTG					

FIGURE 1N

4599	4608	4617	4626	4635	4644
TTG GTC AAT TAA GGC CAG AAA GGG AAA TTG TTA ATT TAG CTC TGG TGC TTT GGT					
4653	4662	4671	4680	4689	4698
TTA CAG GAA CAT AAC TCT TAA CTG ACA TCT GAC ATC ATG ATA GCC ATA TGT GCT					
4707	4716	4725	4734	4743	4752
CAG CTC TGG GTA GAG TTT CTG CAG TTA CTC ATC TGA ACT AAT GAA CAA TAA CTG					
4761	4770	4779	4788	4797	4806
ACC ACT AGT CAC TTT ATG CCG TGT AAC TAG CTC TAG GCC ATA CTT TCA CTG GTT					
4815	4824	4833	4842	4851	4860
ACT GGT GTG AAA GCT GAA ATT CAT TTT GTT ACA TTC TGG TGA AGA CCC CTC TTG					
4869	4878	4887	4896	4905	4914
ATA ATG GGA ATG TTT TAA CTC TCT TGA TGA AAA AAT AAT CTG TAT TTG TGT TGA					
4923	4932	4941	4950	4959	4968
TGT TCA CAT TTC TGT AGC ACA TTT CTT ATC CTT TTG GTT GAA TGA AAA GAT CTT					
4977	4986	4995	5004	5013	5022
GTA TAG GGG TGT GGA GAT GGG GAG TGG GTA GAA GTG TGT GAA GGA CGC TTT GCA					

FIGURE 10

5031	5040	5049	5058	5067	5076
TTT GGG ATC TGT TCA CAA ACA GCC ATA TGA GTG TGT TAA TGA ATG TCA GCC AGT					
5085	5094	5103	5112	5121	5130
TAC CAA CCC TGC TGG TTG TTA TGG GTT GTT TTG AGA AGT TGG CAA CCA GGC ATC					
5139	5148	5157	5166	5175	5184
TAA GAT GTT GCC TGG TAC AGG CCT CTT TTC TTC CCT GAG GCC CAT GAC ATT TCT					
5193	5202	5211	5220	5229	5238
CTG TTA CTC TAG AGG GTT TCT CAG ATG GCC AGT AGG CTC CTC CCT GCT TAG GGT					
5247	5256	5265	5274	5283	5292
CTC ATT TCT CTG AAA AGA GGA TGA ACT GAA AAG CAG GTA GTT CCA GAA GCT AAT					
5301	5310	5319	5328	5337	5346
TGC TGC TTT CCA TCA TAA TTA TTT TTC TTG TGA GAA CAT TTC TCT TTT AAT TAG					
5355	5364	5373	5382	5391	5400
CTA GTG ATT TTG ATT AAG ACT AAT TCA CTA AAC ATA CCC TTC CCT CAA ATC ACC					
5409	5418	5427	5436	5445	5454
TCA GGT AGC AAT CTG TAA GTA ACT AAA AGC ATT GAA AAA CAC ACA AGA AAC ATT					

FIGURE 1P

5463	5472	5481	5490	5499	5508
TTT AAA AAC TAT TTT TAA AGG CCT GGC CGG GTG CAG TGG CTC ACT CCT GTA ATC					
5517	5526	5535	5544	5553	5562
CCA GCA CTT TGG GAG GCC GAG ACG GGT GGA TCA CCT GAG GTC AGG AAT TTG AGA					
5571	5580	5589	5598	5607	5616
CTA GCA TGG CCA ACA TGG TGA AAC CCC GTG TCT ACT AAA AAT ACA AAA ATT AGC					
5625	5634	5643	5652	5661	5670
TGG GCG TGG TGG CAG ACG CCT GTA ATC CCA GCT ACT CAG GGA GGC TGA GGC ACG					
5679	5688	5697	5706	5715	5724
AGA ATC GCT TGA ACC TGG GAG GCG GAG GTT GCA GTG AGC CAA GAT TGC GCC ACT					
5733	5742	5751	5760	5769	5778
GCA CTC CAA CCT GGG TGA CAA GAG CAA GAC TCC GTC TCA CAA AAA ACA AAC AAA					
5787	5796	5805	5814	5823	5832
ACT GAC TGA TTG AAT ATA CTA AAC CAA ACT AAA ATC ATA TTC TTT TGA TTA AGT					
5841	5850	5859	5868	5877	5886
TTA TCC ATG GCT GTA TTC TAT GAA TTC TAT CAT TTA ATT CTT CCA CGA TTA					

FIGURE 1Q

5895	5904	5913	5922	5931	5940
TCT TCT CCT GTA AAT ACA TCA CAG GAG TTA GAA TTC TCT ACC CAT CAG CTG TAC					
5949	5958	5967	5976	5985	5994
CAT GTC GCA GAA TTC ATG CAG GCA CAA AGT TGG AGT TAC AGA GAT GGG TTG ACA					
6003	6012	6021	6030	6039	6048
GCA GGC AAA CTT GGC CTA TGT ATT ATA ACC ACA ACT TCA AGT TCT TAC CTC ATG					
6057	6066	6075	6084	6093	6102
TGA ATA TTC ACC CTT TCT TTA TTA GTC TTC CAA GGC AAA CAG CCC CGT CTC ATC ACC					
6111	6120	6129	6138	6147	6156
AGA TGA GCA AGG TCT TGA TAT GGC ATA GCA GAT CTC CCT AGA CAC AGA TCA TGA					
6165	6174	6183	6192	6201	6210
GAA AAG ATG GAA GAG ACT TAG GGA TTC AGG CAT CAG ATG AAG TTG GCT TTT CCC					
6219	6228	6237	6246	6255	6264
TTT TAT GCC TTG TTT GTA TTT ACC CTG TCT TCT AAT ACA CTA AGG ATA CTT ACT CAT					
6273	6282	6291	6300	6309	6318
TGT ACT TGC AGC TCA ATA TGT CTT TGC TGT TCA GAT ACT AAA ATG TAC CTC TGA					

FIGURE 1R

6327	6336	6345	6354	6363	6372
GTC ATT GTG AGC TGT GTG GTA GGT TGG ACA TTG GCA TAG TTG GTG ATG GGA CTC					
6381	6390	6399	6408	6417	6426
AAA ATG AAA AGG TGG TCT CTT TAC CAG GTC ACA GAC TGT AGC AGA TTG TGC TTG					
6435	6444	6453	6462	6471	6480
TTA TCT GAC AAT GAC TGT CAC TTT GAG GGT CGT TGA TTT GCA TGC ACT ACT CTG					
6489	6498	6507	6516	6525	6534
GGG CCT TGT ATT GGA GCC TTT AAA AAA AAT AAA ATC TGA GAT AGA GGT TGG					
6543	6552	6561	6570	6579	6588
GGG TGT GTG TGT CTG TGT GCA CAT CAT GTG TTA CAA GTG AGA ATC ATC AGA TGA CAT					
6597	6606	6615	6624	6633	6642
CCC TTC TCC TTT CTT GAT GAC AAC CAT CTG AGT ATC AGA ATA GTT CCA GCA CCT					
6651	6660	6669	6678	6687	6696
GTG TTG TTT GTC TGG TTA AGG CCT CTG GAA AAA ATG AAG GTC ACT GGG TTC TGA					
6705	6714	6723	6732	6741	6750
ACA GGG GAT AGA TAC GGG TTC CAG TTC TGC CCT TAT TCC CAG TTA TTC CTG CAG					

FIGURE 1S

6759	TGC	TGG	TTA	AAT	GAA	CAG	TTT	TAC	AGA	AGT	ATA	GTG	CAG	ATC	CCT	TAA	TGA	TCC	6804
6813	TAT	CAT	CCC	AGT	TTC	ACA	CTG	GAA	GAA	ACT	GAG	GCT	CAG	AGA	TTG	AGG	AAC	CTT	6858
6867	GTG	ACT	GCC	ATC	TGT	GCC	AGT	CAC	CAC	CTC	TGC	ATG	ACC	ACA	TTT	CCC	TCC	ATT	6912
6921	AGC	ACT	ACC	AGC	ATG	CCT	GTG	AGG	TAG	TTA	CTC	AGC	TGT	TCA	TCT	GAC	CCC	AGA	6966
6975	CGT	AGA	AAA	GTT	AAG	AGA	GAT	TAG	CCT	GGC	TTA	GGC	CAC	ACA	GCC	AGT	CAG	TGG	7020
7029	TGG	AAT	GAG	GGT	TTA	AAT	CCA	GAT	CTG	CCT	GAC	TTA	ATT	TGC	CCT	CAG	TAA	TAA	7074
7083	GTA	GCT	GGG	TCA	GGT	AGT	GGT	GTG	GTG	GTG	GGT	GGT	GCT	CAA	CCA	TTG	TGT	CAA	7128
7137	GCT	CAA	AGA	GGG	CAG	AAA	AGG	GCA	ATG	GGG	AAT	GAG	GTT	GGG	TGC	TGC	TGA	GCT	7182

FIGURE 1T

7191	7200	7209	7218	7227	7236
GGT TTC CGA TAG TGA CTG CCT GAG TCT GTG AAA CTG AGA CCT CCT TAG GGC CTT					
7245	7254	7263	7272	7281	7290
AAG AAT ATA TAG ATT CTT AAA CCA GGA GAG TAT AAT ATC TAG TGC TCA CTG TAG					
7299	7308	7317	7326	7335	7344
GCT AGA CTG AGT GCT AAG TAT TAC GCA TGG CTT ACC TCA GTC TTC CCA ACA ACC					
7353	7362	7371	7380	7389	7398
CAT TTA CAC ATG CAG AAA TGA GTG AGT GGT GAA GTT AAG CTT GCT TAG GGT TGC					
7407	7416	7425	7434	7443	7452
ACT GCT GGG TTT CCA GCC TAG GTC TGT CTG ACT GCA AAA CCC AAG CTG CCC TAG					
7461	7470	7479	7488	7497	7506
ATT GTG TTG TAT GTG GAC ACC CAT GTT TCT AAT GTG GAA TGG GGT GTT TCA TCC					
7515	7524	7533	7542	7551	7560
CTA GCA AGT GCC TCC TGT GGA CTT TTA AGA TGA AAA ACT TCT GCC TAA CTC TTC					
7569	7578	7587	7596	7605	7614
AGG AAG CCT AGC GTA GTT GTA AAA ATA CTT CAG AAA GCA CTT CTG GTT TTA GCT					

FIGURE 1U

7623	CAG ACA CGT AGA GTT TGA AAG TTA TCC TAT CCT TAC CAC AAG AAA AAG CTG GGC	7632	7641	7650	7659	7668
7677	AAA CTC AAA AGC ATT GGC CTT TCT TGG TCC CAT TAG AGA GCT GAG TGT GCA GGT	7686	7695	7704	7713	7722
7731	CAA ACT GCC ATC CCA AAA TCT AGA GAG AGG TGA GTC CAG AGA GTG AGC AAG ATC	7740	7749	7758	7767	7776
7785	TAA TTG CCT GGA GCA GAA GCC ACG GGA GCC ATA GCT AGT AGG AAA ACT TAA ATG	7794	7803	7812	7821	7830
7839	GTA ATT TTG ATG AAC TGC TGG AGG TTG AGT GTG GAC TAG CAT GGG AGG GAG CAA	7848	7857	7866	7875	7884
7893	CTC CCC CTC ACC CCC AAC GCT TTC CTG GGT TTT ACC CTA CTT GGT TCT ATT GGT	7902	7911	7920	7929	7938
7947	GAA GAG CTG AGG AAG ACC CTC TGG TGA CTC TGG CAG GGG AAA GGG AGA ATC ATC	7956	7965	7974	7983	7992
8001	TTT GTA ATC AGA GCC TTC TCC ATA ATA AA 3'	8010	8019			

FIGURE 1V

5'			9			18			27			36			45			54
	GCC	TGC	CGA	GTT	CCG	AGC	GAC	CGA	TGG	AGA	TGG	CGG	CTG	CGG	CTG	AGT	GAC	GGA
			63			72			81			90			99			108
	CGG	TGG	AGG	CCC	AGA	GCC	CGG	GCC	TGA	AGG	GGG	GGA	CAA	ACC	TGG	GTG	CCC	GCA
			117			126			135			144			153			162
	GGA	GCC	CGG	CAG	GGT	GTC	TTA	CAA	GTA	TCA	AGA	ACT	TAC	TAT	ATG	TGG	TTG	AAT
			171			180			189			198			207			216
	AAA	CAA	TCA	AGG	TAA	AGA	GCA	TCA	AGT	AAA	AAC	TTC	TGC	TTG	TTG	ATA	AGT	ACT
			225			234			243			252			261			270
	TCA	GAC	ATT	CCC	CCA	GTG	GCT	GAA	GTG	GCA	TAT	GAA	TTA	TGA	AGT	TGG	ATC	ATT
			279			288			297			306			315			324
	TGG	AAT	GAA	TGT	AAG	AGA	ATT	GCC	AAG	GGC	TCC	TCC	TAC	TCC	AGA	GAG	GAA	ACC
			333			342			351			360			369			378
	TCA	TCC	AGG	GCC	ATG	AAG	CCA	CTT	CCT	CAC	CAT	CTG	TGT	GCT	GCT	TAA	GCT	AAT
			387			396			405			414			423			432
	GCT	GCG	GGA	ACC	ATG	GTT	CCT	TGG	GAG	GAA	TCA	AGC	TGA	CTC	TTG	GCA	TGA	GAT