



US00RE40948E

(19) **United States**
(12) **Reissued Patent**
Vogelstein et al.

(10) **Patent Number:** **US RE40,948 E**
(45) **Date of Reissued Patent:** ***Oct. 27, 2009**

(54) **APC ANTIBODIES**

(75) Inventors: **Bert Vogelstein**, Baltimore, MD (US);
Kenneth W. Kinzler, Bel Air, MD (US);
Hans Albertsen, Cupertino, CA (US);
Rakesh Anand, Sandbach (GB); **Mary Carlson**, Salt Lake City, UT (US);
Joanna Groden, Salt Lake City, UT (US); **Philip Hedge**, Winsford (GB);
Geoff Joslyn, Salt Lake City, UT (US);
Alexander Fred Markham, Crewe (GB); **Yusuke Nakumura**, Tokyo (JP);
Andrew Thliveris, Salt Lake City, UT (US); **Raymond White**, Salt Lake City, UT (US)

(73) Assignees: **The Johns Hopkins University**, Baltimore; **Astrazeneca United Kingdom, Ltd.**, London (GB); **Cancer Institute, Japanese, Foundation for Cancer Research**, Tokyo (JP); **The University of Utah**, Salt Lake City, UT (US)

(*) Notice: This patent is subject to a terminal disclaimer.

(21) Appl. No.: **09/983,543**

(22) Filed: **Oct. 24, 2001**

Related U.S. Patent Documents

Reissue of:

(64) Patent No.: **5,691,454**
Issued: **Nov. 25, 1997**
Appl. No.: **08/452,654**
Filed: **May 25, 1995**

U.S. Applications:

(60) Continuation of application No. 09/442,489, filed on Nov. 18, 1999, which is a division of application No. 08/289,548, filed on Aug. 12, 1994, now Pat. No. 5,648,212, which is a division of application No. 07/741,940, filed on Aug. 8, 1991, now Pat. No. 5,352,775.

(30) **Foreign Application Priority Data**

Jan. 16, 1991 (GB) 9100962.1
Jan. 16, 1991 (GB) 9100963.9
Jan. 16, 1991 (GB) 9100974.6
Jan. 16, 1991 (GB) 9100975.3

(51) **Int. Cl.**
C07K 16/30 (2006.01)
A61K 39/395 (2006.01)

(52) **U.S. Cl.** **530/387.9**; 530/387.1; 530/387.7;
530/828; 530/806; 424/130.1; 424/138.1;
424/139.1; 424/174.1

(58) **Field of Classification Search** 530/388.2,
530/387.9, 387.7, 388.8, 389.7, 806, 827,
530/843, 844, 387.1; 536/23.5; 424/130.1,
424/138.1, 141.1, 174.1; 435/240.27
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,098,823 A 3/1992 Bodmer et al.
5,137,806 A 8/1992 LeMaistre et al.
5,244,801 A * 9/1993 Tobi

FOREIGN PATENT DOCUMENTS

WO WO 89/01481 8/1988

OTHER PUBLICATIONS

Kinzler et al., "Identification of FAP Locus Genes from Chromosome 5q21", *Science* 253: 661-665, 1991.
Groden et al., "Identification and Characterization of the familial Adenomatous Polyposis Coli Gene", *Cell* 66:589-600.
Joslyn et al. "Identification of Deletion Mutations and Three New Genes at the Familial Polyposis Locus" *Cell*, 66:601-613 (1991).
Nishisho et al., "Mutations of Chromosome 5q21 Genes in FAP and Colorectal Cancer Patients" *Science*, 253:665-669 (1991).
Orita et al., *Genomics*, vol. 5, pp. 874-879, 1989.
Stanbridge et al., "Identifying Tumor Suppressor Genes in Human Colorectal Cancer", *Science* 247:12-13 (1990).
Fearon et al., "Identification of a Chromosome 18q Gene that is Altered in Colorectal Cancer", *Science* 247:49-56 (1990).
Baker et al., "Chromosome 17 Deletions and p53 Gene Mutations in Colorectal Carcinomas", *Science*, 244:217-221 (1989).
Bodmer et al. "Localization of the Gene for familial Adenomatous Polyposis of Chromosome 5" *Nature* 328:614-616 (1987).
Kinzler et al, *Science* 253: 661-665, 1991.*

* cited by examiner

Primary Examiner—Eileen B O'Hara

Assistant Examiner—F. Pierre VanderVegt

(74) *Attorney, Agent, or Firm*—Banner & Witcoff, LTD

(57) **ABSTRACT**

A human gene termed APC is disclosed. Methods and kits are provided for assessing mutations of the APC gene in human tissues and body samples. APC mutations are found in familial adenomatous polyposis patients as well as in sporadic colorectal cancer patients. APC is expressed in most normal tissues. These results suggest that APC is a tumor suppressor.

1 Claim, 40 Drawing Sheets

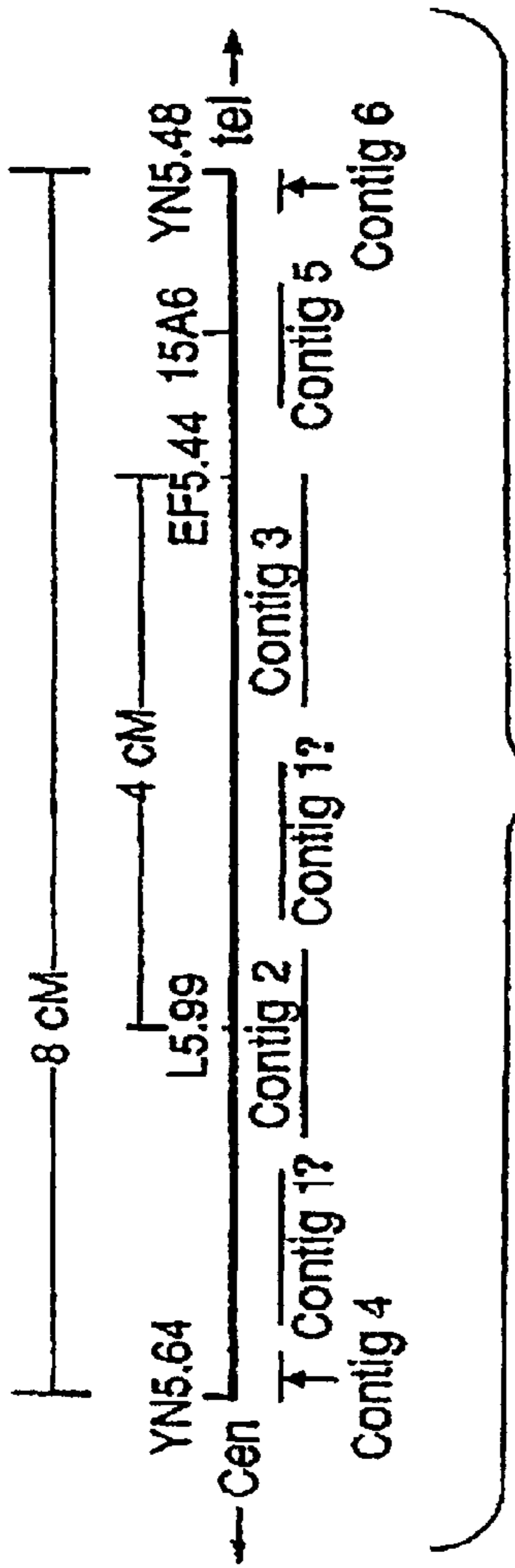


FIG. 1A

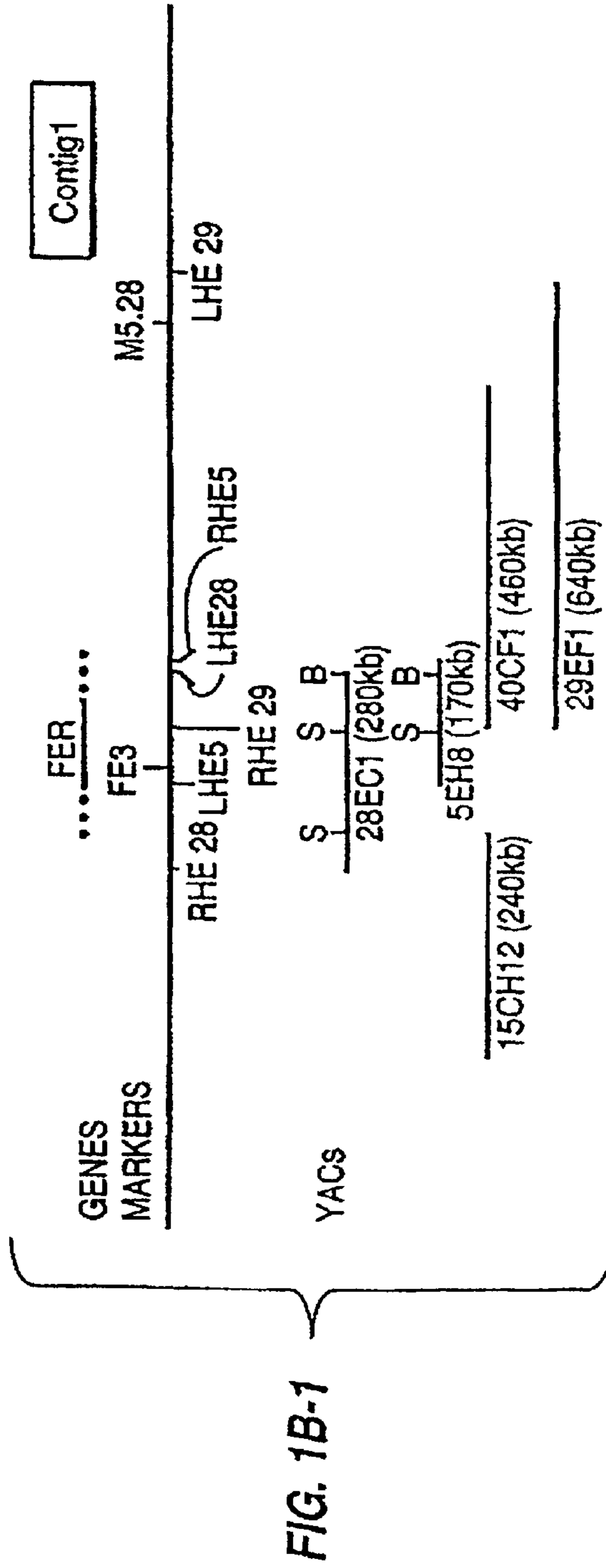


FIG. 1B-1

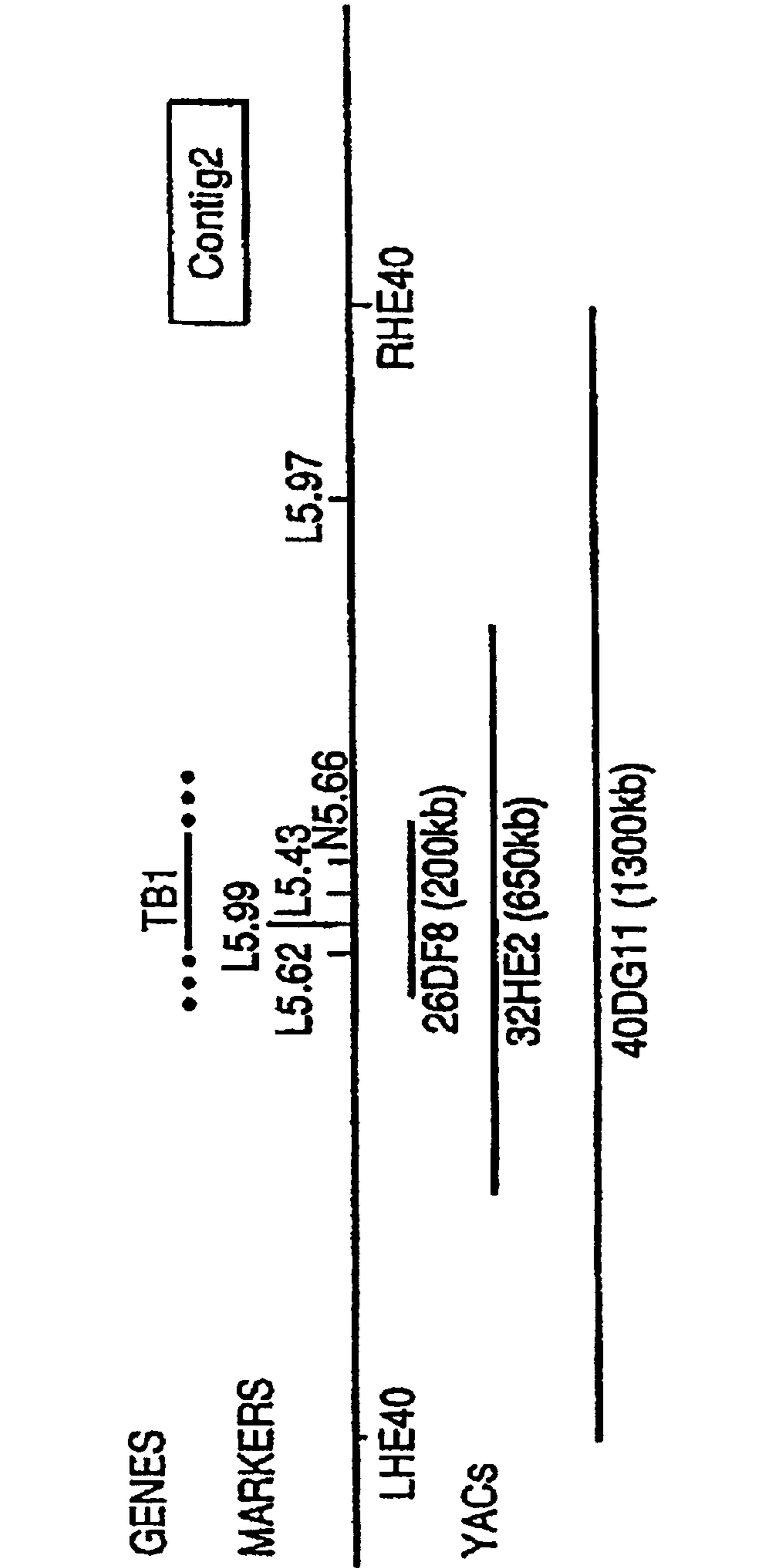


FIG. 1B-2

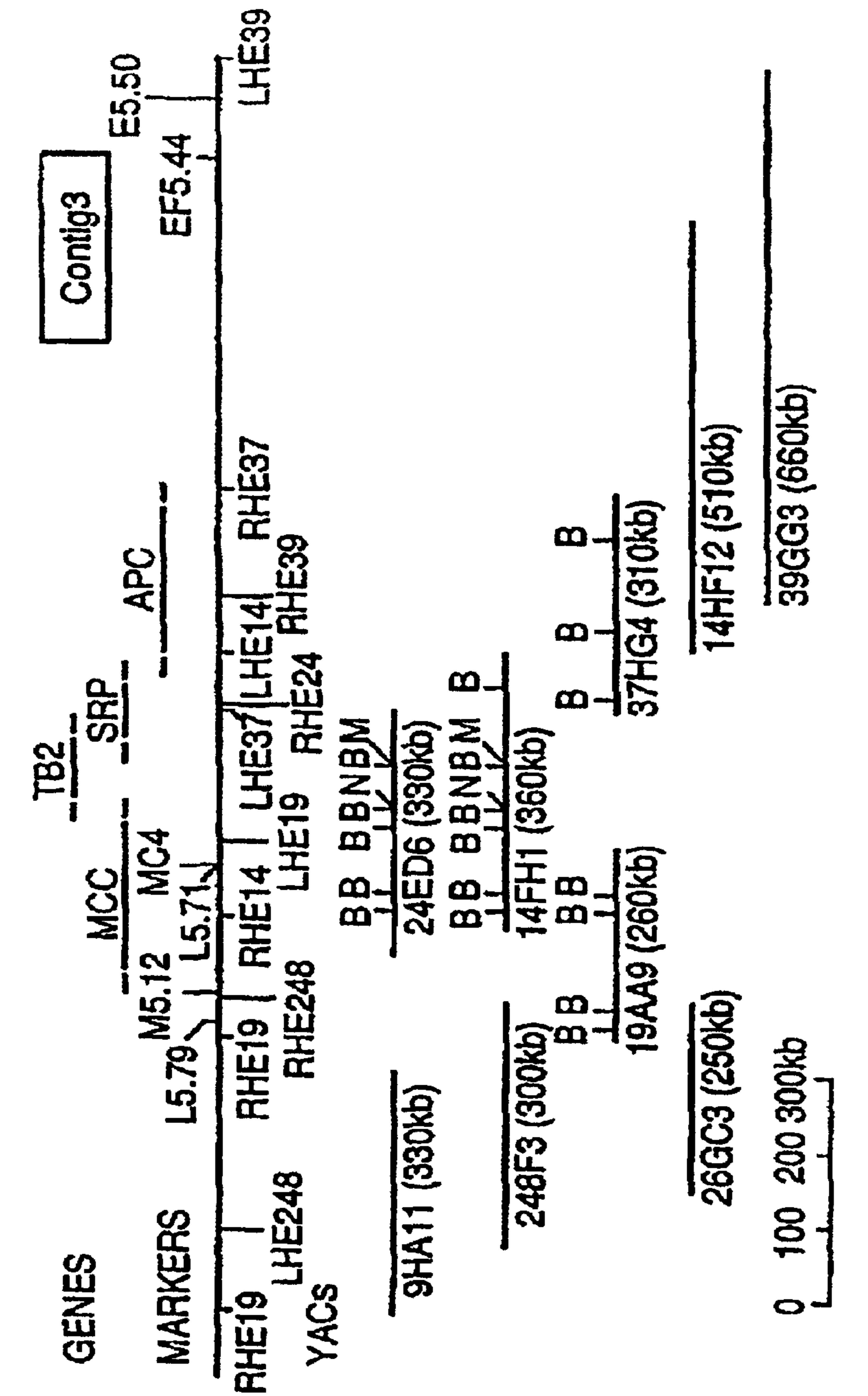


FIG. 1B-3

FIG. 2A

TB1 AMINO ACID SEQUENCE

VAPVYVGSGR APRHPAPAAM HPRRPOGFDG LGYRGGARDE QGFGGAFPAR SFSTGSDLGH 60
WVTTPPDIPG SRNLHWGEKS PPYGVPTTST PYEGPTEEPF SGGGGSVQG OSSEQLNRFA 120
GFGIGLASLF TENVLAHPCI VLRROCOVNY HAQHYHLTPF TVINIMYSFN KTOGPRALWK 180
GMGSTFIYOG VTLGAEGIIS EFTPLPREVL HKWSPKOIGE HLLKSLTYV VAMPFYSASL 240
IETVQSEIR DNTGILECVK EGIGRVIGMG VPHSKRLPL LSLIFPTVLH GVLHYIISV 300
IOKFVLLIK RKTYNSHLAE STSPVQSHLD AYFPELIANF AASLCSOVIL YPLETVLHRL 360
HIQGTRTIID NTOLGYEVLV INTQYEGMRD CINTIRQEEG VFGFYKGFGA VIIQYTLHAA 420
VLOITKIIYS TLLO 434

FIG. 2B

TB2 AMINO ACID SEQUENCE

ELRRFDRFLH EKNCHTDLLA KLEAKTGVNR SFIALGVIGL VALYLVFGYG ASLLCNLIGF 60
GYPAYISIKA IESPNKEDDT QWLTYNVVYVYG VFSIAEFFSD IFLSWFFYY ILKCGFLLWC 120
MAPSPSNGAE LLYKRIIRPF FLKHESQMS VVKDLKDKAK ETADAITKEA KKATVNLIGE 180
EKKST 185

FIG. 3A

Met Ala Ala Ser Tyr Asp Gln Leu Leu Lys Gln Val Glu Ala Leu
1 5 10 15
Lys Met Glu Asn Ser Asn Leu Arg Gln Glu Leu Glu Asp Asn Ser Asn
20 25 30
His Leu Thr Lys Leu Glu Thr Glu Ala Ser Asn Met Lys Glu Val Leu
35 40 45
Lys Gln Leu Gln Gly Ser Ile Glu Asp Glu Ala Met Ala Ser Ser Gly
50 55 60
Gln Ile Asp Leu Leu Glu Arg Leu Lys Glu Leu Asn Leu Asp Ser Ser
65 70 75 80
Asn Phe Pro Gly Val Lys Leu Arg Ser Lys Met Ser Leu Arg Ser Tyr
85 90 95
Gly Ser Arg Glu Gly Ser Val Ser Ser Arg Ser Gly Glu Cys Ser Pro
100 105 110

FIG. 3C

Glu	Lys	Asp	Ile	Leu	Arg	Ile	Arg	Gln	Leu	Leu	Gln	Ser	Gln	Ala	Thr
225					230					235					240
Glu	Ala	Glu	Arg	Ser	Ser	Gln	Asn	Lys	His	Glu	Thr	Gly	Ser	His	Asp
				245					250					255	
Ala	Glu	Arg	Gln	Asn	Glu	Gly	Gln	Gly	Val	Gly	Glu	Ile	Asn	Met	Ala
			260					265					270		
Thr	Ser	Gly	Asn	Gly	Gln	Gly	Ser	Thr	Thr	Arg	Met	Asp	His	Glu	Thr
		275					280						285		
Ala	Ser	Val	Leu	Ser	Ser	Ser	Thr	His	Ser	Ala	Pro	Arg	Arg	Leu	
					295					300					
Thr	Ser	His	Leu	Gly	Thr	Lys	Val	Glu	Met	Val	Tyr	Ser	Leu	Leu	Ser
305					310					315					320
Met	Leu	Gly	Thr	His	Asp	Lys	Asp	Asp	Met	Ser	Arg	Thr	Leu	Leu	Ala
					325				330						

FIG. 3D

Met Ser Ser Ser Gln Asp Ser Cys Ile Ser Met Arg Gln Ser Gly Cys
 340 345 350
 Leu Pro Leu Leu Ile Gln Leu Leu His Gly Asn Asp Lys Asp Ser Val
 355 360 365
 Leu Leu Gly Asn Ser Arg Gly Ser Lys Glu Ala Arg Ala Arg Ala Ser
 370 375 380
 Ala Ala Leu His Asn Ile Ile His Ser Gln Pro Asp Asp Lys Arg Gly
 385 390 395 400
 Arg Arg Glu Ile Arg Val Leu His Leu Leu Glu Gln Ile Arg Ala Tyr
 405 410 415
 Cys Glu Thr Cys Trp Glu Trp Gln Glu Ala His Glu Pro Gly Met Asp
 420 425 430
 Gln Asp Lys Asn Pro Met Pro Ala Pro Val Glu His Gln Ile Cys Pro
 435 440 445

FIG. 3E

Ala Val Cys Val Leu Met Lys Leu Ser Phe Asp Glu Glu His Arg His	
450	460
Ala Met Asn Glu Leu Gly Gly Leu Gln Ala Ile Ala Glu Leu Leu Gln	
465	475
Val Asp Cys Glu Met Tyr Gly Leu Thr Asn Asp His Tyr Ser Ile Thr	
485	490
Leu Arg Arg Tyr Ala Gly Met Ala Leu Thr Asn Leu Thr Phe Gly Asp	
500	510
Val Ala Asn Lys Ala Thr Leu Cys Ser Met Lys Gly Cys Met Arg Ala	
515	525
Leu Val Ala Gln Leu Lys Ser Glu Ser Glu Asp Leu Gln Gln Val Ile	
530	540
Ala Ser Val Leu Arg Asn Leu Ser Trp Arg Ala Asp Val Asn Ser Lys	
545	555
	560

FIG. 3F

Lys Thr Leu Arg Glu Val Gly Ser Val Lys Ala Leu Met Glu Cys Ala	575
	570
Leu Glu Val Lys Lys Glu Ser Thr Leu Lys Ser Val Leu Ser Ala Leu	590
	585
Trp Asn Leu Ser Ala His Cys Thr Glu Asn Lys Ala Asp Ile Cys Ala	605
	600
Val Asp Gly Ala Leu Ala Phe Leu Val Gly Thr Leu Thr Tyr Arg Ser	620
	615
Gln Thr Asn Thr Leu Ala Ile Ile Glu Ser Gly Gly Gly Ile Leu Arg	640
	635
Asn Val Ser Ser Leu Ile Ala Thr Asn Glu Asp His Arg Gln Ile Leu	655
	650
Arg Glu Asn Asn Cys Leu Gln Thr Leu Leu Gln His Leu Lys Ser His	670
	665

FIG. 3H

His Arg Ser Lys Gln Arg His Lys Gln Ser Leu Tyr Gly Asp Tyr Val
785 790 795 800

Phe Asp Thr Asn Arg His Asp Asp Asn Arg Ser Asp Asn Phe Asn Thr
805 810

Gly Asn Met Thr Val Leu Ser Pro Tyr Leu Asn Thr Thr Val Leu Pro
820 825

Ser Ser Ser Ser Arg Gly Ser Leu Asp Ser Ser Arg Ser Glu Lys
835 840

Asp Arg Ser Leu Glu Arg Glu Arg Gly Ile Gly Leu Gly Asn Tyr His
850 855 860

Pro Ala Thr Glu Asn Pro Gly Thr Ser Ser Lys Arg Gly Leu Gln Ile
865 870 875

Ser Thr Thr Ala Ala Gln Ile Ala Lys Val Met Glu Glu Val Ser Ala
880 885 890

FIG. 3J

His Ser Ala Asn His Met Asp Asp Asn Asp Gly Glu Leu Asp Thr Pro	
1010	1020
Ile Asn Tyr Ser Leu Lys Tyr Ser Asp Glu Gln Leu Asn Ser Gly Arg	
1025	1035
Gln Ser Pro Ser Gln Asn Glu Arg Trp Ala Arg Pro Lys His Ile Ile	
1045	1050
Glu Asp Glu Ile Lys Gln Ser Glu Gln Arg Gln Ser Arg Asn Gln Ser	
1060	1070
Thr Thr Tyr Pro Val Tyr Thr Glu Ser Thr Asp Asp Lys His Leu Lys	
1075	1085
Phe Gln Pro His Phe Gly Gln Gln Glu Cys Val Ser Pro Tyr Arg Ser	
1090	1100
Arg Gly Ala Asn Gly Ser Glu Thr Asn Arg Val Gly Ser Asn His Gly	
1105	1115
	1120

FIG. 3K

Ile	Asn	Gln	Asn	Val	Ser	Gln	Ser	Leu	Cys	Gln	Glu	Asp	Asp	Tyr	Glu	
				1125					1130						1135	
Asp	Asp	Lys	Pro	Thr	Asn	Tyr	Ser	Glu	Arg	Tyr	Ser	Glu	Glu	Glu	Gln	
			1140				1145								1150	
His	Glu	Glu	Glu	Glu	Arg	Pro	Thr	Asn	Tyr	Ser	Ile	Lys	Tyr	Asn	Glu	
		1155					1160						1165			
Glu	Lys	Arg	His	Val	Asp	Gln	Pro	Ile	Asp	Tyr	Ser	Leu	Lys	Tyr	Ala	
		1170				1175						1180				
Thr	Asp	Ile	Pro	Ser	Ser	Gln	Lys	Gln	Ser	Phe	Ser	Phe	Ser	Lys	Ser	
						1190				1195					1200	
Ser	Ser	Gly	Gln	Ser	Ser	Lys	Thr	Glu	His	Met	Ser	Ser	Ser	Ser	Glu	
				1205						1210					1215	
Asn	Thr	Ser	Thr	Pro	Ser	Ser	Asn	Ala	Lys	Arg	Gln	Asn	Gln	Leu	His	
				1220					1225						1230	

FIG. 3L

Pro Ser Ser Ala Gln Ser Arg Ser Gly Gln Pro Gln Lys Ala Ala Thr
1235 1240 1245

Cys Lys Val Ser Ser Ile Asn Gln Glu Thr Ile Gln Thr Tyr Cys Val
1250 1255 1260

Glu Asp Thr Pro Ile Cys Phe Ser Arg Cys Ser Ser Leu Ser Ser Leu
1265 1270 1275 1280

Ser Ser Ala Glu Asp Glu Ile Gly Cys Asn Gln Thr Thr Gln Glu Ala
1285 1290 1295

Asp Ser Ala Asn Thr Leu Gln Ile Ala Glu Ile Lys Gly Lys Ile Gly
1300 1305 1310

Thr Arg Ser Ala Glu Asp Pro Val Ser Glu Val Pro Ala Val Ser Gln
1315 1320 1325

His Pro Arg Thr Lys Ser Ser Arg Leu Gln Gly Ser Ser Leu Ser Ser
1330 1335 1340

FIG. 3M

Glu Ser Ala Arg His Lys Ala Val Glu Phe Pro Ser Gly Ala Lys Ser
1345 1350 1355 1360
Pro Ser Lys Ser Gly Ala Gln Thr Pro Lys Ser Pro Pro Glu His Tyr
1365 1370 1375
Val Gln Glu Thr Pro Leu Met Phe Ser Arg Cys Thr Ser Val Ser Ser
1380 1385 1390
Leu Asp Ser Phe Glu Ser Arg Ser Ile Ala Ser Ser Val Gln Ser Glu
1395 1400 1405
Pro Cys Ser Gly Met Val Ser Gly Ile Ile Ser Pro Ser Asp Leu Pro
1410 1415 1420
Asp Ser Pro Gly Gln Thr Met Pro Pro Ser Arg Ser Lys Thr Pro Pro
1425 1430 1435 1440
Pro Pro Pro Gln Thr Ala Gln Thr Lys Arg Glu Val Pro Lys Asn Lys
1445 1450 1455

FIG. 3N

Ala Pro Thr Ala Glu Lys Arg Glu Ser Gly Pro Lys Gln Ala Ala Val
1460 1465 1470
Asn Ala Ala Val Gln Arg Val Gln Val Leu Pro Asp Ala Asp Thr Leu
1475 1480 1485
Leu His Phe Ala Thr Glu Ser Thr Pro Asp Gly Phe Ser Cys Ser Ser
1490 1495 1500
Ser Leu Ser Ala Leu Ser Leu Asp Glu Pro Phe Ile Gln Lys Asp Val
1505 1510 1515 1520
Glu Leu Arg Ile Met Pro Pro Val Gln Glu Asn Asp Asn Gly Asn Glu
1525 1530 1535
Thr Glu Ser Glu Gln Pro Lys Glu Ser Asn Glu Asn Gln Glu Lys Glu
1540 1545 1550
Ala Glu Lys Thr Ile Asp Ser Glu Lys Asp Leu Leu Asp Asp Ser Asp
1555 1560 1565

FIG. 30

Asp Asp Ile Glu Ile Leu Glu Glu Cys Ile Ile Ser Ala Met Pro
 1570 1575 1580
 Thr Lys Ser Ser Arg Lys Gly Lys Lys Pro Ala Gln Thr Ala Ser Lys
 1585 1590 1595 1600
 Leu Pro Pro Val Ala Arg Lys Pro Ser Gln Leu Pro Val Tyr Lys
 1605 1610 1615
 Leu Leu Pro Ser Gln Asn Arg Leu Gln Pro Gln Lys His Val Ser Phe
 1620 1625 1630
 Thr Pro Gly Asp Asp Met Pro Arg Val Tyr Cys Val Glu Gly Thr Pro
 1635 1640 1645
 Ile Asn Phe Ser Thr Ala Thr Ser Leu Ser Asp Leu Thr Ile Glu Ser
 1650 1655 1660
 Pro Pro Asn Glu Leu Ala Ala Gly Glu Gly Val Arg Gly Gly Ala Gln
 1665 1670 1675 1680

FIG. 3P

Ser Gly Glu Phe Glu Lys Arg Asp Thr Ile Pro Thr Glu Gly Arg Ser
1685 1690
Thr Asp Glu Ala Gln Gly Gly Lys Thr Ser Ser Val Thr Ile Pro Glu
1700 1705
Leu Asp Asp Asn Lys Ala Glu Glu Gly Asp Ile Leu Ala Glu Cys Ile
1715 1720 1725
Asn Ser Ala Met Pro Lys Gly Lys Ser His Lys Pro Phe Arg Val Lys
1730 1735 1740
Lys Ile Met Asp Gln Val Gln Gln Ala Ser Ala Ser Ser Ala Pro
1745 1750 1755 1760
Asn Lys Asn Gln Leu Asp Gly Lys Lys Lys Pro Thr Ser Pro Val
1765 1770 1775
Lys Pro Ile Pro Gln Asn Thr Glu Tyr Arg Thr Arg Val Arg Lys Asn
1780 1785 1790

FIG. 3Q

Ala Asp Ser Lys Asn Asn Leu Asn Ala Glu Arg Val Phe Ser Asp Asn
1795 1800
Lys Asp Ser Lys Lys Gln Asn Leu Lys Asn Asn Ser Lys Asp Phe Asn
1810 1815 1820
Asp Lys Leu Pro Asn Asn Glu Asp Arg Val Arg Gly Ser Phe Ala Phe
1825 1830 1835
Asp Ser Pro His His Tyr Thr Pro Ile Glu Gly Thr Pro Tyr Cys Phe
1845 1850
Ser Arg Asn Asp Ser Leu Ser Ser Leu Asp Phe Asp Asp Asp Val
1860 1865 1870
Asp Leu Ser Arg Glu Lys Ala Glu Leu Arg Lys Ala Lys Glu Asn Lys
1875 1880 1885
Glu Ser Glu Ala Lys Val Thr Ser His Thr Glu Leu Thr Ser Asn Gln
1890 1900

FIG. 3R

Gln Ser Ala Asn Lys Thr Gln Ala Ile Ala Lys Gln Pro Ile Asn Arg
1905 1910 1915 1920
Gly Gln Pro Lys Pro Ile Leu Gln Lys Gln Ser Thr Phe Pro Gln Ser
1925 1930 1935
Ser Lys Asp Ile Pro Asp Arg Gly Ala Ala Thr Asp Glu Lys Leu Gln
1940 1945 1950
Asn Phe Ala Ile Glu Asn Thr Pro Val Cys Phe Ser His Asn Ser Ser
1955 1960 1965
Leu Ser Ser Leu Ser Asp Ile Asp Gln Glu Asn Asn Asn Lys Glu Asn
1970 1975 1980
Glu Pro Ile Lys Glu Thr Glu Pro Pro Asp Ser Gln Gly Glu Pro Ser
1985 1990 1995 2000
Lys Pro Gln Ala Ser Gly Tyr Ala Pro Lys Ser Phe His Val Glu Asp
2005 2010 2015

FIG. 3S

Thr Pro Val Cys Phe Ser Arg Asn Ser Ser Leu Ser Ser Leu Ser Ile
2020 2025 2030
Asp Ser Glu Asp Asp Leu Leu Gln Glu Cys Ile Ser Ser Ala Met Pro
2035 2040 2045
Lys Lys Lys Lys Pro Ser Arg Leu Lys Gly Asp Asn Glu Lys His Ser
2050 2055 2060
Pro Arg Asn Met Gly Gly Ile Leu Gly Glu Asp Leu Thr Leu Asp Leu
2065 2070 2075 2080
Lys Asp Ile Gln Arg Pro Asp Ser Glu His Gly Leu Ser Pro Asp Ser
2085 2090 2095
Glu Asn Phe Asp Trp Lys Ala Ile Gln Glu Gly Ala Asn Ser Ile Val
2100 2105 2110
Ser Ser Leu His Gln Ala Ala Ala Cys Leu Ser Arg Gln Ala
2115 2120 2125

FIG. 3T

Ser Ser Asp Ser Asp Ser Ile Leu Ser Ser Leu Lys Ser Gly Ile Ser Leu
2130 2135 2140
Gly Ser Pro Phe His Leu Thr Pro Asp Gln Glu Glu Lys Pro Phe Thr
2145 2150 2155 2160
Ser Asn Lys Gly Pro Arg Ile Leu Lys Pro Gly Glu Lys Ser Thr Leu
2165 2170 2175
Glu Thr Lys Lys Ile Glu Ser Glu Ser Lys Gly Ile Lys Gly Gly Lys
2180 2185 2190
Lys Val Tyr Lys Ser Leu Ile Thr Gly Lys Val Arg Ser Asn Ser Glu
2195 2200 2205
Ile Ser Gly Gln Met Lys Gln Pro Leu Gln Ala Asn Met Pro Ser Ile
2210 2215 2220
Ser Arg Gly Arg Thr Met Ile His Ile Pro Gly Val Arg Asn Ser
2225 2230 2235 2240

FIG. 3U

Ser Ser Thr Ser Pro Val Ser Lys Lys Gly Pro Pro Leu Lys Thr Pro
2245 2250 2255

Ala Ser Lys Ser Pro Ser Glu Gly Gln Thr Ala Thr Ser Pro Arg
2260 2265 2270

Gly Ala Lys Pro Ser Val Lys Ser Glu Leu Ser Pro Val Ala Arg Gln
2275 2280 2285

Thr Ser Gln Ile Gly Gly Ser Ser Lys Ala Pro Ser Arg Ser Gly Ser
2290 2295 2300

Arg Asp Ser Thr Pro Ser Arg Pro Ala Gln Pro Leu Ser Arg Pro
2305 2310 2315 2320

Ile Gln Ser Pro Gly Arg Asn Ser Ile Ser Pro Gly Arg Asn Gly Ile
2325 2330 2335

Ser Pro Pro Asn Lys Leu Ser Gln Leu Pro Arg Thr Ser Ser Pro Ser
2340 2345 2350

FIG. 3V

Thr Ala Ser Thr Lys Ser Ser Gly Ser Gly Lys Met Ser Tyr Thr Ser
 2355 2360 2365
 Pro Gly Arg Gln Met Ser Gln Gln Asn Leu Thr Lys Gln Thr Gly Leu
 2370 2375 2380
 Ser Lys Asn Ala Ser Ser Ile Pro Arg Ser Glu Ser Ala Ser Lys Gly
 2385 2390 2395 2400
 Leu Asn Gln Met Asn Asn Gly Asn Gly Ala Asn Lys Lys Val Glu Leu
 2405 2410 2415
 Ser Arg Met Ser Ser Thr Lys Ser Ser Gly Ser Glu Ser Asp Arg Ser
 2420 2425 2430
 Glu Arg Pro Val Leu Val Arg Gln Ser Thr Phe Ile Lys Glu Ala Pro
 2435 2440 2445
 Ser Pro Thr Leu Arg Arg Lys Leu Glu Glu Ser Ala Ser Phe Glu Ser
 2450 2455 2460

FIG. 3W

Leu Ser Pro Ser Ser Arg Pro Ala Ser Pro Thr Arg Ser Gln Ala Gln 2480
 2465 2470 2475
 Thr Pro Val Leu Ser Pro Ser Leu Pro Asp Met Ser Leu Ser Thr His 2495
 2485 2490
 Ser Ser Val Gln Ala Gly Gly Trp Arg Lys Leu Pro Pro Asn Leu Ser 2510
 2500 2505
 Pro Thr Ile Glu Tyr Asn Asp Gly Arg Pro Ala Lys Arg His Asp Ile 2525
 2515 2520
 Ala Arg Ser His Ser Glu Ser Pro Ser Arg Leu Pro Ile Asn Arg Ser 2540
 2530 2535
 Gly Thr Trp Lys Arg Glu His Ser Lys His Ser Ser Ser Leu Pro Arg 2560
 2545 2550 2555
 Val Ser Thr Trp Arg Arg Thr Gly Ser Ser Ser Ser Ile Leu Ser Ala 2575
 2565 2570

FIG. 3X

Ser Ser Glu Ser Ser Glu Lys Ala Lys Ser Glu Asp Glu Lys His Val	
	2580
	2585
Asn Ser Ile Ser Gly Thr Lys Gln Ser Lys Glu Asn Gln Val Ser Ala	
	2595
	2600
	2605
Lys Gly Thr Trp Arg Lys Ile Lys Glu Asn Glu Phe Ser Pro Thr Asn	
	2610
	2615
	2620
Ser Thr Ser Gln Thr Val Ser Ser Gly Ala Thr Asn Gly Ala Glu Ser	
	2625
	2630
	2635
Lys Thr Leu Ile Tyr Gln Met Ala Pro Ala Val Ser Lys Thr Glu Asp	
	2645
	2650
	2655
Val Trp Val Arg Ile Glu Asp Cys Pro Ile Asn Asn Pro Arg Ser Gly	
	2660
	2665
	2670
Arg Ser Pro Thr Gly Asn Thr Pro Pro Val Ile Asp Ser Val Ser Glu	
	2675
	2680
	2685

FIG. 3Z

Arg Pro Ser Gln Ile Pro Thr Pro Val Asn Asn Asn Thr Lys Lys Arg
2805
Asp Ser Lys Thr Asp Ser Thr Glu Ser Ser Gly Thr Gln Ser Pro Lys
2820 2825 2830
Arg His Ser Gly Ser Tyr Leu Val Thr Ser Val
2835 2840

FIG. 4A

APC	203	LGTCODMEKRAORRIARIOOIEKDILRROL	233
		::: :	
RAL2	576	LTGAKGLOLRALRRRIARIEQGGTAISPTSPL	606

FIG. 4B

APC	453	MKLSFDEEHRHAMNELGGLOAIAELLOVD	481	
		: : : : :		
M3	HACHR	249	LYWRIYKETEKRTELKELAGLOASGTEAETE	277
		: :		
MCC		220	LYPNLAEERSRWEKELAGLREENESLTAM	248
		: : : :		
APC	453	MKLSFDEEHRHAMNELGGLOAIAELLOVD	481	

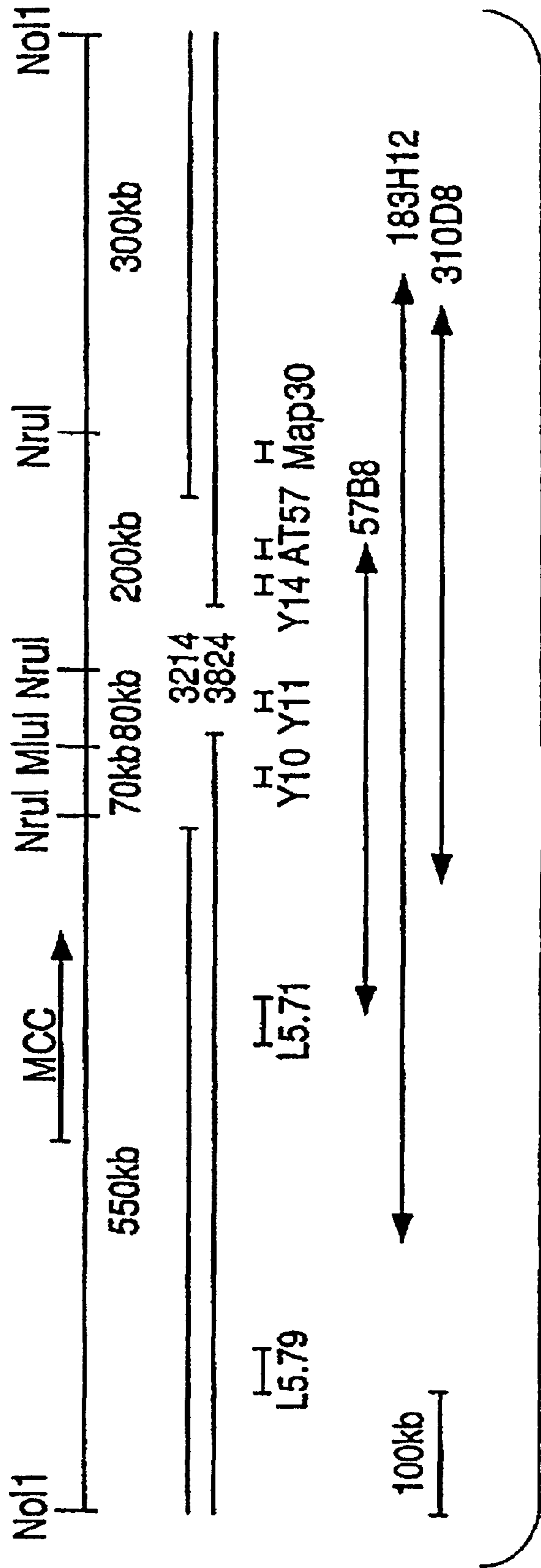


FIG. 5

FIG. 6A

GCA	GTC	GCC	GCT	CCA	GTC	TAT	CCG	GCA	CTA	GGA	ACA	GCC	CCG	CCG	GGN	GGC	GAG	ACG	55
Ala	Val	Ala	Ala	Pro	Val	Tyr	Pro	Ala	Leu	Gly	Thr	Ala	Pro	Gly	Gly	Gly	Glu	Thr	109
GTC	CCC	GCC	ATG	TCT	GCG	GCC	ATG	AGG	GAG	AGG	TTC	GAC	CGG	TTC	CTG	CAC	GAG	GAG	163
Val	Pro	Ala	MET	Ser	Ala	Ala	MET	Arg	Glu	Arg	Phe	Asp	Arg	Phe	Leu	His	Glu	Glu	163
AAG	AAC	TGC	ATG	ACT	GAC	CTT	CTG	GCC	AAG	CTC	GAG	GCC	AAA	ACC	GGC	GTG	AAC	AAC	190
Lys	Asn	Cys	MET	Thr	Asp	Leu	Leu	Ala	Lys	Leu	Glu	Ala	Lys	Thr	Gly	Val	Asn	Asn	217
AGG	AGC	TTC	ATC	GCT	CTT	GGT	GTC	ATC	GGA	CTG	GTG	GCC	TTG	TAC	CTG	GTG	TTC	TTC	271
Arg	Ser	Phe	Ile	Ala	Leu	Gly	Val	Ile	Gly	Leu	Val	Ala	Leu	Tyr	Leu	Val	Phe	Phe	271
GGT	TAT	GGA	GCC	TCT	CTC	CTC	TGC	AAC	CTG	ATA	GGA	TTT	GGC	TAC	CCA	GCC	TAC	TAC	271
Gly	Tyr	Gly	Ala	Ser	Leu	Leu	Cys	Asn	Leu	Ile	Gly	Phe	Gly	Tyr	Pro	Ala	Tyr	Tyr	325
ATC	TCA	ATT	AAA	GCT	ATA	GAG	AGT	CCC	AAC	AAA	GAA	GAT	GAT	ACC	CAG	TGG	CTG	CTG	379
Ile	Ser	Ile	Lys	Ala	Ile	Glu	Ser	Pro	Asn	Lys	Glu	Asp	Asp	Thr	Gln	Trp	Leu	Leu	379
ACC	TAC	TGG	GTA	GTG	TAT	GGT	GTG	TTC	AGC	ATT	GCT	GAA	TTC	TTC	TCT	GAT	ATC	ATC	433
Thr	Tyr	Trp	Val	Val	Tyr	Gly	Val	Phe	Ser	Ile	Ala	Glu	Phe	Phe	Ser	Asp	Ile	Ile	433
TTC	CTG	TCA	TGG	TTC	CCC	TTC	TAC	TAC	ATG	CTG	AAG	TGT	GGC	TTC	CTG	TTG	TGG	TGG	487
Phe	Leu	Ser	Trp	Phe	Pro	Phe	Tyr	Tyr	MET	Leu	Lys	Cys	Gly	Phe	Leu	Leu	Trp	Trp	487
TGC	ATG	GCC	CCG	AGC	CCT	TCT	AAT	GGG	GCT	GAA	CTG	CTC	TAC	AAG	CGC	ATC	ATC	ATC	541
Cys	MET	Ala	Pro	Ser	Pro	Ser	Asn	Gly	Ala	Glu	Leu	Leu	Tyr	Lys	Arg	Ile	Ile	Ile	541
CGT	CCT	TTC	TTC	CTG	AAG	CAC	GAG	TCC	CAG	ATG	GAC	AGT	GTG	GTC	AAG	GAC	CTT	CTT	541
Arg	Pro	Phe	Phe	Leu	Lys	His	Glu	Ser	Gln	MET	Asp	Ser	Val	Val	Lys	Asp	Leu	Leu	541

FIG. 6B

AAA GAC AAG TCC AAA GAG ACT GCA GAT GCC ATC ACT AAA GAA GCG AAG AAA GCT	595
Lys Asp Lys Ser Lys Glu Thr Ala Asp Ala Ile Thr Lys Glu Ala Lys Lys Ala	
ACC GTG AAT TTA CTG GGT GAA GAA AAG AAG AGC ACC TAA ACC AGA	622
Thr Val Asn Leu Leu Gly Glu Glu Lys Lys Ser Thr	
CTAAACCAGA CTGGATGGAA ACTTCCTGCC CTCCTGTAC CTCCTACTG GAGCTTGATG	640 650 660 670 680 690
710 720 730 740 750 760	770
GACTGTGGTA TAATTATTTT AATAATGTTG CCTTGGAAAC ATTTTGGAGA TATTAAGAT	780 790 800 810 820 830
TGTAAGTTTC TTTGCTTACT TTTACTGTCT ATATATATAG GGAGCACTTT AAACCTTAATG	840 850 860 870 880 890
TGTCACCGTT TTTGGAAAT GTATTTTCCC TCTGGGTAGG AAAAGATGTA TGTGCTATC	900 910 920 930 940 950 960 970
TATAAACTTA AAATAAAATT ATATACCCCA CAGGCTGTGT ACTTTACTGG GCTCTCCCTG	980 990 1000 1010 1020 1030 1040 1050
CTCTGTAGTT ACATTTAGGR TAATCTTTAT GGTCTACTT CCTRTAATGT ACAATTTTAT	1060 1070 1080 1090 1100 1110 1120
AATGTTTTTA ATGTATTTGT GCACATGTAC ATATGGAAAT GTTACTGTCT GACTACANCA	1130 1140 1150 1160 1170 1180 1190
TCATGGGGAG GGAGCAGGGG AAGGTTGTAT GTGTCATTA TAACTTCTGT ACAGTAAGAC	1200 1210 1220 1230 1240 1250 1260
AAGCTGGAGG AACCAITGTG CTGGTGTGGT CTACTAAATA ATACTTTAGG AAATACGTGA	1270 1280 1290 1300 1310 1320 1330
AGTGAACAAA GTGAGAAATG AAATCGAATG GAGATTGGCC TGGTTGTTTC CGTAGTATAT	1340 1350 1360 1370 1380 1390 1400

FIG. 6C

TACCAGGATA	GCTTTATAAA	GCAGTTAGTT	AGTTAGTTAC	TCACCTCTAGT	GATAAATCGG	GAAATTTACA
1410	1420	1430	1440	1450	1460	1470
CACACACACA	CACACACACA	CACACACACA	CACACACACA	CACACACACA	GAGTACCCTG	TAACTCTCAA
1480	1490	1500	1510	1520	1530	1540
TTCCCTGAAA	AAC TAGTAAT	ACTGTCCTAT	CTGCTATAAA	CTTTACATAT	TTGTCCTATTG	TCAAGATGCT
1550	1560	1570	1580	1590	1600	1610
ACANTGGAMN	CCATTTC TGG	TTTTATCTTC	ANAGSGGAGA	NACATGTTGA	TTTAGTCTTC	TTTCCCAATC
1620	1630	1640	1650	1660	1670	1680
TTCTTTTTTA	AMCCAGTTTN	AGGMNCTTCT	GRAGATTTGY	CCACCCTCTGA	TTACATGTAT	GTTCTYGTIT
1690	1700	1710	1720	1730	1740	1750
GTATCATKAG	CAACAACATG	CTAATGRCGA	CACCTAGCTC	TRAGMGCAAT	TCTGGGAGAN	TGARAGGNWG
1760	1770	1780	1790	1800	1810	1820
TATARAGTMN	CCATAATCT	GCTTGGCAAT	AGTTAAGTCA	ATCTATCTTC	AGTTTTTCTC	TGGCCTTTAA
1830	1840	1850	1860	1870	1880	1890
GGTCAAACAC	AAGAGGCTTC	CCTAGTTTAC	AAGTCAGAGT	CACCTGTAGT	CCATTTTAAAT	GCCCTCATCC
1900	1910	1920	1930	1940	1950	1960
GTATTCTTTG	TGTTGATAAG	CTGCACAKGA	CTACATAGTA	AGTACAGANC	AGTAAAGTTA	ANNCGGATGT
1970	1980	1990	2000	2010	2020	2030
CTCCATTGAT	CTGCCAANTC	GNTATAGAGA	GCAATTTGTC	TGGACTAGAA	AATCTGAGTT	TTACACCCATA
2040	2050	2060	2070	2080	2090	2100
CTGTTAAGAG	TCCTTTTGAA	TTAAACTAGA	CTAAAACAAG	TGTATAACTA	AACTAACAAAG	ATTAATAATC
2110	2120	2130	2140	2150	2160	2170
CAGCCAGTAC	AGTATTTTTT	AAGGCAAATA	AAGATGATTA	GCTCACCTTG	AGNTAACAAAT	CAGGTAAGAT
2180	2190	2200	2210	2220	2230	2240
CATNACAATG	TCTCATGATG	TNAANAATAT	TAAAGATATC	AATACTAAGT	GACAGTATCA	CNNCTAATAT

FIG. 6D

2250 2260 2270 2280 2290 2300 2310
 AATATGGATC AGAGCATTTA TTTTGGGGAG GAAACACAGTG GTGATTACCG GCATTTTATT AAACITAAA
 2320 2330 2340 2350 2360 2370 2380
 CTTGTAGAA AGCAAACAAA ATTGTTCTTG GGAGAAAATC AACTTT TAGA TTAATAAAAT TTTAAGTAWC
 2390 2400 2410 2420 2430 2440 2450
 TAGGAGTATT TAAATCCTTT TCCCATAAAT AAAAGTACAG TTTTCTTGGT GGACAGAAATGA AAATCAGCAA
 2460 2470 2480 2490 2500 2510 2520
 CNTCTAGCAT ATAGACTATA TAATCAGATT GACAGCATAT AGAATATATT ATCAGACAAG ATGAGGAGGT
 2530 2540 2550 2560 2570 2580 2590
 ACAAAAAGTTA CTATTGCTCA TAATGACTTA CAGGCTAAA NTAGNTNTAA AATACTATAT TAAATTTCTGA
 2600 2610 2620 2630 2640 2650 2660
 ATGCAATTTT TTTTGTGCC CTTGAGACCA AAATTTAAGT TAACTGTGC TGGCAGTCTA AGTGTAATG
 2670 2680 2690 2700 2710 2720 2730
 TTAACAGCAG GAGAAGTTAA GAATTGAGCA GTTCTGTGC ATGATTTCCC AAATGAAATA CTGCCTTGGC
 2740 2750 2760 2770 2780 2790 2800
 TAGAGTTTGA AAAACTAATT GAGCCTGTGC CTGGCTAGAA AACAAAGCGTT TATTTGAATG TGAATAGTGT
 2810 2820 2830 2840 2850 2860 2870
 TTCAAAGGTA TGTAGTTACA GAATTCCTAC CAAACAGCCTT AAATCTTCA AGAAAGAATT CCTGCAGCAG
 2880 2890 2900 2910 2920 2930 2940
 TTATTCCTT ACCTGAAGC TTCAATCATT TGGATCAACA ACTGCTACTC TCGGGAAGAC TCCTCTACTC
 2950 2960 2970 2980 2990 3000 3010
 ACAGCTGAG AAAATGAGCA CACCCTTAC ACTGTTATCA CCTATCCTGA AGATGTGATA CACTGAATGG
 3020 3030 3040 3050 3060 3070 3080
 AATAAATAG ATGTAATAA AATTGAGWTC TCATTTAAA AAAACCATGT GCCCAATGGG AAAATGACCCT
 3090 3100 3110 3120 3130 3140 3150
 CATGTTGTG TTTAACAAGC AACTGCACCC ACTAGCACAG CCCATTGAGC TANCCTATAT ATACATCTCT
 3160
 GTCAGTGCCC CTC

FIG. 7A

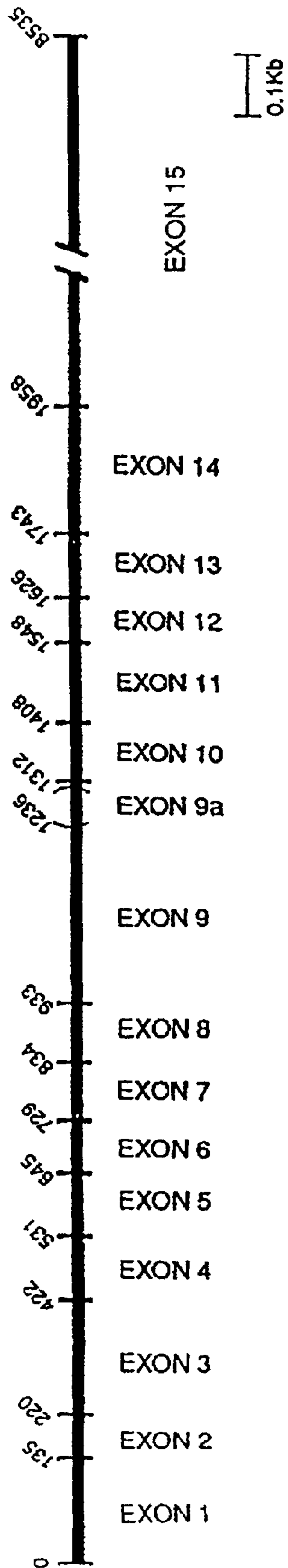


FIG. 7B-1

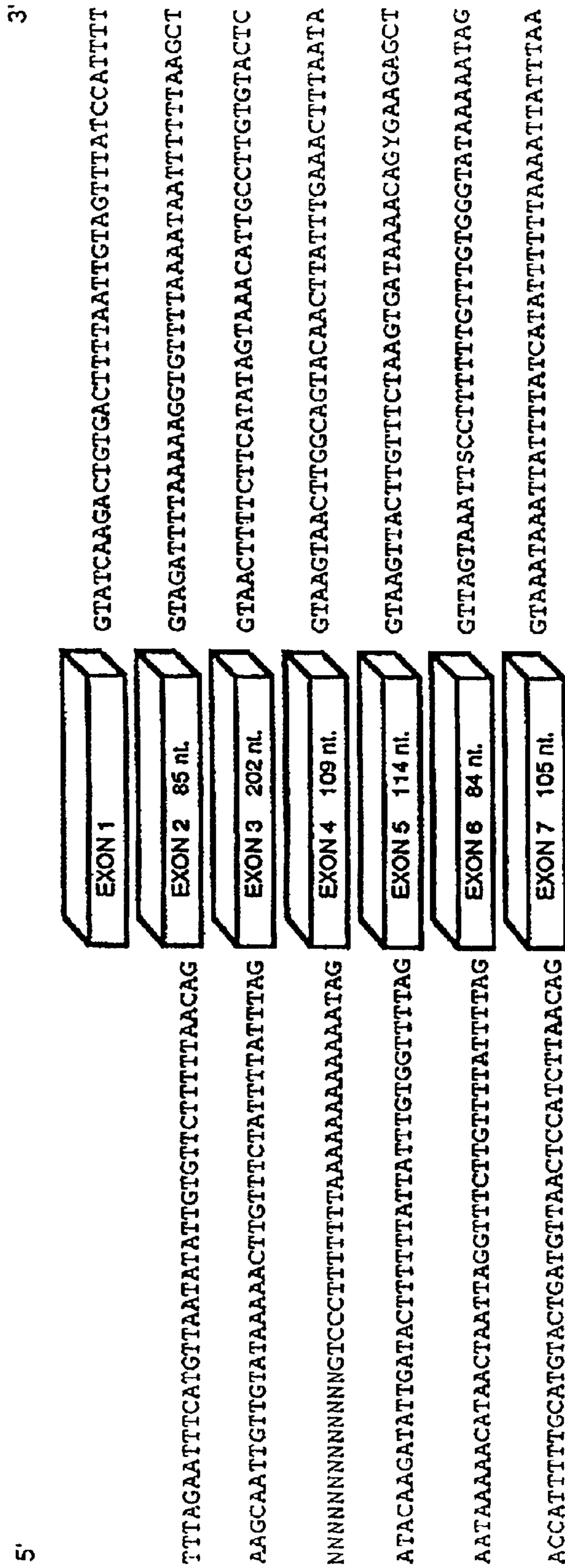
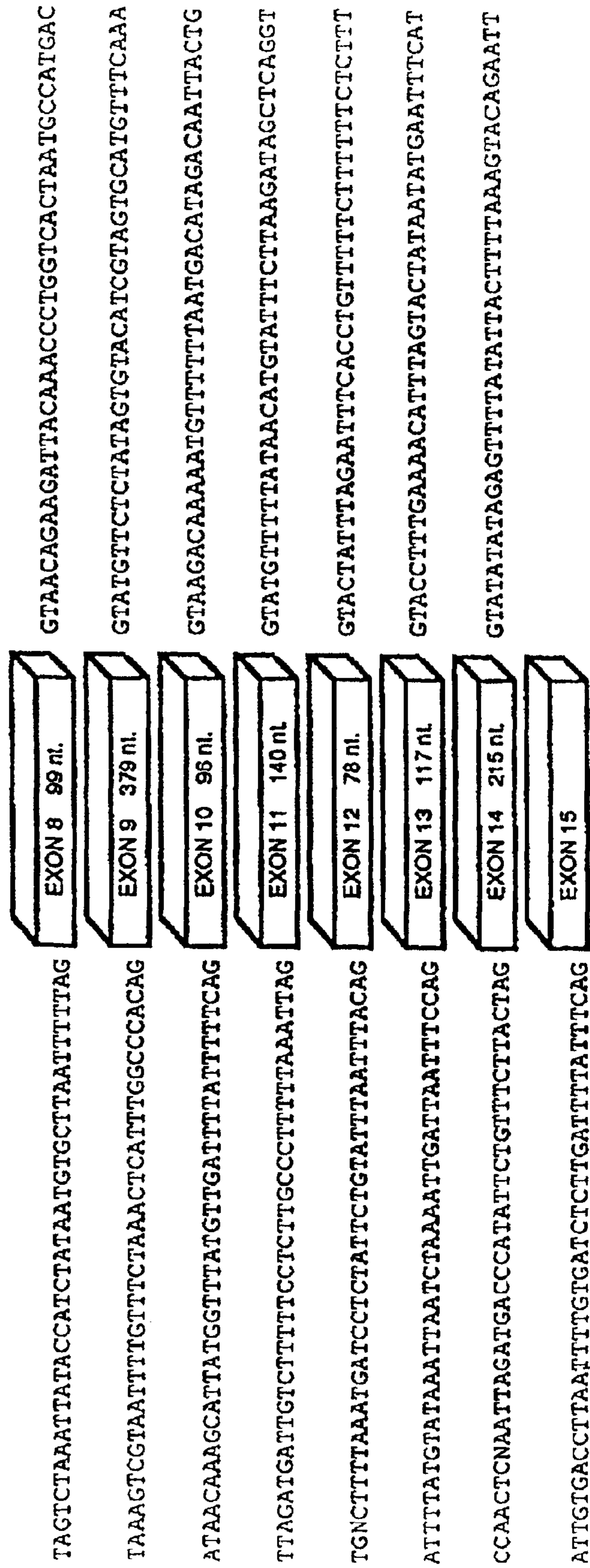


FIG. 7B-2



APC ANTIBODIES

Matter enclosed in heavy brackets [] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

Notice: More than one reissue application has been filed for the reissue of Pat. No. 5,691,454. The reissue applications are Ser. Nos. 09/983,543 (the present application), which is a continuation reissue of Pat. No. 5,691,454, and 09/442,489.

This application is a continuation of application of Ser. No. 09/442,489, filed Nov. 18, 1999, which issued as RE38916 and which is a Reissue Application of application Ser. No. 08/452,654, filed May 25, 1995, which issued as Pat. No. 5,691,454, which is a division, of application Ser. No. 08/289,548, filed Aug. 12, 1994, which issued as U.S. Pat. No. 5,648,212, which is a division of application Ser. No. 07/741,940 filed Aug. 8, 1991 (issued as U.S. Pat. No. 5,352,775).

The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of grants awarded by the National Institutes of Health.

TECHNICAL AREA OF THE INVENTION

The invention relates to the area of cancer diagnostics and therapeutics. More particularly, the invention relates to detection of the germline and somatic alterations of wild-type APC genes. In addition, it relates to therapeutic intervention to restore the function of APC gene product.

BACKGROUND OF THE INVENTION

According to the model of Knudson for tumorigenesis (Cancer Research, Vol. 45, p. 1482, 1985), there are tumor suppressor genes in all normal cells which, when they become non-functional due to mutation, cause neoplastic development. Evidence for this model has been found in the cases of retinoblastoma and colorectal tumors. The implicated suppressor genes in those tumors, RB, p53, DCC and MCC, were found to be deleted or altered in many cases of the tumors studied. (Hansen and Cavenee, Cancer Research, Vol. 47, pp: 5518-5527 (1987); Baker et al., Science, Vol. 244, p. 217 (1989); Fearon et al., Science, Vol. 247, p. 49 (1990); Kinzler et al. Science Vol. 251. p. 1366 (1991).)

In order to fully understand the pathogenesis of tumors, it will be necessary to identify the other suppressor genes that play a role in the tumorigenesis process. Prominent among these is the one(s) presumptively located at 5q21. Cytogenetic (Herrera et al., Am J. Med. Genet., Vol. 25, p. 473 (1986) and linkage (Leppert et al., Science, Vol. 238, p. 1411 (1987); Bodmer et al., Nature, Vol. 328, p. 614 (1987)) studies have shown that this chromosome region harbors the gene responsible for familial adenomatous polyposis (FAP) and Gardner's Syndrome (GS). FAP is an autosomal-dominant, inherited disease in which affected individuals develop hundreds to thousands of adenomatous polyps, some of which progress to malignancy. GS is a variant of FAP in which desmold tumors, osteomas and other soft tissue tumors occur together with multiple adenomas of the colon and rectum. A less severe form of polyposis has been identified in which only a few (2-40) polyps develop. This condition also is familial and is linked to the same chromosomal markers as FAP and GS (Leppert et al., New England

Journal of Medicine, Vol. 322, pp. 904-908, 1990.) Additionally, this chromosomal region is often deleted from the adenomas (Vogelstein et al., N. Engl. J. Med., Vol. 319, p. 525 (1988)) and carcinomas (Vogelstein et al., N. Engl. J. Med., Vol. 319, p. 525 (1988); Solomon et al., Nature, Vol. 328, p. 616 (1987); Sasaki et al., Cancer Research, Vol. 49, p. 4402 (1989); Delattre et al., Lancet, Vol. 2, p. 353 (1989); and Ashton-Rickardt et al., Oncogene, Vol. 4, p. 1169 (1989)) of patients without FAP (sporadic tumors). Thus, a putative suppressor gene on chromosome 5q21 appears to play a role in the early stages of colorectal neoplasia in both sporadic and familial tumors.

Although the MCC gene has been identified on 5q21 as a candidate suppressor gene, it does not appear to be altered in FAP or GS patients. Thus there is a need in the art for investigations of this chromosomal region to identify genes and to determine if any of such genes are associated with FAP and/or GS and the process of tumorigenesis.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method for diagnosing and prognosing a neoplastic tissue of a human.

It is another object of the invention to provide a method of detecting genetic predisposition to cancer.

It is another object of the invention to provide a method of supplying wild-type APC gene function to a cell which has lost said gene function.

It is yet another object of the invention to provide a kit for determination of the nucleotide sequence of APC alleles by the polymerase chain reaction.

It is still another object of the invention to provide nucleic acid probes for detection of mutations in the human APC gene.

It is still another object of the invention to provide a cDNA molecule encoding the APC gene product.

It is yet another object of the invention to provide a preparation of the human APC protein.

It is another object of the invention to provide a method of screening for genetic predisposition to cancer.

It is an object of the invention to provide methods of testing therapeutic agents for the ability to suppress neoplasia.

It is still another object of the invention to provide animals carrying mutant APC alleles.

These and other objects of the invention are provided by one or more of the embodiments which are described below. In one embodiment of the present invention a method of diagnosing or prognosing a neoplastic tissue of a human is provided comprising: detecting somatic alteration of wild-type APC genes of their expression products in a sporadic colorectal cancer tissue, said alteration indicating neoplasia of the tissue.

In yet another embodiment a method is provided of detecting genetic predisposition to cancer in a human including familial adenomatous polyposis (FAP) and Gardner's Syndrome (GS), comprising: isolating a human sample selected from the group consisting of blood and fetal tissue; detecting alteration of wild-type APC gene coding sequences or their expression products from the sample, said alteration indicating genetic predisposition to cancer.

In another embodiment of the present invention a method is provided for supplying wild-type APC gene function to a cell which has lost said gene function by virtue of a mutation in the APC gene, comprising: introducing a wild-type APC

gene into a cell which has lost said gene function such that said wild-type gene is expressed in the cell.

In another embodiment a method of supplying wild-type APC gene function to a cell is provided comprising: introducing a portion of a wild-type APC gene into a cell which has lost said gene function such that said portion is expressed in the cell, said portion encoding a part of the APC protein which is required for non-neoplastic growth of said cell. APC protein can also be applied to cells or administered to animals to remediate for mutant APC genes. Synthetic peptides or drugs can also be used to mimic APC function in cells which have altered APC expression.

In yet another embodiment a pair of single stranded primers is provided for determination of the nucleotide sequence of the APC gene by polymerase chain reaction. The sequence of said pair of single stranded DNA primers is derived from chromosome 5q band 21, said pair of primers allowing synthesis of APC gene coding sequences.

In still another embodiment of the invention a nucleic acid probe is provided which is complementary to human wild-type APC gene coding sequences and which can form mismatches with mutant APC genes, thereby allowing their detection by enzymatic or chemical cleavage or by shifts in electrophoretic mobility.

In another embodiment of the invention a method is provided for detecting the presence of a neoplastic tissue in a human. The method comprises isolating a body sample from a human; detecting in said sample alteration of a wild-type APC gene sequence or wild-type APC expression product, said alteration indicating the presence of a neoplastic tissue in the human.

In still another embodiment a cDNA molecule is provided which comprises the coding sequence of the APC gene.

In even another embodiment a preparation of the human APC protein is provided which is substantially free of other human proteins. The amino acid sequence of the protein is shown in FIG. 3 (SEQ ID NOS. 7 and 2).

In yet another embodiment of the invention a method is provided for screening for genetic predisposition to cancer, including familial adenomatous polyposis (FAP) and Gardner's Syndrome (GS), in a human. The method comprises: detecting among kindred persons the presence of a DNA polymorphism which is linked to a mutant APC allele in an individual having a genetic predisposition to cancer, said kindred being genetically related to the individual, the presence of said polymorphism suggesting a predisposition to cancer.

In another embodiment of the invention a method of testing therapeutic agents for the ability to suppress a neoplastically transformed phenotype is provided. The method comprises: applying a test substance to a cultured epithelial cell which carries a mutation in an APC allele; and determining whether said test substance suppresses the neoplastically transformed phenotype of the cell.

In another embodiment of the invention a method of testing therapeutic agents for the ability to suppress a neoplastically transformed phenotype is provided. The method comprises: administering a test substance to an animal which carries a mutant APC allele; and determining whether said test substance prevents or suppresses the growth of tumors.

In still other embodiments of the invention transgenic animals are provided. The animals carry a mutant APC allele from a second animal species or have been genetically engineered to contain an insertion mutation which disrupts an APC allele.

The present invention provides the art with the information that the APC gene, a heretofore unknown gene is, in fact, a target of mutational alterations or chromosome 5q21 and that these alterations are associated with the process of tumorigenesis. This information allows highly specific assays to be performed to assess the neoplastic status of a particular tissue or the predisposition to cancer of an individual. This invention has applicability to Familial Adenomatous Polyposis, sporadic colorectal cancers, Gardner's Syndrome, as well as the less severe familial polyposis discussed above.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A shows an overview of yeast artificial chromosome (YAC) contigs. Genetic distances between selected RFLP markers from within the contigs are shown in centi-Morgans.

FIGS. 1B-1, 1B-2 and 1B-3 show a detailed map of the three central contigs. The position of the six identified genes from within the FAP region is shown; the 5' and 3' ends of the transcripts from these genes have in general not yet been isolated, as indicated by the string of dots surrounding the bars denoting the genes' positions. Selected restriction endonuclease recognition sites are indicated. B, BssH2; S, SstII; M, MluI; N, NruI.

FIGS. 2A and 2B show the sequence of TB1 (FIG. 2A, SEQ ID NO:5) and TB2 (FIG. 2B, SEQ ID NO:6) [genes] proteins. The cDNA sequence of the TB1 gene was determined from the analysis of 11 cDNA clones derived from normal colon and liver, as described in the text. A total of 2314 bp were contained within the overlapping cDNA clones, defining an ORF of 424 amino acids beginning at nucleotide 1. Only the predicted amino acids from the ORF are shown. The carboxy-terminal end of the ORF has apparently been identified, but the 5' end of the TB1 transcript has not yet been precisely determined.

The cDNA sequence of the TB2 gene was determined from the YS-39 clone derived as described in the text. This clone consisted of 2300 bp and defined an ORF of 185 amino acids beginning at nucleotide 1. Only the predicted amino acids are shown. The carboxy terminal end of the ORF has apparently been identified, but the 5' end of the TB2 transcript has not been precisely determined.

FIGS. 3A-3F show the sequence of the APC gene product (SEQ ID NO:7). The cDNA sequence was determined through the analysis of 87 cDNA clones derived from normal colon, liver, and brain. A total of 8973 bp were contained within overlapping cDNA clones, defining an ORF of 2842 amino acids. In frame stop codons surrounded this ORF, as described in the text, suggesting that the entire APC gene product was represented in the ORF illustrated. Only the predicted amino acids are shown.

FIGS. 4A and 4B show the local similarity between human APC (SEQ ID NO:2) and *ral2* (SEQ ID NO:8) of yeast. FIG. 4A shows amino acids 203 to 233 of APC, and FIG. 4B shows amino acids 453 to 481 of APC. Local similarity among the APC (SEQ ID NO:2) and MCC genes (SEQ ID NO:10) genes and the m3 muscarinic acetylcholine receptor (SEQ ID NO:9) is shown. The region of the mAChR shown corresponds to that responsible for coupling the receptor to G protein. The connecting lines indicate identities; dots indicate related amino acids residues.

FIG. 5 shows the genomic map of the 1200 kb NotI fragment at the FAP locus. The NotI fragment is shown as a bold line. Relevant parts of the deletion chromosomes from patients 3214 and 3824 are shown as stippled lines. Probes

used to characterize the NotI fragment and the deletions, and three YACs from which subclones were obtained, are shown below the restriction map. The chimeric end of YAC 183H12 is indicated by a dotted line. The orientation and approximate position of MCC are indicated above the map.

FIG. 6A–6D show the DNA sequence (SEQ ID NO:3) and predicted amino acid sequence of DP1 (TB2) (SEQ ID NO:4). The nucleotide numbering begins at the most 5' nucleotide isolated. A proposed initiation methionine (base 77) is indicated in bold type. The entire coding sequence is presented.

FIG. 7A, FIG. 7B-1, and FIG. 7B-2 show the arrangement of exons in DP2.5 (APC). (A) Exon 9 corresponds to nucleotides 933–1312; exon 9a corresponds to nucleotides 1236–1312. The stop codon in the cDNA is at nucleotide 8535. (B) Partial intronic sequence surrounding each exon is shown (SEQ ID NO:11–38). 5' intron sequences of exons 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15 are shown in SEQ ID NOS: 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, respectively. 3' intron sequences of exons 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 are shown in SEQ ID NOS: 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, respectively.

DETAILED DESCRIPTION

It is a discovery of the present invention that mutational events associated with tumorigenesis occur in a previously unknown gene on chromosome 5q named here the APC (Adenomatous Polyposis Coil) gene. Although it was previously known that deletion of alleles on chromosome 5q were common in certain types of cancers, it was not known that a target gene of these deletions was the APC gene. Further it was not known that other types of mutational events in the APC gene are also associated with cancers. The mutations of the APC gene can involve gross rearrangements, such as insertions and deletions. Point mutations have also been observed.

According to the diagnostic and prognostic method of the present invention, alteration of the wild-type APC gene is detected. "Alteration of a wild-type gene" according to the present invention encompasses all forms of mutations—including deletions. The alteration may be due to either rearrangements such as insertions, inversions, and deletions, or to point mutations. Deletions may be of the entire gene or only a portion of the gene. Somatic mutations are those which occur only in certain tissues, e.g., in the tumor tissue, and are not inherited in the germline. Germline mutations can be found in any of a body's tissues. If only a single allele is somatically mutated, an early neoplastic state is indicated. However, if both alleles are mutated then a late neoplastic state is indicated. The finding of APC mutations thus provides both diagnostic and prognostic information. An APC allele which is not deleted (e.g., that on the sister chromosome to a chromosome carrying an APC deletion) can be screened for other mutations, such as insertions, small deletions, and point mutations. It is believed that many mutations found in tumor tissues will be those leading to decreased expression of the APC gene product. However, mutations leading to non-functional gene products would also lead to a cancerous state. Point mutational events may occur in regulatory regions, such as in the promoter of the gene, leading to loss or diminution of expression of the mRNA. Point mutations may also abolish proper RNA processing, leading to loss of expression of the APC gene product.

In order to detect the alteration of the wild-type APC gene in a tissue, it is helpful to isolate the tissue free from sur-

rounding normal tissues. Means for enriching a tissue preparation for tumor cells are known in the art. For example, the tissue may be isolated from paraffin or cryostat sections. Cancer cells may also be separated from normal cells by flow cytometry. These as well as other techniques for separating tumor from normal cells are well known in the art. If the tumor tissue is highly contaminated with normal cells, detection of mutations is more difficult.

Detection of point mutations may be accomplished by molecular cloning of the APC allele (or alleles) and sequencing that allele(s) using techniques well known in the art. Alternatively, the polymerase chain reaction (PCR) can be used to simplify gene sequences directly from a genomic DNA preparation from the tumor tissue. The DNA sequence of the amplified sequences can then be determined. The polymerase chain reaction itself is well known in the art. See, e.g., Saiki et al., *Science*, Vol. 239, p. 487, 1988; U.S. Pat. No. 4,683,203; and U.S. Pat. No. 4,683,195. Specific primers which can be used in order to amplify the gene will be discussed in more detail below. The ligase chain reaction, which is known in the art, can also be used to amplify APC sequences. See Wu et al., *Genomics*, Vol. 4, pp. 560–569 (1989). In addition, a technique known as allele specific PCR can be used. (See Ruano and Kidd, *Nucleic Acids Research*, Vol. 17, p. 8392, 1989.) According to this technique, primers are used which hybridize at their 3' ends to a particular APC mutation. If the particular APC mutation is not present, an amplification product is not observed. Amplification Refractory Mutation System (ARMS) can also be used as disclosed in European Patent Application Publication No. 0332435 and in Newton et al., *Nucleic Acids Research*, Vol. 17, p.7, 1989. Insertions and deletions of genes can also be detected by cloning, sequencing and amplification. In addition, restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score alteration of an allele or an insertion in a polymorphic fragment. Such a method is particularly useful for screening among kindred persons of an affected individual for the presence of the APC mutation found in that individual. Single stranded conformation polymorphism (SSCP) analysis can also be used to detect base change variants of an allele. (Orita et al., *Proc. Natl. Acad. Sci. USA* Vol. 86, pp. 2766–2770, 1989, and *Genomics*, Vol. 5, pp. 874–879, 1989.) Other techniques for detecting insertions and deletions as are known in the art can be used.

Alterations of wild-type genes can also be detected on the basis of the alteration of a wild-type expression product of the gene. Such expression products include both the APC mRNA as well as the APC protein product. The sequences of these products are shown in FIG. 3. Point mutations may be detected by amplifying and sequencing the mRNA or via molecular cloning of cDNA made from the mRNA. The sequence of the cloned cDNA can be determined using DNA sequencing techniques which are well known in the art. The cDNA can also be sequenced via the polymerase chain reaction (PCR) which will be discussed in more detail below.

Mismatches, according to the present invention are hybridized nucleic acid duplexes which are not 100% homologous. The lack of total homology may be due to deletions, insertions, inversions, substitutions or frameshift mutations. Mismatch detection can be used to detect point mutations in the gene or its mRNA product. While these techniques are less sensitive than sequencing, they are simpler to perform on a large number of tumor samples. An example of a mismatch cleavage technique is the RNase protection method, which is described in detail in Winter et al., *Proc. Natl. Acad. Sci. USA*, Vol. 82, p. 7575, 1985 and

Meyers et al., Science, Vol. 230, p. 1242, 1985. In the practice of the present invention the method involves the use of a labeled riboprobe which is complementary to the human wild-type APC gene coding sequence. The riboprobe and either mRNA or DNA isolated from the tumor tissue are annealed (hybridized) together and subsequently digested with the enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected by RNase A, it cleaves at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which is smaller than the full-length duplex RNA for the riboprobe and the mRNA or DNA. The riboprobe need not be the full length of the APC mRNA or gene but can be a segment of either. If the riboprobe comprises only a segment of the APC mRNA or gene it will be desirable to use a number of these probes to screen the whole mRNA sequence for mismatches.

In similar fashion, DNA probes can be used to detect mismatches, through enzymatic or chemical cleavage. See, e.g., Cotton et al., Proc. Natl. Acad. Sci. USA, Vol. 85, 4397 1988; and Shenk et al., Proc. Natl. Acad. Sci. USA, Vol. 72, p. 989, 1975. Alternatively, mismatches can be detected by shifts in the electrophoretic mobility of mismatched duplexes relative to matched duplexes. See, e.g., Cariello, Human Genetics, Vol. 42, p. 726, 1988. With either riboprobes or DNA probes, the cellular mRNA or DNA which might contain a mutation can be amplified using PCR (see below) before hybridization. Changes in DNA of the APC gene can also be detected using Southern hybridization, especially if the changes are gross rearrangements, such as deletions and insertions.

DNA sequences of the APC gene which have been amplified by use of polymerase chain reaction may also be screened using allele-specific probes. These probes are nucleic acid oligomers, each of which contains a region of the APC gene sequence harboring a known mutation. For example, one oligomer may be about 30 nucleotides in length, corresponding to a portion of the APC gene sequence. By use of a battery of such allele-specific probes, PCR amplification products can be screened to identify the presence of a previously identified mutation in the APC gene. Hybridization of allele-specific probes with amplified APC sequences can be performed, for example, on a nylon filter. Hybridization to a particular probe under stringent hybridization conditions indicates the presence of the same mutation in the tumor tissue as in the allele-specific probe.

Alterations of APC mRNA expression can be detected by any technique known in the art. These include Northern blot analysis, PCR amplification and RNase protection. Diminished mRNA expression indicates an alteration of the wild-type APC gene. Alteration of wild-type APC genes can also be detected by screening for alteration of wild-type APC protein. For example, monoclonal antibodies immunoreactive with APC can be used to screen a tissue. Lack of cognate antigen would indicate an APC mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant APC gene product. Such immunological assays can be done in any convenient format known in the art. These include Western blots, immunohistochemical assays and ELISA assays. Any means for detecting an altered APC protein can be used to detect alteration of wild-type APC genes. Functional assays can be used, such as protein binding determinations. For example, it is believed that APC protein oligomerizes to itself and/or MCC protein or binds to a G protein. Thus, an assay for the ability to bind

to wild type APC or MCC protein or that G protein can be employed. In addition, assays can be used which detect APC biochemical function. It is believed that APC is involved in phospholipid metabolism. Thus, assaying the enzymatic products of the involved phospholipid metabolic pathway can be used to determine APC activity. Finding a mutant APC gene product indicates alteration of a wild-type APC gene.

Mutant APC genes or gene products can also be detected in other human body samples, such as, serum, stool, urine and sputum. The same techniques discussed above for detection of mutant APC genes or gene products in tissues can be applied to other body samples. Cancer cells are sloughed off from tumors and appear in such body samples. In addition, the APC gene product itself may be secreted into the extracellular space and found in these body samples even in the absence of cancer cells. By screening such body samples, a simple early diagnosis can be achieved for many types of cancers. In addition, the progress of chemotherapy or radiotherapy can be monitored more easily by testing such body samples for mutant APC genes or gene products.

The methods of diagnosis of the present invention are applicable to any tumor in which APC has a role in tumorigenesis. Deletions of chromosome arm 5q have been observed in tumors of lung, breast, colon, rectum, bladder, liver, sarcomas, stomach and prostate, as well as in leukemias and lymphomas. Thus these are likely to be tumors in which APC has a role. The diagnostic method of the present invention is useful for clinicians so that they can decide upon an appropriate course of treatment. For example, a tumor displaying alteration of both APC alleles might suggest a more aggressive therapeutic regimen than a tumor displaying alteration of only one APC allele.

The primer pairs of the present invention are useful for determination of the nucleotide sequence of a particular APC allele using the polymerase chain reaction. The pairs of single stranded DNA primers can be annealed to sequences within or surrounding the APC gene on chromosome 5q in order to prime amplifying DNA synthesis of the APC gene itself. A complete set of these primers allows synthesis of all of the nucleotides of the APC gene coding sequences, i.e., the exons. The set of primers preferably allows synthesis of both intron and exon sequences. Allele specific primers can also be used. Such primers anneal only to particular APC mutant alleles, and thus will only amplify a product in the presence of the mutant allele as a template.

In order to facilitate subsequent cloning of amplified sequences, primers may have restriction enzyme site sequences appended to their 5' ends. Thus, all nucleotides of the primers are derived from APC sequences or sequences adjacent to APC except the few nucleotides necessary to form a restriction enzyme site. Such enzymes and sites are well known in the art. The primers themselves can be synthesized using techniques which are well known in the art. Generally, the primers can be made using oligonucleotide synthesizing machines which are commercially available. Given the sequence of the APC open reading frame shown in FIG. 3 (SEQ ID NO:1), design of particular primers is well within the skill of the art.

The nucleic acid probes provided by the present invention are useful for a number of purposes. They can be used in Southern hybridization to genomic DNA and in the RNase protection method for detecting point mutations already discussed above. The probes can be used to detect PCR amplification products. They may also be used to detect mismatches with the APC gene or mRNA using other

techniques. Mismatches can be detected using either enzymes (e.g., S1 nuclease), chemicals (e.g., hydroxylamine or osmium tetroxide and piperidine), or changes in electrophoretic mobility of mismatched hybrids as compared to totally matched hybrids. These techniques are known in the art. See, Cotton, supra, Shenk, supra, Myers, supra, Winter, supra, and Novack et al., Proc. Natl. Acad. Sci. USA, Vol. 83, p. 586, 1986. Generally, the probes are complementary to APC gene coding sequences, although probes to certain introns are also contemplated. An entire battery of nucleic acid probes is used to compose a kit for detecting alteration of wild-type APC genes. The kit allows for hybridization to the entire APC gene. The probes may overlap with each other or be contiguous.

If a riboprobe is used to detect mismatches with mRNA, it is complementary to the mRNA of the human wild-type APC gene. The riboprobe thus is an anti-sense probe in that it does not code for the APC protein because it is of the opposite polarity to the sense strand. The riboprobe generally will be labeled with a radioactive, colorimetric, or fluorometric material, which can be accomplished by any means known in the art. If the riboprobe is used to detect mismatches with DNA it can be of either polarity, sense or anti-sense. Similarly, DNA probes also may be used to detect mismatches.

Nucleic acid probes may also be complementary to mutant alleles of the APC gene. These are useful to detect similar mutations in other patients on the basis of hybridization rather than mismatches. These are discussed above and referred to as allele-specific probes. As mentioned above, the APC probes can also be used in Southern hybridizations to genomic DNA to detect gross chromosomal changes such as deletions and insertions. The probes can also be used to select cDNA clones of APC genes from tumor and normal tissues. In addition, the probes can be used to detect APC mRNA in tissues to determine if expression is diminished as a result of alteration of wild-type APC genes.

According to the present invention a method is also provided of supplying wild-type APC function to a cell which carries mutant APC alleles. Supplying such function should suppress neoplastic growth of the recipient cells. The wild-type APC gene or a part of the gene may be introduced into the cell in a vector such that the gene remains extrachromosomal. In such a situation the gene will be expressed by the cell from the extrachromosomal location. If a gene portion is introduced and expressed in a cell carrying a mutant APC allele, the gene portion should encode a part of the APC protein which is required for non-neoplastic growth of the cell. More preferred is the situation where the wild-type APC gene or a part of it is introduced into the mutant cell in such a way that it recombines with the endogenous mutant APC gene present in the cell. Such recombination requires a double recombination event which results in the correction of the APC gene mutation. Vectors for introduction of genes both for recombination and for extrachromosomal maintenance are known in the art and any suitable vector may be used. Methods for introducing DNA into cells such as electroporation, calcium phosphate co-precipitation and viral transduction are known in the art and the choice of method is within the competence of the routineer. Cells transformed with the wild-type APC gene can be used as model systems to study cancer remission and drug treatments which promote such remission.

Similarly, cells and animals which carry a mutant APC allele can be used as model systems to study and test for substances which have potential as therapeutic agents. The cells are typically cultured epithelial cells. These may be

isolated from individuals with APC mutations, either somatic or germline. Alternatively, the cell line can be engineered to carry the mutation in the APC allele. After a test substance is applied to the cells, the neoplastically transformed pheno-type of the cell will be determined. Any trait of neoplastically transformed cells can be assessed, including anchorage-independent growth, tumorigenicity in nude mice, invasiveness of cells, and growth factor dependence. Assays for each of these traits are known in the art.

Animals for testing therapeutic agents can be selected after mutagenesis of whole animals or after treatment of germline cells or zygotes. Such treatments include insertion of mutant APC alleles, usually from a second animal species, as well as insertion of disrupted homologous genes. Alternatively, the endogenous APC gene(s) of the animals may be disrupted by insertion or deletion mutation. After test substances have been administered to the animals, the growth of tumors must be assessed. If the test substance prevents or suppresses the growth of tumors, then the test substance is a candidate therapeutic agent for the treatment of FAP and/or sporadic cancers.

Polypeptides which have APC activity can be supplied to cells which carry mutant or missing APC alleles. The sequence of the APC protein is disclosed in FIG. 3 (SEQ ID NO:7). These two sequences differ slightly and appear to indicate the existence of two different forms of the APC protein. Protein can be produced by expression of the cDNA sequence in bacteria, for example, using known expression vectors. Alternatively, APC can be extracted from APC-producing mammalian cells such as brain cells. In addition, the techniques of synthetic chemistry can be employed to synthesize APC protein. Any of such techniques can provide the preparation of the present invention which comprises the APC protein. The preparation is substantially free of other human proteins. This is most readily accomplished by synthesis in a microorganism or in vitro.

Active APC molecules can be introduced into cells by microinjection or by use of liposomes, for example. Alternatively, some such active molecules may be taken up by cells, actively or by diffusion. Extracellular application of APC gene product may be sufficient to affect tumor growth. Supply of molecules with APC activity should lead to a partial reversal of the neoplastic state. Other molecules with APC activity may also be used to effect such a reversal, for example peptides, drugs, or organic compounds.

The present invention also provides a preparation of antibodies immunoreactive with a human APC protein. The antibodies may be polyclonal or monoclonal and may be raised against native APC protein, APC fusion proteins, or mutant APC proteins. The antibodies should be immunoreactive with APC epitopes, preferably epitopes not present on other human proteins. In a preferred embodiment of the invention the antibodies will immunoprecipitate APC proteins from solution as well as react with APC protein on Western or immunoblots of polyacrylamide gels. In another preferred embodiment, the antibodies will detect APC proteins in paraffin or frozen tissue sections, using immunocytochemical techniques. Techniques for raising and purifying antibodies are well known in the art and any such techniques may be chosen to achieve the preparation of the invention.

Predisposition to cancers as in FAP and GS can be ascertained by testing any tissue of a human for mutations of the APC gene. For example, a person who has inherited a germline APC mutation would be prone to develop cancers. This can be determined by testing DNA from any tissue of the person's body. Most simply, blood can be drawn and DNA

extracted from the cells of the blood. In addition, prenatal diagnosis can be accomplished by testing fetal cells, placental cells, or amniotic fluid for mutations of the APC gene. Alteration of a wild-type APC allele, whether for example, by point mutation or by deletion, can be detected by any of the means discussed above.

Molecules of cDNA according to the present invention are intron-free, APC gene coding molecules. They can be made by reverse transcriptase using the APC mRNA as a template. These molecules can be propagated in vectors and cell lines as is known in the art. Such molecules have the sequence shown in SEQ ID NO:3. The cDNA can also be made using the techniques of synthetic chemistry given the sequence disclosed herein.

A short region of homology has been identified between APC and the human m3 muscarinic acetylcholine receptor (mAChR). This homology was largely confined to 29 residues in which 6 out of 7 amino acids (EL(GorA)GLQA) were identified (see FIG. 4 (SEQ ID NO:9)). Initially, it was not known whether this homology was significant, because many other proteins had higher levels of global homology (though few had six out of seven contiguous amino acids in common). However, a study on the sequence elements controlling G protein activation by mAChR subtypes (Lechleiter et al., *EMBO J.*, p. 4381 (1990)) has shown that a 21 amino acid region from the m3 mAChR completely mediated G protein specificity when substituted for the 21 amino acids of m2 mAChR at the analogous protein position. These 21 residues overlap the 19 amino acid homology between APC and m3 mAChR.

This connection between APC and the G protein activating region of mAChR is intriguing in light of previous investigations relating G proteins to cancer. For example, the RAS oncogenes, which are often mutated in colorectal cancers (Vogelstein, et al., *N. Engl. J. Med.*, Vol. 319, p. 525 (1988); Bos et al., *Nature* Vol. 327, p. 293 (1987)), are members of the (1 protein family (Bourne, et al, *Nature*, Vol. 348, p. 125 (1990)) as is an in vitro transformation suppressor (Noda et al., *Proc. Natl. Acad. Sci. USA*, Vol. 86, p. 162 (1989)) and genes mutated in hormone producing tumors (Candis et al., *Nature*, Vol. 340, p. 692 (1989); Lyons et al., *Science*, Vol. 249, p. 655 (1990)). Additionally, the gene responsible for neurofibromatosis (presumably a tumor suppressor gene) has been shown to activate the GTPase activity of RAS (Xu et al., *Cell*, Vol. 63, p. 835 (1990); Martin et al., *Cell*, Vol. 63, p. 843 (1990); Ballester et al., *Cell*, Vol. 63, p. 851 (1990)). Another interesting link between G proteins and colon cancer involves the drug sulindac. This agent has been shown to inhibit the growth of benign colon tumors in patients with FAP, presumably by virtue of its activity as a cyclooxygenase inhibitor (Waddell et al., *J. Surg. Oncology* 24(1), 83 (1983); Wadell, et al., *Am. J. Surg.*, 157(1), 175 (1989); Charneau et al., *Gastroenterologie Clinique at Biologique* 14(2), 153 (1990)). Cyclooxygenase is required to convert arachidonic acid to prostaglandins and other biologically active molecules. G proteins are known to regulate phospholipase A2 activity, which generates arachidonic acid from phospholipids (Role et al., *Proc. Natl. Acad. Sci. USA*, Vol. 84, p. 3623 (1987); Kurachi et al., *Nature*, Vol. 337, 12 555 (1989)). Therefore we propose that wild-type APC protein functions by interacting with a G protein and is involved in phospholipid metabolism.

The following are provided for exemplification purposes only and are not intended to limit the scope of the invention which has been described in broad terms above.

EXAMPLE 1

This example demonstrates the isolation of a 5.5 Mb region of human DNA linked to the FAP locus. Six genes are

identified in this region, all of which are expressed in normal colon cells and in colorectal, lung, and bladder tumors.

The cosmid markers YN5.64 and YN5.48 have previously been shown to delimit an 8 cM region containing the locus for FAP (Nakamura et al., *Am. J. Hum. Genet.* Vol. 43, p. 638 (1988)). Further linkage and pulse-field gel electrophoresis (PFGE) analysis with additional markers has shown that the FAP locus is contained within a 4 cM region bordered by cosmids EF5.44 and L5.99. In order to isolate clones representing a significant portion of this locus, a yeast artificial chromosome (YAC) library was screened with various 5p21 markers. Twenty-one YAC clones, distributed within six contigs and including 5.5 Mb from the region between YN5.64 and YN5.48, were obtained (FIG. 1A).

Three contigs encompassing approximately 4 Mb were contained within the central portion of this region. The YAC's constituting these contigs, together with the markers used for their isolation and orientations, are shown in FIG. 1. These YAC contigs were obtained in the following way. To initiate each contig, the sequence of a genomic marker cloned from chromosome 5q21 was determined and used to design primers for PCR. PCR was then carried out on pools of YAC clones distributed in microtiter trays as previously described (Anand et al., *Nucleic Acids Research*, Vol. 18, p. 1951 (1980)). Individual YAC clones from the positive pools were identified by further PCR or hybridization based assays, and the YAC sizes were determined by PFGE.

To extend the areas covered by the original YAC clones, "chromosomal walking" was performed. For this purpose, YAC termini were isolated by a PCR based method and sequenced (Riley et al., *Nucleic Acids Research*, Vol. 18, p. 2887 (1990)). PCR primers based on these sequences were then used to rescreen the YAC library. For example, the sequence from an intron of the FER gene (Hao et al., *Mol. Cell. Biol.*, Vol. 9, p. 1587 (1989)) was used to design PCR primers for isolation of the 28EC1 and 5EH8 YACs. The termini of the 28EC1 YAC were sequenced to derive markers RHE28 and LHE28, respectively. The sequences of these two markers were then used to isolate YAC clones 15CH12 (from RHE28) and 40CF1 and 29EF1 (from LHE28). These five YAC's formed a contig encompassing 1200 kb (contig 1, FIG. 1B).

Similarly, contig 2 was initiated using cosmid N5.66 sequences, and contig 3 was initiated using sequences both from the MCC gene and from cosmid EF5.44. A walk in the telomeric direction from YAC 14FH1 and a walk in the opposite direction from YAC 39GG3 allowed connection of the initial contig 3 clones through YAC 37HG4 (FIG. 1B). YAC37HG4 was deposited at the National Collection of Industrial and Marine Bacteria (NCIMB), P.O. Box 31, 23 St. Machar Drive, Aberdeen AB2 1RY, Scotland, under Accession No. 40353 on Dec. 17, 1990.

Multipoint linkage analysis with the various markers used to define the contigs, combined with PFGE analysis, showed that contigs 1 and 2 were centromeric to contig 3. These contigs were used as tools to orient and/or identify genes which might be responsible for FAP. Six genes were found to lie within this cluster of YAC's, as follows:

Contig #1: FER—The FER gene was discovered through its homology to the viral oncogene ABL (Hao et al., supra). It has an intrinsic tyrosine kinase activity, and in situ hybridization with an FER probe showed that the gene was located at 5q11-23 (Morris et al., *Cytogenet. Cell. Genet.*, Vol. 53, p. 4, (1990)). Because of the potential role of this oncogene-related gene in neoplasia, we decided to evaluate it further with regards to the FAP locus. A human genomic clone from

FER was isolated (MF 2.3) and used to define a restriction fragment length polymorphism (RFLP), and the RFLP in turn used to map FER by linkage analysis using a panel of three generation families. This showed that FER was very tightly linked to previously defined polymorphic markers for the FAP locus. The genetic mapping of FER was complemented by physical mapping using the YAC clones derived from FER sequences (FIG. 1B). Analysis of YAC contig 1 showed that FER was within 600 kb of cosmid marker M5.28, which maps to within 1.5 Mb of cosmid L5.99 by PFGE of human genomic DNA. Thus, the YAC mapping results were consistent with the FER linkage data and PFGE analyses.

Contig 2: TB1—TB1 was identified through a cross-hybridization approach. Exons of genes are often evolutionarily conserved while introns and intergenic regions are much less conserved. Thus, it a human probe cross-hybridizes strongly to the DNA from non-primate species, there is a reasonable chance that it contains exon sequences. Subclones of the cosmids shown in FIG. 1 were used to screen Southern blots containing rodent DNA samples. A subclone of cosmid N5.66 (p 5.66—4) was shown to strongly hybridize to rodent DNA, and this clone was used to screen cDNA libraries derived from normal adult colon and fetal liver. The ends of the initial cDNA clones obtained in this screen were then used to extend the cDNA sequence. Eventually, 11 cDNA clones were isolated, covering 2314 bp. The gene detected by these clones was named TB1. Sequence analysis of the overlapping clones revealed an open reading frame (ORF) that extended for 1302 bp starting from the most 5' sequence data obtained (FIG. 2A). If this entire open reading frame were translated, it would encode 434 amino acids (SEQ ID NO:5). The product of this gene was not globally homologous to any other sequence in the current database but showed two significant local similarities to a family of ADP, ATP carrier/translocator proteins and mitochondrial brown fat uncoupling proteins which are widely distributed from yeast to mammals. These conserved regions of TB1 (underlined in FIG. 2A) may define a predictive motif for this sequence family. In addition, TB1 appeared to contain a signal peptide (or mitochondrial targeting sequence) as well as at least 7 transmembrane domains.

Contig 3: MCC, TB2, SRP and APC—The MCC gene was also discovered through a cross-hybridization approach, as described previously (Kinzler et al., *Science* Vol. 251, p. 1366 (1991)). The MCC gene was considered a candidate for causing FAP by virtue of its tight genetic linkage to FAP susceptibility and its somatic mutation in sporadic colorectal carcinomas. However, mapping experiments suggested that the coding region of MCC was approximately 50 kb proximal to the centromeric end of a 200 kb deletion found in an FAP patient. MCC cDNA probes detected a 10 kb mRNA transcript on Northern blot analysis of which 4151 bp, including the entire open reading frame, have been cloned. Although the 3' non-translated portion or an alternatively spliced form of MCC might have extended into this deletion, it was possible that the deletion did not affect the MCC gene product. We therefore used MCC sequences to initiate a YAC contig, and subsequently used the YAC clones to identify genes 50 to 250 kb distal to MCC that might be contained within the deletion.

In a first approach, the insert from YAC24ED6 (FIG. 1B) was radiolabelled and hybridized to a cDNA library from normal colon. One of the cDNA clones (YS39) identified in this manner detected a 3.1 kb mRNA transcript when used as a probe for Northern blot hybridization. Sequence analysis of the YS39 clone revealed that it encompassed 2283 nucleotides and contained an ORF that extended for 555 bp from the most 5' sequence data obtained. If all of this ORF were

translated, it would encode 185 amino acids (SEQ ID NO:6) (FIG. 2B). The gene detected by YS39 was named TB2. Searches of nucleotide and protein databases revealed that the TB2 gene was not identical to any previously reported sequences nor were there any striking similarities.

Another clone (YS11) identified through the YAC 24ED6 screen appeared to contain portions of two distinct genes. Sequences from one end of YS11 were identical to at least 180 bp of the signal recognition particle protein SRP19 (Lingelbach et al. *Nucleic Acids Research*, Vol. 16, p. 9431 (1988)). A second ORF, from the opposite end of clone YS11, proved to be identical to 78 bp of a novel gene which was independently identified through a second YAC-based approach. For the latter, DNA from yeast cells containing YAC 14FH1 (FIG. 1B) was digested with EcoRI and subcloned into a plasmid vector. Plasmids that contained human DNA fragments were selected by colony hybridization using total human DNA as a probe. These clones were then used to search for cross-hybridizing sequences as described above for TB1, and the cross-hybridizing clones were subsequently used to screen cDNA libraries. One of the cDNA clones discovered in this way (FH38) contained a long ORF (2496 bp), 78 bp of which were identical to the above-noted sequences in YS11. The ends of the FH38 cDNA clone were then used to initiate cDNA walking to extend the sequence. Eventually, 85 cDNA clones were isolated from normal colon, brain and liver cDNA libraries and found to encompass 8973 nucleotides of contiguous transcript. The gene corresponding to this transcript was named APC. When used as probes for Northern blot analysis, APC cDNA clones hybridized to a single transcript of approximately 9.5 kb, suggesting that the great majority of the gene product was represented in the cDNA clones obtained. Sequences from the 5' end of the APC gene were found in YAC 37HG4 but not in YAC 14FH1. However, the 3' end of the APC gene was found in 14FH1 as well as 37HG4. Analogously, the 5' end of the MCC coding region was found in YAC clones 19AA9 and 266C3 but not 24ED6 or 14FH1, while the 3' end displayed the opposite pattern. Thus, MCC and APC transcription units pointed in opposite directions, with the direction of transcription going from centromeric to telomeric in the case of MCC, and telomeric to centromeric in the case of APC. PFGE analysis of YAC DNA digested with various restriction endonucleases showed that TB2 and SRP were between MCC and APC, and that the 3' ends of the coding regions of MCC and APC were separated by approximately 150 kb (FIG. 1B).

Sequence analysis of the APC cDNA clones revealed an open reading frame of 8,535 nucleotides. The 5' end of the ORF contained a methionine codon (codon 1) that was preceded by an in-frame stop codon 9 bp upstream, and the 3' end was followed by several in-frame stop codons. The protein produced by initiation at codon 1 would contain 2,842 amino acids (SEQ ID NO:7) (FIG. 3). The results of database searching with the APC gene product were quite complex due to the presence of large segments with locally biased amino acid compositions. In spite of this, APC could be roughly divided into two domains. The N-terminal 25% of the protein had a high content of leucine residues (12%) and showed local sequence similarities to myosins, various intermediate filament proteins (e.g., desmin, vimentin, neurofilaments) and *Drosophila* armadillo/human plakoglobin. The latter protein is a component of adhesive junctions (desmosomes) joining epithelial cells (Franke et al., *Proc. Natl. Acad. Sci. U.S.A.*, Vol. 86, p. 4027 (1989); Perfer et al., *Cell*, Vol. 63, p. 1167 (1990)). The C-terminal 75% of APC (residues 731–2832) is 17% serine by composition with serine residues more or less uniformly distributed. This large domain also contains local concentrations of charged (mostly acidic) and proline residues. There was no indication of potential signal peptides, transmembrane regions, or nuclear targeting signals in APC, suggesting a cytoplasmic localization.

To detect short similarities to APC, a database search was performed using the PAM-40 matrix (Altschul. J. Mol. Bio., Vol. 219, p. 555 (1991)). Potentially interesting matches to several proteins were found. The most suggestive of these involved the *ral2* gene product of yeast, which is implicated in the regulation of ras activity (Fukul et al., Mol. Cell. Biol., Vol. 9, p. 5617 (1989)). Little is known about how *ral2* might interact with ras but it is interesting to note the positively-charged character of this region in the context of the negatively-charged GAP interaction region of ras. A specific electrostatic interaction between ras and GAP-related proteins has been proposed.

Because of the proximity of the MCC and APC genes, and the fact that both [am] are implicated in colorectal tumorigenesis, we searched for similarities between the two predicted proteins. Bourne has previously noted that MCC has the potential to form alpha helical coiled coils (Nature, Vol. 351, p. 188 (1991)). Lupas and colleagues have recently developed a program for predicting coiled coil potential from primary sequence data (Science, Vol. 252, p. 1162 (1991)) and we have used their program to analyze both MCC and APC. Analysis of MCC indicated a discontinuous pattern of coiled-coil domains separated by putative "hinge" or ["sparer"] "spacer" regions similar to those seen in laminin and other intermediate filament proteins. Analysis of the APC sequence revealed two regions in the N-terminal domain which had strong coiled coil-forming potential, and these regions corresponded to those that showed local similarities with myosin and IF proteins on database searching. In addition, one other putative coiled coil region was identified in the central region of APC. The potential for both APC and MCC to form coiled coils is interesting in that such structures often mediate homo- and hetero-oligomerization.

Finally, it had previously been noted that MCC shared a short similarity with the region of the m3 muscarinic acetylcholine receptor (mAChR) known to regulate specificity of G-protein coupling. The APC gene also contained a local similarity to the region of the m3 mAChR (SEQ ID NO:9) that overlapped with the MCC similarity (SEQ ID NO:10) (FIG. 4B). Although the similarities to *ral2* (SEQ ID NO:8) (FIG. 4A) and m3 mAChR (SEQ ID NO:9) (FIG. 4B) were not statistically significant, they were intriguing in light of previous observations relating G-proteins to neoplasia.

Each of the six genes described above was expressed in normal colon mucosa, as indicated by their representation in colon cDNA libraries. To study expression of the genes in neoplastic colorectal epithelium, we employed reverse transcription-polymerase chain reaction (PCR) assays. Primers based on the sequences of FER, TB1, TB2, MCC, and APC were each used to design primers for PCR performed with cDNA templates. Each of these genes was found to be expressed in normal colon, in each of ten cell lines derived from colorectal cancers, and in tumor cell lines derived from lung and bladder tumors. The ten colorectal cancer cell lines included eight from patients with sporadic CRC and two from patients with FAP.

EXAMPLE 2

This example demonstrates a genetic analysis of the role of the FER gene in FAP and sporadic colorectal cancers.

We considered FER as a candidate because of its proximity to the FAP locus as judged by physical and genetic criteria (see Example 1), and its homology to known tyrosine kinases with oncogenic potential. Primers were designed to PCR-amplify the complete coding sequence of FER from the RNA of two colorectal cancer cell lines derived from FAP patients, cDNA was generated from RNA and used as a template for PCR. The primers used were 5'-AGAAGGATC CCTTGTGCAGTGTGGA-3' (SEQ ID NO:95) and

5'-GACAGGATCCTGAAGCTGAGTTTG-3' (SEQ ID NO:96). The underlined nucleotides were altered from the true FER sequence to create BamHI sites. The cell lines used were JW and Difi, both derived from colorectal cancers of FAP patients. (C. Paraskeva, B. G. Buckle, D. Sheer, C. B. Wigley, Int. J. Cancer 34, 49 (1984); M. E. Gross et al., Cancer Res. 51, 1452 (1991)). The resultant 2554 basepair fragments were cloned and sequenced in their entirety. The PCR products were cloned in the BamHI site of Bluescript SK (Stratagene) and pools of at least 50 clones were sequenced en masse using T7 polymerase, as described in Nigro et al., Nature 342, 705 (1989).

Only a single conservative amino acid change (GTG→CTG, creating a val to leu substitution at codon 439) was observed. The region surrounding this codon was then amplified from the DNA of individuals without FAP and this substitution was found to be a common polymorphism, not specifically associated with FAP. Based on these results, we considered it unlikely (though still possible) the FER gene was responsible for FAP. To amplify the regions surrounding codon 439, the following primers were used: 5'-TCAGAAAGTGCTGAAGAG-3' (SEQ ID NO:97) and 5'-GGAATAATTAGGTCTCCAA-3' (SEQ ID NO:98). PCR products were digested with PstI, which yields a 50 bp fragment if codon 439 is leucine, but 26 and 24 bp fragments if it is valine. The primers used for sequencing were chosen from the FER cDNA sequence in Hao et al., supra.

EXAMPLE 3

This example demonstrates the genetic analysis of MCC, TB2, SRP and APC in FAP and sporadic colorectal tumors. Each of these genes is linked and encompassed by contig 3 (see FIG. 1).

Several lines of evidence suggested that this contig was of particular interest. First, at least three of the four genes in this contig were within the deleted region identified in two FAP patients. (See Example 5 infra). Second, allelic deletions of chromosome 5q21 in sporadic cancers appeared to be centered in this region. (Ashton-Rickardt et al., Oncogene, in press; and Miki et al., Jpn. J. Cancer Res., in press.) Some tumors exhibited loss of proximal RFLP markers (up to and potentially including the 5' end of MCC), but no loss of markers distal to MCC. Other tumors exhibited loss of markers distal to and perhaps including the 3' end of MCC, but no loss of sequences proximal to MCC. This suggested either that different ends of MCC were affected by loss in all such cases, or alternatively, that two genes (one proximal to and perhaps including MCC, the other distal to MCC) were separate targets of deletion. Third, clones from each of the six FAP region genes were used as probes on Southern blots containing tumor DNA from patients with Sporadic CRC. Only two examples of somatic changes were observed in over 200 tumors studied: a rearrangement/deletion whose centrometric end was located within the MCC gene (Kinzler et al., supra) and an 800 bp insertion within the APC gene between nucleotides 4424 and 5584. Fourth, point mutations of MCC were observed in two tumors (Kinzler et al.) supra strongly suggesting that MCC was a target of mutation in at least some sporadic colorectal cancers.

Based on these results, we attempted to search for subtle alterations of contig 3 genes in patients with FAP. We chose to examine MCC and APC, rather than TB2 or SRP, because of the somatic mutations in MCC and APC noted above. To facilitate the identification of subtle alterations, the genomic sequences of MCC and APC exons were determined (see Table I, SEQ ID NO:24-38).

TABLE I

APC EXONS		
EXON	NUCLEOTIDES ¹	EXON BOUNDARY SEQUENCE ²
822 to 930	<u>catgatgttatctgtatttacctatagctaaattataccatctataatgtgcttaatttttag</u> /GGTTCA. . .	(SEQ ID NO: 24)
	. . .ACCAAG/gtaacagaagattacaaccctgtcactaatgcatgactactttgctaag	(SEQ ID NO: 25)
931 to 1039	<u>ggatattaaagtcgtaattttgttctaaactcattggcccacag</u> /GTGGAA. . .	(SEQ ID NO: 26)
	. . .ATCCAA/gtatgttctctatagtgatcacgtagtgcatg	(SEQ ID NO: 27)
1310 to 1405	<u>catcattgctcttcaaataacaagaacattatggtttatggtttatgttttttcag</u> /TGCCAG. . .	(SEQ ID NO: 28)
	. . .AACTAG/gtaagacaaaaatgttttataatgacatagacaactactggtg	(SEQ ID NO: 29)
1406 to 1545	<u>tagatgattgtcttttctctgtcccttttaaatag</u> /GGGGAC. . .	(SEQ ID NO: 30)
	. . .ACAAG/gtatgtttataacatgtatttctaaaggatagctcaggtctga	(SEQ ID NO: 31)
1546 to 1623	<u>gcttggctcaagttgcttttaaatgacacctattctgtatttaattacag</u> /GCTACG. . .	(SEQ ID NO: 32)
	. . .CAGCAG/gtactatttagaattcacctgttttcttttctcttttctttgagcagggctcactctg	(SEQ ID NO: 33)
1624 to 1740	<u>gcaactagatgattttatgataaataactaaaattgattaattgacag</u> /GTTATT. . .	(SEQ ID NO: 34)
	. . .AAAAG/gtaccttgaacacttagtactataatagaattcatgt	(SEQ ID NO: 35)
1741 to 1955	<u>caacttaattagatgacccatattcagaacttag</u> /GAATCA. . .	(SEQ ID NO: 36)
	. . .CCACAG/gtatatatagagtttatattacttttaagtacagaattcactctcaaaaa	(SEQ ID NO: 37)
1956 to 8973	tcttgattttatttcag/GCAAAT. . .	(SEQ ID NO: 38)
	. . .GGTATTTATGCAAAAAAATGTTTTTGT	(SEQ ID NO: 1)

¹Relative to predicted translation initiation site

²Small case letters represent introns, large case letters represent exons

The entire 3' end of the cloned APC cDNA (nt 1956-8973) appeared to be encoded in this exon, as indicated by restriction endonuclease mapping and sequencing of the cloned genomic DNA. The ORF ended at nt 8535. The extreme 3' end of the APC transcript has not yet been identified.

These sequences were used to design primers for PCR analysis of constitutional DNA from FAP patients.

We first amplified eight exons and surrounding introns of the MCC gene in affected individuals from 90 different FAP kindreds. The PCR products were analyzed by a ribonuclease (RNase) protein assay. In brief, the PCR products were hybridized to in vitro transcribed RNA probes representing the normal genomic sequences. The hybrids were digested with RNase A, which can cleave at single base pair mismatches within DNA-RNA hybrids, and the cleavage products were visualized following denaturing gel electrophoresis. Two separate RNase protection analyses were performed for each exon, one with the sense and one with the antisense strand. Under these conditions, approximately 40% of all mismatches are detectable. Although some amino acid variants of MCC were observed in FAP patients, all such variants were found in a small percentage of normal individuals. These variants were thus unlikely to be responsible for the inheritance of FAP.

We next examined three exons of the [A PC] APC gene. The three exons examined included those containing nt 822-930, 931-1309, and the first 300 nt of the most distal exon (nt 1956-2256). PCR and RNase protection analysis were performed as described in Kinzler et al. supra, using the primers underlined in Table I (SEQ ID NO:24-38). The primers for nt 1956-2256 were 5'-GCAAATCCTAAGAGAGAACAA-3' (SEQ ID NO:99) and 5'-GATGGCAAGCTTGAGCCAG-3' (SEQ ID NO:100).

In 90 kindreds, the RNase protection method was used to screen for mutations and in an additional 13 kindreds, the PCR products were cloned and sequenced to search for mutations not detectable by RNase protection. PCR products were cloned into a Bluescript vector modified as described in T. A. Holton and M. W. Graham, Nucleic Acids Res. 19, 1156 (1991). A minimum of 100 clones were pooled and sequenced. Five variants were detected among the 103 kindreds analyzed. Cloning and subsequent DNA sequencing of the PCR product of patient P21 indicated a C to T transition in codon 413 that resulted in a change from arginine to cysteine. This amino acid variant was not observed in any of 200 DNA samples from individuals without FAP. Cloning

and sequencing of the PCR product from patients P24 and P34, who demonstrated the same abnormal RNase protection pattern indicated that both had a C to T transition at codon 801 that resulted in a change from arginine (CGA) to a stop codon (TGA). This change was not present in 200 individuals without FAP. As this point mutation resulted in the predicted loss of the recognition site for the enzyme Taq I, appropriate PCR products could be digested with Taq I to detect the mutation. This allowed us to determine that the stop codon co-segregated with disease phenotype in members of the family of P24. The inheritance of this change in affected members of the pedigree provides additional evidence for the importance of the mutation.

Cloning and sequencing of the PCR product from FAP patient P93 indicated a C to G transversion at codon 279, also resulting in a stop codon (change from TCA to TGA). This mutation was not present in 200 individuals without FAP. Finally, one additional mutation resulting in a serine (TCA) to stop codon (TGA) at codon 712 was detected in a single patient with FAP (patient P60).

The five germline mutations identified are summarized in Table IIA, as well as four others discussed in Example 9.

TABLE IIA

Germline mutations of the APC gene in FAP and GS Patients					
EXTRA-COLONIC PATIENT DISEASE	CODON	NUCLEOTIDE CHANGE	AMINO ACID CHANGE	AGE	
93	279	TCA->TGA	Ser->Stop	39	Mandibular
<u>Osteoma</u>					
24	301	CGA->TGA	Arg->Stop	46	None
34	301	CGA->TGA	Arg->Stop	27	Desmoid
<u>Tumor</u>					
21	413	CGC->TGC	Arg->Cys	24	Mandibular

TABLE IIA-continued

Germline mutations of the APC gene in FAP and GS Patients					
EXTRA-COLO-NIC PATIENT DISEASE	CODON	NUCLEOTIDE CHANGE	AMINO ACID CHANGE	AGE	
Osteoma					
60	712	TCA-> <u>T</u> GA	Ser->Stop	37	Mandibular
Osteoma					
3746	243	CAGAG->CAG	splice-junction		
3460	301	CGA-> <u>T</u> GA	Arg->Stop		
3827	456	CTTCA-> <u>C</u> TTCA	frameshift		
3712	500	T-> <u>G</u>	Tyr->Stop		

* The mutated nucleotides are underlined.

In addition to these germline mutations, we identified several somatic mutations of MCC and APC in sporadic CRC's. Seventeen MCC exons were examined in 90 sporadic colorectal cancers by RNase protection analysis. In each case where an abnormal RNase protection pattern was observed, the corresponding PCR products were cloned and sequenced. This led to the identification of six point mutations (two described previously) (Kinzler et al., supra), each of which was not found in the germline of these patients (Table IIB).

TABLE IIB

Somatic Mutations in Sporadic CRC Patients			
PATIENT	CODON	NUCLEOTIDE CHANGE	AMINO ACID CHANGE
T35	MCC 12	GAG/gtaaga->GAG/gtaaaa	(Splice Donor)
T16	MCC 145	ctcag/GGA->atcag/GGA	(Splice Acceptor)
T47	MCC 267	CGG-> <u>C</u> TG	Arg->Leu
T81	MCC 490	TCG-> <u>T</u> TG	Ser->Leu
T35	MCC 506	CGG-> <u>C</u> AG	Arg->Gln
T91	MCC 698	GCT-> <u>G</u> TT	Ala->Val
T34	APC 288	CCAGT-> <u>CCCAGCC</u> AGT	(Insertion)
T27	APC 331	CGA-> <u>T</u> GA	Arg->Stop
T135	APC 437	CAA/gtaa->CAA/gcaa	(Splice Donor)
T20I	APC 1338	CAG-> <u>T</u> AG	Gln->Stop

For splice site mutations, the codon nearest to the mutation is listed. The underlined nucleotides were mutant; small case letters represent introns, large case letters represent exons.

Four of the mutations resulted in amino acid substitutions and two resulted in the alteration of splice site consensus elements. Mutations at analogous splice site positions in other genes have been shown to alter RNA processing in vivo and in vitro.

Three exons of APC were also evaluated in sporadic tumors. Sixty tumors were screened by RNase protection, and an additional 98 tumors were evaluated by sequencing. The exons examined included nt 822-930, 931-1309, and 1406-1545 (Table I). A total of three mutations were identified, each of which proved to be somatic. Tumor T27 contained a somatic mutation of CGA (arginine) to TGA (stop codon) at codon 33. Tumor T135 contained a GT to GC change at a splice donor site. Tumor T34 contained a 5 bp insertion (CAGCC between codons 288 and 289) resulting in a stop at codon 291 due to a frameshift. We serendipitously discovered one additional somatic mutation in a col-

orectal cancer. During our attempt to define the sequences and splice patterns of the MCC and APC gene products in colorectal epithelial cells, we cloned cDNA from the colorectal cancer cell line SW480. The amino acid sequence of the MCC gene from SW480 was identical to that previously found in clones from human brain. The sequence of APC in SW480 cells, however, differed significantly, in that a transition at codon 1338 resulted in a change from glutamine (CAG) to a stop codon (TAG). To determine if this mutation was somatic, we recovered DNA from archival paraffin blocks of the original surgical specimen (T201) from which the tumor cell line was derived 28 years ago.

DNA was purified from paraffin sections as described in S. E. Goetz, S. R. Hamilton, and B. Vogelstein. *Biochem. Biophys. Res. Comm.* 130, 118 (1985). PCR was performed as described in reference 24, using the primers 5'-GTTCCAGCAGTGTACACAG-3' (SEQ ID NO:101) and 5'-GGGAGATTCGCTCCTGA-3' (SEQ ID NO:102). A PCR product containing codon 1338 was amplified from the archival DNA and used to show that the stop codon represented a somatic mutation present in the original primary tumor and in cell lines derived from the primary and metastatic tumor sites, but not from normal tissue of the patient.

The ten point mutations in the MCC and APC genes so far discovered in sporadic CRCs are summarized in Table IIB. Analysis of the number of mutant and wild-type PCR clones obtained from each of these tumors showed that in eight of the ten cases, the wild-type sequence was present in approximately equal proportions to the mutant. This was confirmed by RFLP analysis using flanking markers from chromosome 5q which demonstrated that only two of the ten tumors (T135 and T201) exhibited an allelic deletion on chromosome 5q. These results are consistent with previous observations showing that 20-40% of sporadic colorectal tumors had allelic deletions of chromosome 5q. Moreover, these data suggest that mutations of 5q21 genes are not limited to those colorectal tumors which contain allelic deletions of this chromosome.

EXAMPLE 4

This example characterizes small, nested deletions in DNA from two unrelated FAP patients.

DNA from 40 FAP patients was screened with cosmids that has been mapped into a region near the APC locus to identify small deletions or rearrangements. Two of these cosmids, L5.71 and L5.79, hybridized with a 1200 kb NotI fragment in DNAs from most of the FAP patients screened.

The DNA of one FAP patient, 3214, showed only a 940 kb NotI fragment instead of the expected 1200 kb fragment. DNA was analyzed from four other members of the patient's immediate family; the 940 kb fragment was present in her affected mother (4711), but not in the other, unaffected family members. The mother also carried a normal 1200 kb NotI fragment that was transmitted to her two unaffected offspring. These observations indicated that the mutant polyposis allele is on the same chromosome as the 940 kb NotI fragment. A simple interpretation is that APC patients 3214 and 4711 each carry a 260 kb deletion within the APC locus.

If a deletion were present, then other enzymes might also be expected to produce fragments with altered mobilities. Hybridization of L5.79 to NruI-digested DNAs from both affected members of the family revealed a novel NruI fragment of 1300 kb, in addition to the normal 1200 kb NruI fragment. Furthermore, MluI fragments in patients 3214 and 4711 also showed an increase in size consistent with the deletion of an MluI site. The two chromosome 5 homologs

of patient 3214 were segregated in somatic cell hybrid lines; HHW1155 (deletion hybrid) carried the abnormal homolog and HHW1159 (normal hybrid) carried the normal homolog.

Because patient 8214 showed a 940 kb NotI fragment, she had not inherited the 1200 kb fragment present in the unaffected father's DNA. This observation suggests that he must be heterozygous for, and have transmitted, either a deletion of the L5.79 probe region or a variant NotI fragment too large to resolve on the gel system. As expected, the hybrid cell line HHW1159, which carries the paternal homolog, revealed no resolved NotI fragment when probed with L5.79. However, probing of HHW1159 DNA with L5.79 following digestion with other enzymes did reveal restriction fragments, demonstrating the presence of DNA homologous to the probe. The father is, therefore, interpreted as heterozygous for a polymorphism at the NotI site, with one chromosome 5 having a 1200 kb NotI fragment and the other having a fragment too large to resolve consistently on the gel. The latter was transmitted to patient 3214.

When double digests were used to order restriction sites within the 1200 kb NotI fragment, L5.71 and L5.79 were both found to lie on a 550 kb NotI-NruI fragment and, therefore, on the same side of an NruI site in the 1200 kb NotI fragment. To obtain genomic representation of sequences present over the entire 1200 kb NotI fragment, we constructed a library of small-fragment inserts enriched for sequences from this fragment. DNA from the somatic cell hybrid HHW141, which contains about 40% of chromosome 5, was digested with NotI and electrophoresed under pulsed-field gel (PFGE) conditions; EcoRI fragments from the 1200 kb region of this gel were cloned into a phage vector. Probe Map30 was isolated from this library. In normal individuals probe Map30 hybridizes to the 1200 kb NotI fragment and to a 200 kb NruI fragment. This latter hybridization places Map30 distal, with respect to the locations of L5.71 and L5.79, to the NruI site of the 550 kb NotI-NruI fragment.

Because Map30 hybridized to the abnormal, 1300 kb NruI fragment of patient 3214, the locus defined by Map30 lies outside the hypothesized deletion. Furthermore, in normal chromosomes Map30 identified a 200 kb NruI fragment and L5.79 identified a 1200 kb NruI fragment; the hypothesized deletion must, therefore, be removing an NruI site, or sites, lying between Map30 and L5.79, and these two probes must flank the hypothesized deletion. A restriction map of the genomic region, showing placement of these probes, is shown in FIG. 5.

A NotI digest of DNA from another FAP patient, 3824 was probed with L5.79. In addition to the 1200 kb normal NotI fragment, a fragment of approximately 1100 kb was observed, consistent with the presence of a 100 kb deletion in one chromosome 5. In this case, however, digestion with NruI and MluI did not reveal abnormal bands, indicating that if a deletion were present, its boundaries must lie distal to the NruI and MluI sites of the fragments identified by L5.79. Consistent with this expectation, hybridization of Map30 to DNA from patient 3824 identified a 760 kb MluI fragment in addition to the expected 860 kb fragment, supporting the interpretation of a 100 kb deletion in this patient. The two chromosome 5 homologs of patient 3824 were segregated in somatic cell hybrid lines; HHW1291 was found to carry only the abnormal homolog and HHW1290 only the normal homolog.

That the 860 kb MluI fragment identified by Map30 is distinct from the 830 kb MluI fragment identified previously by L5.79 was demonstrated by hybridization of Map30 and L5.79 to a NotI-MluI double digest of DNA from the hybrid

cell (HHW1159) containing the nondeleted chromosome 5 homolog of patient 3214. As previously indicated, this hybrid is interpreted as missing one of the NotI sites that define the 1200 kb fragment. A 620 kb NotI-MluI fragment was seen with probe L5.79, and an 860 kb fragment was seen with Map30. Therefore, the 830 kb MluI fragment recognized by probe L5.79 must contain a NotI site in HHW1159 DNA; because the 860 kb MluI fragment remains intact, it does not carry this NotI site and must be distinct from the 830 kb MluI fragment.

EXAMPLE 5

This example demonstrates the isolation of human sequences which span the region deleted in the two unrelated FAP patients characterized in Example 4.

A strong prediction of the hypothesis that patients 8214 and 3824 carry deletions is that some sequences present on normal chromosome 5 homologs would be missing from the hypothesized deletion homologs. Therefore, to develop genomic probes that might confirm the deletions, as well as to identify genes from the region, YAC clones from a contig seeded by cosmid L5.79 were localized from a library containing seven haploid human genome equivalents (Albertsen et al., Proc. Natl. Acad. Sci. U.S.A., Vol. 87, pp. 4256-4260 (1990)) with respect to the hypothesized deletions. Three clones, YACs 57B8, 310D8, and 183H12, were found to overlap the deleted region.

Importantly, one end of YAC 57B8 (clone AT57) was found to lie within the patient 3214 deletion. Inverse polymerase chain reaction (PCR) defined the end sequences of the insert of YAC 57B8. PCR primers based on one of these end sequences repeatedly failed to amplify DNA from the somatic cell hybrid (HHW1155) carrying the deleted homolog of patient 3214, but did amplify a product of the expected size from the somatic cell hybrid (HHW1159) carrying the normal chromosome 5 homolog. This result supported the interpretation that the abnormal restriction fragments found in the DNA of patient 3214 result from a deletion.

Additional support for the hypothesis of deletion in DNA from patient 3214 came from subcloned fragments of YAC 183H12, which spans the region in question. Y11, an EcoRI fragment cloned from YAC 183H12, hybridized to the normal, 1200 kb NotI fragment of patient 4711, but failed to hybridize to the abnormal, 940 kb NotI fragment of 4711 or to DNA from deletion cell line HHW1155. This result confirmed the deletion in patient 3214.

Two additional EcoRI fragments from YAC 183H12, Y10 and Y14, were localized within the patient 3214 deletion by their failure to hybridize to DNA from HHW1155. Probe Y10 hybridizes to a 150 kb NruI fragment in normal chromosome 5 homologs. Because the 3214 deletion creates the 1300 kb NruI fragment seen with the probes L5.79 and Map30 that flank the deletion, these NruI sites and the 150 kb NruI fragment lying between must be deleted in patient 3214. Furthermore, probe Y10 hybridizes to the same 620 kb NotI-MluI fragment seen with probe L5.79 in normal DNA, indicating its location as L5.79-proximal to the deleted MluI site and placing it between the MluI site and the L5.79-proximal NruI site. The MluI site must, therefore, lie between the NruI sites that define the 150 kb NruI fragment (see FIG. 5).

Probe Y11 also hybridized to the 150 kb NruI fragment in the normal chromosome 5 homolog, but failed to hybridize to the 620 kb NotI-MluI fragment, placing it L5.79-distal to the MluI site, but proximal to the second NruI site. Hybrid-

ization to the same (860 kb) MluI fragment as Map30 confirmed the localization of probe Y11 L5.79-distal to the MluI site.

Probe Y14 was shown to be L5.79-distal to both deleted NruI sites by virtue of its hybridization to the same 200 kb NruI fragment of the normal chromosome 5 seen with Map30. Therefore, the order of these EcoRI fragments derived from YAC 183H12 and deleted in patient 3214, with respect to L5.79 and Map30, is L5.79-Y10-Y11-Y14-Map30.

The 100 kb deletion of patient 3824 was confirmed by the failure of aberrant restriction fragments in this DNA to hybridize with probe Y11, combined with positive hybridizations to probes Y10 and/or Y14. Y10 and Y14 each hybridized to the 1100 kb NotI fragment of patient 3824 as well as to the normal 1200 kb NotI fragment, but Y11 hybridized to the 1200 kb fragment only. In the MluI digest, probe Y14 hybridized to the 860 kb and 760 kb fragments of patient 3824 DNA, but probe Y11 hybridized only to the 860 k13 fragment. We conclude that the basis for the alteration in fragment size in DNA from patient 3824 is, indeed, a deletion. Furthermore, because probes Y10 and Y14 are missing from the deleted 3214 chromosome, but present on the deleted 3824 chromosome, and they have been shown to flank probe Y11, the deletion in patient 3824 must be nested within the patient 3214 deletion.

Probes Y10, Y11, Y14 and Map30 each hybridized to YAC 310D8, indicating that this YAC spanned the patient 3824 deletion and at a minimum, most of the 3214 deletion. The YAC characterizations, therefore, confirmed the presence of deletions in the patients and provided physical representation of the deleted region.

EXAMPLE 6

This example demonstrates that the MCC coding sequence maps outside of the region deleted in the two FAP patients characterized in Example 4.

An intriguing FAP candidate gene, MCC, recently was ascertained with cosmid L5.71 and was shown to have undergone mutation in colon carcinomas (Kinzler et al., supra). It was therefore of interest to map this gene with respect to the deletions in APC patients. Hybridization of MCC probes with an overlapping series of YAC clones extending in either direction from L5.71 showed that the 3' end of MCC must be oriented toward the region of the two APC deletions.

Therefore, two 3' cDNA clones from MCC were mapped with respect, to the deletions: clone 1CI (bp 2378–4181) and clone 7 (bp 2890–3560). Clone 1CI contains sequences from the C-terminal end of the open reading frame, which stops at nucleotide 2708, as well as 3' untranslated sequence. Clone 7 contains sequence that is entirely 3' to the open reading frame. Importantly, the entire 3' untranslated sequence contained in the cDNA clones consists of a single 2.5 kb exon. These two clones were hybridized to DNAs from the YACs spanning the FAP region. Clone 7 fails to hybridize to YAC 310D8, although it does hybridize to YACs 183H12 and 57B8; the same result was obtained with the cDNA 1CI. Furthermore, these probes did show hybridization to DNAs from both hybrid cell lines (HWW1159 and HWW1155) and the lymphoblastoid cell line from patient 3214, confirming their locations outside the deleted region. Additional mapping experiments suggested that the 3' end of the MCC cDNA clone contig is likely to be located more than 45 kb from the deletion of patient 3214 and, therefore, more than 100 kb from the deletion of patient 3824.

EXAMPLE 7

This example identifies three genes within the deleted region of chromosome 5 in the two unrelated FAP patients characterized in Example 4.

Genomic clones were used to screen cDNA libraries in three separate experiments. One screening was done with a phage clone derived from YAC 310D8 known to span the 260 kb deletion of patient 3214. A large-insert phage library was constructed from this YAC; screening with Y11 identified λ 205, which mapped within both deletions. When clone λ 205 was used to probe a random-, plus oligo(dT)-, primed fetal brain cDNA library (approximately 300,000 phage), six cDNA clones were isolated and each of them mapped entirely within both deletions. Sequence analysis of these six clones formed a single cDNA contig, but did not reveal an extended open reading frame. One of the six cDNAs was used to isolate more cDNA clones, some of which crossed the L5.71-proximal breakpoint of the 3824 deletion, as indicated by hybridization to both chromosome of this patient. These clones also contained an open reading frame, indicating a transcriptional orientation proximal to distal with respect to L5.71. This gene was named DP1 (deleted in polyposis 1). This gene is identical to TB2 described above.

cDNA walks yielded a cDNA contig of 3.0–3.5 kb, and included two clones containing terminal poly(A) sequences. This size corresponds to the 3.5 kb band seen by Northern analysis. Sequencing of the first 3163 bp of the cDNA contig revealed an open reading frame extending from the first base to nucleotide 631, followed by a 2.5 kb 3' untranslated region. The sequence surrounding the methionine codon at base 77 conforms to the Kozak consensus of an initiation methionine (Kozak, 1984). Failed attempts to walk farther, coupled with the similarity of the lengths of isolated cDNA and mRNA, suggested that the NH₂-terminus of the DP1 protein had been reached. Hybridization to a combination of genomic and YAC DNAs cut with various enzymes indicated the genomic coverage of DP1 to be approximately 30 kb.

Two additional probes for the locus, YS-11 and YS-39, which had been ascertained by screening of a cDNA library with an independent YAC probe identified with MCC sequences adjacent to L5.71, were mapped into the deletion region. YS-39 was shown to be a cDNA identical in sequence to DP1. Partial characterization of YS-11 had shown that 200 bp of DNA sequence at one end was identical to sequence coding for the 19 kb protein of the ribosomal signal recognition particle, SRP19 (Lingelbach et al., supra). Hybridization experiments mapped YS-11 within both deletions. The sequence of this clone, however, was found to be complex. Although 454 bp of the 1032 bp sequence of YS-11 were identical to the GenBank entry for the SRP19 gene, another 578 bp appended 5' to the SRP19 sequence was found to consist of previously unreported sequence containing no extended open reading frame. This suggested that YS-11 was either a chimeric clone containing two independent inserts or a clone of an incompletely processed or aberrant message. If YS-11 were a conventional chimeric clone, the independent segments would not be expected to map to the same physical region. The segments resulting from anomalous processing of a continuous transcript, however, would map to a single chromosomal region.

Inverse PCR with primers specific to the two ends of YS-11, the SRP19, end and the unidentified region, verified that both sequences map within the YAC 310D8; therefore, YS-11 is most likely a clone of an immature or anomalous mRNA species. Subsequently, both ends were shown to lie with the deleted region of patient 3824, and YS-11 was used to screen for additional cDNA clones.

Of the 14 cDNA clones selected from the fetal brain library, one clone, V5, was of particular interest in that it contained an open reading frame throughout, although it included only a short identity to the first 78 5' bases of the YS-11 sequence. Following the 78 bp of identical sequence, the two cDNA sequences diverged at an AG. Furthermore, divergence from genomic sequence was also seen after these 78 bp, suggesting the presence of a splice junction, and supporting the view that YS-11 represents an irregular message.

Starting with V5, successive 5' and 3' walks were performed; the resulting cDNA contig consisted of more than 100 clones, which defined a new transcript, DP2. Clones walking in the 5' directions crossed the 3824 deletion breakpoint farthest from L5.71; since its 3' end is closer to this cosmid than its 5' end, the transcriptional orientation of DP2 is opposite to that of MCC and DP1.

The third screening approach relied on hybridization with a 120 kb MluI fragment from YAC 57B8. This fragment hybridizes with probe Y11 and completely spans the 100 kb deletion in patient 3824, the fragment was purified on two preparative PFGs, labeled, and used to screen a fetal brain cDNA library. A number of cDNA clones previously identified in the development of the DP1 and DP2 contigs were reascertained. However, 19 new cDNA clones mapped into the patient 3824 deletion. Analysis indicated that these 19 formed a new contig, DP3, containing a large open reading frame.

A clone from the 5' end of this new cDNA contig hybridized to the same EcoRI fragment as the 3' end of DP2. Subsequently, the DP2 and DP3 contigs were connected by a single 5' walking step from DP3, to form the single contig DP2.5. The complete nucleotide sequence of DP2.5 is shown in FIG. 9.

The consensus cDNA sequence of DP2.5 suggests that the entire coding sequence of DP2.5 has been obtained and is 8532 bp long. The most 5' ATG codon occurs two codons from an in-frame stop and conforms to the Kozak initiation

consensus (Kozak, Nucl. Acids. Res., Vol. 12, p. 857-872 1984). The 3' open reading frame breaks down over the final 1.8 kb, giving multiple stops in all frames. A poly(A) sequence was found in one clone approximately 1 kb into the 3' untranslated region, associated with a polyadenylation signal 33 bp upstream (position 9530). The open reading frame is almost identical to that identified as APC above.

An alternatively spliced exon at nucleotide 934 of the DP2.5 transcript is of potential interest, it was first discovered by noting that two classes of cDNA had been isolated. The more abundant cDNA class contains a 303 bp exon not included in the other. The presence in vivo of the two transcripts was verified by an exon connection experiment. Primers flanking the alternatively spliced exon were used to amplify, by PCR, cDNA prepared from various adult tissues. Two PCR products that differed in size by approximately 300 bases were amplified from all the tissues tested; the larger product was always more abundant than the smaller.

EXAMPLE 8

This example demonstrates the primers used to identify subtle mutations in DP1, SRP19, and DP25.

To obtain DNA sequence adjacent to the exons of the genes DP1, DP2.5, and SRP19, sequencing substrate was obtained by inverse PCR amplification of DNAs from two YACs, 310D8 and 183H12, that span the deletions. Ligation at low concentration cyclized the restriction enzyme-digested YAC DNAs. Oligonucleotides with sequencing tails, designed in inverse orientation at intervals along the cDNAs, primed PCR amplification from the cyclized templates. Comparison of these DNA sequences with the cDNA sequences placed exon boundaries at the divergence points. SRP19 and DP1 were each shown to have five exons. DP2.5 consisted of 15 exons. The sequences of the oligonucleotides synthesized to provide PCR amplification primers for the exons of each of these genes are listed in Table III SEQ ID NO:39-94.

TABLE III

Sequences of Primers Used for SSCP Analyses

Exon	Primer 1	Primer 2
		<u>DP1</u>
	UP-TCCCCGCCTGCCGCTCTC	RP-GCAGCGGCGGCTCCCGTG
	UP-GTGAACGGCTCTCATGCTGC	RP-ACGTGCGGGGAGGAATGGA
	UP-ATGATATCTTACCAAATGATATAC	RP-TTATTCCTACTTCTTCTATACAG
	UP-TACCCATGCTGGCTCTTTTTC	RP-TGGGGCCATCTGTTCCTGA
	UP-ACATTAGGCACAAAGCTTGCAA	RP-ATCAAGCTCCAGTAAGAAGGTA
		<u>SRP19</u>
	UP-TGCGGCTCCTGGGTTGTTG	RP-GCCCCCTTCCTTTCTGAGGAC
	UP-TTTCTCCTGCCTCTTACTGC	RP-ATGACACCCCCCATTCCTC
	UP-CCACTTAAAGCACATATATTTAGT	RP-GTATGGAAAATAGTGAAGAACC
	UP-TTCTTAAGTCCTGTTTTTCTTTTG	RP-TTTAGAACCTTTTTTGTGTTGTG
	UP-CTCAGATTATACTAAGCCTAAC	RP-CATGTCTTTACAGTAGTACCA
		<u>DP2.5</u>
	UP-AGGTCCAAGGGTAGCCAAGG*	RP-TAAAAATGGATAAACTACAATTAAAAG
	UP-AAATACAGAATCATGTCTTGAAGT	RP-ACACCTAAAGATGACAATTTGAG
	UP-TAACTTAGATAGCAGTAATTTCCC*	RP-ACAATAAACTGGAGTACACAAGG
	UP-ATAGGTCATTGCTTCTTGCTGAT*	RP-TGAATTTAATGGATTACCTAGGT
	UP-CTTTTTTGTCTTTTACTGATTAACG	RP-TGTAATTCATTTTATTCCTAATACCTC
	UP-GGTAGCCATAGTATGATTATTTCT	RP-CTACCTATTTTATACCCACAAAC
	UP-AAGAAAGCCTACACCATTTTTC	RP-GATCATTCTTAGAACCATCTTGC
	UP-ACCTATAGTCTAAATTATACCATC	RP-GTCATGGCATTACTGACCAG
	UP-AGTCGTAATTTGTTTCTAAACTC	RP-TGAAGGACTCCGATTTACCCC*
	UP-TCATTCACCTCACAGCCTGATGAC*	RP-GCTTTGAAACATGCACTACGAT
	UP-AAACATCATTGCTCTTCAAATAAC	RP-TACCATGATTTAAAATCCACCAG

TABLE III-continued

Sequences of Primers Used for SSCP Analyses	
Exon Primer 1	Primer 2
UP-GATGATTGTCTTTTCTCTTTGC	RP-CTGAGCTATCTTAAGAAATCACTG
UP-TTTTAAATGATCCTCTATTCTGTAT	RP-ACAGAGTCAGACCCTCCCTCAAAG
UP-TTTCTATTCTTACTGCTAGCATT	RP-ATACACAGGTAAGAAATTAGGA
UP-TAGATGACCCATATTCTCTTC	RP-CAATTAGGTCTTTTTGAGAGTA
3-A UP-GTTACTGCATACACATTGTGAC	RP-GCTTTTTGTTTCGTAACATGAAG*
-B UP-AGTACAAGGATGCCAATATTATG*	RP-ACTTCTATCTTTTTCAGAACGAG*
-C UP-ATTTGAATACTACAGTGTTACCC*	RP-CTTGTATTCTAATTTGGCATAAGG*
-D UP-CTGCCCATACACATTCAAACAC*	RP-TGTTTTCGCTCTTGCCCATCTT*
-E UP-AGTCTTAAATATTCAGATGAGCAG*	RP-GTTTCTCTTCATTATATTTTATGCTA*
-F UP-AAGCCTACCAATTATAGTGAACG*	RP-AGCTGATGACAAAGATGATAATC*
-G UP-AAGAAACAATACAGACTTATTGTG*	RP-ATGAGTGGGGTCTCCTGAAC*
-H UPATCTCCCTCCAAAAGTGGTGC*	RP-TCCATCTGGAGTACTTTCTGTG*
-I UP-AGTAAATGCTGCAGTTCAGAGG*	RP-CCGTGGCATAATCATCCCC*
-J UP-CCCAGACTGCTTCAAATTACC*	RP-GAGCCTCATCTGTACTTCTGC*
-K UP-CCCTCCAAATGAGTTAGCTGC*	RP-TTGTGGTATAGGTTTTACTGGTG*
-L UP-ACCCAACAAAATCAGTTAGATG*	RP-GTGGCTGGTAACTTTAGCCTC*
-N UP-ATGATGTTGACCTTTCCAGGG*	RP-ATTGTGTAACTTTTCATCAGTTGC*
-M UP-AAAGACATAACCAGACAGAGGG*	RP-CTTTTTTGGCATTGCGGAGCT*
-O UP-AAGATGACCTGTTGCAGGAATG*	RP-GAATCAGACCAAGCTTGTCTAGAT*
-P UP-CAATAGTAAGTAGTTTACATCAAG*	RP-AAACAGGACTTGTACTGTAGGA*
-Q UP-CAGCCCCTTCAAGCAAACATC*	RP-GAGGACTTATTCCATTCTACC*
-R UP-CAGTCTCCTGGCCGAAACTC*	RP-GTTGACTGGCGTACTAATACAG*
-S UP-TGGTAATGGAGCCAATAAAAAGG*	RP-TGGGACTTTTCGCCATCCAC*
-T UP-TGTCTCTATCCACACATTTCGTC*	RP-ATGTTTTTTCATCCTCCTTTTTTGC*
-U UP-GGAGAAGAAGTGAAGTTCATC*	RP-TTGAATCTTAAATGTTTGGATTTGC*
-V UP-TCTCCACAGGTAATACTCCC	RP-GCTACAAGTGAATGGGGTACG
-W UP-CAGGACAAAATAATCCTGTCCC	RP-ATTTTCTTACTTTCATTCTCCTC

All primers are read in the 5' to 3' direction, the first primer in each pair lies 5' of the exon it amplifies; the second primer lies 3' of the exon it amplifies. Primers that lie within the exons are identified by an asterisk. UP represents the -21M13 universal primer sequence; RP represents the M13 reverse primer sequence.

With the exception of exons 1, 3, 4, 9, and 15 of DP2.5 (see below), the primer sequences were located in intron sequences flanking the exons. The 5' primer of exon 1 is complementary to the cDNA sequence, but extends just into the 5' Kozak consensus sequence for the initiator methionine, allowing a survey of the translated sequences. The 5' primer of exon 3 is actually in the 5' coding sequences of this exon, as three separate intronic primers simply would not amplify. The 5' primer of exon 4 just overlaps the 5' end of this exon, and we thus fail to survey the 19 most 5' bases of this exon. For exon 9, two overlapping primer sets were used, such that each had one end within the exon. For exon 15, the large 3' exon of DP2.5, overlapping primer pairs were placed along the length of the exon; each pair amplified a product of 250–400 bases.

EXAMPLE 9

This example demonstrates the use of single stranded conformation polymorphism (SSCP) analysis as described by Orita et al. Proc. Natl. Acad. Sci. U.S.A., Vol. 86, pp. 2766–70 (1989) and Genomics, Vol. 5, pp. 874–879 (1989) as applied to DP1, SRP19 and DP2.5.

SSCP analysis identifies most single- or multiple-base changes in DNA fragments up to 400 bases in length. Sequence alterations are detected as shifts in electrophoretic mobility of single-stranded DNA on nondenaturing acrylamide gels; the two complementary strands of a DNA segment usually resolve as two SSCP conformers of distinct mobilities. However, if the sample is from an individual heterozygous for a base-pair variant within the amplified segment, often three or more bands are seen. In some cases, even the sample from a homozygous individual will show multiple bands. Base-pair-change variants are identified by differences in pattern among the DNAs of the sample set.

Exons of the candidate genes were amplified by PCR from the DNAs of 61 unrelated FAP patients and a control set of 12 normal individuals. The five exons from DP1 revealed no unique conformers in the FAP patients, although common conformers were observed with exons 2 and 3 in some individuals of both affected and control sets, indicating the presence of DNA sequence polymorphisms. Likewise, none of the five exons of SRP19 revealed unique conformers in DNA from FAP patients in the test panel.

Testing of exons 1 through 14 and primer sets A through N of exon 15, of the DP2.5 gene, however, revealed variant conformers specific to FAP patients in exons 7, 8, 10, 11, and 15. These variants were in the unrelated patients 3746, 3460, 3827, 3712, and 3751, respectively. The PCR-SSCP procedure was repeated for each of these exons in the five affected individuals and in an expanded set of 48 normal controls. The variant bands were reproducible in the FAP patients but were not observed in any of the control DNA samples. Additional variant conformers in exons 11 and 15 of the DP2.5 gene were seen; however, each of these was found in both the affected and control DNA sets. The five sets of conformers unique to the FAP patients were sequenced to determine the nucleotide changes responsible for their altered mobilities. The normal conformers from the host individuals were sequenced also. Bands were cut from the dried acrylamide gels, and the DNA was eluted. PCR amplification of these DNAs provided template for sequencing.

The sequences of the unique conformers from exons 7, 8, 10, and 11 of DP2.5 revealed dramatic mutations in the DP2.5 gene. The sequence of the new mutation creating the exon 7 conformer in patient 3746 was shown to contain a deletion of two adjacent nucleotides, at positions 730 and 731 in the cDNA sequence (FIG. 7, SEQ ID NO:1). The normal sequence at this splice junction is CAGGGTCA

(intronic sequence underlined), with the intron-exon boundary between the two repetitions of AG. The mutant allele in this patient has the sequence CAGGTCA. Although this change is at the 5' splice site, comparison with known consensus sequences of splice junctions would suggest that a functional splice junction is maintained. If this new splice junction were functional, the mutation would introduce a frameshift that creates a stop codon 15 nucleotides downstream. If the new splice junction were not functional, messenger processing would be significantly altered.

To confirm the 2-base deletion, the PCR product from FAP patient 3746 and a control DNA were electrophoresed on an acrylamide-urea denaturing gel, along with the products of a sequencing reaction. The sample from patient 3746 showed two bands differing in size by 2 nucleotides, with the larger band identical in mobility to the control sample; this result was independent confirmation that patient, 3746 is heterozygous for a 2 bp deletion.

The unique conformer found in exon 8 of patient 3460 was found to carry a C-T transition, at position 904 in the cDNA sequence of DP2.5 (shown in FIG. 7), which replaced the normal sequence of CGA with TGA. This point mutation, when read in frame, results in a stop codon replacing the normal arginine codon. This single-base change had occurred within the context of a CG dimer, a potential hot spot for mutation (Barker et al., 1984).

The conformer unique to FAP patient 3827 in exon 10 was found to contain a deletion of one nucleotide (1367, 1368, or 1369) when compared to the normal sequence found in the other bands on the SSCP gel. This deletion, occurring within a set of three T's, changed the sequence from CTTTCA to CTTCA; this 1 base frameshift creates a downstream stop within 30 bases. The PCR product amplified from this patient's DNA also was electrophoresed on an acrylamide-urea denaturing gel, along with the PCR product from a control DNA and products from a sequencing reaction. The patient's PCR product showed two bands differing by 1 bp in length, with the larger identical in mobility to the PCR product from the normal DNA; this result confirmed the presence of a 1 bp deletion in patient 3827.

Sequence analysis of the variant conformer of exon 11 from patient 3712 revealed the substitution of a T by a G at position changing the normal tyrosine codon to a stop codon.

The pair of conformers observed in exon 15 of the DP2.5 gene for FAP patient 3751 also was sequenced. These conformers were found to carry a nucleotide substitution of C to G at position 5253, the third base of a valine codon. No amino acid change resulted from this substitution, suggesting that this conformer reflects a genetically silent polymorphism.

The observation of distinct inactivating mutations in the DP2.5 gene in four unrelated patients strongly suggested that DP2.5 is the gene involved in FAP. These mutations are summarized in Table IIA.

EXAMPLE 10

This example demonstrates that the mutations identified in the DP2.5 (APC) gene segregate with the FAP phenotype.

Patient 3746, described above as carrying an APC allele with a frameshift mutation, is an affected offspring of two normal parents. Colonoscopy revealed no polyps in either parent nor among the patient's three siblings.

DNA samples from both parents, from the patient's wife, and from their three children were examined. SSCP analysis of DNA from both of the patient's parents displayed the

normal pattern of conformers for exon 7, as did DNA from the patient's wife and one of his off-spring. The two other children, however, displayed the same new conformers as their affected father. Testing of the patient and his parents with highly polymorphic VNTR (variable number of tandem repeat) markers showed a 99.98% likelihood that they are his biological parents.

These observations confirmed that this novel conformer, known to reflect a 2 bp deletion mutation in the DP2.5 gene, appeared spontaneously with FAP in this pedigree and was transmitted to two of the children of the affected individual.

EXAMPLE 11

This example demonstrates polymorphisms in the APC gene which appear to be unrelated to disease (FAP).

Sequencing of variant conformers found among controls as well as individuals with APC has revealed the following polymorphisms in the APC gene; first, in exon 11, at position 1458, a substitution of T to C creating an RsaI restriction site but no amino acid change; and second, in exon 15, at positions 5037 and 5271, substitutions of A to G and G to T, respectively, neither resulting in amino acid substitutions. These nucleotide polymorphisms in the APC gene sequence may be useful for diagnostic purposes.

EXAMPLE 12

This example shows the structure of the APC gene.

The structure of the APC gene is schematically shown in FIG. 8, with flanking intron sequences indicated (SEQ ID NO:11-38).

The continuity of the very large (6.5 kb), most 3' exon in DP2.5 was shown in two ways. First, inverse PCR with primers spanning the entire length of this exon revealed no divergence of the cDNA sequence from the genomic sequence. Second, PCR amplification with converging primers placed at intervals along the exon generated products of the same size whether amplified from the originally isolated cDNA, cDNA from various tissues, or genomic template. Two forms of exon 9 were found in DP2.5: one is the complete exon; and the other, labeled exon 9A, is the result of a splice into the interior of the exon that deletes bases 934 to 1236 in the mRNA and removes 101 amino acids from the predicted protein (see FIG. 3, SEQ ID NO:1 and 2).

EXAMPLE 13

This example demonstrates the mapping of the FAP deletions with respect to the APC exons.

Somatic cell hybrids carrying the segregated chromosomes 5 from the 100 kb (HHW1291) and 260 kb (HHW1155) deletion patients were used to determine the distribution of the APC genes exons across the deletions. DNAs from these cell lines were used as template, along with genomic DNA from a normal control, for PCR-based amplification of the APC exons.

PCR analysis of the hybrids from the 260 kb deletion of patient 3214 showed that all but one (exon 1) of the APC exons are removed by this deletion. PCR analysis of the somatic cell hybrid HHW1291, carrying the chromosome 5 homolog with the 100 kb deletion from patient 3824, revealed that exons 1 through 9 are present but exons 10 through 15 are missing. This result placed the deletion breakpoint either between exons 9 and 10 or within exon 10.

EXAMPLE 14

This example demonstrates the expression of alternately spliced APC messenger in normal tissues and in cancer cell lines.

Tissues that express the APC gene were identified by PCR amplification of cDNA made to mRNA with primers located within adjacent APC exons. In addition, PCR primers that flank the alternatively spliced exon 9 were chosen so that the expression pattern of both splice forms could be assessed. All tissue types tested (brain, lung, aorta, spleen, heart, kidney, liver, stomach, placenta, and colonic mucosa) and cultured cell lines (lymphoblasts, HL60, and choriocarcinoma) expressed both splice forms of the APC gene. We note, however, that expression by lymphocytes normally residing in some tissues, including colon, prevents unequivocal assessment of expression. The large mRNA, containing the complete exon 9 rather than only exon 9A, appears to be the more abundant message.

Northern analysis of poly(A)-selected RNA from lymphoblasts revealed a single band of approximately 10 kb, consistent with the size of the sequenced cDNA.

EXAMPLE 15

This example discusses structural features of the APC protein predicted from the sequence.

The cDNA consensus sequence of APC predicts that the longer, more abundant form of the message codes for a 2842 or 2844 amino acid peptide with a mass of 311.8 kd. This predicted APC peptide was compared with the current data bases of protein and DNA sequences using both Intelligenetics and GCG software packages. No genes with a high degree of amino acid sequence similarity were found. Although many short (approximately 20 amino acid) regions

of sequence similarity were uncovered, none was sufficiently strong to reveal which, if any, might represent functional homology. Interestingly, multiple similarities to myosins and keratins did appear. The APC gene also was scanned for sequence motifs of known function; although multiple glycosylation, phosphorylation, and myristoylation sites were seen, their significance is uncertain.

Analysis of the APC peptide sequence did identify features important in considering potential protein structure. Hydropathy plots (Kyte and Doolittle, *J. Mol. Biol.* Vol. 157, pp. 105–132 (1982)) indicate that the APC protein is notably hydrophilic. No hydrophobic domains suggesting a signal peptide or a membrane-spanning domain were found. Analysis of the first 1000 residues indicates that α -helical rods may form (Cohen and Parry, *Trends Biochem. Sci.* Vol. 77, pp. 245–248 (1986)); there is a scarcity of proline residues and, there are a number of regions containing heptad repeats (apolar-X-X-apolar-X-X-X). Interestingly, in exon 9A, the deleted form of exon 9, two heptad repeat regions are reconnected in the proper heptad repeat frame, deleting the intervening peptide region. After the first 1000 residues, the high proline content of the remainder of the peptide suggests a compact rather than a rod-like structure.

The prominent feature of the second 1000 residues is a 20 amino acid repeat that is iterated seven times with semiregular spacing (Table 4). The intervening sequences between the seven repeat regions contained 114, 116, 151, 205, 107, and 58 amino acids, respectively. Finally, residues 2200–24000 contain a 200 amino acid basic domain.

TABLE IV

Seven Different Versions of the 20-Amino Acid Repeat																					
Consensus:	F	*	V	E	*	T	P	*	C	F	S	R	*	S	S	L	S	S	L	S	(SEQ ID NO: 147)
1262:	Y	C	V	E	D	T	P	I	C	F	S	R	C	S	S	L	S	S	L	S	(SEQ ID NO: 148)
1376:	H	T	V	Q	E	T	P	L	M	F	S	R	C	T	S	V	S	S	L	D	(SEQ ID NO: 149)
1492:	F	A	T	E	S	T	P	D	G	F	S	C	S	S	S	L	S	A	L	S	(SEQ ID NO: 150)
1643:	Y	C	V	E	G	T	P	I	N	F	S	T	A	T	S	L	S	D	L	T	(SEQ ID NO: 151)
1848:	T	P	I	E	G	T	P	Y	C	F	S	R	N	D	S	L	S	S	L	D	(SEQ ID NO: 152)
1953:	F	A	I	E	N	T	P	V	C	P	S	H	N	S	S	L	S	S	L	S	(SEQ ID NO: 153)
2013:	R	H	V	E	D	T	P	V	C	F	S	R	N	S	S	L	S	S	L	S	(SEQ ID NO: 154)

Numbers denote the first amino acid of each repeat. The consensus sequence at the top reflects a majority amino acid at a given position. In the consensus sequence, "*" indicates "Xaa."

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: [102] 154

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9606 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Homo sapiens*

(vii) IMMEDIATE SOURCE:

(B) CLONE: DP2.5 (APC)

-continued

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 34..8562

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

GGACTCGGAA ATGAGGTCCA AGGGTAGCCA AGG ATG GCT GCA GCT TCA TAT GAT	54
Met Ala Ala Ala Ser Tyr Asp	
1 5	
CAG TTG TTA AAG CAA GTT GAG GCA CTG AAG ATG GAG AAC TCA AAT CTT	102
Gln Leu Leu Lys Gln Val Glu Ala Leu Lys Met Glu Asn Ser Asn Leu	
10 15 20	
CGA CAA GAG CTA GAA GAT AAT TCC AAT CAT CTT ACA AAA CTG GAA ACT	150
Arg Gln Glu Leu Glu Asp Asn Ser Asn His Leu Thr Lys Leu Glu Thr	
25 30 35	
GAG GCA TCT AAT ATG AAG GAA GTA CTT AAA CAA CTA CAA GGA AGT ATT	198
Glu Ala Ser Asn Met Lys Glu Val Leu Lys Gln Leu Gln Gly Ser Ile	
40 45 50 55	
GAA GAT GAA GCT ATG GCT TCT TCT GGA CAG ATT GAT TTA TTA GAG CGT	246
Glu Asp Glu Ala Met Ala Ser Ser Gly Gln Ile Asp Leu Leu Glu Arg	
60 65 70	
CTT AAA GAG CTT AAC TTA GAT AGC AGT AAT TTC CCT GGA GTA AAA CTG	294
Leu Lys Glu Leu Asn Leu Asp Ser Ser Asn Phe Pro Gly Val Lys Leu	
75 80 85	
CGG TCA AAA ATG TCC CTC CGT TCT TAT GGA AGC CGG GAA GGA TCT GTA	342
Arg Ser Lys Met Ser Leu Arg Ser Tyr Gly Ser Arg Glu Gly Ser Val	
90 95 100	
TCA AGC CGT TCT GGA GAG TGC AGT CCT GTT CCT ATG GGT TCA TTT CCA	390
Ser Ser Arg Ser Gly Glu Cys Ser Pro Val Pro Met Gly Ser Phe Pro	
105 110 115	
AGA AGA GGG TTT GTA AAT GGA AGC AGA GAA AGT ACT GGA TAT TTA GAA	438
Arg Arg Gly Phe Val Asn Gly Ser Arg Glu Ser Thr Gly Tyr Leu Glu	
120 125 130 135	
GAA CTT GAG AAA GAG AGG TCA TTG CTT CTT GCT GAT CTT GAC AAA GAA	486
Glu Leu Glu Lys Glu Arg Ser Leu Leu Leu Ala Asp Leu Asp Lys Glu	
140 145 150	
GAA AAG GAA AAA GAC TGG TAT TAC GCT CAA CTT CAG AAT CTC ACT AAA	534
Glu Lys Glu Lys Asp Trp Tyr Tyr Ala Gln Leu Gln Asn Leu Thr Lys	
155 160 165	
AGA ATA GAT AGT CTT CCT TTA ACT GAA AAT TTT TCC TTA CAA ACA GAT	582
Arg Ile Asp Ser Leu Pro Leu Thr Glu Asn Phe Ser Leu Gln Thr Asp	
170 175 180	
TTG ACC AGA AGG CAA TTG GAA TAT GAA GCA AGG CAA ATC AGA GTT GCG	630
Leu Thr Arg Arg Gln Leu Glu Tyr Glu Ala Arg Gln Ile Arg Val Ala	
185 190 195	
ATG GAA GAA CAA CTA GGT ACC TGC CAG GAT ATG GAA AAA CGA GCA CAG	678
Met Glu Glu Gln Leu Gly Thr Cys Gln Asp Met Glu Lys Arg Ala Gln	
200 205 210 215	
CGA AGA ATA GCC AGA ATT CAG CAA ATC GAA AAG GAC ATA CTT CGT ATA	726
Arg Arg Ile Ala Arg Ile Gln Gln Ile Glu Lys Asp Ile Leu Arg Ile	
220 225 230	
CGA CAG CTT TTA CAG TCC CAA GCA ACA GAA GCA GAG AGG TCA TCT CAG	774
Arg Gln Leu Leu Gln Ser Gln Ala Thr Glu Ala Glu Arg Ser Ser Gln	
235 240 245	
AAC AAG CAT GAA ACC GGC TCA CAT GAT GCT GAG CGG CAG AAT GAA GGT	822
Asn Lys His Glu Thr Gly Ser His Asp Ala Glu Arg Gln Asn Glu Gly	
250 255 260	
CAA GGA GTG GGA GAA ATC AAC ATG GCA ACT TCT GGT AAT GGT CAG GGT	870
Gln Gly Val Gly Glu Ile Asn Met Ala Thr Ser Gly Asn Gly Gln Gly	
265 270 275	

-continued

ACT	GAG	AAT	AAA	GCT	GAT	ATA	TGT	GCT	GTA	GAT	GGT	GCA	CTT	GCA	TTT	1878
Thr	Glu	Asn	Lys	Ala	Asp	Ile	Cys	Ala	Val	Asp	Gly	Ala	Leu	Ala	Phe	
600					605					610					615	
TTG	GTT	GGC	ACT	CTT	ACT	TAC	CGG	AGC	CAG	ACA	AAC	ACT	TTA	GCC	ATT	1926
Leu	Val	Gly	Thr	Leu	Thr	Tyr	Arg	Ser	Gln	Thr	Asn	Thr	Leu	Ala	Ile	
				620					625						630	
ATT	GAA	AGT	GGA	GGT	GGG	ATA	TTA	CGG	AAT	GTG	TCC	AGC	TTG	ATA	GCT	1974
Ile	Glu	Ser	Gly	Gly	Gly	Ile	Leu	Arg	Asn	Val	Ser	Ser	Leu	Ile	Ala	
			635					640							645	
ACA	AAT	GAG	GAC	CAC	AGG	CAA	ATC	CTA	AGA	GAG	AAC	AAC	TGT	CTA	CAA	2022
Thr	Asn	Glu	Asp	His	Arg	Gln	Ile	Leu	Arg	Glu	Asn	Asn	Cys	Leu	Gln	
		650						655					660			
ACT	TTA	TTA	CAA	CAC	TTA	AAA	TCT	CAT	AGT	TTG	ACA	ATA	GTC	AGT	AAT	2070
Thr	Leu	Leu	Gln	His	Leu	Lys	Ser	His	Ser	Leu	Thr	Ile	Val	Ser	Asn	
	665						670					675				
GCA	TGT	GGA	ACT	TTG	TGG	AAT	CTC	TCA	GCA	AGA	AAT	CCT	AAA	GAC	CAG	2118
Ala	Cys	Gly	Thr	Leu	Trp	Asn	Leu	Ser	Ala	Arg	Asn	Pro	Lys	Asp	Gln	
680						685				690					695	
GAA	GCA	TTA	TGG	GAC	ATG	GGG	GCA	GTT	AGC	ATG	CTC	AAG	AAC	CTC	ATT	2166
Glu	Ala	Leu	Trp	Asp	Met	Gly	Ala	Val	Ser	Met	Leu	Lys	Asn	Leu	Ile	
				700					705						710	
CAT	TCA	AAG	CAC	AAA	ATG	ATT	GCT	ATG	GGA	AGT	GCT	GCA	GCT	TTA	AGG	2214
His	Ser	Lys	His	Lys	Met	Ile	Ala	Met	Gly	Ser	Ala	Ala	Ala	Leu	Arg	
			715					720							725	
AAT	CTC	ATG	GCA	AAT	AGG	CCT	GCG	AAG	TAC	AAG	GAT	GCC	AAT	ATT	ATG	2262
Asn	Leu	Met	Ala	Asn	Arg	Pro	Ala	Lys	Tyr	Lys	Asp	Ala	Asn	Ile	Met	
			730					735							740	
TCT	CCT	GGC	TCA	AGC	TTG	CCA	TCT	CTT	CAT	GTT	AGG	AAA	CAA	AAA	GCC	2310
Ser	Pro	Gly	Ser	Ser	Leu	Pro	Ser	Leu	His	Val	Arg	Lys	Gln	Lys	Ala	
		745					750				755					
CTA	GAA	GCA	GAA	TTA	GAT	GCT	CAG	CAC	TTA	TCA	GAA	ACT	TTT	GAC	AAT	2358
Leu	Glu	Ala	Glu	Leu	Asp	Ala	Gln	His	Leu	Ser	Glu	Thr	Phe	Asp	Asn	
760						765				770					775	
ATA	GAC	AAT	TTA	AGT	CCC	AAG	GCA	TCT	CAT	CGT	AGT	AAG	CAG	AGA	CAC	2406
Ile	Asp	Asn	Leu	Ser	Pro	Lys	Ala	Ser	His	Arg	Ser	Lys	Gln	Arg	His	
				780						785					790	
AAG	CAA	AGT	CTC	TAT	GGT	GAT	TAT	GTT	TTT	GAC	ACC	AAT	CGA	CAT	GAT	2454
Lys	Gln	Ser	Leu	Tyr	Gly	Asp	Tyr	Val	Phe	Asp	Thr	Asn	Arg	His	Asp	
				795				800							805	
GAT	AAT	AGG	TCA	GAC	AAT	TTT	AAT	ACT	GGC	AAC	ATG	ACT	GTC	CTT	TCA	2502
Asp	Asn	Arg	Ser	Asp	Asn	Phe	Asn	Thr	Gly	Asn	Met	Thr	Val	Leu	Ser	
		810						815							820	
CCA	TAT	TTG	AAT	ACT	ACA	GTG	TTA	CCC	AGC	TCC	TCT	TCA	TCA	AGA	GGA	2550
Pro	Tyr	Leu	Asn	Thr	Thr	Val	Leu	Pro	Ser	Ser	Ser	Ser	Ser	Arg	Gly	
		825					830								835	
AGC	TTA	GAT	AGT	TCT	CGT	TCT	GAA	AAA	GAT	AGA	AGT	TTG	GAG	AGA	GAA	2598
Ser	Leu	Asp	Ser	Ser	Arg	Ser	Glu	Lys	Asp	Arg	Ser	Leu	Glu	Arg	Glu	
840						845				850					855	
CGC	GGA	ATT	GGT	CTA	GGC	AAC	TAC	CAT	CCA	GCA	ACA	GAA	AAT	CCA	GGA	2646
Arg	Gly	Ile	Gly	Leu	Gly	Asn	Tyr	His	Pro	Ala	Thr	Glu	Asn	Pro	Gly	
				860						865					870	
ACT	TCT	TCA	AAG	CGA	GGT	TTG	CAG	ATC	TCC	ACC	ACT	GCA	GCC	CAG	ATT	2694
Thr	Ser	Ser	Lys	Arg	Gly	Leu	Gln	Ile	Ser	Thr	Thr	Ala	Ala	Gln	Ile	
				875					880						885	
GCC	AAA	GTC	ATG	GAA	GAA	GTG	TCA	GCC	ATT	CAT	ACC	TCT	CAG	GAA	GAC	2742
Ala	Lys	Val	Met	Glu	Glu	Val	Ser	Ala	Ile	His	Thr	Ser	Gln	Glu	Asp	
		890						895							900	
AGA	AGT	TCT	GGG	TCT	ACC	ACT	GAA	TTA	CAT	TGT	GTG	ACA	GAT	GAG	AGA	2790
Arg	Ser	Ser	Gly	Ser	Thr	Thr	Glu	Leu	His	Cys	Val	Thr	Asp	Glu	Arg	

-continued

905	910	915	
AAT GCA CTT AGA AGA AGC TCT GCT GCC CAT ACA CAT TCA AAC ACT TAC Asn Ala Leu Arg Arg Ser Ser Ala Ala His Thr His Ser Asn Thr Tyr 920 925 930 935			2838
AAT TTC ACT AAG TCG GAA AAT TCA AAT AGG ACA TGT TCT ATG CCT TAT Asn Phe Thr Lys Ser Glu Asn Ser Asn Arg Thr Cys Ser Met Pro Tyr 940 945 950			2886
GCC AAA TTA GAA TAC AAG AGA TCT TCA AAT GAT AGT TTA AAT AGT GTC Ala Lys Leu Glu Tyr Lys Arg Ser Ser Asn Asp Ser Leu Asn Ser Val 955 960 965			2934
AGT AGT AAT GAT GGT TAT GGT AAA AGA GGT CAA ATG AAA CCC TCG ATT Ser Ser Asn Asp Gly Tyr Gly Lys Arg Gly Gln Met Lys Pro Ser Ile 970 975 980			2982
GAA TCC TAT TCT GAA GAT GAT GAA AGT AAG TTT TGC AGT TAT GGT CAA Glu Ser Tyr Ser Glu Asp Asp Glu Ser Lys Phe Cys Ser Tyr Gly Gln 985 990 995			3030
TAC CCA GCC GAC CTA GCC CAT AAA ATA CAT AGT GCA AAT CAT ATG GAT Tyr Pro Ala Asp Leu Ala His Lys Ile His Ser Ala Asn His Met Asp 1000 1005 1010 1015			3078
GAT AAT GAT GGA GAA CTA GAT ACA CCA ATA AAT TAT AGT CTT AAA TAT Asp Asn Asp Gly Glu Leu Asp Thr Pro Ile Asn Tyr Ser Leu Lys Tyr 1020 1025 1030			3126
TCA GAT GAG CAG TTG AAC TCT GGA AGG CAA AGT CCT TCA CAG AAT GAA Ser Asp Glu Gln Leu Asn Ser Gly Arg Gln Ser Pro Ser Gln Asn Glu 1035 1040 1045			3174
AGA TGG GCA AGA CCC AAA CAC ATA ATA GAA GAT GAA ATA AAA CAA AGT Arg Trp Ala Arg Pro Lys His Ile Ile Glu Asp Glu Ile Lys Gln Ser 1050 1055 1060			3222
GAG CAA AGA CAA TCA AGG AAT CAA AGT ACA ACT TAT CCT GTT TAT ACT Glu Gln Arg Gln Ser Arg Asn Gln Ser Thr Thr Tyr Pro Val Tyr Thr 1065 1070 1075			3270
GAG AGC ACT GAT GAT AAA CAC CTC AAG TTC CAA CCA CAT TTT GGA CAG Glu Ser Thr Asp Asp Lys His Leu Lys Phe Gln Pro His Phe Gly Gln 1080 1085 1090 1095			3318
CAG GAA TGT GTT TCT CCA TAC AGG TCA CGG GGA GCC AAT GGT TCA GAA Gln Glu Cys Val Ser Pro Tyr Arg Ser Arg Gly Ala Asn Gly Ser Glu 1100 1105 1110			3366
ACA AAT CGA GTG GGT TCT AAT CAT GGA ATT AAT CAA AAT GTA AGC CAG Thr Asn Arg Val Gly Ser Asn His Gly Ile Asn Gln Asn Val Ser Gln 1115 1120 1125			3414
TCT TTG TGT CAA GAA GAT GAC TAT GAA GAT GAT AAG CCT ACC AAT TAT Ser Leu Cys Gln Glu Asp Asp Tyr Glu Asp Asp Lys Pro Thr Asn Tyr 1130 1135 1140			3462
AGT GAA CGT TAC TCT GAA GAA GAA CAG CAT GAA GAA GAA GAG AGA CCA Ser Glu Arg Tyr Ser Glu Glu Glu Gln His Glu Glu Glu Glu Arg Pro 1145 1150 1155			3510
ACA AAT TAT AGC ATA AAA TAT AAT GAA GAG AAA CGT CAT GTG GAT CAG Thr Asn Tyr Ser Ile Lys Tyr Asn Glu Glu Lys Arg His Val Asp Gln 1160 1165 1170 1175			3558
CCT ATT GAT TAT AGT TTA AAA TAT GCC ACA GAT ATT CCT TCA TCA CAG Pro Ile Asp Tyr Ser Leu Lys Tyr Ala Thr Asp Ile Pro Ser Ser Gln 1180 1185 1190			3606
AAA CAG TCA TTT TCA TTC TCA AAG AGT TCA TCT GGA CAA AGC AGT AAA Lys Gln Ser Phe Ser Phe Ser Lys Ser Ser Ser Gly Gln Ser Ser Lys 1195 1200 1205			3654
ACC GAA CAT ATG TCT TCA AGC AGT GAG AAT ACG TCC ACA CCT TCA TCT Thr Glu His Met Ser Ser Ser Ser Glu Asn Thr Ser Thr Pro Ser Ser 1210 1215 1220			3702
AAT GCC AAG AGG CAG AAT CAG CTC CAT CCA AGT TCT GCA CAG AGT AGA			3750

-continued

Asn	Ala	Lys	Arg	Gln	Asn	Gln	Leu	His	Pro	Ser	Ser	Ala	Gln	Ser	Arg		
	1225					1230						1235					
AGT	GGT	CAG	CCT	CAA	AAG	GCT	GCC	ACT	TGC	AAA	GTT	TCT	TCT	ATT	AAC		3798
Ser	Gly	Gln	Pro	Gln	Lys	Ala	Ala	Thr	Cys	Lys	Val	Ser	Ser	Ile	Asn		
	1240				1245					1250					1255		
CAA	GAA	ACA	ATA	CAG	ACT	TAT	TGT	GTA	GAA	GAT	ACT	CCA	ATA	TGT	TTT		3846
Gln	Glu	Thr	Ile	Gln	Thr	Tyr	Cys	Val	Glu	Asp	Thr	Pro	Ile	Cys	Phe		
				1260					1265					1270			
TCA	AGA	TGT	AGT	TCA	TTA	TCA	TCT	TTG	TCA	TCA	GCT	GAA	GAT	GAA	ATA		3894
Ser	Arg	Cys	Ser	Ser	Leu	Ser	Ser	Leu	Ser	Ser	Ala	Glu	Asp	Glu	Ile		
			1275				1280						1285				
GGA	TGT	AAT	CAG	ACG	ACA	CAG	GAA	GCA	GAT	TCT	GCT	AAT	ACC	CTG	CAA		3942
Gly	Cys	Asn	Gln	Thr	Thr	Gln	Glu	Ala	Asp	Ser	Ala	Asn	Thr	Leu	Gln		
	1290					1295						1300					
ATA	GCA	GAA	ATA	AAA	GGA	AAG	ATT	GGA	ACT	AGG	TCA	GCT	GAA	GAT	CCT		3990
Ile	Ala	Glu	Ile	Lys	Gly	Lys	Ile	Gly	Thr	Arg	Ser	Ala	Glu	Asp	Pro		
	1305					1310					1315						
GTG	AGC	GAA	GTT	CCA	GCA	GTG	TCA	CAG	CAC	CCT	AGA	ACC	AAA	TCC	AGC		4038
Val	Ser	Glu	Val	Pro	Ala	Val	Ser	Gln	His	Pro	Arg	Thr	Lys	Ser	Ser		
	1320				1325					1330					1335		
AGA	CTG	CAG	GGT	TCT	AGT	TTA	TCT	TCA	GAA	TCA	GCC	AGG	CAC	AAA	GCT		4086
Arg	Leu	Gln	Gly	Ser	Ser	Leu	Ser	Ser	Glu	Ser	Ala	Arg	His	Lys	Ala		
				1340					1345					1350			
GTT	GAA	TTT	CCT	TCA	GGA	GCG	AAA	TCT	CCC	TCC	AAA	AGT	GGT	GCT	CAG		4134
Val	Glu	Phe	Pro	Ser	Gly	Ala	Lys	Ser	Pro	Ser	Lys	Ser	Gly	Ala	Gln		
			1355					1360					1365				
ACA	CCC	AAA	AGT	CCA	CCT	GAA	CAC	TAT	GTT	CAG	GAG	ACC	CCA	CTC	ATG		4182
Thr	Pro	Lys	Ser	Pro	Pro	Glu	His	Tyr	Val	Gln	Glu	Thr	Pro	Leu	Met		
		1370					1375					1380					
TTT	AGC	AGA	TGT	ACT	TCT	GTC	AGT	TCA	CTT	GAT	AGT	TTT	GAG	AGT	CGT		4230
Phe	Ser	Arg	Cys	Thr	Ser	Val	Ser	Ser	Leu	Asp	Ser	Phe	Glu	Ser	Arg		
	1385					1390					1395						
TCG	ATT	GCC	AGC	TCC	GTT	CAG	AGT	GAA	CCA	TGC	AGT	GGA	ATG	GTA	AGT		4278
Ser	Ile	Ala	Ser	Ser	Val	Gln	Ser	Glu	Pro	Cys	Ser	Gly	Met	Val	Ser		
	1400				1405					1410				1415			
GGC	ATT	ATA	AGC	CCC	AGT	GAT	CTT	CCA	GAT	AGC	CCT	GGA	CAA	ACC	ATG		4326
Gly	Ile	Ile	Ser	Pro	Ser	Asp	Leu	Pro	Asp	Ser	Pro	Gly	Gln	Thr	Met		
				1420					1425					1430			
CCA	CCA	AGC	AGA	AGT	AAA	ACA	CCT	CCA	CCA	CCT	CCT	CAA	ACA	GCT	CAA		4374
Pro	Pro	Ser	Arg	Ser	Lys	Thr	Pro	Pro	Pro	Pro	Pro	Pro	Gln	Thr	Ala	Gln	
			1435					1440						1445			
ACC	AAG	CGA	GAA	GTA	CCT	AAA	AAT	AAA	GCA	CCT	ACT	GCT	GAA	AAG	AGA		4422
Thr	Lys	Arg	Glu	Val	Pro	Lys	Asn	Lys	Ala	Pro	Thr	Ala	Glu	Lys	Arg		
	1450						1455						1460				
GAG	AGT	GGA	CCT	AAG	CAA	GCT	GCA	GTA	AAT	GCT	GCA	GTT	CAG	AGG	GTC		4470
Glu	Ser	Gly	Pro	Lys	Gln	Ala	Ala	Val	Asn	Ala	Ala	Val	Gln	Arg	Val		
	1465					1470					1475						
CAG	GTT	CTT	CCA	GAT	GCT	GAT	ACT	TTA	TTA	CAT	TTT	GCC	ACA	GAA	AGT		4518
Gln	Val	Leu	Pro	Asp	Ala	Asp	Thr	Leu	Leu	His	Phe	Ala	Thr	Glu	Ser		
	1480				1485					1490					1495		
ACT	CCA	GAT	GGA	TTT	TCT	TGT	TCA	TCC	AGC	CTG	AGT	GCT	CTG	AGC	CTC		4566
Thr	Pro	Asp	Gly	Phe	Ser	Cys	Ser	Ser	Ser	Leu	Ser	Ala	Leu	Ser	Leu		
				1500					1505					1510			
GAT	GAG	CCA	TTT	ATA	CAG	AAA	GAT	GTG	GAA	TTA	AGA	ATA	ATG	CCT	CCA		4614
Asp	Glu	Pro	Phe	Ile	Gln	Lys	Asp	Val	Glu	Leu	Arg	Ile	Met	Pro	Pro		
			1515				1520						1525				
GTT	CAG	GAA	AAT	GAC	AAT	GGG	AAT	GAA	ACA	GAA	TCA	GAG	CAG	CCT	AAA		4662
Val	Gln	Glu	Asn	Asp	Asn	Gly	Asn	Glu	Thr	Glu	Ser	Glu	Gln	Pro	Lys		
	1530						1535						1540				

-continued

GAA TCA AAT GAA AAC CAA GAG AAA GAG GCA GAA AAA ACT ATT GAT TCT Glu Ser Asn Glu Asn Gln Glu Lys Glu Ala Glu Lys Thr Ile Asp Ser 1545 1550 1555	4710
GAA AAG GAC CTA TTA GAT GAT TCA GAT GAT GAT GAT ATT GAA ATA CTA Glu Lys Asp Leu Leu Asp Asp Ser Asp Asp Asp Asp Ile Glu Ile Leu 1560 1565 1570 1575	4758
GAA GAA TGT ATT ATT TCT GCC ATG CCA ACA AAG TCA TCA CGT AAA GGC Glu Glu Cys Ile Ile Ser Ala Met Pro Thr Lys Ser Ser Arg Lys Gly 1580 1585 1590	4806
AAA AAG CCA GCC CAG ACT GCT TCA AAA TTA CCT CCA CCT GTG GCA AGG Lys Lys Pro Ala Gln Thr Ala Ser Lys Leu Pro Pro Pro Val Ala Arg 1595 1600 1605	4854
AAA CCA AGT CAG CTG CCT GTG TAC AAA CTT CTA CCA TCA CAA AAC AGG Lys Pro Ser Gln Leu Pro Val Tyr Lys Leu Leu Pro Ser Gln Asn Arg 1610 1615 1620	4902
TTG CAA CCC CAA AAG CAT GTT AGT TTT ACA CCG GGG GAT GAT ATG CCA Leu Gln Pro Gln Lys His Val Ser Phe Thr Pro Gly Asp Asp Met Pro 1625 1630 1635	4950
CGG GTG TAT TGT GTT GAA GGG ACA CCT ATA AAC TTT TCC ACA GCT ACA Arg Val Tyr Cys Val Glu Gly Thr Pro Ile Asn Phe Ser Thr Ala Thr 1640 1645 1650 1655	4998
TCT CTA AGT GAT CTA ACA ATC GAA TCC CCT CCA AAT GAG TTA GCT GCT Ser Leu Ser Asp Leu Thr Ile Glu Ser Pro Pro Asn Glu Leu Ala Ala 1660 1665 1670	5046
GGA GAA GGA GTT AGA GGA GGA GCA CAG TCA GGT GAA TTT GAA AAA CGA Gly Glu Gly Val Arg Gly Gly Ala Gln Ser Gly Glu Phe Glu Lys Arg 1675 1680 1685	5094
GAT ACC ATT CCT ACA GAA GGC AGA AGT ACA GAT GAG GCT CAA GGA GGA Asp Thr Ile Pro Thr Glu Gly Arg Ser Thr Asp Glu Ala Gln Gly Gly 1690 1695 1700	5142
AAA ACC TCA TCT GTA ACC ATA CCT GAA TTG GAT GAC AAT AAA GCA GAG Lys Thr Ser Ser Val Thr Ile Pro Glu Leu Asp Asp Asn Lys Ala Glu 1705 1710 1715	5190
GAA GGT GAT ATT CTT GCA GAA TGC ATT AAT TCT GCT ATG CCC AAA GGG Glu Gly Asp Ile Leu Ala Glu Cys Ile Asn Ser Ala Met Pro Lys Gly 1720 1725 1730 1735	5238
AAA AGT CAC AAG CCT TTC CGT GTG AAA AAG ATA ATG GAC CAG GTC CAG Lys Ser His Lys Pro Phe Arg Val Lys Lys Ile Met Asp Gln Val Gln 1740 1745 1750	5286
CAA GCA TCT GCG TCG TCT TCT GCA CCC AAC AAA AAT CAG TTA GAT GGT Gln Ala Ser Ala Ser Ser Ser Ala Pro Asn Lys Asn Gln Leu Asp Gly 1755 1760 1765	5334
AAG AAA AAG AAA CCA ACT TCA CCA GTA AAA CCT ATA CCA CAA AAT ACT Lys Lys Lys Lys Pro Thr Ser Pro Val Lys Pro Ile Pro Gln Asn Thr 1770 1775 1780	5382
GAA TAT AGG ACA CGT GTA AGA AAA AAT GCA GAC TCA AAA AAT AAT TTA Glu Tyr Arg Thr Arg Val Arg Lys Asn Ala Asp Ser Lys Asn Asn Leu 1785 1790 1795	5430
AAT GCT GAG AGA GTT TTC TCA GAC AAC AAA GAT TCA AAG AAA CAG AAT Asn Ala Glu Arg Val Phe Ser Asp Asn Lys Asp Ser Lys Lys Gln Asn 1800 1805 1810 1815	5478
TTG AAA AAT AAT TCC AAG GAC TTC AAT GAT AAG CTC CCA AAT AAT GAA Leu Lys Asn Asn Ser Lys Asp Phe Asn Asp Lys Leu Pro Asn Asn Glu 1820 1825 1830	5526
GAT AGA GTC AGA GGA AGT TTT GCT TTT GAT TCA CCT CAT CAT TAC ACG Asp Arg Val Arg Gly Ser Phe Ala Phe Asp Ser Pro His His Tyr Thr 1835 1840 1845	5574
CCT ATT GAA GGA ACT CCT TAC TGT TTT TCA CGA AAT GAT TCT TTG AGT Pro Ile Glu Gly Thr Pro Tyr Cys Phe Ser Arg Asn Asp Ser Leu Ser 1850 1855 1860	5622

-continued

TCT CTA GAT TTT GAT GAT GAT GAT GTT GAC CTT TCC AGG GAA AAG GCT	5670
Ser Leu Asp Phe Asp Asp Asp Asp Val Asp Leu Ser Arg Glu Lys Ala	
1865 1870 1875	
GAA TTA AGA AAG GCA AAA GAA AAT AAG GAA TCA GAG GCT AAA GTT ACC	5718
Glu Leu Arg Lys Ala Lys Glu Asn Lys Glu Ser Glu Ala Lys Val Thr	
1880 1885 1890 1895	
AGC CAC ACA GAA CTA ACC TCC AAC CAA CAA TCA GCT AAT AAG ACA CAA	5766
Ser His Thr Glu Leu Thr Ser Asn Gln Gln Ser Ala Asn Lys Thr Gln	
1900 1905 1910	
GCT ATT GCA AAG CAG CCA ATA AAT CGA GGT CAG CCT AAA CCC ATA CTT	5814
Ala Ile Ala Lys Gln Pro Ile Asn Arg Gly Gln Pro Lys Pro Ile Leu	
1915 1920 1925	
CAG AAA CAA TCC ACT TTT CCC CAG TCA TCC AAA GAC ATA CCA GAC AGA	5862
Gln Lys Gln Ser Thr Phe Pro Gln Ser Ser Lys Asp Ile Pro Asp Arg	
1930 1935 1940	
GGG GCA GCA ACT GAT GAA AAG TTA CAG AAT TTT GCT ATT GAA AAT ACT	5910
Gly Ala Ala Thr Asp Glu Lys Leu Gln Asn Phe Ala Ile Glu Asn Thr	
1945 1950 1955	
CCA GTT TGC TTT TCT CAT AAT TCC TCT CTG AGT TCT CTC AGT GAC ATT	5958
Pro Val Cys Phe Ser His Asn Ser Ser Leu Ser Ser Leu Ser Asp Ile	
1960 1965 1970 1975	
GAC CAA GAA AAC AAC AAT AAA GAA AAT GAA CCT ATC AAA GAG ACT GAG	6006
Asp Gln Glu Asn Asn Asn Lys Glu Asn Glu Pro Ile Lys Glu Thr Glu	
1980 1985 1990	
CCC CCT GAC TCA CAG GGA GAA CCA AGT AAA CCT CAA GCA TCA GGC TAT	6054
Pro Pro Asp Ser Gln Gly Glu Pro Ser Lys Pro Gln Ala Ser Gly Tyr	
1995 2000 2005	
GCT CCT AAA TCA TTT CAT GTT GAA GAT ACC CCA GTT TGT TTC TCA AGA	6102
Ala Pro Lys Ser Phe His Val Glu Asp Thr Pro Val Cys Phe Ser Arg	
2010 2015 2020	
AAC AGT TCT CTC AGT TCT CTT AGT ATT GAC TCT GAA GAT GAC CTG TTG	6150
Asn Ser Ser Leu Ser Ser Leu Ser Ile Asp Ser Glu Asp Asp Leu Leu	
2025 2030 2035	
CAG GAA TGT ATA AGC TCC GCA ATG CCA AAA AAG AAA AAG CCT TCA AGA	6198
Gln Glu Cys Ile Ser Ser Ala Met Pro Lys Lys Lys Lys Pro Ser Arg	
2040 2045 2050 2055	
CTC AAG GGT GAT AAT GAA AAA CAT AGT CCC AGA AAT ATG GGT GGC ATA	6246
Leu Lys Gly Asp Asn Glu Lys His Ser Pro Arg Asn Met Gly Gly Ile	
2060 2065 2070	
TTA GGT GAA GAT CTG ACA CTT GAT TTG AAA GAT ATA CAG AGA CCA GAT	6294
Leu Gly Glu Asp Leu Thr Leu Asp Leu Lys Asp Ile Gln Arg Pro Asp	
2075 2080 2085	
TCA GAA CAT GGT CTA TCC CCT GAT TCA GAA AAT TTT GAT TGG AAA GCT	6342
Ser Glu His Gly Leu Ser Pro Asp Ser Glu Asn Phe Asp Trp Lys Ala	
2090 2095 2100	
ATT CAG GAA GGT GCA AAT TCC ATA GTA AGT AGT TTA CAT CAA GCT GCT	6390
Ile Gln Glu Gly Ala Asn Ser Ile Val Ser Ser Leu His Gln Ala Ala	
2105 2110 2115	
GCT GCT GCA TGT TTA TCT AGA CAA GCT TCG TCT GAT TCA GAT TCC ATC	6438
Ala Ala Ala Cys Leu Ser Arg Gln Ala Ser Ser Asp Ser Asp Ser Ile	
2120 2125 2130 2135	
CTT TCC CTG AAA TCA GGA ATC TCT CTG GGA TCA CCA TTT CAT CTT ACA	6486
Leu Ser Leu Lys Ser Gly Ile Ser Leu Gly Ser Pro Phe His Leu Thr	
2140 2145 2150	
CCT GAT CAA GAA GAA AAA CCC TTT ACA AGT AAT AAA GGC CCA CGA ATT	6534
Pro Asp Gln Glu Glu Lys Pro Phe Thr Ser Asn Lys Gly Pro Arg Ile	
2155 2160 2165	
CTA AAA CCA GGG GAG AAA AGT ACA TTG GAA ACT AAA AAG ATA GAA TCT	6582
Leu Lys Pro Gly Glu Lys Ser Thr Leu Glu Thr Lys Lys Ile Glu Ser	

-continued

2170	2175	2180	
GAA AGT AAA GGA ATC AAA GGA GGA AAA AAA GTT TAT AAA AGT TTG ATT Glu Ser Lys Gly Ile Lys Gly Gly Lys Lys Val Tyr Lys Ser Leu Ile 2185 2190 2195			6630
ACT GGA AAA GTT CGA TCT AAT TCA GAA ATT TCA GGC CAA ATG AAA CAG Thr Gly Lys Val Arg Ser Asn Ser Glu Ile Ser Gly Gln Met Lys Gln 2200 2205 2210 2215			6678
CCC CTT CAA GCA AAC ATG CCT TCA ATC TCT CGA GGC AGG ACA ATG ATT Pro Leu Gln Ala Asn Met Pro Ser Ile Ser Arg Gly Arg Thr Met Ile 2220 2225 2230			6726
CAT ATT CCA GGA GTT CGA AAT AGC TCC TCA AGT ACA AGT CCT GTT TCT His Ile Pro Gly Val Arg Asn Ser Ser Ser Ser Thr Ser Pro Val Ser 2235 2240 2245			6774
AAA AAA GGC CCA CCC CTT AAG ACT CCA GCC TCC AAA AGC CCT AGT GAA Lys Lys Gly Pro Pro Leu Lys Thr Pro Ala Ser Lys Ser Pro Ser Glu 2250 2255 2260			6822
GGT CAA ACA GCC ACC ACT TCT CCT AGA GGA GCC AAG CCA TCT GTG AAA Gly Gln Thr Ala Thr Thr Ser Pro Arg Gly Ala Lys Pro Ser Val Lys 2265 2270 2275			6870
TCA GAA TTA AGC CCT GTT GCC AGG CAG ACA TCC CAA ATA GGT GGG TCA Ser Glu Leu Ser Pro Val Ala Arg Gln Thr Ser Gln Ile Gly Gly Ser 2280 2285 2290 2295			6918
AGT AAA GCA CCT TCT AGA TCA GGA TCT AGA GAT TCG ACC CCT TCA AGA Ser Lys Ala Pro Ser Arg Ser Gly Ser Arg Asp Ser Thr Pro Ser Arg 2300 2305 2310			6966
CCT GCC CAG CAA CCA TTA AGT AGA CCT ATA CAG TCT CCT GGC CGA AAC Pro Ala Gln Gln Pro Leu Ser Arg Pro Ile Gln Ser Pro Gly Arg Asn 2315 2320 2325			7014
TCA ATT TCC CCT GGT AGA AAT GGA ATA AGT CCT CCT AAC AAA TTA TCT Ser Ile Ser Pro Gly Arg Asn Gly Ile Ser Pro Pro Asn Lys Leu Ser 2330 2335 2340			7062
CAA CTT CCA AGG ACA TCA TCC CCT AGT ACT GCT TCA ACT AAG TCC TCA Gln Leu Pro Arg Thr Ser Ser Pro Ser Thr Ala Ser Thr Lys Ser Ser 2345 2350 2355			7110
GGT TCT GGA AAA ATG TCA TAT ACA TCT CCA GGT AGA CAG ATG AGC CAA Gly Ser Gly Lys Met Ser Tyr Thr Ser Pro Gly Arg Gln Met Ser Gln 2360 2365 2370 2375			7158
CAG AAC CTT ACC AAA CAA ACA GGT TTA TCC AAG AAT GCC AGT AGT ATT Gln Asn Leu Thr Lys Gln Thr Gly Leu Ser Lys Asn Ala Ser Ser Ile 2380 2385 2390			7206
CCA AGA AGT GAG TCT GCC TCC AAA GGA CTA AAT CAG ATG AAT AAT GGT Pro Arg Ser Glu Ser Ala Ser Lys Gly Leu Asn Gln Met Asn Asn Gly 2395 2400 2405			7254
AAT GGA GCC AAT AAA AAG GTA GAA CTT TCT AGA ATG TCT TCA ACT AAA Asn Gly Ala Asn Lys Lys Val Glu Leu Ser Arg Met Ser Ser Thr Lys 2410 2415 2420			7302
TCA AGT GGA AGT GAA TCT GAT AGA TCA GAA AGA CCT GTA TTA GTA CGC Ser Ser Gly Ser Glu Ser Asp Arg Ser Glu Arg Pro Val Leu Val Arg 2425 2430 2435			7350
CAG TCA ACT TTC ATC AAA GAA GCT CCA AGC CCA ACC TTA AGA AGA AAA Gln Ser Thr Phe Ile Lys Glu Ala Pro Ser Pro Thr Leu Arg Arg Lys 2440 2445 2450 2455			7398
TTG GAG GAA TCT GCT TCA TTT GAA TCT CTT TCT CCA TCA TCT AGA CCA Leu Glu Glu Ser Ala Ser Phe Glu Ser Leu Ser Pro Ser Ser Arg Pro 2460 2465 2470			7446
GCT TCT CCC ACT AGG TCC CAG GCA CAA ACT CCA GTT TTA AGT CCT TCC Ala Ser Pro Thr Arg Ser Gln Ala Gln Thr Pro Val Leu Ser Pro Ser 2475 2480 2485			7494
CTT CCT GAT ATG TCT CTA TCC ACA CAT TCG TCT GTT CAG GCT GGT GGA			7542

-continued

Leu	Pro	Asp	Met	Ser	Leu	Ser	Thr	His	Ser	Ser	Val	Gln	Ala	Gly	Gly		
		2490					2495					2500					
TGG	CGA	AAA	CTC	CCA	CCT	AAT	CTC	AGT	CCC	ACT	ATA	GAG	TAT	AAT	GAT		7590
Trp	Arg	Lys	Leu	Pro	Pro	Asn	Leu	Ser	Pro	Thr	Ile	Glu	Tyr	Asn	Asp		
		2505				2510					2515						
GGA	AGA	CCA	GCA	AAG	CGC	CAT	GAT	ATT	GCA	CGG	TCT	CAT	TCT	GAA	AGT		7638
Gly	Arg	Pro	Ala	Lys	Arg	His	Asp	Ile	Ala	Arg	Ser	His	Ser	Glu	Ser		
		2520			2525					2530					2535		
CCT	TCT	AGA	CTT	CCA	ATC	AAT	AGG	TCA	GGA	ACC	TGG	AAA	CGT	GAG	CAC		7686
Pro	Ser	Arg	Leu	Pro	Ile	Asn	Arg	Ser	Gly	Thr	Trp	Lys	Arg	Glu	His		
				2540					2545					2550			
AGC	AAA	CAT	TCA	TCA	TCC	CTT	CCT	CGA	GTA	AGC	ACT	TGG	AGA	AGA	ACT		7734
Ser	Lys	His	Ser	Ser	Ser	Leu	Pro	Arg	Val	Ser	Thr	Trp	Arg	Arg	Thr		
			2555					2560					2565				
GGA	AGT	TCA	TCT	TCA	ATT	CTT	TCT	GCT	TCA	TCA	GAA	TCC	AGT	GAA	AAA		7782
Gly	Ser	Ser	Ser	Ser	Ile	Leu	Ser	Ala	Ser	Ser	Glu	Ser	Ser	Glu	Lys		
			2570					2575					2580				
GCA	AAA	AGT	GAG	GAT	GAA	AAA	CAT	GTG	AAC	TCT	ATT	TCA	GGA	ACC	AAA		7830
Ala	Lys	Ser	Glu	Asp	Glu	Lys	His	Val	Asn	Ser	Ile	Ser	Gly	Thr	Lys		
		2585					2590						2595				
CAA	AGT	AAA	GAA	AAC	CAA	GTA	TCC	GCA	AAA	GGA	ACA	TGG	AGA	AAA	ATA		7878
Gln	Ser	Lys	Glu	Asn	Gln	Val	Ser	Ala	Lys	Gly	Thr	Trp	Arg	Lys	Ile		
		2600				2605				2610					2615		
AAA	GAA	AAT	GAA	TTT	TCT	CCC	ACA	AAT	AGT	ACT	TCT	CAG	ACC	GTT	TCC		7926
Lys	Glu	Asn	Glu	Phe	Ser	Pro	Thr	Asn	Ser	Thr	Ser	Gln	Thr	Val	Ser		
				2620					2625					2630			
TCA	GGT	GCT	ACA	AAT	GGT	GCT	GAA	TCA	AAG	ACT	CTA	ATT	TAT	CAA	ATG		7974
Ser	Gly	Ala	Thr	Asn	Gly	Ala	Glu	Ser	Lys	Thr	Leu	Ile	Tyr	Gln	Met		
				2635					2640					2645			
GCA	CCT	GCT	GTT	TCT	AAA	ACA	GAG	GAT	GTT	TGG	GTG	AGA	ATT	GAG	GAC		8022
Ala	Pro	Ala	Val	Ser	Lys	Thr	Glu	Asp	Val	Trp	Val	Arg	Ile	Glu	Asp		
			2650					2655					2660				
TGT	CCC	ATT	AAC	AAT	CCT	AGA	TCT	GGA	AGA	TCT	CCC	ACA	GGT	AAT	ACT		8070
Cys	Pro	Ile	Asn	Asn	Pro	Arg	Ser	Gly	Arg	Ser	Pro	Thr	Gly	Asn	Thr		
						2670						2675					
CCC	CCG	GTG	ATT	GAC	AGT	GTT	TCA	GAA	AAG	GCA	AAT	CCA	AAC	ATT	AAA		8118
Pro	Pro	Val	Ile	Asp	Ser	Val	Ser	Glu	Lys	Ala	Asn	Pro	Asn	Ile	Lys		
						2685				2690					2695		
GAT	TCA	AAA	GAT	AAT	CAG	GCA	AAA	CAA	AAT	GTG	GGT	AAT	GGC	AGT	GTT		8166
Asp	Ser	Lys	Asp	Asn	Gln	Ala	Lys	Gln	Asn	Val	Gly	Asn	Gly	Ser	Val		
					2700				2705					2710			
CCC	ATG	CGT	ACC	GTG	GGT	TTG	GAA	AAT	CGC	CTG	ACC	TCC	TTT	ATT	CAG		8214
Pro	Met	Arg	Thr	Val	Gly	Leu	Glu	Asn	Arg	Leu	Thr	Ser	Phe	Ile	Gln		
				2715					2720					2725			
GTG	GAT	GCC	CCT	GAC	CAA	AAA	GGA	ACT	GAG	ATA	AAA	CCA	GGA	CAA	AAT		8262
Val	Asp	Ala	Pro	Asp	Gln	Lys	Gly	Thr	Glu	Ile	Lys	Pro	Gly	Gln	Asn		
			2730					2735					2740				
AAT	CCT	GTC	CCT	GTA	TCA	GAG	ACT	AAT	GAA	AGT	CCT	ATA	GTG	GAA	CGT		8310
Asn	Pro	Val	Pro	Val	Ser	Glu	Thr	Asn	Glu	Ser	Pro	Ile	Val	Glu	Arg		
						2745			2750				2755				
ACC	CCA	TTC	AGT	TCT	AGC	AGC	TCA	AGC	AAA	CAC	AGT	TCA	CCT	AGT	GGG		8358
Thr	Pro	Phe	Ser	Ser	Ser	Ser	Ser	Ser	Lys	His	Ser	Ser	Pro	Ser	Gly		
						2765				2770					2775		
ACT	GTT	GCT	GCC	AGA	GTG	ACT	CCT	TTT	AAT	TAC	AAC	CCA	AGC	CCT	AGG		8406
Thr	Val	Ala	Ala	Arg	Val	Thr	Pro	Phe	Asn	Tyr	Asn	Pro	Ser	Pro	Arg		
					2780					2785				2790			
AAA	AGC	AGC	GCA	GAT	AGC	ACT	TCA	GCT	CGG	CCA	TCT	CAG	ATC	CCA	ACT		8454
Lys	Ser	Ser	Ala	Asp	Ser	Thr	Ser	Ala	Arg	Pro	Ser	Gln	Ile	Pro	Thr		
				2795					2800					2805			

-continued

530			535			540									
Ala	Ser	Val	Leu	Arg	Asn	Leu	Ser	Trp	Arg	Ala	Asp	Val	Asn	Ser	Lys
545					550					555					560
Lys	Thr	Leu	Arg	Glu	Val	Gly	Ser	Val	Lys	Ala	Leu	Met	Glu	Cys	Ala
				565					570					575	
Leu	Glu	Val	Lys	Lys	Glu	Ser	Thr	Leu	Lys	Ser	Val	Leu	Ser	Ala	Leu
			580					585					590		
Trp	Asn	Leu	Ser	Ala	His	Cys	Thr	Glu	Asn	Lys	Ala	Asp	Ile	Cys	Ala
		595					600					605			
Val	Asp	Gly	Ala	Leu	Ala	Phe	Leu	Val	Gly	Thr	Leu	Thr	Tyr	Arg	Ser
	610					615					620				
Gln	Thr	Asn	Thr	Leu	Ala	Ile	Ile	Glu	Ser	Gly	Gly	Gly	Ile	Leu	Arg
625					630					635					640
Asn	Val	Ser	Ser	Leu	Ile	Ala	Thr	Asn	Glu	Asp	His	Arg	Gln	Ile	Leu
				645					650					655	
Arg	Glu	Asn	Asn	Cys	Leu	Gln	Thr	Leu	Leu	Gln	His	Leu	Lys	Ser	His
			660					665					670		
Ser	Leu	Thr	Ile	Val	Ser	Asn	Ala	Cys	Gly	Thr	Leu	Trp	Asn	Leu	Ser
		675					680					685			
Ala	Arg	Asn	Pro	Lys	Asp	Gln	Glu	Ala	Leu	Trp	Asp	Met	Gly	Ala	Val
	690					695					700				
Ser	Met	Leu	Lys	Asn	Leu	Ile	His	Ser	Lys	His	Lys	Met	Ile	Ala	Met
705					710					715					720
Gly	Ser	Ala	Ala	Ala	Leu	Arg	Asn	Leu	Met	Ala	Asn	Arg	Pro	Ala	Lys
				725					730					735	
Tyr	Lys	Asp	Ala	Asn	Ile	Met	Ser	Pro	Gly	Ser	Ser	Leu	Pro	Ser	Leu
			740					745					750		
His	Val	Arg	Lys	Gln	Lys	Ala	Leu	Glu	Ala	Glu	Leu	Asp	Ala	Gln	His
	755						760					765			
Leu	Ser	Glu	Thr	Phe	Asp	Asn	Ile	Asp	Asn	Leu	Ser	Pro	Lys	Ala	Ser
	770					775					780				
His	Arg	Ser	Lys	Gln	Arg	His	Lys	Gln	Ser	Leu	Tyr	Gly	Asp	Tyr	Val
785					790					795					800
Phe	Asp	Thr	Asn	Arg	His	Asp	Asp	Asn	Arg	Ser	Asp	Asn	Phe	Asn	Thr
			805						810					815	
Gly	Asn	Met	Thr	Val	Leu	Ser	Pro	Tyr	Leu	Asn	Thr	Thr	Val	Leu	Pro
			820					825					830		
Ser	Ser	Ser	Ser	Ser	Arg	Gly	Ser	Leu	Asp	Ser	Ser	Arg	Ser	Glu	Lys
		835					840					845			
Asp	Arg	Ser	Leu	Glu	Arg	Glu	Arg	Gly	Ile	Gly	Leu	Gly	Asn	Tyr	His
	850					855					860				
Pro	Ala	Thr	Glu	Asn	Pro	Gly	Thr	Ser	Ser	Lys	Arg	Gly	Leu	Gln	Ile
865					870					875					880
Ser	Thr	Thr	Ala	Ala	Gln	Ile	Ala	Lys	Val	Met	Glu	Glu	Val	Ser	Ala
			885						890					895	
Ile	His	Thr	Ser	Gln	Glu	Asp	Arg	Ser	Ser	Gly	Ser	Thr	Thr	Glu	Leu
		900						905					910		
His	Cys	Val	Thr	Asp	Glu	Arg	Asn	Ala	Leu	Arg	Arg	Ser	Ser	Ala	Ala
		915					920					925			
His	Thr	His	Ser	Asn	Thr	Tyr	Asn	Phe	Thr	Lys	Ser	Glu	Asn	Ser	Asn
	930					935						940			
Arg	Thr	Cys	Ser	Met	Pro	Tyr	Ala	Lys	Leu	Glu	Tyr	Lys	Arg	Ser	Ser
945					950					955					960

-continued

Asn Asp Ser Leu Asn Ser Val Ser Ser Asn Asp Gly Tyr Gly Lys Arg
 965 970 975

Gly Gln Met Lys Pro Ser Ile Glu Ser Tyr Ser Glu Asp Asp Glu Ser
 980 985 990

Lys Phe Cys Ser Tyr Gly Gln Tyr Pro Ala Asp Leu Ala His Lys Ile
 995 1000 1005

His Ser Ala Asn His Met Asp Asp Asn Asp Gly Glu Leu Asp Thr Pro
 1010 1015 1020

Ile Asn Tyr Ser Leu Lys Tyr Ser Asp Glu Gln Leu Asn Ser Gly Arg
 1025 1030 1035 1040

Gln Ser Pro Ser Gln Asn Glu Arg Trp Ala Arg Pro Lys His Ile Ile
 1045 1050 1055

Glu Asp Glu Ile Lys Gln Ser Glu Gln Arg Gln Ser Arg Asn Gln Ser
 1060 1065 1070

Thr Thr Tyr Pro Val Tyr Thr Glu Ser Thr Asp Asp Lys His Leu Lys
 1075 1080 1085

Phe Gln Pro His Phe Gly Gln Gln Glu Cys Val Ser Pro Tyr Arg Ser
 1090 1095 1100

Arg Gly Ala Asn Gly Ser Glu Thr Asn Arg Val Gly Ser Asn His Gly
 1105 1110 1115 1120

Ile Asn Gln Asn Val Ser Gln Ser Leu Cys Gln Glu Asp Asp Tyr Glu
 1125 1130 1135

Asp Asp Lys Pro Thr Asn Tyr Ser Glu Arg Tyr Ser Glu Glu Glu Gln
 1140 1145 1150

His Glu Glu Glu Glu Arg Pro Thr Asn Tyr Ser Ile Lys Tyr Asn Glu
 1155 1160 1165

Glu Lys Arg His Val Asp Gln Pro Ile Asp Tyr Ser Leu Lys Tyr Ala
 1170 1175 1180

Thr Asp Ile Pro Ser Ser Gln Lys Gln Ser Phe Ser Phe Ser Lys Ser
 1185 1190 1195 1200

Ser Ser Gly Gln Ser Ser Lys Thr Glu His Met Ser Ser Ser Ser Glu
 1205 1210 1215

Asn Thr Ser Thr Pro Ser Ser Asn Ala Lys Arg Gln Asn Gln Leu His
 1220 1225 1230

Pro Ser Ser Ala Gln Ser Arg Ser Gly Gln Pro Gln Lys Ala Ala Thr
 1235 1240 1245

Cys Lys Val Ser Ser Ile Asn Gln Glu Thr Ile Gln Thr Tyr Cys Val
 1250 1255 1260

Glu Asp Thr Pro Ile Cys Phe Ser Arg Cys Ser Ser Leu Ser Ser Leu
 1265 1270 1275 1280

Ser Ser Ala Glu Asp Glu Ile Gly Cys Asn Gln Thr Thr Gln Glu Ala
 1285 1290 1295

Asp Ser Ala Asn Thr Leu Gln Ile Ala Glu Ile Lys Gly Lys Ile Gly
 1300 1305 1310

Thr Arg Ser Ala Glu Asp Pro Val Ser Glu Val Pro Ala Val Ser Gln
 1315 1320 1325

His Pro Arg Thr Lys Ser Ser Arg Leu Gln Gly Ser Ser Leu Ser Ser
 1330 1335 1340

Glu Ser Ala Arg His Lys Ala Val Glu Phe Pro Ser Gly Ala Lys Ser
 1345 1350 1355 1360

Pro Ser Lys Ser Gly Ala Gln Thr Pro Lys Ser Pro Pro Glu His Tyr
 1365 1370 1375

-continued

Val	Gln	Glu	Thr	Pro	Leu	Met	Phe	Ser	Arg	Cys	Thr	Ser	Val	Ser	Ser	1380	1385	1390	
Leu	Asp	Ser	Phe	Glu	Ser	Arg	Ser	Ile	Ala	Ser	Ser	Val	Gln	Ser	Glu	1395	1400	1405	
Pro	Cys	Ser	Gly	Met	Val	Ser	Gly	Ile	Ile	Ser	Pro	Ser	Asp	Leu	Pro	1410	1415	1420	
Asp	Ser	Pro	Gly	Gln	Thr	Met	Pro	Pro	Ser	Arg	Ser	Lys	Thr	Pro	Pro	1425	1430	1435	1440
Pro	Pro	Pro	Gln	Thr	Ala	Gln	Thr	Lys	Arg	Glu	Val	Pro	Lys	Asn	Lys	1445	1450	1455	
Ala	Pro	Thr	Ala	Glu	Lys	Arg	Glu	Ser	Gly	Pro	Lys	Gln	Ala	Ala	Val	1460	1465	1470	
Asn	Ala	Ala	Val	Gln	Arg	Val	Gln	Val	Leu	Pro	Asp	Ala	Asp	Thr	Leu	1475	1480	1485	
Leu	His	Phe	Ala	Thr	Glu	Ser	Thr	Pro	Asp	Gly	Phe	Ser	Cys	Ser	Ser	1490	1495	1500	
Ser	Leu	Ser	Ala	Leu	Ser	Leu	Asp	Glu	Pro	Phe	Ile	Gln	Lys	Asp	Val	1505	1510	1515	1520
Glu	Leu	Arg	Ile	Met	Pro	Pro	Val	Gln	Glu	Asn	Asp	Asn	Gly	Asn	Glu	1525	1530	1535	
Thr	Glu	Ser	Glu	Gln	Pro	Lys	Glu	Ser	Asn	Glu	Asn	Gln	Glu	Lys	Glu	1540	1545	1550	
Ala	Glu	Lys	Thr	Ile	Asp	Ser	Glu	Lys	Asp	Leu	Leu	Asp	Asp	Ser	Asp	1555	1560	1565	
Asp	Asp	Asp	Ile	Glu	Ile	Leu	Glu	Glu	Cys	Ile	Ile	Ser	Ala	Met	Pro	1570	1575	1580	
Thr	Lys	Ser	Ser	Arg	Lys	Gly	Lys	Lys	Pro	Ala	Gln	Thr	Ala	Ser	Lys	1585	1590	1595	1600
Leu	Pro	Pro	Pro	Val	Ala	Arg	Lys	Pro	Ser	Gln	Leu	Pro	Val	Tyr	Lys	1605	1610	1615	
Leu	Leu	Pro	Ser	Gln	Asn	Arg	Leu	Gln	Pro	Gln	Lys	His	Val	Ser	Phe	1620	1625	1630	
Thr	Pro	Gly	Asp	Asp	Met	Pro	Arg	Val	Tyr	Cys	Val	Glu	Gly	Thr	Pro	1635	1640	1645	
Ile	Asn	Phe	Ser	Thr	Ala	Thr	Ser	Leu	Ser	Asp	Leu	Thr	Ile	Glu	Ser	1650	1655	1660	
Pro	Pro	Asn	Glu	Leu	Ala	Ala	Gly	Glu	Gly	Val	Arg	Gly	Gly	Ala	Gln	1665	1670	1675	1680
Ser	Gly	Glu	Phe	Glu	Lys	Arg	Asp	Thr	Ile	Pro	Thr	Glu	Gly	Arg	Ser	1685	1690	1695	
Thr	Asp	Glu	Ala	Gln	Gly	Gly	Lys	Thr	Ser	Ser	Val	Thr	Ile	Pro	Glu	1700	1705	1710	
Leu	Asp	Asp	Asn	Lys	Ala	Glu	Glu	Gly	Asp	Ile	Leu	Ala	Glu	Cys	Ile	1715	1720	1725	
Asn	Ser	Ala	Met	Pro	Lys	Gly	Lys	Ser	His	Lys	Pro	Phe	Arg	Val	Lys	1730	1735	1740	
Lys	Ile	Met	Asp	Gln	Val	Gln	Gln	Ala	Ser	Ala	Ser	Ser	Ser	Ala	Pro	1745	1750	1755	1760
Asn	Lys	Asn	Gln	Leu	Asp	Gly	Lys	Lys	Lys	Lys	Pro	Thr	Ser	Pro	Val	1765	1770	1775	
Lys	Pro	Ile	Pro	Gln	Asn	Thr	Glu	Tyr	Arg	Thr	Arg	Val	Arg	Lys	Asn	1780	1785	1790	
Ala	Asp	Ser	Lys	Asn	Asn	Leu	Asn	Ala	Glu	Arg	Val	Phe	Ser	Asp	Asn				

-continued

1795			1800			1805									
Lys	Asp	Ser	Lys	Lys	Gln	Asn	Leu	Lys	Asn	Asn	Ser	Lys	Asp	Phe	Asn
1810					1815				1820						
Asp	Lys	Leu	Pro	Asn	Asn	Glu	Asp	Arg	Val	Arg	Gly	Ser	Phe	Ala	Phe
1825				1830					1835						1840
Asp	Ser	Pro	His	His	Tyr	Thr	Pro	Ile	Glu	Gly	Thr	Pro	Tyr	Cys	Phe
			1845						1850					1855	
Ser	Arg	Asn	Asp	Ser	Leu	Ser	Ser	Leu	Asp	Phe	Asp	Asp	Asp	Val	
		1860						1865					1870		
Asp	Leu	Ser	Arg	Glu	Lys	Ala	Glu	Leu	Arg	Lys	Ala	Lys	Glu	Asn	Lys
	1875						1880						1885		
Glu	Ser	Glu	Ala	Lys	Val	Thr	Ser	His	Thr	Glu	Leu	Thr	Ser	Asn	Gln
	1890						1895							1900	
Gln	Ser	Ala	Asn	Lys	Thr	Gln	Ala	Ile	Ala	Lys	Gln	Pro	Ile	Asn	Arg
1905					1910					1915					1920
Gly	Gln	Pro	Lys	Pro	Ile	Leu	Gln	Lys	Gln	Ser	Thr	Phe	Pro	Gln	Ser
			1925						1930					1935	
Ser	Lys	Asp	Ile	Pro	Asp	Arg	Gly	Ala	Ala	Thr	Asp	Glu	Lys	Leu	Gln
		1940						1945						1950	
Asn	Phe	Ala	Ile	Glu	Asn	Thr	Pro	Val	Cys	Phe	Ser	His	Asn	Ser	Ser
		1955						1960						1965	
Leu	Ser	Ser	Leu	Ser	Asp	Ile	Asp	Gln	Glu	Asn	Asn	Asn	Lys	Glu	Asn
	1970						1975							1980	
Glu	Pro	Ile	Lys	Glu	Thr	Glu	Pro	Pro	Asp	Ser	Gln	Gly	Glu	Pro	Ser
1985					1990					1995					2000
Lys	Pro	Gln	Ala	Ser	Gly	Tyr	Ala	Pro	Lys	Ser	Phe	His	Val	Glu	Asp
			2005						2010					2015	
Thr	Pro	Val	Cys	Phe	Ser	Arg	Asn	Ser	Ser	Leu	Ser	Ser	Leu	Ser	Ile
			2020					2025						2030	
Asp	Ser	Glu	Asp	Asp	Leu	Leu	Gln	Glu	Cys	Ile	Ser	Ser	Ala	Met	Pro
		2035					2040							2045	
Lys	Lys	Lys	Lys	Pro	Ser	Arg	Leu	Lys	Gly	Asp	Asn	Glu	Lys	His	Ser
	2050						2055				2060				
Pro	Arg	Asn	Met	Gly	Gly	Ile	Leu	Gly	Glu	Asp	Leu	Thr	Leu	Asp	Leu
2065					2070					2075					2080
Lys	Asp	Ile	Gln	Arg	Pro	Asp	Ser	Glu	His	Gly	Leu	Ser	Pro	Asp	Ser
			2085						2090					2095	
Glu	Asn	Phe	Asp	Trp	Lys	Ala	Ile	Gln	Glu	Gly	Ala	Asn	Ser	Ile	Val
			2100					2105						2110	
Ser	Ser	Leu	His	Gln	Ala	Ala	Ala	Ala	Ala	Cys	Leu	Ser	Arg	Gln	Ala
		2115					2120						2125		
Ser	Ser	Asp	Ser	Asp	Ser	Ile	Leu	Ser	Leu	Lys	Ser	Gly	Ile	Ser	Leu
		2130					2135				2140				
Gly	Ser	Pro	Phe	His	Leu	Thr	Pro	Asp	Gln	Glu	Glu	Lys	Pro	Phe	Thr
2145					2150					2155					2160
Ser	Asn	Lys	Gly	Pro	Arg	Ile	Leu	Lys	Pro	Gly	Glu	Lys	Ser	Thr	Leu
			2165						2170					2175	
Glu	Thr	Lys	Lys	Ile	Glu	Ser	Glu	Ser	Lys	Gly	Ile	Lys	Gly	Gly	Lys
		2180						2185						2190	
Lys	Val	Tyr	Lys	Ser	Leu	Ile	Thr	Gly	Lys	Val	Arg	Ser	Asn	Ser	Glu
		2195						2200						2205	
Ile	Ser	Gly	Gln	Met	Lys	Gln	Pro	Leu	Gln	Ala	Asn	Met	Pro	Ser	Ile
		2210					2215				2220				

-continued

Ser Arg Gly Arg Thr Met Ile His Ile Pro Gly Val Arg Asn Ser Ser
 2225 2230 2235 2240
 Ser Ser Thr Ser Pro Val Ser Lys Lys Gly Pro Pro Leu Lys Thr Pro
 2245 2250 2255
 Ala Ser Lys Ser Pro Ser Glu Gly Gln Thr Ala Thr Thr Ser Pro Arg
 2260 2265 2270
 Gly Ala Lys Pro Ser Val Lys Ser Glu Leu Ser Pro Val Ala Arg Gln
 2275 2280 2285
 Thr Ser Gln Ile Gly Gly Ser Ser Lys Ala Pro Ser Arg Ser Gly Ser
 2290 2295 2300
 Arg Asp Ser Thr Pro Ser Arg Pro Ala Gln Gln Pro Leu Ser Arg Pro
 2305 2310 2315 2320
 Ile Gln Ser Pro Gly Arg Asn Ser Ile Ser Pro Gly Arg Asn Gly Ile
 2325 2330 2335
 Ser Pro Pro Asn Lys Leu Ser Gln Leu Pro Arg Thr Ser Ser Pro Ser
 2340 2345 2350
 Thr Ala Ser Thr Lys Ser Ser Gly Ser Gly Lys Met Ser Tyr Thr Ser
 2355 2360 2365
 Pro Gly Arg Gln Met Ser Gln Gln Asn Leu Thr Lys Gln Thr Gly Leu
 2370 2375 2380
 Ser Lys Asn Ala Ser Ser Ile Pro Arg Ser Glu Ser Ala Ser Lys Gly
 2385 2390 2395 2400
 Leu Asn Gln Met Asn Asn Gly Asn Gly Ala Asn Lys Lys Val Glu Leu
 2405 2410 2415
 Ser Arg Met Ser Ser Thr Lys Ser Ser Gly Ser Glu Ser Asp Arg Ser
 2420 2425 2430
 Glu Arg Pro Val Leu Val Arg Gln Ser Thr Phe Ile Lys Glu Ala Pro
 2435 2440 2445
 Ser Pro Thr Leu Arg Arg Lys Leu Glu Glu Ser Ala Ser Phe Glu Ser
 2450 2455 2460
 Leu Ser Pro Ser Ser Arg Pro Ala Ser Pro Thr Arg Ser Gln Ala Gln
 2465 2470 2475 2480
 Thr Pro Val Leu Ser Pro Ser Leu Pro Asp Met Ser Leu Ser Thr His
 2485 2490 2495
 Ser Ser Val Gln Ala Gly Gly Trp Arg Lys Leu Pro Pro Asn Leu Ser
 2500 2505 2510
 Pro Thr Ile Glu Tyr Asn Asp Gly Arg Pro Ala Lys Arg His Asp Ile
 2515 2520 2525
 Ala Arg Ser His Ser Glu Ser Pro Ser Arg Leu Pro Ile Asn Arg Ser
 2530 2535 2540
 Gly Thr Trp Lys Arg Glu His Ser Lys His Ser Ser Ser Leu Pro Arg
 2545 2550 2555 2560
 Val Ser Thr Trp Arg Arg Thr Gly Ser Ser Ser Ser Ile Leu Ser Ala
 2565 2570 2575
 Ser Ser Glu Ser Ser Glu Lys Ala Lys Ser Glu Asp Glu Lys His Val
 2580 2585 2590
 Asn Ser Ile Ser Gly Thr Lys Gln Ser Lys Glu Asn Gln Val Ser Ala
 2595 2600 2605
 Lys Gly Thr Trp Arg Lys Ile Lys Glu Asn Glu Phe Ser Pro Thr Asn
 2610 2615 2620
 Ser Thr Ser Gln Thr Val Ser Ser Gly Ala Thr Asn Gly Ala Glu Ser
 2625 2630 2635 2640

-continued

Lys Thr Leu Ile Tyr Gln Met Ala Pro Ala Val Ser Lys Thr Glu Asp
 2645 2650 2655

Val Trp Val Arg Ile Glu Asp Cys Pro Ile Asn Asn Pro Arg Ser Gly
 2660 2665 2670

Arg Ser Pro Thr Gly Asn Thr Pro Pro Val Ile Asp Ser Val Ser Glu
 2675 2680 2685

Lys Ala Asn Pro Asn Ile Lys Asp Ser Lys Asp Asn Gln Ala Lys Gln
 2690 2695 2700

Asn Val Gly Asn Gly Ser Val Pro Met Arg Thr Val Gly Leu Glu Asn
 2705 2710 2715 2720

Arg Leu Thr Ser Phe Ile Gln Val Asp Ala Pro Asp Gln Lys Gly Thr
 2725 2730 2735

Glu Ile Lys Pro Gly Gln Asn Asn Pro Val Pro Val Ser Glu Thr Asn
 2740 2745 2750

Glu Ser Pro Ile Val Glu Arg Thr Pro Phe Ser Ser Ser Ser Ser Ser
 2755 2760 2765

Lys His Ser Ser Pro Ser Gly Thr Val Ala Ala Arg Val Thr Pro Phe
 2770 2775 2780

Asn Tyr Asn Pro Ser Pro Arg Lys Ser Ser Ala Asp Ser Thr Ser Ala
 2785 2790 2795 2800

Arg Pro Ser Gln Ile Pro Thr Pro Val Asn Asn Asn Thr Lys Lys Arg
 2805 2810 2815

Asp Ser Lys Thr Asp Ser Thr Glu Ser Ser Gly Thr Gln Ser Pro Lys
 2820 2825 2830

Arg His Ser Gly Ser Tyr Leu Val Thr Ser Val
 2835 2840

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3172 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:

- (B) CLONE: DP1(TB2)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..630

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

GCA GTC GCC GCT CCA GTC TAT CCG GCA CTA GGA ACA GCC CCG GGN GGC 48
 Ala Val Ala Ala Pro Val Tyr Pro Ala Leu Gly Thr Ala Pro Gly Gly
 1 5 10 15

GAG ACG GTC CCC GCC ATG TCT GCG GCC ATG AGG GAG AGG TTC GAC CGG 96
 Glu Thr Val Pro Ala Met Ser Ala Ala Met Arg Glu Arg Phe Asp Arg
 20 25 30

TTC CTG CAC GAG AAG AAC TGC ATG ACT GAC CTT CTG GCC AAG CTC GAG 144
 Phe Leu His Glu Lys Asn Cys Met Thr Asp Leu Leu Ala Lys Leu Glu
 35 40 45

GCC AAA ACC GGC GTG AAC AGG AGC TTC ATC GCT CTT GGT GTC ATC GGA 192
 Ala Lys Thr Gly Val Asn Arg Ser Phe Ile Ala Leu Gly Val Ile Gly
 50 55 60

CTG GTG GCC TTG TAC CTG GTG TTC GGT TAT GGA GCC TCT CTC CTC TGC 240

-continued

CATTTAAATG CCCTCATCCG TATTCTTTGT GTTGATAAGC TGCACAKGAC TACATAGTAA 1940
 GTACAGANCA GTAAAGTTAA NNCGGATGTC TCCATTGATC TGCCAANTCG NTATAGAGAG 2000
 CAATTTGTCT GGACTAGAAA ATCTGAGTTT TACACCATAC TGTTAAGAGT CCTTTTGAAT 2060
 TAAACTAGAC TAAAACAAGT GTATAACTAA ACTAACAAGA TTAAATATCC AGCCAGTACA 2120
 GTATTTTTTA AGGCAAATAA AGATGATTAG CTCACCTTGA GNTAACAATC AGGTAAGATC 2180
 ATNACAATGT CTCATGATGT NAANAATATT AAAGATATCA ATACTAAGTG ACAGTATCAC 2240
 NNCTAATATA ATATGGATCA GAGCATTAT TTTGGGGAGG AAAACAGTGG TGATTACCGG 2300
 CATTTTATTA AACTTAAAAC TTTGTAGAAA GCAAACAAAA TTGTTCTTGG GAGAAAATCA 2360
 ACTTTTAGAT TAAAAAATT TTAAGTAWCT AGGAGTATTT AAATCCTTTT CCCATAAATA 2420
 AAAGTACAGT TTTCTTGGTG GCAGAATGAA AATCAGCAAC NTCTAGCATA TAGACTATAT 2480
 AATCAGATTG ACAGCATATA GAATATATTA TCAGACAAGA TGAGGAGGTA CAAAAGTTAC 2540
 TATTGCTCAT AATGACTTAC AGGCTAAAAN TAGNTNTAAA ATACTATATT AAATTCTGAA 2600
 TGCAATTTTT TTTTGTCCC TTGAGACCAA AATTTAAGTT AACTGTTGCT GGCAGTCTAA 2660
 GTGTAAATGT TAACAGCAGG AGAAGTTAAG AATTGAGCAG TTCTGTTGCA TGATTTCCCA 2720
 AATGAAATAC TGCCTTGGCT AGAGTTTGAA AACTAATTG AGCCTGTGCC TGGCTAGAAA 2780
 ACAAGCGTTT ATTTGAATGT GAATAGTGTT TCAAAGGTAT GTAGTTACAG AATTCCTACC 2840
 AACAGCTTA AATCCTCAA GAAAGAATTC CTGCAGCAGT TATTCCTTA CCTGAAGGCT 2900
 TCAATCATTT GGATCAACAA CTGCTACTCT CGGGAAGACT CCTCTACTCA CAGCTGAAGA 2960
 AAATGAGCAC ACCCTTCACA CTGTTATCAC CTATCCTGAA GATGTGATAC ACTGAATGGA 3020
 AATAAATAGA TGTAATAAA ATTGAGWTCT CATTAAAAA AAACCATGTG CCCAATGGGA 3080
 AAATGACCTC ATGTTGTGGT TTAACAGCA ACTGCACCCA CTAGCACAGC CCATTGAGCT 3140
 ANCCTATATA TACATCTCTG TCAGTGCCCC TC 3172

(2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 210 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Ala Val Ala Ala Pro Val Tyr Pro Ala Leu Gly Thr Ala Pro Gly Gly
 1 5 10 15
 Glu Thr Val Pro Ala Met Ser Ala Ala Met Arg Glu Arg Phe Asp Arg
 20 25 30
 Phe Leu His Glu Lys Asn Cys Met Thr Asp Leu Leu Ala Lys Leu Glu
 35 40 45
 Ala Lys Thr Gly Val Asn Arg Ser Phe Ile Ala Leu Gly Val Ile Gly
 50 55 60
 Leu Val Ala Leu Tyr Leu Val Phe Gly Tyr Gly Ala Ser Leu Leu Cys
 65 70 75 80
 Asn Leu Ile Gly Phe Gly Tyr Pro Ala Tyr Ile Ser Ile Lys Ala Ile
 85 90 95
 Glu Ser Pro Asn Lys Glu Asp Asp Thr Gln Trp Leu Thr Tyr Trp Val
 100 105 110
 Val Tyr Gly Val Phe Ser Ile Ala Glu Phe Phe Ser Asp Ile Phe Leu
 115 120 125

-continued

Ser Trp Phe Pro Phe Tyr Tyr Met Leu Lys Cys Gly Phe Leu Leu Trp
 130 135 140

Cys Met Ala Pro Ser Pro Ser Asn Gly Ala Glu Leu Leu Tyr Lys Arg
 145 150 155 160

Ile Ile Arg Pro Phe Phe Leu Lys His Glu Ser Gln Met Asp Ser Val
 165 170 175

Val Lys Asp Leu Lys Asp Lys Ser Lys Glu Thr Ala Asp Ala Ile Thr
 180 185 190

Lys Glu Ala Lys Lys Ala Thr Val Asn Leu Leu Gly Glu Glu Lys Lys
 195 200 205

Ser Thr
 210

(2) INFORMATION FOR SEQ ID NO: 5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 434 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
 (B) CLONE: TB1

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

Val Ala Pro Val Val Val Gly Ser Gly Arg Ala Pro Arg His Pro Ala
 1 5 10 15

Pro Ala Ala Met His Pro Arg Arg Pro Asp Gly Phe Asp Gly Leu Gly
 20 25 30

Tyr Arg Gly Gly Ala Arg Asp Glu Gln Gly Phe Gly Gly Ala Phe Pro
 35 40 45

Ala Arg Ser Phe Ser Thr Gly Ser Asp Leu Gly His Trp Val Thr Thr
 50 55 60

Pro Pro Asp Ile Pro Gly Ser Arg Asn Leu His Trp Gly Glu Lys Ser
 65 70 75 80

Pro Pro Tyr Gly Val Pro Thr Thr Ser Thr Pro Tyr Glu Gly Pro Thr
 85 90 95

Glu Glu Pro Phe Ser Ser Gly Gly Gly Gly Ser Val Gln Gly Gln Ser
 100 105 110

Ser Glu Gln Leu Asn Arg Phe Ala Gly Phe Gly Ile Gly Leu Ala Ser
 115 120 125

Leu Phe Thr Glu Asn Val Leu Ala His Pro Cys Ile Val Leu Arg Arg
 130 135 140

Gln Cys Gln Val Asn Tyr His Ala Gln His Tyr His Leu Thr Pro Phe
 145 150 155 160

Thr Val Ile Asn Ile Met Tyr Ser Phe Asn Lys Thr Gln Gly Pro Arg
 165 170 175

Ala Leu Trp Lys Gly Met Gly Ser Thr Phe Ile Val Gln Gly Val Thr
 180 185 190

Leu Gly Ala Glu Gly Ile Ile Ser Glu Phe Thr Pro Leu Pro Arg Glu
 195 200 205

Val Leu His Lys Trp Ser Pro Lys Gln Ile Gly Glu His Leu Leu Leu
 210 215 220

-continued

Lys Ser Leu Thr Tyr Val Val Ala Met Pro Phe Tyr Ser Ala Ser Leu
 225 230 235 240
 Ile Glu Thr Val Gln Ser Glu Ile Ile Arg Asp Asn Thr Gly Ile Leu
 245 250 255
 Glu Cys Val Lys Glu Gly Ile Gly Arg Val Ile Gly Met Gly Val Pro
 260 265 270
 His Ser Lys Arg Leu Leu Pro Leu Leu Ser Leu Ile Phe Pro Thr Val
 275 280 285
 Leu His Gly Val Leu His Tyr Ile Ile Ser Ser Val Ile Gln Lys Phe
 290 295 300
 Val Leu Leu Ile Leu Lys Arg Lys Thr Tyr Asn Ser His Leu Ala Glu
 305 310 315 320
 Ser Thr Ser Pro Val Gln Ser Met Leu Asp Ala Tyr Phe Pro Glu Leu
 325 330 335
 Ile Ala Asn Phe Ala Ala Ser Leu Cys Ser Asp Val Ile Leu Tyr Pro
 340 345 350
 Leu Glu Thr Val Leu His Arg Leu His Ile Gln Gly Thr Arg Thr Ile
 355 360 365
 Ile Asp Asn Thr Asp Leu Gly Tyr Glu Val Leu Pro Ile Asn Thr Gln
 370 375 380
 Tyr Glu Gly Met Arg Asp Cys Ile Asn Thr Ile Arg Gln Glu Glu Gly
 385 390 395 400
 Val Phe Gly Phe Tyr Lys Gly Phe Gly Ala Val Ile Ile Gln Tyr Thr
 405 410 415
 Leu His Ala Ala Val Leu Gln Ile Thr Lys Ile Ile Tyr Ser Thr Leu
 420 425 430
 Leu Gln

(2) INFORMATION FOR SEQ ID NO: 6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 185 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:

- (B) CLONE: YS-39(TB2)

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

Glu Leu Arg Arg Phe Asp Arg Phe Leu His Glu Lys Asn Cys Met Thr
 1 5 10 15
 Asp Leu Leu Ala Lys Leu Glu Ala Lys Thr Gly Val Asn Arg Ser Phe
 20 25 30
 Ile Ala Leu Gly Val Ile Gly Leu Val Ala Leu Tyr Leu Val Phe Gly
 35 40 45
 Tyr Gly Ala Ser Leu Leu Cys Asn Leu Ile Gly Phe Gly Tyr Pro Ala
 50 55 60
 Tyr Ile Ser Ile Lys Ala Ile Glu Ser Pro Asn Lys Glu Asp Asp Thr
 65 70 75 80
 Gln Trp Leu Thr Tyr Trp Val Val Tyr Gly Val Phe Ser Ile Ala Glu
 85 90 95

-continued

Phe Phe Ser Asp Ile Phe Leu Ser Trp Phe Pro Phe Tyr Tyr Ile Leu
 100 105 110

Lys Cys Gly Phe Leu Leu Trp Cys Met Ala Pro Ser Pro Ser Asn Gly
 115 120 125

Ala Glu Leu Leu Tyr Lys Arg Ile Ile Arg Pro Phe Phe Leu Lys His
 130 135 140

Glu Ser Gln Met Asp Ser Val Val Lys Asp Leu Lys Asp Lys Ala Lys
 145 150 155 160

Glu Thr Ala Asp Ala Ile Thr Lys Glu Ala Lys Lys Ala Thr Val Asn
 165 170 175

Leu Leu Gly Glu Glu Lys Lys Ser Thr
 180 185

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: [2842] 2843 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:

(B) CLONE: APC

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

Met Ala Ala Ala Ser Tyr Asp Gln Leu Leu Lys Gln Val Glu Ala Leu
 1 5 10 15

Lys Met Glu Asn Ser Asn Leu Arg Gln Glu Leu Glu Asp Asn Ser Asn
 20 25 30

His Leu Thr Lys Leu Glu Thr Glu Ala Ser Asn Met Lys Glu Val Leu
 35 40 45

Lys Gln Leu Gln Gly Ser Ile Glu Asp Glu Ala Met Ala Ser Ser Gly
 50 55 60

Gln Ile Asp Leu Leu Glu Arg Leu Lys Glu Leu Asn Leu Asp Ser Ser
 65 70 75 80

Asn Phe Pro Gly Val Lys Leu Arg Ser Lys Met Ser Leu Arg Ser Tyr
 85 90 95

Gly Ser Arg Glu Gly Ser Val Ser Ser Arg Ser Gly Glu Cys Ser Pro
 100 105 110

Val Pro Met Gly Ser Phe Pro Arg Arg Gly Phe Val Asn Gly Ser Arg
 115 120 125

Glu Ser Thr Gly Tyr Leu Glu Glu Leu Glu Lys Glu Arg Ser Leu Leu
 130 135 140

Leu Ala Asp Leu Asp Lys Glu Glu Lys Glu Lys Asp Trp Tyr Tyr Ala
 145 150 155 160

Gln Leu Gln Asn Leu Thr Lys Arg Ile Asp Ser Leu Pro Leu Thr Glu
 165 170 175

Asn Phe Ser Leu Gln Thr Asp Met Thr Arg Arg Gln Leu Glu Tyr Glu
 180 185 190

Ala Arg Gln Ile Arg Val Ala Met Glu Glu Gln Leu Gly Thr Cys Gln
 195 200 205

Asp Met Glu Lys Arg Ala Gln Arg Arg Ile Ala Arg Ile Gln Gln Ile
 210 215 220

-continued

Glu	Lys	Asp	Ile	Leu	Arg	Ile	Arg	Gln	Leu	Leu	Gln	Ser	Gln	Ala	Thr	225	230	235	240
Glu	Ala	Glu	Arg	Ser	Ser	Gln	Asn	Lys	His	Glu	Thr	Gly	Ser	His	Asp	245	250	255	
Ala	Glu	Arg	Gln	Asn	Glu	Gly	Gln	Gly	Val	Gly	Glu	Ile	Asn	Met	Ala	260	265	270	
Thr	Ser	Gly	Asn	Gly	Gln	Gly	Ser	Thr	Thr	Arg	Met	Asp	His	Glu	Thr	275	280	285	
Ala	Ser	Val	Leu	Ser	Ser	Ser	Ser	Thr	His	Ser	Ala	Pro	Arg	Arg	Leu	290	295	300	
Thr	Ser	His	Leu	Gly	Thr	Lys	Val	Glu	Met	Val	Tyr	Ser	Leu	Leu	Ser	305	310	315	320
Met	Leu	Gly	Thr	His	Asp	Lys	Asp	Asp	Met	Ser	Arg	Thr	Leu	Leu	Ala	325	330	335	
Met	Ser	Ser	Ser	Gln	Asp	Ser	Cys	Ile	Ser	Met	Arg	Gln	Ser	Gly	Cys	340	345	350	
Leu	Pro	Leu	Leu	Ile	Gln	Leu	Leu	His	Gly	Asn	Asp	Lys	Asp	Ser	Val	355	360	365	
Leu	Leu	Gly	Asn	Ser	Arg	Gly	Ser	Lys	Glu	Ala	Arg	Ala	Arg	Ala	Ser	370	375	380	
Ala	Ala	Leu	His	Asn	Ile	Ile	His	Ser	Gln	Pro	Asp	Asp	Lys	Arg	Gly	385	390	395	400
Arg	Arg	Glu	Ile	Arg	Val	Leu	His	Leu	Leu	Glu	Gln	Ile	Arg	Ala	Tyr	405	410	415	
Cys	Glu	Thr	Cys	Trp	Glu	Trp	Gln	Glu	Ala	His	Glu	Pro	Gly	Met	Asp	420	425	430	
Gln	Asp	Lys	Asn	Pro	Met	Pro	Ala	Pro	Val	Glu	His	Gln	Ile	Cys	Pro	435	440	445	
Ala	Val	Cys	Val	Leu	Met	Lys	Leu	Ser	Phe	Asp	Glu	Glu	His	Arg	His	450	455	460	
Ala	Met	Asn	Glu	Leu	Gly	Gly	Leu	Gln	Ala	Ile	Ala	Glu	Leu	Leu	Gln	465	470	475	480
Val	Asp	Cys	Glu	Met	Tyr	Gly	Leu	Thr	Asn	Asp	His	Tyr	Ser	Ile	Thr	485	490	495	
Leu	Arg	Arg	Tyr	Ala	Gly	Met	Ala	Leu	Thr	Asn	Leu	Thr	Phe	Gly	Asp	500	505	510	
Val	Ala	Asn	Lys	Ala	Thr	Leu	Cys	Ser	Met	Lys	Gly	Cys	Met	Arg	Ala	515	520	525	
Leu	Val	Ala	Gln	Leu	Lys	Ser	Glu	Ser	Glu	Asp	Leu	Gln	Gln	Val	Ile	530	535	540	
Ala	Ser	Val	Leu	Arg	Asn	Leu	Ser	Trp	Arg	Ala	Asp	Val	Asn	Ser	Lys	545	550	555	560
Lys	Thr	Leu	Arg	Glu	Val	Gly	Ser	Val	Lys	Ala	Leu	Met	Glu	Cys	Ala	565	570	575	
Leu	Glu	Val	Lys	Lys	Glu	Ser	Thr	Leu	Lys	Ser	Val	Leu	Ser	Ala	Leu	580	585	590	
Trp	Asn	Leu	Ser	Ala	His	Cys	Thr	Glu	Asn	Lys	Ala	Asp	Ile	Cys	Ala	595	600	605	
Val	Asp	Gly	Ala	Leu	Ala	Phe	Leu	Val	Gly	Thr	Leu	Thr	Tyr	Arg	Ser	610	615	620	
Gln	Thr	Asn	Thr	Leu	Ala	Ile	Ile	Glu	Ser	Gly	Gly	Gly	Ile	Leu	Arg	625	630	635	640
Asn	Val	Ser	Ser	Leu	Ile	Ala	Thr	Asn	Glu	Asp	His	Arg	Gln	Ile	Leu				

-continued

645					650					655					
Arg	Glu	Asn	Asn	Cys	Leu	Gln	Thr	Leu	Leu	Gln	His	Leu	Lys	Ser	His
			660					665					670		
Ser	Leu	Thr	Ile	Val	Ser	Asn	Ala	Cys	Gly	Thr	Leu	Trp	Asn	Leu	Ser
		675					680					685			
Ala	Arg	Asn	Pro	Lys	Asp	Gln	Glu	Ala	Leu	Trp	Asp	Met	Gly	Ala	Val
	690					695					700				
Ser	Met	Leu	Lys	Asn	Leu	Ile	His	Ser	Lys	His	Lys	Met	Ile	Ala	Met
705					710					715					720
Gly	Ser	Ala	Ala	Ala	Leu	Arg	Asn	Leu	Met	Ala	Asn	Arg	Pro	Ala	Lys
				725					730					735	
Tyr	Lys	Asp	Ala	Asn	Ile	Met	Ser	Pro	Gly	Ser	Ser	Leu	Pro	Ser	Leu
			740					745					750		
His	Val	Arg	Lys	Gln	Lys	Ala	Leu	Glu	Ala	Glu	Leu	Asp	Ala	Gln	His
		755					760					765			
Leu	Ser	Glu	Thr	Phe	Asp	Asn	Ile	Asp	Asn	Leu	Ser	Pro	Lys	Ala	Ser
	770					775					780				
His	Arg	Ser	Lys	Gln	Arg	His	Lys	Gln	Ser	Leu	Tyr	Gly	Asp	Tyr	Val
785					790					795					800
Phe	Asp	Thr	Asn	Arg	His	Asp	Asp	Asn	Arg	Ser	Asp	Asn	Phe	Asn	Thr
				805					810					815	
Gly	Asn	Met	Thr	Val	Leu	Ser	Pro	Tyr	Leu	Asn	Thr	Thr	Val	Leu	Pro
			820					825					830		
Ser	Ser	Ser	Ser	Ser	Arg	Gly	Ser	Leu	Asp	Ser	Ser	Arg	Ser	Glu	Lys
			835				840					845			
Asp	Arg	Ser	Leu	Glu	Arg	Glu	Arg	Gly	Ile	Gly	Leu	Gly	Asn	Tyr	His
	850					855					860				
Pro	Ala	Thr	Glu	Asn	Pro	Gly	Thr	Ser	Ser	Lys	Arg	Gly	Leu	Gln	Ile
865					870					875					880
Ser	Thr	Thr	Ala	Ala	Gln	Ile	Ala	Lys	Val	Met	Glu	Glu	Val	Ser	Ala
				885					890					895	
Ile	His	Thr	Ser	Gln	Glu	Asp	Arg	Ser	Ser	Gly	Ser	Thr	Thr	Glu	Leu
			900					905						910	
His	Cys	Val	Thr	Asp	Glu	Arg	Asn	Ala	Leu	Arg	Arg	Ser	Ser	Ala	Ala
		915					920					925			
His	Thr	His	Ser	Asn	Thr	Tyr	Asn	Phe	Thr	Lys	Ser	Glu	Asn	Ser	Asn
		930				935					940				
Arg	Thr	Cys	Ser	Met	Pro	Tyr	Ala	Lys	Leu	Glu	Tyr	Lys	Arg	Ser	Ser
945					950					955					960
Asn	Asp	Ser	Leu	Asn	Ser	Val	Ser	Ser	Ser	Asp	Gly	Tyr	Gly	Lys	Arg
				965					970					975	
Gly	Gln	Met	Lys	Pro	Ser	Ile	Glu	Ser	Tyr	Ser	Glu	Asp	Asp	Glu	Ser
			980					985					990		
Lys	Phe	Cys	Ser	Tyr	Gly	Gln	Tyr	Pro	Ala	Asp	Leu	Ala	His	Lys	Ile
		995					1000					1005			
His	Ser	Ala	Asn	His	Met	Asp	Asp	Asn	Asp	Gly	Glu	Leu	Asp	Thr	Pro
						1015					1020				
Ile	Asn	Tyr	Ser	Leu	Lys	Tyr	Ser	Asp	Glu	Gln	Leu	Asn	Ser	Gly	Arg
1025					1030					1035					1040
Gln	Ser	Pro	Ser	Gln	Asn	Glu	Arg	Trp	Ala	Arg	Pro	Lys	His	Ile	Ile
				1045					1050					1055	
Glu	Asp	Glu	Ile	Lys	Gln	Ser	Glu	Gln	Arg	Gln	Ser	Arg	Asn	Gln	Ser
			1060					1065					1070		

-continued

Thr Thr Tyr Pro Val Tyr Thr Glu Ser Thr Asp Asp Lys His Leu Lys
 1075 1080 1085

Phe Gln Pro His Phe Gly Gln Gln Glu Cys Val Ser Pro Tyr Arg Ser
 1090 1095 1100

Arg Gly Ala Asn Gly Ser Glu Thr Asn Arg Val Gly Ser Asn His Gly
 1105 1110 1115 1120

Ile Asn Gln Asn Val Ser Gln Ser Leu Cys Gln Glu Asp Asp Tyr Glu
 1125 1130 1135

Asp Asp Lys Pro Thr Asn Tyr Ser Glu Arg Tyr Ser Glu Glu Glu Gln
 1140 1145 1150

His Glu Glu Glu Glu Arg Pro Thr Asn Tyr Ser Ile Lys Tyr Asn Glu
 1155 1160 1165

Glu Lys Arg His Val Asp Gln Pro Ile Asp Tyr Ser Leu Lys Tyr Ala
 1170 1175 1180

Thr Asp Ile Pro Ser Ser Gln Lys Gln Ser Phe Ser Phe Ser Lys Ser
 1185 1190 1195 1200

Ser Ser Gly Gln Ser Ser Lys Thr Glu His Met Ser Ser Ser Ser Glu
 1205 1210 1215

Asn Thr Ser Thr Pro Ser Ser Asn Ala Lys Arg Gln Asn Gln Leu His
 1220 1225 1230

Pro Ser Ser Ala Gln Ser Arg Ser Gly Gln Pro Gln Lys Ala Ala Thr
 1235 1240 1245

Cys Lys Val Ser Ser Ile Asn Gln Glu Thr Ile Gln Thr Tyr Cys Val
 1250 1255 1260

Glu Asp Thr Pro Ile Cys Phe Ser Arg Cys Ser Ser Leu Ser Ser Leu
 1265 1270 1275 1280

Ser Ser Ala Glu Asp Glu Ile Gly Cys Asn Gln Thr Thr Gln Glu Ala
 1285 1290 1295

Asp Ser Ala Asn Thr Leu Gln Ile Ala Glu Ile Lys Glu Lys Ile Gly
 1300 1305 1310

Thr Arg Ser Ala Glu Asp Pro Val Ser Glu Val Pro Ala Val Ser Gln
 1315 1320 1325

His Pro Arg Thr Lys Ser Ser Arg Leu Gln Gly Ser Ser Leu Ser Ser
 1330 1335 1340

Glu Ser Ala Arg His Lys Ala Val Glu Phe Ser Ser Gly Ala Lys Ser
 1345 1350 1355 1360

Pro Ser Lys Ser Gly Ala Gln Thr Pro Lys Ser Pro Pro Glu His Tyr
 1365 1370 1375

Val Gln Glu Thr Pro Leu Met Phe Ser Arg Cys Thr Ser Val Ser Ser
 1380 1385 1390

Leu Asp Ser Phe Glu Ser Arg Ser Ile Ala Ser Ser Val Gln Ser Glu
 1395 1400 1405

Pro Cys Ser Gly Met Val Ser Gly Ile Ile Ser Pro Ser Asp Leu Pro
 1410 1415 1420

Asp Ser Pro Gly Gln Thr Met Pro Pro Ser Arg Ser Lys Thr Pro Pro
 1425 1430 1435 1440

Pro Pro Pro Gln Thr Ala Gln Thr Lys Arg Glu Val Pro Lys Asn Lys
 1445 1450 1455

Ala Pro Thr Ala Glu Lys Arg Glu Ser Gly Pro Lys Gln Ala Ala Val
 1460 1465 1470

Asn Ala Ala Val Gln Arg Val Gln Val Leu Pro Asp Ala Asp Thr Leu
 1475 1480 1485

-continued

Leu His Phe Ala Thr Glu Ser Thr Pro Asp Gly Phe Ser Cys Ser Ser
 1490 1495 1500
 Ser Leu Ser Ala Leu Ser Leu Asp Glu Pro Phe Ile Gln Lys Asp Val
 1505 1510 1515 1520
 Glu Leu Arg Ile Met Pro Pro Val Gln Glu Asn Asp Asn Gly Asn Glu
 1525 1530 1535
 Thr Glu Ser Glu Gln Pro Lys Glu Ser Asn Glu Asn Gln Glu Lys Glu
 1540 1545 1550
 Ala Glu Lys Thr Ile Asp Ser Glu Lys Asp Leu Leu Asp Asp Ser Asp
 1555 1560 1565
 Asp Asp Asp Ile Glu Ile Leu Glu Glu Cys Ile Ile Ser Ala Met Pro
 1570 1575 1580
 Thr Lys Ser Ser Arg Lys Ala Lys Lys Pro Ala Gln Thr Ala Ser Lys
 1585 1590 1595 1600
 Leu Pro Pro Pro Val Ala Arg Lys Pro Ser Gln Leu Pro Val Tyr Lys
 1605 1610 1615
 Leu Leu Pro Ser Gln Asn Arg Leu Gln Pro Gln Lys His Val Ser Phe
 1620 1625 1630
 Thr Pro Gly Asp Asp Met Pro Arg Val Tyr Cys Val Glu Gly Thr Pro
 1635 1640 1645
 Ile Asn Phe Ser Thr Ala Thr Ser Leu Ser Asp Leu Thr Ile Glu Ser
 1650 1655 1660
 Pro Pro Asn Glu Leu Ala Ala Gly Glu Gly Val Arg Gly Gly Ala Gln
 1665 1670 1675 1680
 Ser Gly Glu Phe Glu Lys Arg Asp Thr Ile Pro Thr Glu Gly Arg Ser
 1685 1690 1695
 Thr Asp Glu Ala Gln Gly Gly Lys Thr Ser Ser Val Thr Ile Pro Glu
 1700 1705 1710
 Leu Asp Asp Asn Lys Ala Glu Glu Gly Asp Ile Leu Ala Glu Cys Ile
 1715 1720 1725
 Asn Ser Ala Met Pro Lys Gly Lys Ser His Lys Pro Phe Arg Val Lys
 1730 1735 1740
 Lys Ile Met Asp Gln Val Gln Gln Ala Ser Ala Ser Ser Ser Ala Pro
 1745 1750 1755 1760
 Asn Lys Asn Gln Leu Asp Gly Lys Lys Lys Lys Pro Thr Ser Pro Val
 1765 1770 1775
 Lys Pro Ile Pro Gln Asn Thr Glu Tyr Arg Thr Arg Val Arg Lys Asn
 1780 1785 1790
 Ala Asp Ser Lys Asn Asn Leu Asn Ala Glu Arg Val Phe Ser Asp Asn
 1795 1800 1805
 Lys Asp Ser Lys Lys Gln Asn Leu Lys Asn Asn Ser Lys Asp Phe Asn
 1810 1815 1820
 Asp Lys Leu Pro Asn Asn Glu Asp Arg Val Arg Gly Ser Phe Ala Phe
 1825 1830 1835 1840
 Asp Ser Pro His His Tyr Thr Pro Ile Glu Gly Thr Pro Tyr Cys Phe
 1845 1850 1855
 Ser Arg Asn Asp Ser Leu Ser Ser Leu Asp Phe Asp Asp Asp Val
 1860 1865 1870
 Asp Leu Ser Arg Glu Lys Ala Glu Leu Arg Lys Ala Lys Glu Asn Lys
 1875 1880 1885
 Glu Ser Glu Ala Lys Val Thr Ser His Thr Glu Leu Thr Ser Asn Gln
 1890 1895 1900
 Gln Ser Ala Asn Lys Thr Gln Ala Ile Ala Lys Gln Pro Ile Asn Arg

-continued

1905	1910	1915	1920
Gly Gln Pro Lys	Pro Ile Leu Gln Lys	Gln Ser Thr Phe Pro Gln Ser	
	1925	1930	1935
Ser Lys Asp Ile Pro Asp Arg Gly Ala Ala Thr Asp Glu Lys Leu Gln	1940	1945	1950
Asn Phe Ala Ile Glu Asn Thr Pro Val Cys Phe Ser His Asn Ser Ser	1955	1960	1965
Leu Ser Ser Leu Ser Asp Ile Asp Gln Glu Asn Asn Asn Lys Glu Asn	1970	1975	1980
Glu Pro Ile Lys Glu Thr Glu Pro Pro Asp Ser Gln Gly Glu Pro Ser	1985	1990	1995
Lys Pro Gln Ala Ser Gly Tyr Ala Pro Lys Ser Phe His Val Glu Asp	2005	2010	2015
Thr Pro Val Cys Phe Ser Arg Asn Ser Ser Leu Ser Ser Leu Ser Ile	2020	2025	2030
Asp Ser Glu Asp Asp Leu Leu Gln Glu Cys Ile Ser Ser Ala Met Pro	2035	2040	2045
Lys Lys Lys Lys Pro Ser Arg Leu Lys Gly Asp Asn Glu Lys His Ser	2050	2055	2060
Pro Arg Asn Met Gly Gly Ile Leu Gly Glu Asp Leu Thr Leu Asp Leu	2065	2070	2075
Lys Asp Ile Gln Arg Pro Asp Ser Glu His Gly Leu Ser Pro Asp Ser	2085	2090	2095
Glu Asn Phe Asp Trp Lys Ala Ile Gln Glu Gly Ala Asn Ser Ile Val	2100	2105	2110
Ser Ser Leu His Gln Ala Ala Ala Ala Ala Cys Leu Ser Arg Gln Ala	2115	2120	2125
Ser Ser Asp Ser Asp Ser Ile Leu Ser Leu Lys Ser Gly Ile Ser Leu	2130	2135	2140
Gly Ser Pro Phe His Leu Thr Pro Asp Gln Glu Glu Lys Pro Phe Thr	2145	2150	2155
Ser Asn Lys Gly Pro Arg Ile Leu Lys Pro Gly Glu Lys Ser Thr Leu	2165	2170	2175
Glu Thr Lys Lys Ile Glu Ser Glu Ser Lys Gly Ile Lys Gly Gly Lys	2180	2185	2190
Lys Val Tyr Lys Ser Leu Ile Thr Gly Lys Val Arg Ser Asn Ser Glu	2195	2200	2205
Ile Ser Gly Gln Met Lys Gln Pro Leu Gln Ala Asn Met Pro Ser Ile	2210	2215	2220
Ser Arg Gly Arg Thr Met Ile His Ile Pro Gly Val Arg Asn Ser Ser	2225	2230	2235
Ser Ser Thr Ser Pro Val Ser Lys Lys Gly Pro Pro Leu Lys Thr Pro	2245	2250	2255
Ala Ser Lys Ser Pro Ser Glu Gly Gln Thr Ala Thr Thr Ser Pro Arg	2260	2265	2270
Gly Ala Lys Pro Ser Val Lys Ser Glu Leu Ser Pro Val Ala Arg Gln	2275	2280	2285
Thr Ser Gln Ile Gly Gly Ser Ser Lys Ala Pro Ser Arg Ser Gly Ser	2290	2295	2300
Arg Asp Ser Thr Pro Ser Arg Pro Ala Gln Gln Pro Leu Ser Arg Pro	2305	2310	2315
Ile Gln Ser Pro Gly Arg Asn Ser Ile Ser Pro Gly Arg Asn Gly Ile	2325	2330	2335

-continued

Ser Pro Pro Asn Lys Leu Ser Gln Leu Pro Arg Thr Ser Ser Pro Ser
 2340 2345 2350

Thr Ala Ser Thr Lys Ser Ser Gly Ser Gly Lys Met Ser Tyr Thr Ser
 2355 2360 2365

Pro Gly Arg Gln Met Ser Gln Gln Asn Leu Thr Lys Gln Thr Gly Leu
 2370 2375 2380

Ser Lys Asn Ala Ser Ser Ile Pro Arg Ser Glu Ser Ala Ser Lys Gly
 2385 2390 2395 2400

Leu Asn Gln Met Asn Asn Gly Asn Gly Ala Asn Lys Lys Val Glu Leu
 2405 2410 2415

Ser Arg Met Ser Ser Thr Lys Ser Ser Gly Ser Glu Ser Asp Arg Ser
 2420 2425 2430

Glu Arg Pro Val Leu Val Arg Gln Ser Thr Phe Ile Lys Glu Ala Pro
 2435 2440 2445

Ser Pro Thr Leu Arg Arg Lys Leu Glu Glu Ser Ala Ser Phe Glu Ser
 2450 2455 2460

Leu Ser Pro Ser Ser Arg Pro Ala Ser Pro Thr Arg Ser Gln Ala Gln
 2465 2470 2475 2480

Thr Pro Val Leu Ser Pro Ser Leu Pro Asp Met Ser Leu Ser Thr His
 2485 2490 2495

Ser Ser Val Gln Ala Gly Gly Trp Arg Lys Leu Pro Pro Asn Leu Ser
 2500 2505 2510

Pro Thr Ile Glu Tyr Asn Asp Gly Arg Pro Ala Lys Arg His Asp Ile
 2515 2520 2525

Ala Arg Ser His Ser Glu Ser Pro Ser Arg Leu Pro Ile Asn Arg Ser
 2530 2535 2540

Gly Thr Trp Lys Arg Glu His Ser Lys His Ser Ser Ser Leu Pro Arg
 2545 2550 2555 2560

Val Ser Thr Trp Arg Arg Thr Gly Ser Ser Ser Ser Ile Leu Ser Ala
 2565 2570 2575

Ser Ser Glu Ser Ser Glu Lys Ala Lys Ser Glu Asp Glu Lys His Val
 2580 2585 2590

Asn Ser Ile Ser Gly Thr Lys Gln Ser Lys Glu Asn Gln Val Ser Ala
 2595 2600 2605

Lys Gly Thr Trp Arg Lys Ile Lys Glu Asn Glu Phe Ser Pro Thr Asn
 2610 2615 2620

Ser Thr Ser Gln Thr Val Ser Ser Gly Ala Thr Asn Gly Ala Glu Ser
 2625 2630 2635 2640

Lys Thr Leu Ile Tyr Gln Met Ala Pro Ala Val Ser Lys Thr Glu Asp
 2645 2650 2655

Val Trp Val Arg Ile Glu Asp Cys Pro Ile Asn Asn Pro Arg Ser Gly
 2660 2665 2670

Arg Ser Pro Thr Gly Asn Thr Pro Pro Val Ile Asp Ser Val Ser Glu
 2675 2680 2685

Lys Ala Asn Pro Asn Ile Lys Asp Ser Lys Asp Asn Gln Ala Lys Gln
 2690 2695 2700

Asn Val Gly Asn Gly Ser Val Pro Met Arg Thr Val Gly Leu Glu Asn
 2705 2710 2715 2720

Arg Leu Asn Ser Phe Ile Gln Val Asp Ala Pro Asp Gln Lys Gly Thr
 2725 2730 2735

Glu Ile Lys Pro Gly Gln Asn Asn Pro Val Pro Val Ser Glu Thr Asn
 2740 2745 2750

-continued

Glu Ser Ser Ile Val Glu Arg Thr Pro Phe Ser Ser Ser Ser Ser Ser
 2755 2760 2765

Lys His Ser Ser Pro Ser Gly Thr Val Ala Ala Arg Val Thr Pro Phe
 2770 2775 2780

Asn Tyr Asn Pro Ser Pro Arg Lys Ser Ser Ala Asp Ser Thr Ser Ala
 2785 2790 2795 2800

Arg Pro Ser Gln Ile Pro Thr Pro Val Asn Asn Asn Thr Lys Lys Arg
 2805 2810 2815

Asp Ser Lys Thr Asp Ser Thr Glu Ser Ser Gly Thr Gln Ser Pro Lys
 2820 2825 2830

Arg His Ser Gly Ser Tyr Leu Val Thr Ser Val
 2835 2840

(2) INFORMATION FOR SEQ ID NO: 8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 31 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (vii) IMMEDIATE SOURCE:
 (B) CLONE: ral2(yeast)

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

Leu Thr Gly Ala Lys Gly Leu Gln Leu Arg Ala Leu Arg Arg Ile Ala
 1 5 10 15

Arg Ile Glu Gln Gly Gly Thr Ala Ile Ser Pro Thr Ser Pro Leu
 20 25 30

(2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 29 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

- (vii) IMMEDIATE SOURCE:
 (B) CLONE: m3(mAChR)

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

Leu Tyr Trp Arg Ile Tyr Lys Glu Thr Glu Lys Arg Thr Lys Glu Leu
 1 5 10 15

Ala Gly Leu Gln Ala Ser Gly Thr Glu Ala Glu Thr Glu
 20 25

(2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 29 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

- (vii) IMMEDIATE SOURCE:
 (B) CLONE: MCC

-continued

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

Leu Tyr Pro Asn Leu Ala Glu Glu Arg Ser Arg Trp Glu Lys Glu Leu
 1 5 10 15

Ala Gly Leu Arg Glu Glu Asn Glu Ser Leu Thr Ala Met
 20 25

(2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 40 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

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

GTATCAAGAC TGTGACTTTT AATTGTAGTT TATCCATTTT 40

(2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 40 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

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

TTTAGAATTT CATGTTAATA TATTGTGTTT TTTTAAACAG 40

(2) INFORMATION FOR SEQ ID NO: 13:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 40 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

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

GTAGATTTTA AAAAGGTGTT TTAAATAAT TTTTAAAGCT 40

(2) INFORMATION FOR SEQ ID NO: 14:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 40 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

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

AAGCAATTGT TGTATAAAAA CTTGTTTCTA TTTTATTTAG 40

-continued

(2) INFORMATION FOR SEQ ID NO: 15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

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

GTAACITTTTC TTCATATAGT AACATTGCC TTGTGTACTC 40

(2) INFORMATION FOR SEQ ID NO: 16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

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

NNNNNNNNNN NNNGTCCCTT TTTTAAAAA AAAAAAATAG 40

(2) INFORMATION FOR SEQ ID NO: 17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

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

GTAAGTAACT TGGCAGTACA ACTTATTTGA AACTTTAATA 40

(2) INFORMATION FOR SEQ ID NO: 18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

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

ATACAAGATA TTGATACTTT TTTATTATTT GTGGTTTTAG 40

(2) INFORMATION FOR SEQ ID NO: 19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid

-continued

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

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:
GTAAGTTACT TGTTTCTAAG TGATAAAACA GYGAAGAGCT 40

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:
AATAAAAACA TAACTAATTA GGTTTCTTGT TTTATTTTAG 40

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:
GTTAGTAAAT TSCCTTTTTT GTTTGTGGGT ATAAAAATAG 40

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:
ACCATTTTTG CATGTACTGA TGTTAACTCC ATCTTAACAG 40

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

-continued

-
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:
 GTAAATAAAT TATTTTATCA TATTTTAA AATTATTTAA 40
- (2) INFORMATION FOR SEQ ID NO: 24:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 64 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:
 CATGATGTTA TCTGTATTTA CCTATAGTCT AAATTATACC ATCTATAATG TGCTTAATTT 60
 TTAG 64
- (2) INFORMATION FOR SEQ ID NO: 25:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 52 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:
 GTAACAGAAG ATTACAAACC CTGGTCACTA ATGCCATGAC TACTTTGCTA AG 52
- (2) INFORMATION FOR SEQ ID NO: 26:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 46 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:
 GGATATTAAA GTCGTAATTT TGTTTCTAAA CTCATTTGGC CCACAG 46
- (2) INFORMATION FOR SEQ ID NO: 27:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 40 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:
 GTATGTTCTC TATAGTGTAC ATCGTAGTGC ATGTTTCAAA 40

-continued

(2) INFORMATION FOR SEQ ID NO: 28:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 56 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

CATCATTGCT CTTCAAATAA CAAAGCATTG TGGTTTATGT TGATTTTATT TTTCAG 56

(2) INFORMATION FOR SEQ ID NO: 29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 43 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

GTAAGACAAA AATGTTTTTT AATGACATAG ACAATTACTG GTG 43

(2) INFORMATION FOR SEQ ID NO: 30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 40 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

TTAGATGATT GTCTTTTCC TCTTGCCCTT TTAAATTAG 40

(2) INFORMATION FOR SEQ ID NO: 31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 44 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

GTATGTTTTT ATAACATGTA TTTCTTAAGA TAGCTCAGGT ATGA 44

(2) INFORMATION FOR SEQ ID NO: 32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 54 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

-continued

-
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:
- GCTTGGCTTC AAGTTGNCTT TTTAATGATC CTCTATTCTG TATTTAATTT ACAG 54
- (2) INFORMATION FOR SEQ ID NO: 33:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 65 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:
- GTACTATTTA GAATTCACC TGTTTTTCTT TTTTCTCTT TTCTTTGAGG CAGGGTCTCA 60
CTCTG 65
- (2) INFORMATION FOR SEQ ID NO: 34:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:
- GCAACTAGTA TGATTTTATG TATAAATTAA TCTAAAATTG ATTAATTTCC AG 52
- (2) INFORMATION FOR SEQ ID NO: 35:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:
- GTACCTTTGA AACATTTAG TACTATAATA TGAATTTTCAT GT 42
- (2) INFORMATION FOR SEQ ID NO: 36:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:

-continued

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:
 CCAACTCNA A TTAGATGACC CATATTCAGA AACTTACTAG 40

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 54 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:
 GTATATATAG AGTTTTATAT TACTTTTAAA GTACAGAATT CATACTCTCA AAAA 54

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 41 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:
 ATTGTGACCT TAATTTTGTG ATCTCTTGAT TTTTATTTCA G 41

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:
 TCCCCGCCTG CCGCTCTC 18

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:
 GCAGCGGCGG CTCCCGTG 18

-continued

(2) INFORMATION FOR SEQ ID NO: 41:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

GTGAACGGCT CTCATGCTGC

20

(2) INFORMATION FOR SEQ ID NO: 42:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

ACGTGCGGGG AGGAATGGA

19

(2) INFORMATION FOR SEQ ID NO: 43:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

ATGATATCTT ACCAAATGAT ATAC

24

(2) INFORMATION FOR SEQ ID NO: 44:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

TTATTCCTAC TTCTTCTATA CAG

23

(2) INFORMATION FOR SEQ ID NO: 45:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

-continued

-
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:
TACCCATGCT GGCTCTTTT C 21
- (2) INFORMATION FOR SEQ ID NO: 46:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:
TGGGGCCATC TTGTTCTGA 20
- (2) INFORMATION FOR SEQ ID NO: 47:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:
ACATTAGGCA CAAAGCTTGC AA 22
- (2) INFORMATION FOR SEQ ID NO: 48:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:
ATCAAGCTCC AGTAAGAAGG TA 22
- (2) INFORMATION FOR SEQ ID NO: 49:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

-continued

TGCGGCTCCT GGGTTGTTG

19

(2) INFORMATION FOR SEQ ID NO: 50:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

GCCCTTCCT TTCTGAGGAC

20

(2) INFORMATION FOR SEQ ID NO: 51:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

TTTTCTCCTG CCTCTTACTG C

21

(2) INFORMATION FOR SEQ ID NO: 52:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

ATGACACCCC CCATTCCTC

20

(2) INFORMATION FOR SEQ ID NO: 53:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

CCACTTAAAG CACATATATT TAGT

24

(2) INFORMATION FOR SEQ ID NO: 54:

- (i) SEQUENCE CHARACTERISTICS:

-continued

-
- (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:
 GTATGGAAAA TAGTGAAGAA CC 22
- (2) INFORMATION FOR SEQ ID NO: 55:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:
 TTCTTAAGTC CTGTTTTTCT TTTG 24
- (2) INFORMATION FOR SEQ ID NO: 56:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:
 TTTAGAACCT TTTTGTGTT GTG 23
- (2) INFORMATION FOR SEQ ID NO: 57:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:
 CTCAGATTAT AACTAAGCC TAAC 24
- (2) INFORMATION FOR SEQ ID NO: 58:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

-continued

-
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:
 CATGTCCTTT ACAGTAGTAC CA 22
- (2) INFORMATION FOR SEQ ID NO: 59:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:
 AGGTCCAAGG GTAGCCAAGG 20
- (2) INFORMATION FOR SEQ ID NO: 60:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 27 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:
 TAAAAATGGA TAAACTACAA TTAAAAG 27
- (2) INFORMATION FOR SEQ ID NO: 61:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:
 AAATACAGAA TCATGTCTTG AAGT 24
- (2) INFORMATION FOR SEQ ID NO: 62:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:
 ACACCTAAAG ATGACAATTT GAG 23

-continued

(2) INFORMATION FOR SEQ ID NO: 63:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

TAACTTAGAT AGCAGTAATT TCCC

24

(2) INFORMATION FOR SEQ ID NO: 64:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

ACAATAAACT GGAGTACACA AGG

23

(2) INFORMATION FOR SEQ ID NO: 65:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

ATAGGTCATT GCTTCTTGCT GAT

23

(2) INFORMATION FOR SEQ ID NO: 66:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

TGAATTTTAA TGGATTACCT AGGT

24

(2) INFORMATION FOR SEQ ID NO: 67:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

-continued

-
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:
CTTTTTTGC TTTTACTGAT TAACG 25
- (2) INFORMATION FOR SEQ ID NO: 68:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:
TGTAATTCAT TTTATTCCTA ATA[G]CCTC 27
- (2) INFORMATION FOR SEQ ID NO: 69:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:
GGTAGCCATA GTATGATTAT TTCT 24
- (2) INFORMATION FOR SEQ ID NO: 70:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:
CTACCTATTT TTATACCCAC AAAC 24
- (2) INFORMATION FOR SEQ ID NO: 71:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

-continued

-
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:
AAGAAAGCCT ACACCATTTT TGC 23
- (2) INFORMATION FOR SEQ ID NO: 72:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA
 - (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:
GATCATTCTT AGAACCATCT TGC 23
- (2) INFORMATION FOR SEQ ID NO: 73:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA
 - (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:
ACCTATAGTC TAAATTATAC CATC 24
- (2) INFORMATION FOR SEQ ID NO: 74:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA
 - (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:
GTCATGGCAT TAGTGACCAG 20
- (2) INFORMATION FOR SEQ ID NO: 75:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA
 - (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:
AGTCGTAATT TTGTTTCTAA ACTC 24
- (2) INFORMATION FOR SEQ ID NO: 76:

-continued

-
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:
TGAAGGACTC GGATTTAC[G]C C 21
- (2) INFORMATION FOR SEQ ID NO: 77:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:
TCATTCACTC ACAGCCTGAT GAC 23
- (2) INFORMATION FOR SEQ ID NO: 78:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:
GCTTTGAAAC ATGCACTACG AT 22
- (2) INFORMATION FOR SEQ ID NO: 79:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:
AAACATCATT GCTCTCAA TAAC 24
- (2) INFORMATION FOR SEQ ID NO: 80:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

-continued

-
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:
TACCATGATT TAAAAATCCA CCAG 24
- (2) INFORMATION FOR SEQ ID NO: 81:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:
GATGATTGTC TTTTCCTCT TGC 23
- (2) INFORMATION FOR SEQ ID NO: 82:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:
CTGAGCTATC TTAAGAAATA CATG 24
- (2) INFORMATION FOR SEQ ID NO: 83:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:
TTTAAATGA TCCTCTATTC TGTAT 25
- (2) INFORMATION FOR SEQ ID NO: 84:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:
ACAGAGTCAG ACCCTGCCTC AAAG 24

-continued

(2) INFORMATION FOR SEQ ID NO: 85:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

TTTCTATTCT TACTGCTAGC ATT 23

(2) INFORMATION FOR SEQ ID NO: 86:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

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

ATACACAGGT AAGAAATTAG GA 22

(2) INFORMATION FOR SEQ ID NO: 87:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:

TAGATGACCC ATATTCTGTT TC 22

(2) INFORMATION FOR SEQ ID NO: 88:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 88:

CAATTAGGTC TTTTGGAGAG TA 22

(2) INFORMATION FOR SEQ ID NO: 89:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid

-continued

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

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 89:
GTTACTGCAT ACACATTGTG AC 22

(2) INFORMATION FOR SEQ ID NO: 90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 90:
GCTTTTGTGTT TCCTAACATG AAG 23

(2) INFORMATION FOR SEQ ID NO: 91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 91:
TCTCCACAG GTAATACTCC C 21

(2) INFORMATION FOR SEQ ID NO: 92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 92:
GCTAGAACTG AATGGGGTAC G 21

(2) INFORMATION FOR SEQ ID NO: 93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

-continued

-
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 93:
CAGGACAAA TAATCCTGTC CC 22
- (2) INFORMATION FOR SEQ ID NO: 94:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 94:
ATTTTCTTAG TTTCATTCTT CCTC 24
- (2) INFORMATION FOR SEQ ID NO: 95:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 95:
AGAAGGATCC CTTGTGCAGT GTGGA 25
- (2) INFORMATION FOR SEQ ID NO: 96:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 96:
GACAGGATCC TGAAGCTGAG TTTG 24
- (2) INFORMATION FOR SEQ ID NO: 97:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 97:
TCAGAAAGTG CTGAAGAG 18
- (2) INFORMATION FOR SEQ ID NO: 98:

-continued

-
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:
 GGAATAATTA GGTCTCCAA 19
- (2) INFORMATION FOR SEQ ID NO: 99:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 99:
 GCAAATCCTA AGAGAGAACA A 21
- (2) INFORMATION FOR SEQ ID NO: 100:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 100:
 GATGGCAAGC TTGAGCCAG 19
- (2) INFORMATION FOR SEQ ID NO: 101:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 101:
 GTTCCAGCAG TGTCACAG 18
- (2) INFORMATION FOR SEQ ID NO: 102:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

-continued

-
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 102:
GGGAGATTTC GCTCCTGA 18
- (2) INFORMATION FOR SEQ ID NO: 103:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 103:
AGTACAAGGA TGCCAATATT ATG 23
- (2) INFORMATION FOR SEQ ID NO: 104:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 104:
ACTTCTATCT TTTTCAGAAC GAG 23
- (2) INFORMATION FOR SEQ ID NO: 105:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 105:
ATTGAATAC TACAGTGTTA CCC 23
- (2) INFORMATION FOR SEQ ID NO: 106:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 106:

-continued

CTTGTTATTCT AATTTGGCAT AAGG

24

(2) INFORMATION FOR SEQ ID NO: 107:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 107:

CTGCCCATAC ACATTCAAAC AC

22

(2) INFORMATION FOR SEQ ID NO: 108:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 108:

TGTTTGCCTC TTGCCCATCT T

21

(2) INFORMATION FOR SEQ ID NO: 109:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 109:

AGTCTTAAAT ATTCAGATGA GCAG

24

(2) INFORMATION FOR SEQ ID NO: 110:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 110:

GTTTCTCTTC ATTATATTTT ATGCTA

26

(2) INFORMATION FOR SEQ ID NO: 111:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs

-continued

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

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 111:

AAGCCTACCA ATTATAGTGA ACG

23

(2) INFORMATION FOR SEQ ID NO: 112:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 112:

AGCTGATGAC AAAGATGATA ATC

23

(2) INFORMATION FOR SEQ ID NO: 113:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 113:

AAGAAACAAT ACAGACTTAT TGTG

24

(2) INFORMATION FOR SEQ ID NO: 114:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 114:

ATGAGTGGGG TCTCCTGAAC

20

(2) INFORMATION FOR SEQ ID NO: 115:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

-continued

(A) ORGANISM: *Homo sapiens*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 115:

ATCTCCCTCC AAAAGTGGTG C 21

(2) INFORMATION FOR SEQ ID NO: 116:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Homo sapiens*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 116:

TCCATCTGGA GTACTTTCTG TG 22

(2) INFORMATION FOR SEQ ID NO: 117:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Homo sapiens*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 117:

AGTAAATGCT GCAGTTCAGA GG 22

(2) INFORMATION FOR SEQ ID NO: 118:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Homo sapiens*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 118:

CCGTGGCATA TCATCCCCC 19

(2) INFORMATION FOR SEQ ID NO: 119:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Homo sapiens*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 119:

CCCAGACTGC TTCAAATTA CC 22

-continued

(2) INFORMATION FOR SEQ ID NO: 120:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 120:

GAGCCTCATC TGTACTTCTG C

21

(2) INFORMATION FOR SEQ ID NO: 121:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 121:

CCCTCCAAAT GAGTTAGCTG C

21

(2) INFORMATION FOR SEQ ID NO: 122:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 122:

TTGTGGTATA GGTTTTACTG GTG

23

(2) INFORMATION FOR SEQ ID NO: 123:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 123:

ACCCAACAAA AATCAGTTAG ATG

23

(2) INFORMATION FOR SEQ ID NO: 124:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

-continued

-
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 124:
GTGGCTGGTA ACTTTAGCCT C 21
- (2) INFORMATION FOR SEQ ID NO: 125:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 125:
ATGATGTTGA CCTTCCAGG G 21
- (2) INFORMATION FOR SEQ ID NO: 126:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 126:
ATTGTGTAAC TTTTCATCAG TTGC 24
- (2) INFORMATION FOR SEQ ID NO: 127:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 127:
AAAGACATAC CAGACAGAGG G 21
- (2) INFORMATION FOR SEQ ID NO: 128:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 128:

-continued

CTTTTTTGGC ATTGCGGAGC T

21

(2) INFORMATION FOR SEQ ID NO: 129:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Homo sapiens*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 129:

AAGATGACCT GTTGCAGGAA TG

22

(2) INFORMATION FOR SEQ ID NO: 130:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Homo sapiens*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 130:

GAATCAGACC AAGCTTGTCT AGAT

24

(2) INFORMATION FOR SEQ ID NO: 131:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Homo sapiens*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 131:

CAATAGTAAG TAGTTTACAT CAAG

24

(2) INFORMATION FOR SEQ ID NO: 132:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Homo sapiens*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 132:

AAACAGGACT TGTACTGTAG GA

22

(2) INFORMATION FOR SEQ ID NO: 133:

- (i) SEQUENCE CHARACTERISTICS:

-continued

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 133:

CAGCCCCTTC AAGCAAACAT C

21

(2) INFORMATION FOR SEQ ID NO: 134:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 134:

GAGGACTTAT TCCATTTCTA CC

22

(2) INFORMATION FOR SEQ ID NO: 135:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 135:

CAGTCTCCTG GCCGAAACTC

20

(2) INFORMATION FOR SEQ ID NO: 136:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 136:

GTTGACTGGC GTACTAATAC AG

22

(2) INFORMATION FOR SEQ ID NO: 137:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

-continued

-
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 137:
 TGGTAATGGA GCCAATAAAA AGG 23
- (2) INFORMATION FOR SEQ ID NO: 138:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 20 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 138:
 TGGGACTTTT CGCCATCCAC 20
- (2) INFORMATION FOR SEQ ID NO: 139:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 139:
 TGTCTCTATC CACACATTCG TC 22
- (2) INFORMATION FOR SEQ ID NO: 140:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 140:
 ATGTTTTTCA TCCTCACTTT TTGC 24
- (2) INFORMATION FOR SEQ ID NO: 141:
- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Homo sapiens*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 141:
 GGAGAAGAAC TGGAAGTTCA TC 22

-continued

(2) INFORMATION FOR SEQ ID NO: 142:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 142:

TTGAATCTTT AATGTTTGA TTTGC

25

(2) INFORMATION FOR SEQ ID NO: 143:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 143:

TCTCCACAG GTAATACTCC C

21

(2) INFORMATION FOR SEQ ID NO: 144:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 144:

GCTACAACG AATGGGTAC G

21

(2) INFORMATION FOR SEQ ID NO: 145:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 145:

CAGGACAAA TAATCCTGTC CC

22

(2) INFORMATION FOR SEQ ID NO: 146:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

-continued

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Homo sapiens*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 146:

ATTTTCTTAC TTTCATTCTT CCTC

24

(2) INFORMATION FOR SEQ ID NO: 147:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial sequence (consensus)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 147:

Phe	Xaa	Val	Glu	Xaa	Thr	Pro	Xaa	Cys	Phe	Ser	Arg	Xaa	Ser	Ser	Leu
1			5					10						15	

Ser	Ser	Leu	Ser
			20

(2) INFORMATION FOR SEQ ID NO: 148:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Homo sapiens*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 148:

Tyr	Cys	Val	Glu	Asp	Thr	Pro	Ile	Cys	Phe	Ser	Arg	Cys	Ser	Ser	Leu
1				5					10					15	

Ser	Ser	Leu	Ser
			20

(2) INFORMATION FOR SEQ ID NO: 149:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Homo sapiens*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 149:

His	Thr	Val	Gln	Glu	Thr	Pro	Leu	Met	Phe	Ser	Arg	Cys	Thr	Ser	Val
1				5					10					15	

Ser	Ser	Leu	Asp
			20

(2) INFORMATION FOR SEQ ID NO: 150:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 amino acids

-continued

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 150:

Phe Ala Thr Glu Ser Thr Pro Asp Gly Phe Ser Cys Ser Ser Ser Leu
1 5 10 15

Ser Ala Leu Ser
20

(2) INFORMATION FOR SEQ ID NO: 151:

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

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 151:

Tyr Cys Val Glu Gly Thr Pro Ile Asn Phe Ser Thr Ala Thr Ser Leu
1 5 10 15

Ser Asp Leu Thr
20

(2) INFORMATION FOR SEQ ID NO: 152:

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

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 152:

Thr Pro Ile Glu Gly Thr Pro Tyr Cys Phe Ser Arg Asn Asp Ser Leu
1 5 10 15

Ser Ser Leu Asp
20

(2) INFORMATION FOR SEQ ID NO: 153:

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

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 153:

Phe Ala Ile Glu Asn Thr Pro Val Cys Pro Ser His Asn Ser Ser Leu
1 5 10 15

Ser Ser Leu Ser
20

-continued

(2) INFORMATION FOR SEQ ID NO: 154:

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

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 154:

Arg His Val Glu Asp Thr Pro Val Cys Phe Ser Arg Asn Ser Ser Leu
 1 5 10 15
 Ser Ser Leu Ser
 20

We claim:

[1. A preparation of antibodies which specifically binds to a human APC (adenomatous polyposis coil) protein having an amino acid sequence as shown in SEQ ID NO:1, 2, or 7, and does not specifically bind to other human proteins.]

2. A preparation of antibodies which specifically binds to a human APC protein which is the product of a mutant allele found in a tumor, wherein the antibodies do not specifically bind to other human proteins, and wherein the human APC protein is a mutant form of the amino acid sequence shown in [SEQ ID NOS:2 and] SEQ ID NO: 7, and the mutant allele is a mutant form of the nucleotide sequence shown in SEQ ID NO:1] having the sequence of SEQ ID NO: 7 but for a substitution of Arg→Cys at residue 414.

[3. The preparation of claim 2 wherein the mutant allele contains a mutation selected from the group consisting of

mutations at codons 243, 279, 288, 301, 331, 413, 437, 456, 500, 712, and 1338.]

[4. The preparation of claim 2 wherein the mutant allele contains a premature stop codon.]

[5. The preparation of claim 2 wherein the mutant allele contains a missense mutation.]

[6. The preparation of claim 2 wherein the mutant allele contains a frameshift mutation.]

[7. The preparation of claim 2 wherein the mutant allele contains a splice junction mutation.]

[8. The preparation of claim 2 wherein the mutant allele contains an insertion mutation.]

* * * * *