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Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

E.

S.

SUPERINDUSTRIA Y COMERCIO

Radicacion : 00000434 00000001

Folios: 152

Fecha (AMD): 2000-03-28 16:29:57

Tramite : 002 PATENTES D 1 REGISTRO/ 329 COMPLEMEN
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

Re: P1.-ABBOTT LABORATORIES.-Solicitud de Patente de invención
para "GEN DE LA DESATURASA HUMANA Y SUS USOS".-Clase
C.12N.-Expediente número 00-000.434.-

1. Me refiero a la solicitud de patente arriba nombrada de fecha 5 de Enero de 2000.

2. Anexos me permito presentar a usted:

(a) Copia auténtica debidamente certificada **respecto de la cual reclamo prioridad unionista**, a saber la solicitud estadinense número 09/227.613 del 8 de Enero de 1999, junto con su traducción oficial, **correspondiente a las legalizaciones y reivindicaciones de dicho documento; y**

(b) Copia debidamente autenticada por el Notario Sexto del Circulo de Bogotá, de los documentos que acreditan la calidad de traductor Oficial de la señora Clemencia Paredes, quien elaboró la traducción de la solicitud estadinense número 09/227.613 respecto de la cual se reivindica prioridad en el presente caso.

16A
167

3. Ruego a usted tomar atenta nota de lo anterior y proceder de conformidad.

Señor Superintendente,



JOSE ANTONIO LLOREDA

T.P. 10.784

Código 231

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168

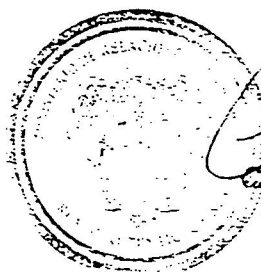
**EL SUSCRITO JEFE DEL AREA DE
LEGALIZACIONES DEL MINISTERIO DE
RELACIONES EXTERIORES**

CERTIFICA

Se revisados los registros que se llevan en la oficina de Legalizaciones aparece la firma de la señora CLEMENCIA PAREDES TEJADA, como traductora e intérprete oficial para los idiomas INGLES-ESPAÑOL, ESPAÑOL-INGLES y FRANCES - ESPAÑOL-FRANCES, según resolución 6694 del 14 de diciembre de 1978.

La anterior certificación se expide a solicitud de la señora CLEMENCIA PAREDES TEJADA, quien se identificó con cédula de ciudadanía 41.438.41 en Bogotá.

Dada en Santafé de Bogotá, D.C., a los once (11) días de junio de 1997.



CARLOS GUILLERMO DIAZ BUITRAGO
Jefe Area de Legalizaciones

COMO NOTARIO SEXTO DE
SANTAFE DE BOGOTA
HAGO CONSTAR
QUE LA FOTOCOPIA COINCIDE CON LA FOTOCOPIA
AUTENTICA QUE HE TENIDO A LA VISTA.
SANTAFE DE BOGOTA

25 SET 1997

COMO NOTARIO SEXTO DE
SANTAFE DE BOGOTA
HAGO CONSTAR
QUE LA FOTOCOPIA COINCIDE CON LA FOTOCOPIA
AUTENTICA QUE HE TENIDO A LA VISTA.
SANTAFE DE BOGOTA

15 FEB 2000

JUAN MANUEL BOTERO MEDINA
NOTARIO

Notaria Voluntaria del Estado de Santafé
Bogotá, como Notaria Voluntaria Hago constar
que esta copia coincide con el documento
original presentado para su autenticación.
Secretaría de las P. C. República de Colombia
23 JUN 1997
María Eugenia Rojas de Puela
NOTARIA VEINTISIETE

171
169

República de Colombia



Ministerio de Justicia y del Derecho

LA SECRETARIA GENERAL DEL MINISTERIO DE JUSTICIA Y DEL DERECHO

CERTIFICA:

COMO NOTARIO SEATO DE
SANTAFE DE BOGOTA
HAGO CONSTAR
QUE LA FOTOCOPIA COINCIDE CON LA FOTOCOPIA
AUTENTICA QUE HE TENIDO A LA VISTA.
SANTAFE DE BOGOTA, 07 MAYO 1998

Que por Resolución 6694 del 14 de diciembre de 1978, le fue expedida licencia para ejercer funciones de traductor e Intérprete oficial en los idiomas **INGLES** **ESPAÑOL**, **ESPAÑOL-INGLES** y **FRANCES -ESPAÑOL** a **CLEMENCIA PAREDES TEJADA** identificada con la cédula de ciudadanía No. 41.433.414 expedida en Bogotá.

Que dicha Resolución se encuentra vigente en la fecha.

Se expide la presente certificación a solicitud de la interesada.

Dada en Santa Fe de Bogotá, D.C., a

06 JUN. 1997

COMO NOTARIO SEATO DE
SANTAFE DE BOGOTA
HAGO CONSTAR
QUE LA FOTOCOPIA COINCIDE CON LA FOTOCOPIA
AUTENTICA QUE HE TENIDO A LA VISTA.
SANTAFE DE BOGOTA, 25 SET. 1997
JUAN MANUEL BOTERO MEDINA
NOTARIO

Maritza Hidalgo Anibal

MARITZA HIDALGO ANIBAL

HAR/mmc

COMO NOTARIO SEATO DE
SANTAFE DE BOGOTA
HAGO CONSTAR
QUE LA FOTOCOPIA COINCIDE CON LA FOTOCOPIA
AUTENTICA QUE HE TENIDO A LA VISTA.
SANTAFE DE BOGOTA, 15 FEB. 2000
JUAN MANUEL BOTERO MEDINA
NOTARIO

Notaria Valenciana del Circulo
Bogotá, Como Notaria Valenciana (BOGOTA)
Que esta copia coincide con documento
original presentado para su autenticación.
Sealada de 194, U. C. Republica de Colombia
23 JUN 1998
María Eugenia Rojas de
NOTARIA VEINTOSIETE

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MM-07091391



MINISTERIO DE JUSTICIA- OFICINA JURIDICA

RESOLUCION NUMERO 6694 DE 1.978

(14 DIC.1978)

Por la cual se expide una licencia para ejercer funciones como Intérprete y Traductora Oficial.

EL MINISTRO DE JUSTICIA,

en ejercicio de la facultad que le confieren los Decretos 382, 22-57 de 1.951 y 576 de 1.974, y

CONSIDERANDO:

Que la señorita CLENE LA PAREDES TEJADA, de nacionalidad Colombiana, portadora de la cédula de ciudadanía número 41.433.414, de Bogotá, solicita de este Ministerio le sea expedida licencia para ejercer funciones de Intérprete y Traductora Oficial, conforme a los Decretos 382 y 2257 de 1.951.

Que para comprobar el cumplimiento de los requisitos exigidos, la peticionaria presentó los siguientes documentos:

1o).- Certificado del Registro Civil de Nacimiento expedido por el Notario Cuarto del Circuito de Bogotá, D.E., que comprueba ser Colombiano de nacimiento y mayor de edad.

2o).- Certificado No. 083, expedido por el Jefe de la Oficina Jurídica del Ministerio de Educación Nacional, con el que se comprueba ser idónea para desempeñar funciones de Intérprete y Traductora en los Idiomas Inglés e Francés.

3o).- Certificado No. 081 expedido por el Jefe de la División de Asesoría Jurídica, Contratos, Personería Jurídica y Asesoría del Ministerio de Educación Nacional, con el que se comprueba ser idónea para desempeñar funciones de Intérprete y Traductora Oficial en los Idiomas Inglés, Francés y Español.

4o).- Certificado expedido por el Jefe de la División de Censura y Control de la Imprenta, con el que se comprueba que el documento es auténtico y que no ha sido alterado.

En consecuencia, se expide la presente licencia para que la señorita CLENE LA PAREDES TEJADA, pueda ejercer las funciones de Intérprete y Traductora Oficial en los Idiomas Inglés, Francés y Español.

En Bogotá, D.C., a 17 de Diciembre de 1978.

HAGO CONSTAR QUE LA FOTOCOPIA QUE HE TENIDO A LA VISTA AUTENTICA QUE HE TENIDO A LA VISTA. SANTA FE DE BOGOTÁ, 07 MAYO 1998

COMO NOTARIO SEXTO DE BOGOTÁ, D.E., HE TENIDO A LA VISTA LA FOTOCOPIA QUE HE TENIDO A LA VISTA. SANTA FE DE BOGOTÁ, 25 SET. 1997

JUAN MANUEL BOTERO MEDINA NOTARIO COMO NOTARIO SEXTO DE BOGOTÁ, D.E., HE TENIDO A LA VISTA LA FOTOCOPIA QUE HE TENIDO A LA VISTA. SANTA FE DE BOGOTÁ, 15 FEB. 2000

Hago constar que esta copia es auténtica y que no ha sido alterada. 122/10/98 SERGIO LEON NOTARIO DEBOGOTÁ

REPUBLICA DE COLOMBIA
27
Marta Lora
de Urusá
NOTARIA

NOTARIO YEINTICRO DEL CIRCUITO DE BOGOTÁ, D.E., HE TENIDO A LA VISTA LA FOTOCOPIA QUE HE TENIDO A LA VISTA. SANTA FE DE BOGOTÁ, 17 DIC. 1998

5
cual la cédula de ciudadanía número 41'433.414 de Bogotá, se en-
cuentra vigente.

50).- Certificado Judicial, registrado bajo el TD. #8000942, expedi-
do por el Departamento Administrativo de Seguridad "DA32", -
según el cual el peticionario no registra antecedentes pe-
nales ni de policía:

60).- Tres declaraciones extrajuicio, rendidas con anuencia del -
Ministerio Público ante el Juzgado Tercero Civil del Circui-
to, sobre conducta y buenos antecedentes del peticionario.
Que acreditados los anteriores requisitos y de acuerdo con los De-
cretos 382, 2257 de 1.951 y 576 de 1.974, corresponde al Ministe-
rio de Justicia expedir a la peticionaria licencia que la habili-
te para desempeñar el cargo de Intérprete y Traductora Oficial en
los idiomas Inglés- Español, Español- Inglés, Francés- Español-Es-
pañol-Francés, en los que es idónea.

RESUELVE:

ARTÍCULO PRIMERO.- Expídesse licencia a la señorita CLEMENCIA PARE-
DES TEJADA, identificada con la cédula de ciu-
dadanía número 41'433.414 expedida en Bogotá, para desempeñar el
cargo de Intérprete y Traductora Oficial en los Idiomas Inglés-Es-
pañol, Español- Inglés y Francés- Español, Español-Francés, previa
inscripción de su nombre ante el Tribunal Superior del Distrito
Judicial correspondiente al territorio donde vaya a actuar.

ARTÍCULO SEGUNDO.- Comuníquese el texto de la presente resolución
a la sub-secretaría de Asuntos Administrati-
vos del Ministerio de Relaciones Exteriores.

ARTÍCULO TERCERO.- La presente resolución rige a partir de la fecha de su expedición.

COMUNIQUESE Y CUMPLASE

Dado en Bogotá, D.E., a

Hago constar que esta copia con-
cide con documento original pre-
sentado por el peticionario.

122-100-1995
Santafé de Bogotá D.C. Colombia
GERGIO FRANCO LEON
NOTARIO PÚBLICO

HUGO ESCOBAR SIERRA

23 JUN 1997

Esta fotocopia coincide con el original
fotocopia autenticada
27-06-1996
NOTARIO

REPUBLICA DE COLOMBIA
MINISTERIO DE RELACIONES EXTERIORES
BOGOTÁ

17B

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MM 08346838



HOJA NUMERO DOS DE LA RESOLUCION NUMERO 3694
DE DICIEMBRE 14 DIC 1978 EL NO-
VECIENTOS SETENTA Y OCHO (1.978). POR LA CUAL-
SE EXPIDE LICENCIA PARA EJERCER FUNCIONES COMO-
INTERPRETE Y TRADUCTORA OFICIAL.

EL SECRETARIO GENERAL,

Gustavo Gutierrez
GUSTAVO GUTIERREZ CALDERA

COMO NOTARIO SEPTO DE
SANTAFE DE BOGOTA
HAGO CONSTAR
QUE LA FOTOCOPIA COINCIDE CON LA FOTOCOPIA
AUTENTICA QUE HE TENIDO A LA VISTA
SANTAFE DE BOGOTA

67 MAYO 1998

JUAN MANUEL BOTERO MEDINA
NOTARIO

COMO NOTARIO SEPTO DE
SANTAFE DE BOGOTA
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AUTENTICA QUE HE TENIDO A LA VISTA
SANTAFE DE BOGOTA

25 SET. 1997

JUAN MANUEL BOTERO MEDINA
NOTARIO

COMO NOTARIO SEPTO DE
SANTAFE DE BOGOTA
HAGO CONSTAR
QUE LA FOTOCOPIA COINCIDE CON LA FOTOCOPIA
AUTENTICA QUE HE TENIDO A LA VISTA
SANTAFE DE BOGOTA

15 FEB 2000

JUAN MANUEL BOTERO MEDINA
NOTARIO

Esta fotocopia coincide con una
fotocopia autenticada que he
tenido a mi vista

23 JUN 1997



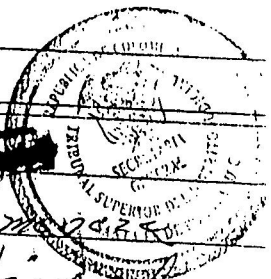
Hago constar que esta copia coin-
cide con documento original pro-
sentado para su autenticación.
122 JUL 1997
Santa Fe de Bogota D.C. - Colombia
SERGIO FRAIGO LEON
NOTARIO DECIMO

DEPARTAMENTO DE JUSTICIA Y DE FERIA
OFICINA DE REGISTRO
BOGOTA

16
Cere. de N° 278

JUDICIAL DE BOGOTÁ

TRIBUNAL SUPERIOR DE BOGOTÁ
SECRETARÍA SALA CIVIL
17 JUN. 1997



Diligencia de posesión de la sede de
Doreos Tejada, Traductor e Intérprete
oficial de los idiomas Inglés - Español, Español
Inglés y francés - Español, Español francés

En Santafé de Bogotá D.C., a los once (11) días
del mes de noviembre de mil novecientos noventa
y tres (1993), compareció al Despacho del señor
Presidente del H. Tribunal Departamental de
Justicia de Santafé de Bogotá, Doreos Tejada,
mencia Doreos Tejada, con cédula de ciudadanía N° 41.433.127 expedida
en Bogotá, con el fin de tomar posesión del cargo
de Traductor e Intérprete oficial, para el cual
fue admitido mediante Resolución N° 6674 de 14
de Diciembre de 1978, emanada del H. Tribunal de
Justicia.

COMO NOTARIO SEXTO DE
SANTAFÉ DE BOGOTÁ
HAGO CONSTAR
QUE LA FOTOCOPIA COINCIDE CON LA FOTOCOPIA
AUTÉNTICA QUE HE TENIDO A LA VISTA
SANTAFÉ DE BOGOTÁ
7 MAYO 1998
JUAN MANUEL BOTERO MEDINA
NOTARIO

COMO NOTARIO SEXTO DE
SANTAFÉ DE BOGOTÁ
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QUE LA FOTOCOPIA COINCIDE CON LA FOTOCOPIA
AUTÉNTICA QUE HE TENIDO A LA VISTA
SANTAFÉ DE BOGOTÁ
25 SET. 1997
JUAN MANUEL BOTERO MEDINA
NOTARIO

Para tal efecto el señor Presidente del H. Tribunal
le recibió el Juramento de posesión por cuya
gravedad prometió cumplir bien y fielmente
con sus deberes.

COMO NOTARIO SEXTO DE
SANTAFÉ DE BOGOTÁ
HAGO CONSTAR
QUE LA FOTOCOPIA COINCIDE CON LA FOTOCOPIA
AUTÉNTICA QUE HE TENIDO A LA VISTA
SANTAFÉ DE BOGOTÁ
15 FEB. 2000
JUAN MANUEL BOTERO MEDINA
NOTARIO

Se firmó por quienes en la diligencia intervinie-
ron, haciendo constar que la copia que se
da a la interesada, le acredita su calidad de
Traductor e Intérprete.

El Presidente,

La Presidencia,

El Secretario,

Abogado

M...



ESTADOS UNIDOS DE AMERICA

DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

OFICINA DE PATENTES Y DE MARCAS REGISTRADAS

DICIEMBRE 13 DE 1999

El presente para certificar que el certificado anexo es una copia verdadera de los records de la Oficina de patentes y de Marcas Registrada de los estados Unidos de la solicitud de patente identificada a continuación que cumple con los requisitos para conceder un archivo de patente de acuerdo al 35 USC 111.

NUMERO DE APLICACIÓN: 09/227,613

FECHA DE ARCHIVO: ENERO 8 DE 1999

FIRMADO POR LA AUTORIDAD DEL COMISIONADO DE PATENTES Y MARCAS
REGISTRADAS

T. WALLACE, OFICIAL QUE CERTIFICA

Clemente D. Lejada
TRADUCTOR DE... AL ESPAÑOL
RES...

— C. Lejada —

DOCUMENTO DE APLICACIÓN PARA PATENTE

ELEMENTOS DE LA APLICACIÓN

1 FORMA PARA PAGO DE HONORARIOS X

2 ESPECIFICACION TOTAL PAGINAS 82

3 DISEÑOS TOTAL PAGINAS 40

PARTES QUE ACOMPAÑAN LA APLICACIÓN

RECIBO DE RETORNO (MPEP 503) X

OTRO: DECLARACION DE NO EJECUCION Y PODER (2PAGINAS)

APLICACIÓN QUE CONTINUA EN PARTE (CIP)

DIRECCION PARA LA CORRESPONDENCIA

NOMBRE STEVEN E. WEINSTOCK

ABBOTT LABORATORIES

DIRECCION D-377/AP6D-2

100 ABBOTT PARK ROAD

CIUDAD ABBOTT PARK CODIGO POSTAL 60064-3500

PAIS ESTADOS UNIDOS DE AMERICA

TELEFONOS (847) 935-1729

FAX (847) 938-238-2623

Clemencia Pareja Lejada
TRADUCTORA OFICIAL INGLÉS-FRANCÉS-ESPAÑOL
RES. 1111, 1111, 1111, 1111, 1111, 1111

[Signature]

~~177~~
175

APPLICANTE PRADIP MUDKERJI et al.

PARA: GENE HUMANO DESATURASA Y SUS USOS.

Examinador: todavía no se ha asignado

Fecha Enero 8 de 1999

Ee835516264US

Asistente Comisionado de Patentes

Washington, D.C. 20231, en

Firmado, Kathleen t. Litz FECHA 1-8-99

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REIVINDICACIONES:

1. Una secuencia aislada de nucleótidos correspondiente a o complementaria a por lo menos alrededor de 50% de la secuencia de nucleótidos representada por SEQ ID NO: 1 (Figura 12).
2. La secuencia de nucleótidos aislados de la reivindicación 1 en donde dicha secuencia está representada por SEQ ID NO 1.
3. La secuencia de nucleótidos aislados de las reivindicaciones 1 o 2 en donde dicha secuencia codifica una desaturasa funcionalmente activa que utiliza un ácido graso poli-insaturado como un substrato.
4. La secuencia de nucleótido de la reivindicación 1 en donde dicha secuencia se deriva de un mamífero.
5. La secuencia de nucleótidos de la reivindicación 4 en donde dicha secuencia se deriva de un humano.
6. Una proteína purificada codificada por dicha secuencia de nucleótidos de las reivindicaciones 1 o 2.
7. Un poli péptido purificado que desatura ácidos grasos poli-insaturados a Carbón 5 y tiene por lo menos alrededor de 50% de similitud aminoácida con la secuencia de deaminoácido de dicha proteína purificada de la reivindicación 6.
8. un método para producir una desaturasa humana Δ5 que comprende los siguientes pasos:

Clemencia Paredes Lejada
 TRADUCTORA OFICIAL INGLÉS-FRANCÉS-ESPAÑOL
 DES. 1001 JUN 1994 10:26 70
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- a) aislamiento de dicha secuencia de nucleótidos representados por SEQ ID NO 1 (Figura 12);
- b) Construcción de un vector que comprende: i) dicha secuencia aislada de nucleótidos operablemente unida a ii) un promotor;
- c) la introducción de dicho vector en una célula huésped bajo tiempo y condiciones suficientes para la expresión de dicha desaturasa $\Delta 5$ humana.

9. El método de la reivindicación 8 en donde dicha célula huésped es una célula eucariótica o una célula procariótica.

10. El método de la reivindicación 9 en donde dicha célula procariótica se selecciona del grupo consistente en cianobacteria E. Coli, y B. Subtilis.

11. El método de la reivindicación 8 en donde dicha célula eucariótica se selecciona del grupo consistente en una célula de mamífero, una célula de insecto, una célula de una planta y una célula de un hongo.

12. El método de la reivindicación 11 en donde dicha célula de hongo es una célula de levadura.

13. El método de la reivindicación 12 en donde dicha célula de levadura se selecciona del grupo consistente en saccharomyces cerevisiae, Saccharomyce carlsbergensis, Candida spp., Lypomyces starkey, Yarrowia lipolytica,

Clementina Dorelos Lejada
TRADUCTORA OFICIAL - INES-70000FS-ESPAÑOL
RES 200. JUN 2001 14 de 13

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Kluyveromyces spp., Hansenula spp., Trichoderma spp y Pichia spp.

14. El método de la reivindicación 13 en donde dicha célula de levadura es saccharomyces cerevisiae.

15. Un vector que comprende: a) una secuencia de nucleótidos como se representa por SEQ ID NO: 1 (Figura 12) operablemente unida a b) un promotor.

16. Una célula huésped que comprende dicho vector de la reivindicación 15.

17. La célula huésped de la reivindicación 16, en donde dicha célula huésped es una célula eucariótica o una célula procariótica.

18. La célula huésped de la reivindicación 17 en donde dicha célula procariótica se selecciona del grupo consistente en cianobacteria E. Coli, y B. Subtilis.

19. La célula huésped de la reivindicación 17 en donde dicha célula eucariótica se selecciona del grupo consistente en una célula de mamífero, una célula de insecto, una célula de una planta y una célula de un hongo.

20. La célula huésped de la reivindicación 19 en donde dicha célula de hongo es una célula de levadura.

21. La célula huésped de la reivindicación 20 en donde dicha célula de levadura se selecciona del grupo consistente en saccharomyces cerevisiae, Saccharomyce carlsbergensis,

Candida spp., Lypomyces starkey, Yarrowia lipolytica,
Kluyveromyces spp., Hansenula spp., Trichoderma spp y Pichia
spp.

22. La célula huésped de la reivindicación 21 en donde dicha célula huésped es saccharomyces cerevisiae.

23. Una célula de planta, una planta o un tejido de planta que comprenda dicho vector de la reivindicación 15, en donde la expresión de dicha secuencia de nucleótidos de dicho vector resulte en la producción de un ácido graso poli-insaturado por dicha célula de la planta o tejido.

24. La célula de la planta, planta o tejido de la planta de la reivindicación 23 en donde dicho ácido graso poli-insaturado es AA o EPA.

25. Uno o más aceites de plantas o ácidos expresados por dicha célula de la planta, planta o tejido de la planta de la reivindicación 23.

26. Una planta transgénica que comprenda dicho vector de la reivindicación 15, en donde la expresión de dicha secuencia de nucleótidos de dicho vector resulte en la producción de un ácido graso poli-insaturado en semillas de dicha planta transgénica.

27. Una célula de mamífero que comprenda dicho vector de la reivindicación 15, en donde la expresión de dicha secuencia de nucleótidos de dicho vector resulte en la producción de

Clementina Paredes Lejada
TRADUCCION DE LOS TEXOS EN ESPAÑOL
RES. 1 JUNIO 1997


niveles alterados de AA o EPA cuando dicha célula crezca en un cultivo que comprenda un ácido graso seleccionado del grupo que consiste en un ácido graso esencial , LA y ALA.

28. Un mamífero no humano, transgénico cuyo genoma comprenda una secuencia de ADN codificando una desaturasa humana $\Delta 5$ operable unida a un promotor.

29. El mamífero no humano, transgénico de la reivindicación 8, en donde dicha secuencia de ADN esté representada por SEQ ID NO: 1 (Figura 12).

30. Un fluido producido por dicho mamífero no humano transgénico de la reivindicación 29 en donde dicho fluido comprenda un nivel detectable de por lo menos desaturasa $\Delta 5$ humana.

31. Un método para producir un ácido graso poli-insaturado que comprenda los pasos de:

- a) aislamiento de dicha secuencia de nucleótidos representados por SEQ ID NO 1 (Figura 12);
- b) Construcción de un vector que comprenda dicha secuencia de nucleótidos aislados;
- c) la introducción de dicho vector en una célula huésped bajo tiempo y condiciones suficientes para la expresión de dicha enzima desaturasa $\Delta 5$ humana.

Clemente Pineda Lejada
TRADUCCIÓN ORIGINAL ES: FRANCÉS - ESPAÑOL
2000-01-10


d) la exposición de dicha enzima desaturasa $\Delta 5$ humana a un substrato de ácido graso poli-insaturado para convertir dicho substrato en un producto de ácido graso poli-insaturado.

32. El método de acuerdo con la reivindicación 31, en donde dicho ácido graso de substrato poli-insaturado es DGLA o 20:4N-3 y dicho producto de ácido graso poli-insaturado es AA o EPA, respectivamente.

33. El método de acuerdo con la reivindicación 31 que comprende además el paso de exponer dicho producto de ácido graso poli-insaturado a una elongasa para convertir dicho producto de ácido graso poli-insaturado en otro ácido graso poli-insaturado.

34. El método de acuerdo con la reivindicación 33 en donde dicho producto de ácido graso poli-insaturado es AA o EPA y dicho otro ácido graso poli-insaturado es ácido adrenico o (n-3) ácido-docosapentaenoico, respectivamente.

35. El método de la reivindicación 33 además comprende los pasos de exposición de dicho otro ácido graso poli-insaturado a una desaturasa adicional para convertir dicho otro ácido graso poli-insaturado en un ácido graso poli-insaturado final.

Clemencia Poreles Dejada
 TRADUCTORA OFICIAL DE LOS LENGÜES ESPAÑOL
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36. El método de la reivindicación 33 en donde dicho ácido graso poli-insaturado final es (n-6) ácido-docosapentaenoico o ácido docosahexaenoico (DHA).

37. Una composición nutricional comprendiendo por lo menos un ácido graso poli-insaturado seleccionado del grupo consistente en dicho producto de ácido graso poli-insaturado producido de acuerdo con el método de la reivindicación 31, dicho otro ácido graso poli-insaturado producido de acuerdo con el método de la reivindicación 33 y dicho ácido graso poli-insaturado final producido de acuerdo con el método de la reivindicación 35.

38. La composición nutricional de la reivindicación 37 en donde dicho producto de ácido graso poli-insaturado es AA o EPA.

39. La composición nutricional de la reivindicación 37 en donde dicho otro ácido graso poli-insaturado es ácido adrenico o (n-3) ácido-docosapentaenoico.

40. La composición nutricional de la reivindicación 37 en donde dicho ácido graso poli-insaturado final es DHA.

41. La composición nutricional de la reivindicación 37 en donde dicha composición nutricional se selecciona del grupo que consiste en una fórmula infante, un suplemento dietario y un substituto dietario.

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42. La composición nutricional de la reivindicación 41 en donde dicha composición nutricional se administra a un humano o a un animal.

43. La composición nutricional de la reivindicación 42 en donde dicha composición nutricional se administra enteralmente o parenteralmente.

44. La composición nutricional de la reivindicación 41 en donde dicha composición nutricional además comprende por lo menos un macro-nutriente seleccionado del grupo consistente en aceite de coco, aceite de soya, aceite de granola, monoglicéridos, diglicéridos, glucosa, lactosa comestible, suero de leche electrodializado, leche desgrasada electrodializada, suero de leche, proteína de soya e hidrolizatos de proteína.

45. La composición nutricional de la reivindicación 44 en donde dicha composición nutricional además comprende por lo menos una vitamina seleccionada del grupo que consiste en vitaminas A, C, D, E y complejo B y por lo menos un mineral seleccionado del grupo que consiste en Magnesio de Calcio, Zinc, Manganeso, Sodio, Potasio, Fósforo, Cobre, Cloruro, Iodo, Selenio y Hierro.

46. Una composición farmacéutica que comprende 1) por lo menos un ácido graso poli-insaturado seleccionado del grupo que consta de dicho producto de ácido poli-insaturado

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producido de acuerdo con el método de la reivindicación 31, dicho otro ácido graso poli-insaturado producido de acuerdo con el método de la reivindicación 33 y dicho ácido graso poli-insaturado final producido de acuerdo con El método de la reivindicación 35 y 2) un portador farmacéuticamente aceptable.

47. La composición farmacéutica de la reivindicación 46 en donde dicha composición farmacéutica se administra a un humano o a un animal.

48. La composición farmacéutica de la reivindicación 46 en donde dicha composición farmacéutica además comprende un elemento seleccionado del grupo que consiste en una vitamina, un mineral, un carbohidrato, un aminoácido, un ácido libre de graso, un fosfolípido, un antioxidante y un compuesto fenólico.

49. Un alimento animal que comprende por lo menos un ácido graso poli-insaturado seleccionado del grupo que consiste en dicho producto de ácido graso poli-insaturado producido de acuerdo con El método de la reivindicación 31, dicho otro ácido graso poli-insaturado producido de acuerdo con el método de la reivindicación 33 y dicho ácido graso poli-insaturado final producido de acuerdo con El método de la reivindicación 35.

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ESPAÑOL
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50. Al comida animal de la reivindicación 49 en donde dicho producto de ácido graso poli-insaturado es AA o EPA.

51. La comida animal de la reivindicación 49 en donde dicho otro ácido graso poli-insaturado es ácido adrenico o (n-3)-ácido docosapentaenoico.

52. La comida animal de la reivindicación 49 en donde dicho ácido graso poli-insaturado final es (n-6)-ácido-docosapentaenoico o DHA.

53. Un cosmético que comprende un ácido graso poli-insaturado seleccionado del grupo que consiste en dicho producto de ácido graso poli-insaturado producido de acuerdo con el método de la reivindicación 31, dicho otro ácido graso poli-insaturado producido de acuerdo con el método de la reivindicación 33 y dicho ácido graso poli-insaturado final producido de acuerdo con el método de la reivindicación 35.

54. Un método de prevención o de tratamiento de una condición causada por insuficiencia de toma de ácidos grasos poli-insaturados que comprende la administración a dicho paciente de dicha composición nutricional de la reivindicación 37 en una cantidad suficiente para que dicho tratamiento haga efecto.

Clemente S. de la Cruz
 TRADUCTOR DE LOS FRANCÉS - ESPAÑOL
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DECLARACION Y PODER PARA UNA APLICACIÓN DE PATENTE EN LOS

ESTADOS UNIDOS

Como el Inventor, por medio del presente declaro:

La dirección de mi residencia, mi dirección postal y el número de mi código postal están indicados al lado de mi firma al final de este documento. Yo creo que soy el inventor original, primero y conjunto del asunto de la referencia y que se reivindica y para el cual se busca la patente de la invención denominada **GENE HUMANO DESATURASA Y SUS USOS**, de la cual la especificación está anexa al presente.

Por medio de este documento declaro que he revisado y comprendido los contenidos de la especificación antes mencionada, incluyendo las reivindicaciones.

Certifico que tengo el deber de revelar a la Oficina de Patentes y de Marcas Registradas toda la información que yo conozca sobre el material para ser patentado, como se define en el título 37, código de las Regulaciones Federales, §1.56.

Reivindicación para beneficio de la patente extranjera:

Por medio del presente reivindico los beneficios de prioridad en el extranjero según la sección 35 U.S.C. §119 para las siguientes aplicaciones para patente o para el certificado del inventor:

Aplicación Internacional PCT/US98/07422

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Por medio del presente reivindico el beneficio bajo la sección U.S.C. §120 de las siguientes aplicaciones para patentes, Hasta el momento como el sujeto asunto de cada una de las reivindicaciones de la manera requerida por la sección 35 U.S.C. § 112, primer párrafo. Yo acepto el deber de revelar a la Oficina de Patentes y Marca Registradas toda la información que conozco sobre el material para patentar como se define en la sección 37 C.F.R,. que existiese entre la fecha de depósito de las aplicaciones previas y la fecha nacional de depósito o PCT de esta aplicación.

Depósito: Abril 11 de 1997

Por medio del presente certifico también el beneficio bajo el título 3 del Código de los Estados Unidos § 119 (e) de cualquier aplicación provisional en los Estados Unidos enumerada a continuación.

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Por medio del presente designo a los siguientes Abogados y/o Agentes para hacer los trámites de esta aplicación y cualquier continuación o división basados en el sujeto de la referencia y para actuar en todos los negocios en la Oficina de Patentes y de Marcas Registradas conectada con el asunto:

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Ciudadanía

Canadá y Taiwan

Además declaro que las declaraciones contenidas en este instrumento de mi conocimiento propio son correctas y verdaderas y que todas las indicaciones efectuadas, creo que son correctas; además que estas declaraciones las efectué consciente de las implicaciones legales que acarrea un juramento en falso, bajo la sección 1001 del Título 18 del Código de los Estados Unidos de América, y que dicho juramento en falso, acarrearía la invalidez de la aplicación para cualquier patente.

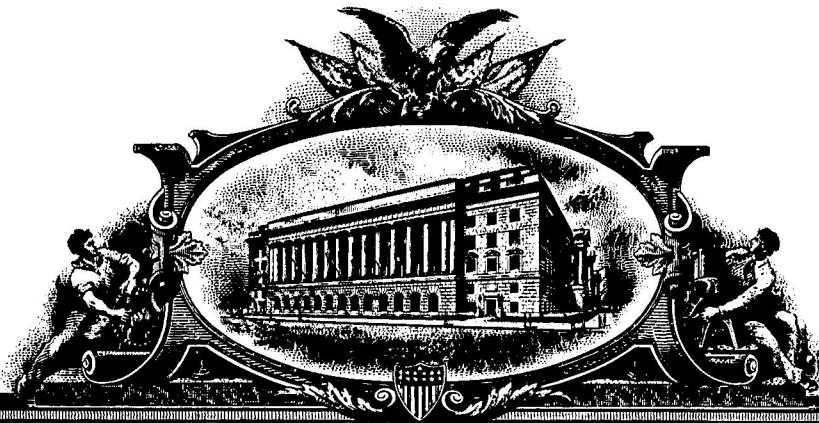
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UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

December 13, 1999

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 09/227,613

FILING DATE: *January 08, 1999*



**By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS**

T. Wallace
T. WALLACE
Certifying Officer

01/08/99

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UTILITY PATENT APPLICATION TRANSMITTAL (Only for new nonprovisional applications under 37 CFR 1.53(b))	Attorney Docket No.	6295.US.P1	Total Pages	127
	First Named Inventor or Application Identifier			
	Pradip Mukerji et al.			
	Express Mail Label No.	EE835516264US		

APPLICATION ELEMENTS See MPEP chapter 600 concerning utility patent application contents.	ADDRESS TO: Assistant Commissioner for Patents, Box Patent Application Washington, DC 20231
1. ☒ Fee Transmittal Form (Submit an original, and a duplicate for fee processing)	6. ☐ Microfiche Computer Program (Appendix)
2. ☒ Specification [Total Pages 82] (preferred arrangement set forth below) - Descriptive title of the invention - Cross References to Related Applications - Statement Regarding Fed sponsored R & D - Reference to Microfiche Appendix - Background of the Invention - Brief Summary of the Invention - Brief Description of the Drawings (if filed) - Detailed Description - Claim(s) - Abstract of the Disclosure	7. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary) a. ☐ Computer Readable Copy b. ☐ Paper Copy (identical to computer copy) c. ☐ Statement verifying identity of above copies
3. ☒ Drawing(s) (35 USC 113) [Total Sheets 40]	ACCOMPANYING APPLICATION PARTS 8. ☐ Assignment Papers (cover sheet & document(s)) 9. ☐ 37 CFR 3.73(b) Statement ☐ Power of Attorney (when there is an assignee) 10. ☐ English Translation Document (if applicable) 11. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations 12. ☐ Preliminary Amendment 13. ☒ Return Receipt Postcard (MPEP 503) (Should be specifically itemized) 14. ☐ Small Entity ☐ Statement filed in prior application, Statement(s) Status still proper and desired 15. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed) 16. ☒ Other: <u>Unexecuted Declaration and</u> <u>Power of Attorney (2 pages)</u>
4. Oath or Declaration [Total Pages] a. ☐ Newly executed (original or copy) b. ☐ Copy from a prior application (37 CFR 1.63(d)) (for continuation/divisional with Box 17 completed) [Note Box 5 below] i. ☐ DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).	
5. ☐ Incorporation By Reference (useable if Box 4b is checked) The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.	

17. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information:
☐ Continuation ☐ Divisional ☒ Continuation-in-part (CIP) of prior application No. See 1 in Addendum

18. CORRESPONDENCE ADDRESS					
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Addendum

1. The subject application is a Continuation-In-Part of pending International Application PCT/US98/07422 filed on April 10, 1998 (which designates the U.S.) which is a Continuation-In-Part of pending U.S. Patent Application Serial No. 08/833,610 filed on April 11, 1997.

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HUMAN DESATURASE GENE AND USES THEREOF

The subject application is a Continuation-In-Part of pending International Application PCT/US98/07422 filed on April 10, 1998 (which designates the U.S.) which is a Continuation-In-Part of pending U.S. Patent Application Serial No. 08/833,610 filed on April 11, 1997.

BACKGROUND OF THE INVENTIONTechnical Field

The subject invention relates to the identification and isolation of a gene that encodes an enzyme (i.e., human $\Delta 5$ -desaturase) involved in the synthesis of polyunsaturated fatty acids and to uses thereof. In particular, $\Delta 5$ -desaturase catalyzes the conversion of, for example, dihomo- γ -linolenic acid (DGLA) to arachidonic acid (AA) and (n-3)-eicosatetraenoic acid (20:4n-3) to eicosapentaenoic acid (20:5n-3). The converted product may then be utilized as a substrate in the production of other polyunsaturated fatty acids (PUFAs). The product or other polyunsaturated fatty acids may be added to pharmaceutical compositions, nutritional composition, animal feeds as well as other products such as cosmetics.

Background Information

Desaturases are critical in the production of long-chain polyunsaturated fatty acids which have many important functions. For example, PUFAs are important components of the plasma membrane of a cell, where they are found in the form of phospholipids. They also serve as precursors to mammalian prostacyclins, eicosanoids, leukotrienes and prostaglandins. Additionally, PUFAs are necessary for the proper development of the developing infant brain as well as for tissue formation and

repair. In view of the biological significance of PUFAs, attempts are being made to produce them, as well as intermediates leading to their production, in an efficient manner.

5 A number of enzymes are involved in PUFA biosynthesis including $\Delta 5$ -desaturase (see Figure 11). For example, elongase (elo) catalyzes the conversion of γ -linolenic acid (GLA) to dihomogamma-linolenic acid (DGLA) and of stearidonic acid (18:4n-3) to (n-3)-eicosatetraenoic acid (20:4n-3). Linoleic acid (LA, 18:2- $\Delta 9,12$ or 18:2n-6) is produced from oleic acid (18:1- $\Delta 9$) by a $\Delta 12$ -desaturase. GLA (18:3- $\Delta 6,9,12$) is produced from linoleic acid by a $\Delta 6$ -desaturase.

10 It must be noted that animals cannot desaturate beyond the $\Delta 9$ position and therefore cannot convert oleic acid into linoleic acid. Likewise, α -linolenic acid (ALA, 18:3- $\Delta 9,12,15$) cannot be synthesized by mammals. However, α -linolenic acid can be converted to stearidonic acid (STA, 18:4- $\Delta 6,9,12,15$) by a $\Delta 6$ -desaturase (see PCT publication WO 96/13591 and The FASEB Journal, Abstracts, Part I, Abstract 3093, page A532 (Experimental Biology 98, San Francisco, CA, April 18-22, 1998) see also U.S. Patent No. 5,552,306), followed by elongation to (n-3)-eicosatetraenoic acid (20:4- $\Delta 8,11,14,17$) in mammals and algae. This polyunsaturated fatty acid (i.e., 20:4- $\Delta 8,11,14,17$) can then be converted to eicosapentaenoic acid (EPA, 20:5- $\Delta 5,8,11,14,17$) by a $\Delta 5$ -desaturase, such as that of the present invention. Other eukaryotes, including fungi and plants, have enzymes which desaturate at carbon 12 (see PCT publication WO 94/11516 and U.S. Patent No. 5,443,974) and carbon 15 (see PCT publication WO 93/11245). The major polyunsaturated fatty acid of animals therefore are either derived from diet and/or from
30 desaturation and elongation of linoleic acid or α -linolenic acid.

In view of these difficulties, it is of significant interest to isolate genes involved in PUFA synthesis from species that naturally produce these fatty acids and to express these genes in a microbial, plant, or animal system which can be altered to provide production of commercial quantities of one or more PUFAs. One of the most important long chain PUFAs, noted above, is arachidonic acid (AA). AA is found in filamentous fungi and can also be purified from mammalian tissues including the liver and adrenal glands. As noted above, AA production from dihomogamma-linolenic acid is catalyzed by a $\Delta 5$ -desaturase. EPA is another important long-chain PUFA. EPA is found in fungi and also in marine oils. As noted above, EPA is produced from (n-3)-eicosatetraenoic acid and is catalyzed by a $\Delta 5$ -desaturase.

In view of the above discussion, there is a definite need for the $\Delta 5$ -desaturase enzyme, the gene encoding this enzyme, as well as recombinant methods of producing this enzyme. Additionally, a need exists for oils containing levels of PUFAs beyond those naturally present as well as those enriched in novel PUFAs. Such oils can only be made by isolation and expression of the $\Delta 5$ -desaturase gene.

All U.S. patents and publications referred to herein are hereby incorporated in their entirety by reference.

SUMMARY OF THE INVENTION

The present invention includes an isolated nucleotide sequence corresponding to or complementary to at least about 50% of the nucleotide sequence shown in SEQ ID NO:1 (Figure 12). The isolated nucleotide sequence may be represented by SEQ ID NO:1. These sequences may encode a functionally active desaturase which utilizes a polyunsaturated fatty acid as a

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substrate. The sequences may be derived from a mammal such as, for example, a human.

The present invention also includes purified proteins encoded by the nucleotide sequences referred to above.

5 Additionally, the present invention includes a purified polypeptide which desaturates polyunsaturated fatty acids at carbon 5 and has at least about 50% amino acid similarity to the amino acid sequence of the purified proteins referred to directly above.

10 Furthermore, the present invention also encompasses a method of producing a human $\Delta 5$ -desaturase. This method comprises the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:1 (Figure 12); b) constructing a vector comprising: i) the isolated nucleotide sequence operably linked to ii) a promoter; and c) introducing the vector into a host cell under time and conditions sufficient for expression of the human $\Delta 5$ -desaturase. The host cell may be, for example, a eukaryotic cell or a prokaryotic cell. In particular, the prokaryotic cell may be, for example, E. coli, cyanobacteria or B. subtilis. The eukaryotic cell may be, for example, a mammalian cell, an insect cell, a plant cell or a fungal cell (e.g., a yeast cell such as Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Candida spp., Lipomyces starkey, Yarrowia lipolytica, Kluyveromyces spp., Hansenula spp., Trichoderma spp. or Pichia spp.).

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Additionally, the present invention also encompasses a vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:1 (Figure 12) operably linked to b) a promoter. The invention also includes a host cell comprising this vector.

30 The host cell may be, for example, a eukaryotic cell or a prokaryotic cell. Suitable eukaryotic cells and prokaryotic cells are as defined above.

Moreover, the present invention also includes a plant cell, plant or plant tissue comprising the above vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid by the plant cell, plant or plant tissue. The polyunsaturated fatty acid may be, for example, selected from the group consisting of AA and EPA. The invention also includes one or more plant oils or acids expressed by the above plant cell, plant or plant tissue.

Additionally, the present invention also encompasses a transgenic plant comprising the above vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in seeds of the transgenic plant.

Also, the invention includes a mammalian cell comprising the above vector wherein expression of the nucleotide sequence of the vector results in production of altered levels of AA or EPA when the cell is grown in a culture media comprising a fatty acid selected from the group consisting of an essential fatty acid, LA and ALA.

It should also be noted that the present invention encompasses a transgenic, non-human mammal whose genome comprises a DNA sequence encoding a human $\Delta 5$ -desaturase operably linked to a promoter. The DNA sequence may be represented by SEQ ID NO:1 (Figure 12). Additionally, the present invention includes a fluid (e.g., milk) produced by the transgenic, non-human mammal wherein the fluid comprises a detectable level of at least human $\Delta 5$ -desaturase.

Additionally, the present invention includes a method (i.e., "first" method) for producing a polyunsaturated fatty acid comprising the steps of: a) isolating the nucleotide sequence represented by SEQ ID NO:1 (Figure 12); b) constructing a vector comprising the isolated nucleotide sequence; c)

introducing the vector into a host cell under time and conditions sufficient for expression of the human $\Delta 5$ -desaturase enzyme; and d) exposing the expressed human $\Delta 5$ -desaturase enzyme to a substrate polyunsaturated fatty acid in order to convert the substrate to a product polyunsaturated fatty acid. The substrate polyunsaturated fatty acid may be, for example, DGLA or 20:4n-3 and the product polyunsaturated fatty acid may be, for example, AA or EPA, respectively. This method may further comprise the step of exposing the product polyunsaturated fatty acid to an elongase in order to convert the product polyunsaturated fatty acid to another polyunsaturated fatty acid (i.e., "second" method). In this method containing the additional step (i.e., "second" method), the product polyunsaturated fatty acid may be, for example, AA or EPA, and the "another" polyunsaturated fatty acid may be adrenic acid or (n-3)-docosapentaenoic acid, respectively. The method containing the additional step may further comprise a step of exposing the another polyunsaturated fatty acid to an additional desaturase in order to convert the another polyunsaturated fatty acid to a final polyunsaturated fatty acid (i.e., "third" method). The final polyunsaturated fatty acid may be, for example, (n-6)-docosapentaenoic acid or docosahexaenoic (DHA) acid.

The present invention also encompasses a nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the "first" method, another polyunsaturated fatty acid produced according to the "second" method, and the final polyunsaturated fatty acid produced according to the "third" method. The product polyunsaturated fatty acid may be, for example, AA or EPA.

The another polyunsaturated fatty acid may be, for example, adrenic acid or (n-3)-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, (n-6)-docosapentaenoic acid or DHA. This nutritional composition, may be, for example, an infant formula, a dietary supplement or a dietary substitute and may be administered to a human or to an animal. It may be administered enterally or parenterally. The nutritional composition may further comprise at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and protein hydrolysates. Additionally, the composition may further comprise at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.

Furthermore, the present invention also includes a pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the "first" method, the another polyunsaturated fatty acid produced according to the "second" method, and the final polyunsaturated fatty acid produced according to the "third" method and 2) a pharmaceutically acceptable carrier. Again, the pharmaceutical composition may be administered to a human or to an animal. The composition may further comprise an element selected from the group consisting of a vitamin, a mineral, a carbohydrate, an amino acid, a free fatty acid, a phospholipid, an antioxidant, and a phenolic compound.

Additionally, the present invention includes an animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the first method, the another

5 polyunsaturated fatty acid produced according to the second method and the final polyunsaturated fatty acid produced according to the third method. The product polyunsaturated fatty acid may be, for example, AA or EPA. The another polyunsaturated fatty acid may be, for example, adrenic acid or
10 (n-3)-docosapentaenoic acid. The final polyunsaturated fatty acid may be, for example, (n-6)-docosapentaenoic acid or DHA.

Moreover, the present invention also includes a cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of the product polyunsaturated fatty acid produced according to the first method, the another polyunsaturated fatty acid produced according to the second method, and the final polyunsaturated fatty acid produced according to the third method.

Additionally, the present invention encompasses a method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to the patient the nutritional composition of above in an amount sufficient to effect prevention or treatment.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 outlines the sections of the *M. alpina* Δ5- and Δ6-desaturases, the clone ID's from the LifeSeq database to which those sections had homology, and the keyword associated with the
30 clone ID's.

Figure 2 represents the contig 2692004.

Figure 3 represents the contig 2153526.

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Figure 17 illustrates the amino acid sequence identity between the putative human desaturase gene in pRAE-7 and the *M. alpina* $\Delta 6$ -desaturase.

Figure 18 illustrates the amino acid sequence identity between the putative human desaturase gene in pRAE-7 and the contig 2535.

5 Figure 19 illustrates the amino acid sequence identity between the putative human desaturase gene in pRAE-7 and the contig 38.

Figure 20 illustrates the amino acid sequence identity between the N-terminus of clone A-1, a representative of Group 1, and the N-terminus of cytochrome b5 gene.

10 Figure 21 illustrates the nucleotide sequence identity between the nucleotide sequence of a portion of clone A-1 and a portion of the GenBank sequence ac004228.

Figure 22 represents the nucleotide sequence identity between the nucleotide sequence of a portion of clone 3-5 of Group 2 and a portion of the GenBank sequence ac004228. Clone 3-5 has an ATG within a *NcoI* site, but translates four stops between the ATG and the *BamHI* site.

Figure 23 represents the nucleotide sequence identity between the nucleotide sequence of a portion of clone A-10 of Group 3 and a portion of the GenBank sequence ac004228. Clone A-10 has an ATG 135 bp upstream of the *BamHI* site, giving an open reading frame of 1267 bp.

Figure 24 represents the nucleotide sequence identity between the nucleotide sequence of a portion of clone A-16 of 25 Group 4 and a portion of the GenBank sequence ac004228. Clone A-16 does not have an ATG; however, there is an ATG (underlined) upstream of where the sequence aligns with ac004228.

Figure 25 represents the nucleotide sequence identity between the nucleotide sequence of a portion of clone A-19 of 30 Group 5 and a portion of the GenBank sequence ac004228. Clone A-19 does not have an ATG; however, this clone matches the ac004228 sequence even upstream of the *BamHI* site.

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Figure 26 represents the partial nucleotide sequence of the GenBank sequence ac004228 and the representative clones from the five Groups.

5 Figure 27 represents the nucleotide sequence identity between the human $\Delta 5$ -desaturase and contig 3381584.

Figure 28 represents the nucleotide sequence identity between the human $\Delta 5$ -desaturase and contig 2153526.

Figure 29 represents the amino acid sequence identity between the human $\Delta 5$ -desaturase and contig 253538a.

10 Figure 30 represents the amino acid sequence identity between the human $\Delta 5$ -desaturase and contig 38.

Figure 31 represents the amino acid sequence identity between the *M. alpina* $\Delta 6$ -desaturase (Ma524) and the human the $\Delta 5$ -desaturase.

5 Figure 32 represents the amino acid sequence identity between the *M. alpina* $\Delta 5$ -desaturase (Ma29) and the human $\Delta 5$ -desaturase.

Figure 33 illustrates the human $\Delta 5$ -desaturase activity of the gene in clone pRAE-28-5, compared to that in pRAE-26-1, pRAE-33, and pRAE-35, when expressed in baker's yeast.

Figure 34 illustrates the substrate specificity of the human $\Delta 5$ -desaturase gene in clone pRAE-28-5, converting DGLA(20:3n-6) to AA(20:4n-6), when the gene is expressed in baker's yeast.

25 DETAILED DESCRIPTION OF THE INVENTION

The subject invention relates to the nucleotide and amino acid sequence of the $\Delta 5$ -desaturase gene derived from humans. Furthermore, the subject invention also includes uses of the gene and of the enzyme encoded by this gene. For example, the
30 gene and corresponding enzyme may be used in the production of polyunsaturated fatty acids such as, for instance, arachidonic

acid, eicosapentaenoic acid, and/or adrenic acid which may be added to pharmaceutical compositions, nutritional compositions and to other valuable products.

5 The Human $\Delta 5$ -Desaturase Gene and Enzyme Encoded Thereby

As noted above, the enzyme encoded by the human $\Delta 5$ -desaturase gene is essential in the production of highly unsaturated polyunsaturated fatty acids having a length greater than 20 carbons. The nucleotide sequence of the isolated human $\Delta 5$ -desaturase gene is shown in Figure 2, and the amino acid sequence of the corresponding purified protein is shown in Figure 3.

As an example, the isolated human $\Delta 5$ -desaturase gene of the present invention converts DGLA to AA or converts 20:4n-3 to EPA. Thus, neither AA nor EPA, for example, can be synthesized without the $\Delta 5$ -desaturase gene (e.g., human or *M. alpina*) and enzyme encoded thereby.

It should be noted that the present invention also encompasses nucleotide sequences (and the corresponding encoded proteins) having sequences corresponding to or complementary to at least about 50%, preferably at least about 60%, and more preferably at least about 70% of the nucleotides in sequence to SEQ ID NO:1 (i.e., the nucleotide sequence of the human $\Delta 5$ -desaturase gene described herein (see Figure 12)). Such sequences may be derived from non-human sources (e.g., *C. elegans* or mouse). Furthermore, the present invention also encompasses fragments and derivatives of the nucleotide sequence of the present invention (i.e., SEQ ID NO:1), as well as of the sequences derived from non-human sources, and having the above-described complementarity or correspondence. Functional equivalents of the above-sequences (i.e., sequences having human

Δ5-desaturase activity) are also encompassed by the present invention. The invention also includes a purified polypeptide which desaturates polyunsaturated fatty acids at the carbon 5 position and has at least about 50% amino acid similarity to the amino acid sequence of the above-noted proteins which are, in turn, encoded by the above-described nucleotide sequences.

The present invention also encompasses an isolated nucleotide sequence which encodes PUFA desaturase activity and that is hybridizable, under moderately stringent conditions, to a nucleic acid having a nucleotide sequence corresponding to or complementary to the nucleotide sequence represented by SEQ ID NO:1 and shown in Figure 12. A nucleic acid molecule is "hybridizable" to another nucleic acid molecule when a single-stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and ionic strength (see Sambrook et al., "Molecular Cloning: A Laboratory Manual, Second Edition (1989), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York)). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. "Hybridization" requires that two nucleic acids contain complementary sequences. However, depending on the stringency of the hybridization, mismatches between bases may occur. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation. Such variables are well known in the art. More specifically, the greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acids having those sequences. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (see Sambrook et al., *supra*). For hybridization with shorter nucleic acids, the position of mismatches becomes more

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important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., *supra*).

Production of the Human $\Delta 5$ -Desaturase Enzyme

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Once the gene encoding the human $\Delta 5$ -desaturase enzyme has been isolated, it may then be introduced into either a prokaryotic or eukaryotic host cell through the use of a vector or construct.

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The vector, for example, a bacteriophage, cosmid or plasmid, may comprise the nucleotide sequence encoding the human $\Delta 5$ -desaturase enzyme as well as any promoter which is functional in the host cell and is able to elicit expression of the human $\Delta 5$ -desaturase encoded by the nucleotide sequence. The promoter is in operable association with or operably linked to the nucleotide sequence. (A promoter is said to be "operably linked" with a coding sequence if the promoter affects transcription or expression of the coding sequence.) Suitable promoters include, for example, those from genes encoding alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglucosomerase, phosphoglycerate kinase, acid phosphatase, T7, TPI, lactase, metallothionein, cytomegalovirus immediate early, whey acidic protein, glucoamylase, and promoters activated in the presence of galactose, for example, GAL1 and GAL10. Additionally, nucleotide sequences which encode other proteins, oligosaccharides, lipids, etc. may also be included within the vector as well as other regulatory sequences such as a polyadenylation signal (e.g., the poly-A signal of SV-40T-antigen, ovalalbumin or bovine growth hormone). The choice of sequences present in the construct is dependent upon the desired expression products as well as the nature of the host cell.

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As noted above, once the vector has been constructed, it may then be introduced into the host cell of choice by methods known to those of ordinary skill in the art including, for example, transfection, transformation and electroporation (see 5 Molecular Cloning: A Laboratory Manual, 2nd ed., Vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press (1989)). The host cell is then cultured under suitable conditions permitting expression of the desired PUFA which is then recovered and purified.

10 Examples of suitable prokaryotic host cells include, for example, bacteria such as Escherichia coli, Bacillus subtilis as well as cyanobacteria such as Spirulina spp. (i.e., blue-green algae). Examples of suitable eukaryotic host cells include, for example, mammalian cells, plant cells, yeast cells such as 5 Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Lipomyces starkey, Candida spp. such as Yarrowia (Candida) lipolytica, Kluyveromyces spp., Pichia spp., Trichoderma spp. or Hansenula spp., or fungal cells such as filamentous fungal cells, for example, Aspergillus, Neurospora and Penicillium. 20 Preferably, Saccharomyces cerevisiae (baker's yeast) cells are utilized.

Expression in a host cell can be accomplished in a transient or stable fashion. Transient expression can occur from introduced constructs which contain expression signals 25 functional in the host cell, but which constructs do not replicate and rarely integrate in the host cell, or where the host cell is not proliferating. Transient expression also can be accomplished by inducing the activity of a regulatable promoter operably linked to the gene of interest, although such 30 inducible systems frequently exhibit a low basal level of expression. Stable expression can be achieved by introduction of a construct that can integrate into the host genome or that

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autonomously replicates in the host cell. Stable expression of the gene of interest can be selected for through the use of a selectable marker located on or transfected with the expression construct, followed by selection for cells expressing the
5 marker. When stable expression results from integration, the site of the construct's integration can occur randomly within the host genome or can be targeted through the use of constructs containing regions of homology with the host genome sufficient to target recombination with the host locus. Where constructs
10 are targeted to an endogenous locus, all or some of the transcriptional and translational regulatory regions can be provided by the endogenous locus.

A transgenic mammal may also be used in order to express the enzyme of interest (i.e., the human $\Delta 5$ -desaturase), and
5 ultimately the PUFA(s) of interest. More specifically, once the above-described construct is created, it may be inserted into the pronucleus of an embryo. The embryo may then be implanted into a recipient female. Alternatively, a nuclear transfer method could also be utilized (Schnieke et al., Science
10 278:2130-2133 (1997)). Gestation and birth are then permitted (see, e.g., U.S. Patent No. 5,750,176 and U.S. Patent No. 5,700,671). Milk, tissue or other fluid samples from the offspring should then contain altered levels of PUFAs, as compared to the levels normally found in the non-transgenic
25 animal. Subsequent generations may be monitored for production of the altered or enhanced levels of PUFAs and thus incorporation of the gene encoding the human $\Delta 5$ -desaturase enzyme into their genomes. The mammal utilized as the host may be selected from the group consisting of, for example, a mouse,
30 a rat, a rabbit, a pig, a goat, a sheep, a horse and a cow. However, any mammal may be used provided it has the ability to incorporate DNA encoding the enzyme of interest into its genome.

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For expression of a human $\Delta 5$ -desaturase polypeptide, functional transcriptional and translational initiation and termination regions are operably linked to the DNA encoding the desaturase polypeptide. Transcriptional and translational

5 initiation and termination regions are derived from a variety of nonexclusive sources, including the DNA to be expressed, genes known or suspected to be capable of expression in the desired system, expression vectors, chemical synthesis, or from an endogenous locus in a host cell. Expression in a plant tissue

10 and/or plant part presents certain efficiencies, particularly where the tissue or part is one which is harvested early, such as seed, leaves, fruits, flowers, roots, etc. Expression can be targeted to that location with the plant by utilizing specific regulatory sequence such as those of U.S. Patent Nos. 5,463,174, 4,943,674, 5,106,739, 5,175,095, 5,420,034, 5,188,958, and 5,589,379. Alternatively, the expressed protein can be an enzyme which produces a product which may be incorporated, either directly or upon further modifications, into a fluid fraction from the host plant. Expression of a human $\Delta 5$ -

15 desaturase gene, or antisense human $\Delta 5$ -desaturase transcripts, can alter the levels of specific PUFAs, or derivatives thereof, found in plant parts and/or plant tissues. The human $\Delta 5$ -desaturase polypeptide coding region may be expressed either by itself or with other genes, in order to produce tissues and/or

25 plant parts containing higher proportions of desired PUFAs or in which the PUFA composition more closely resembles that of human breast milk (Prieto et al., PCT publication WO 95/24494). The termination region may be derived from the 3' region of the gene from which the initiation region was obtained or from a

30 different gene. A large number of termination regions are known to and have been found to be satisfactory in a variety of hosts from the same and different genera and species. The termination

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region usually is selected as a matter of convenience rather than because of any particular property.

As noted above, a plant (e.g., Glycine max (soybean) or Brassica napus (canola)) or plant tissue may also be utilized as a host or host cell, respectively, for expression of the human $\Delta 5$ -desaturase enzyme which may, in turn, be utilized in the production of polyunsaturated fatty acids. More specifically, desired PUFAS can be expressed in seed. Methods of isolating seed oils are known in the art. Thus, in addition to providing a source for PUFAS, seed oil components may be manipulated through the expression of the human $\Delta 5$ -desaturase gene, as well as perhaps other desaturase genes and elongase genes, in order to provide seed oils that can be added to nutritional compositions, pharmaceutical compositions, animal feeds and cosmetics. Once again, a vector which comprises a DNA sequence encoding the human $\Delta 5$ -desaturase operably linked to a promoter, will be introduced into the plant tissue or plant for a time and under conditions sufficient for expression of the human $\Delta 5$ -desaturase gene. The vector may also comprise one or more genes that encode other enzymes, for example, $\Delta 4$ -desaturase, elongase, $\Delta 6$ -desaturase, $\Delta 12$ -desaturase, $\Delta 15$ -desaturase, $\Delta 17$ -desaturase, and/or $\Delta 19$ -desaturase. The plant tissue or plant may produce the relevant substrate (e.g., DGLA, GLA, EPA, 20:4n-3, etc.) upon which the enzymes act or a vector encoding enzymes which produce such substrates may be introduced into the plant tissue, plant cell or plant. In addition, substrate may be sprayed on plant tissues expressing the appropriate enzymes. Using these various techniques, one may produce PUFAS (e.g., n-6 unsaturated fatty acids such as AA, or n-3 fatty acids such as EPA or DHA) by use of a plant cell, plant tissue or plant. It should also be noted that the invention also encompasses a transgenic plant

comprising the above-described vector, wherein expression of the nucleotide sequence of the vector results in production of a polyunsaturated fatty acid in, for example, the seeds of the transgenic plant.

5 The substrates which may be produced by the host cell either naturally or transgenically, as well as the enzymes which may be encoded by DNA sequences present in the vector which is subsequently introduced into the host cell, are shown in Figure 11.

10 In view of the above, the present invention encompasses a method of producing the human $\Delta 5$ -desaturase enzyme comprising the steps of: 1) isolating the nucleotide sequence of the gene encoding human $\Delta 5$ -desaturase enzyme; 2) constructing a vector comprising said nucleotide sequence; and 3) introducing said vector into a host cell under time and conditions sufficient for the production of the desaturase enzyme.

The present invention also encompasses a method of producing polyunsaturated fatty acids comprising exposing an acid to the human $\Delta 5$ -desaturase enzyme such that the desaturase converts the acid to a polyunsaturated fatty acid. For example, when 20:3n-6 is exposed to human $\Delta 5$ -desaturase enzyme, it is converted to AA. AA may then be exposed to elongase which elongates the AA to adrenic acid (i.e., 22:4n-6).

Alternatively, human $\Delta 5$ -desaturase may be utilized to convert
25 20:4n-3 to 20:5n-3 which may be exposed to elongase and converted to (n-3)-docosapentaenoic acid. The (n-3)-docosapentaenoic acid may then be converted to DHA by use of $\Delta 4$ -desaturase. Thus, human $\Delta 5$ -desaturase may be used in the production of polyunsaturated fatty acids which may be used, in
30 turn, for particular beneficial purposes.

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213Uses of the Human $\Delta 5$ -Desaturase Gene and Enzyme Encoded Thereby

As noted above, the isolated human $\Delta 5$ -desaturase gene and the desaturase enzyme encoded thereby have many uses. For example, the gene and corresponding enzyme may be used indirectly or directly in the production of polyunsaturated fatty acids, for example, AA, adrenic acid or EPA. ("Directly" is meant to encompass the situation where the enzyme directly converts the acid to another acid, the latter of which is utilized in a composition (e.g., the conversion of DGLA to AA). "Indirectly" is meant to encompass the situation where an acid is converted to another acid (i.e., a pathway intermediate) by the desaturase (e.g., DGLA to AA) and then the latter acid is converted to another acid by use of a non-desaturase enzyme (e.g., AA to adrenic acid by elongase or by use of another desaturase enzyme (e.g., AA to EPA by $\Delta 17$ -desaturase.)). These polyunsaturated fatty acids (i.e., those produced either directly or indirectly by activity of the desaturase enzyme) may be added to, for example, nutritional compositions, pharmaceutical compositions, cosmetics, and animal feeds, all of which are encompassed by the present invention. These uses are described, in detail, below.

Nutritional Compositions

The present invention includes nutritional compositions. Such compositions, for purposes of the present invention, include any food or preparation for human consumption including for enteral or parenteral consumption, which when taken into the body (a) serve to nourish or build up tissues or supply energy and/or (b) maintain, restore or support adequate nutritional status or metabolic function.

The nutritional composition of the present invention comprises at least one oil or acid produced directly or indirectly by use of the human $\Delta 5$ -desaturase gene, in accordance with the present invention, and may either be in a solid or liquid form. Additionally, the composition may include edible macronutrients, vitamins and minerals in amounts desired for a particular use. The amount of such ingredients will vary depending on whether the composition is intended for use with normal, healthy infants, children or adults having specialized needs such as those which accompany certain metabolic conditions (e.g., metabolic disorders).

Examples of macronutrients which may be added to the composition include but are not limited to edible fats, carbohydrates and proteins. Examples of such edible fats include but are not limited to coconut oil, soy oil, and mono- and diglycerides. Examples of such carbohydrates include but are not limited to glucose, edible lactose and hydrolyzed starch. Additionally, examples of proteins which may be utilized in the nutritional composition of the invention include but are not limited to soy proteins, electrodialysed whey, electrodialysed skim milk, milk whey, or the hydrolysates of these proteins.

With respect to vitamins and minerals, the following may be added to the nutritional compositions of the present invention: calcium, phosphorus, potassium, sodium, chloride, magnesium, manganese, iron, copper, zinc, selenium, iodine, and Vitamins A, E, D, C, and the B complex. Other such vitamins and minerals may also be added.

The components utilized in the nutritional compositions of the present invention will be of semi-purified or purified origin. By semi-purified or purified is meant a material which

has been prepared by purification of a natural material or by synthesis.

Examples of nutritional compositions of the present invention include but are not limited to infant formulas, dietary supplements, dietary substitutes, and rehydration compositions. Nutritional compositions of particular interest include but are not limited to those utilized for enteral and parenteral supplementation for infants, specialist infant formulas, supplements for the elderly, and supplements for those with gastrointestinal difficulties and/or malabsorption.

The nutritional composition of the present invention may also be added to food even when supplementation of the diet is not required. For example, the composition may be added to food of any type including but not limited to margarines, modified butters, cheeses, milk, yogurt, chocolate, candy, snacks, salad oils, cooking oils, cooking fats, meats, fish and beverages.

In a preferred embodiment of the present invention, the nutritional composition is an enteral nutritional product, more preferably, an adult or pediatric enteral nutritional product. This composition may be administered to adults or children experiencing stress or having specialized needs due to chronic or acute disease states. The composition may comprise, in addition to polyunsaturated fatty acids produced in accordance with the present invention, macronutrients, vitamins and minerals as described above. The macronutrients may be present in amounts equivalent to those present in human milk or on an energy basis, i.e., on a per calorie basis.

Methods for formulating liquid or solid enteral and parenteral nutritional formulas are well known in the art. (See also the Examples below.)

The enteral formula, for example, may be sterilized and subsequently utilized on a ready-to-feed (RTF) basis or stored

in a concentrated liquid or powder. The powder can be prepared by spray drying the formula prepared as indicated above, and reconstituting it by rehydrating the concentrate. Adult and pediatric nutritional formulas are well known in the art and are commercially available (e.g., Similac®, Ensure®, Jevity® and Alimentum® from Ross Products Division, Abbott Laboratories, Columbus, Ohio). An oil or acid produced in accordance with the present invention may be added to any of these formulas.

The energy density of the nutritional compositions of the present invention, when in liquid form, may range from about 0.6 Kcal to about 3 Kcal per ml. When in solid or powdered form, the nutritional supplements may contain from about 1.2 to more than 9 Kcals per gram, preferably about 3 to 7 Kcals per gm. In general, the osmolality of a liquid product should be less than 700 mOsm and, more preferably, less than 660 mOsm.

The nutritional formula may include macronutrients, vitamins, and minerals, as noted above, in addition to the PUFAs produced in accordance with the present invention. The presence of these additional components helps the individual ingest the minimum daily requirements of these elements. In addition to the provision of PUFAs, it may also be desirable to add zinc, copper, folic acid and antioxidants to the composition. It is believed that these substance boost a stressed immune system and will therefore provide further benefits to the individual receiving the composition. A pharmaceutical composition may also be supplemented with these elements.

In a more preferred embodiment, the nutritional composition comprises, in addition to antioxidants and at least one PUFA, a source of carbohydrate wherein at least 5 weight percent of the carbohydrate is indigestible oligosaccharide. In a more preferred embodiment, the nutritional composition additionally comprises protein, taurine, and carnitine.

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As noted above, the PUFAs produced in accordance with the present invention, or derivatives thereof, may be added to a dietary substitute or supplement, particularly an infant formula, for patients undergoing intravenous feeding or for preventing or treating malnutrition or other conditions or disease-states. As background, it should be noted that human breast milk has a fatty acid profile comprising from about 0.15% to about 0.36% as DHA, from about 0.03% to about 0.13% as EPA, from about 0.30% to about 0.88% as AA, from about 0.22% to about 0.67% as DGLA, and from about 0.27% to about 1.04% as GLA. Thus, fatty acids such as AA, EPA and/or docosahexaenoic acid (DHA), produced in accordance with the present invention, can be used to alter, for example, the composition of infant formulas in order to better replicate the PUFA content of human breast milk or to alter the presence of PUFAs normally found in a non-human mammal's milk. In particular, a composition for use in a pharmacologic or food supplement, particularly a breast milk substitute or supplement, will preferably comprise one or more of AA, DGLA and GLA. More preferably, the oil will comprise from about 0.3 to 30% AA, from about 0.2 to 30% DGLA, and/or from about 0.2 to about 30% GLA.

Parenteral nutritional compositions comprising from about 2 to about 30 weight percent fatty acids calculated as triglycerides are encompassed by the present invention. The preferred composition has about 1 to about 25 weight percent of the total PUFA composition as GLA (U.S. Patent No. 5,196,198). Other vitamins, particularly fat-soluble vitamins such as vitamin A, D, E and L-carnitine can optionally be included. When desired, a preservative such as alpha-tocopherol may be added in an amount of about 0.1% by weight.

In addition, the ratios of AA, DGLA and GLA can be adapted for a particular given end use. When formulated as a breast

milk supplement or substitute, a composition which comprises one or more of AA, DGLA and GLA will be provided in a ratio of about 1:19:30 to about 6:1:0.2, respectively. For example, the breast milk of animals can vary in ratios of AA:DGLA:GLA ranging from 1:19:30 to 6:1:0.2, which includes intermediate ratios which are preferably about 1:1:1, 1:2:1, 1:1:4. When produced together in a host cell, adjusting the rate and percent of conversion of a precursor substrate such as GLA and DGLA to AA can be used to precisely control the PUFA ratios. For example, a 5% to 10% conversion rate of DGLA to AA can be used to produce an AA to DGLA ratio of about 1:19, whereas a conversion rate of about 75% TO 80% can be used to produce an AA to DGLA ratio of about 6:1. Therefore, whether in a cell culture system or in a host animal, regulating the timing, extent and specificity of human $\Delta 5$ -desaturase expression, as well as the expression of other desaturases and elongases, can be used to modulate PUFA levels and ratios. The PUFAs/acids produced in accordance with the present invention (e.g., AA and EPA) may then be combined with other PUFAs/acids (e.g., GLA) in the desired concentrations and ratios.

Additionally, PUFA produced in accordance with the present invention or host cells containing them may also be used as animal food supplements to alter an animal's tissue or milk fatty acid composition to one more desirable for human or animal consumption.

Pharmaceutical Compositions

The present invention also encompasses a pharmaceutical composition comprising one or more of the acids and/or resulting oils produced using the human $\Delta 5$ -desaturase gene, in accordance with the methods described herein. More specifically, such a

pharmaceutical composition may comprise one or more of the acids and/or oils as well as a standard, well-known, non-toxic pharmaceutically acceptable carrier, adjuvant or vehicle such as, for example, phosphate buffered saline, water, ethanol, polyols, vegetable oils, a wetting agent or an emulsion such as a water/oil emulsion. The composition may be in either a liquid or solid form. For example, the composition may be in the form of a tablet, capsule, ingestible liquid or powder, injectible, or topical ointment or cream. Proper fluidity can be maintained, for example, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. It may also be desirable to include isotonic agents, for example, sugars, sodium chloride and the like. Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening agents, flavoring agents and perfuming agents.

Suspensions, in addition to the active compounds, may comprise suspending agents such as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth or mixtures of these substances.

Solid dosage forms such as tablets and capsules can be prepared using techniques well known in the art. For example, PUFAs produced in accordance with the present invention can be tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders such as acacia, cornstarch or gelatin, disintegrating agents such as potato starch or alginic acid, and a lubricant such as stearic acid or magnesium stearate. Capsules can be prepared by incorporating these excipients into a gelatin capsule along with

antioxidants and the relevant PUFA(s). The antioxidant and PUFA components should fit within the guidelines presented above.

For intravenous administration, the PUFAs produced in accordance with the present invention or derivatives thereof may be incorporated into commercial formulations such as Intralipids™. The typical normal adult plasma fatty acid profile comprises 6.64 to 9.46% of AA, 1.45 to 3.11% of DGLA, and 0.02 to 0.08% of GLA. These PUFAs or their metabolic precursors can be administered alone or in combination with other PUFAs in order to achieve a normal fatty acid profile in a patient. Where desired, the individual components of the formulations may be provided individually, in kit form, for single or multiple use. A typical dosage of a particular fatty acid is from 0.1 mg to 20 g (up to 100 g) daily and is preferably from 10 mg to 1, 2, 5 or 10 g daily.

Possible routes of administration of the pharmaceutical compositions of the present invention include, for example, enteral (e.g., oral and rectal) and parenteral. For example, a liquid preparation may be administered, for example, orally or rectally. Additionally, a homogenous mixture can be completely dispersed in water, admixed under sterile conditions with physiologically acceptable diluents, preservatives, buffers or propellants in order to form a spray or inhalant.

The route of administration will, of course, depend upon the desired effect. For example, if the composition is being utilized to treat rough, dry, or aging skin, to treat injured or burned skin, or to treat skin or hair affected by a disease or condition, it may perhaps be applied topically.

The dosage of the composition to be administered to the patient may be determined by one of ordinary skill in the art and depends upon various factors such as weight of the patient, age of the patient, immune status of the patient, etc.

With respect to form, the composition may be, for example, a solution, a dispersion, a suspension, an emulsion or a sterile powder which is then reconstituted.

The present invention also includes the treatment of various disorders by use of the pharmaceutical and/or nutritional compositions described herein. In particular, the compositions of the present invention may be used to treat restenosis after angioplasty. Furthermore, symptoms of inflammation, rheumatoid arthritis, asthma and psoriasis may also be treated with the compositions of the invention. Evidence also indicates that PUFAs may be involved in calcium metabolism; thus, the compositions of the present invention may, perhaps, be utilized in the treatment or prevention of osteoporosis and of kidney or urinary tract stones.

Additionally, the compositions of the present invention may also be used in the treatment of cancer. Malignant cells have been shown to have altered fatty acid compositions. Addition of fatty acids has been shown to slow their growth, cause cell death and increase their susceptibility to chemotherapeutic agents. Moreover, the compositions of the present invention may also be useful for treating cachexia associated with cancer.

The compositions of the present invention may also be used to treat diabetes (see U.S. Patent No. 4,826,877 and Horrobin et al., Am. J. Clin. Nutr. Vol. 57 (Suppl.) 732S-737S). Altered fatty acid metabolism and composition have been demonstrated in diabetic animals.

Furthermore, the compositions of the present invention, comprising PUFAs produced either directly or indirectly through the use of the human $\Delta 5$ -desaturase enzyme, may also be used in the treatment of eczema, in the reduction of blood pressure, and in the improvement of mathematics examination scores. Additionally, the compositions of the present invention may be

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used in inhibition of platelet aggregation, induction of vasodilation, reduction in cholesterol levels, inhibition of proliferation of vessel wall smooth muscle and fibrous tissue (Brenner et al., Adv. Exp. Med. Biol. Vol. 83, p.85-101, 1976),
5 reduction or prevention of gastrointestinal bleeding and other side effects of non-steroidal anti-inflammatory drugs (see U.S. Patent No. 4,666,701), prevention or treatment of endometriosis and premenstrual syndrome (see U.S. Patent No. 4,758,592), and treatment of myalgic encephalomyelitis and chronic fatigue after
10 viral infections (see U.S. Patent No. 5,116,871).

Further uses of the compositions of the present invention include use in the treatment of AIDS, multiple sclerosis, and inflammatory skin disorders, as well as for maintenance of general health.

Additionally, the composition of the present invention may be utilized for cosmetic purposes. It may be added to pre-existing cosmetic compositions such that a mixture is formed or may be used as a sole composition.

Veterinary Applications

It should be noted that the above-described pharmaceutical and nutritional compositions may be utilized in connection with animals (i.e., domestic or non-domestic), as well as humans, as
25 animals experience many of the same needs and conditions as humans. For example, the oil or acids of the present invention may be utilized in animal feed supplements, animal feed substitutes, animal vitamins or in animal topical ointments.

The present invention may be illustrated by the use of the
30 following non-limiting examples:

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223EXAMPLE IHUMAN DESATURASE GENE SEQUENCES

As described in International Application PCT/US98/07422 (herein incorporated in its entirety by reference), the putative human desaturase gene sequences involved in long chain polyunsaturated fatty acid biosynthesis were isolated based on homology between the human cDNA sequences and *Mortierella alpina* desaturase gene sequences. The three conserved "histidine boxes" known to be conserved among membrane-bound desaturases were found. As with other membrane-bound desaturases, the final HXXHH histidine box motif was found to be QXXHH. The amino acid sequence of the putative human desaturases exhibited homology to *M. alpina* $\Delta 5$ -, $\Delta 6$ -, $\Delta 9$ -, and $\Delta 12$ -desaturases.

The *M. alpina* $\Delta 5$ -desaturase and $\Delta 6$ -desaturase cDNA sequences were used to search the LifeSeq database of Incyte Pharmaceuticals, Inc., Palo Alto, CA. The $\Delta 5$ -desaturase sequence was divided into fragments: 1) amino acid no. 1-150, 2) amino acid no. 151-300, and 3) amino acid no. 301-446. The $\Delta 6$ desaturase sequence was divided into three fragments: 1) amino acid no. 1-150, 2) amino acid no. 151-300, and 3) amino acid no. 301-457. These polypeptide fragments were searched against the database using the "tblastn" algorithm. This algorithm compares a protein query sequence against a nucleotide sequence database dynamically translated in all six reading frames (both strands).

The polypeptide fragments 2 and 3 of *M. alpina* $\Delta 5$ - and $\Delta 6$ -desaturases have homologies with the CloneID sequences as outlined in Figure 1. The CloneID represents an individual sequence from the Incyte LifeSeq database. After the "tblastn" results had been reviewed, Clone Information was searched with the default settings of Stringency of ≥ 50 , and Productscore ≤ 100 for different CloneID numbers. The Clone Information

Results displayed the information including the ClusterID, CloneID, Library, HitID, and Hit Description. When selected, the ClusterID number displayed the clone information of all the clones that belong in that ClusterID. The Assemble command
5 assembled all of the CloneID which comprise the ClusterID. The following default setting were used for GCG (Genetics Computer Group, University of Wisconsin Biotechnology Center, Madison, WI) Assembly:

Word Size: 7; Minimum Overlap: 14; Stringency: 0.8; Minimum
10 Identity: 14; Maximum Gap: 10; Gap Weight: 8; and Length Weight: 2.

GCG Assembly Results displayed the contigs generated on the basis of sequence information within the CloneID. A contig is an alignment of DNA sequences based on areas of homology among
15 these sequences. A new sequence (consensus sequence) was generated based on the aligned DNA sequence within a contig. The contig. containing the CloneID was identified, and the ambiguous sites of the consensus sequence were edited based on the alignment of the CloneIDs (see Figures 2-6) to generate the
20 best possible sequence. The procedure was repeated for all six CloneID listed in Figure 1. This produced five unique contigs. The edited consensus sequences of the 5 contigs were imported into the Sequencher software program (Gene Codes Corporation, Ann Arbor, Michigan). These consensus sequences were assembled.
25 The contig 2511785 overlaps with contig 3506132, and this new contig was called 2535 (Figure 7). The contigs from the Sequencher program were copied into the Sequence Analysis software package of GCG.

Each contig was translated in all six reading frames into
30 protein sequences. The *M. alpina* $\Delta 5$ -desaturase (Ma29) and $\Delta 6$ -desaturase (Ma524) sequences were compared with each of the translated contigs using the FastA search (a Pearson and Lipman

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search for similarity between a query sequence and a group of sequences of the same type (nucleic acid or protein)). Homology among these sequences suggest the open reading frames of each contig as underlined in Figures 3, 5, and 7. The homology among the *M. alpina* $\Delta 5$ - and $\Delta 6$ -desaturase sequences to contigs 2535 and 3854933 were utilized to create the final contig called 253538a (see Figure 8). Figure 9 is the FastA match of the translated sequences of the final contig 253538a and Ma29, and Figure 10 is the FastA match of the translated sequences of the final contig 253538a and Ma524.

Although the open reading frame was generated by merging the two contigs, the contig 2535 shows that there is a unique sequence in the beginning of this contig which does not match with the contig 3854933. Therefore, it is possible that these contigs were generated from independent desaturase-like human genes.

The contig 253538a contains an open reading frame encoding 432 amino acid (Figure 8, underlined). It starts with Gln (CAG) and ends with the stop codon (TGA) (both in bold). The contig 253538a aligns with both *M. alpina* $\Delta 5$ - and $\Delta 6$ -desaturase sequences, suggesting that it could be either of the desaturases, as well as other known desaturases which share homology with each other. The individual contigs listed in Figure 1, as well as the intermediate contig 2535 and the final contig 253538a can be utilized to isolate the complete genes for human desaturases.

Determination of Human $\Delta 5$ -Desaturase Gene Sequence

Primers RO384 and RO388 were designed based on the 5' and 3' sequences, respectively, of contig 2535. The human monocyte cDNA library (Clontech, Palo Alto, CA) was amplified with the vector primer RO329 (5' - CAG ACC AAC TGG TAA TGG TAG - 3') and

RO384 (5' - TCA GGC CCA AGC TGG ATG GCT GCA ACA TG - 3'), and also with the vector primer RO328 (5' - CTC CTG GAG CCC GTC AGT ATC - 3') and RO388 (5' - ATG GTG GGG AAG AGG TGG TGC TCA ATC TG - 3'). Polymerase Chain Reaction (PCR) was carried out in a 100 μ l volume containing: 1 μ l of human monocyte cDNA library, 10 pM each primer, 10 μ l of 10X buffer and 1.0 U of Taq Polymerase. Thermocycler conditions in Perkin Elmer 9600 were as follows: 94 °C for 2 mins, then 30 cycles of 94 °C for 1 min., 58 °C for 2 mins. and 72 °C for 3 mins. PCR was followed by an additional extension at 72 °C for 7 minutes.

The PCR amplified mixture was run on a gel, and the amplified fragments were gel purified. The isolated fragment from PCR amplification with RO329 and RO384 was approximately 900 bp, and that from PCR amplification with RO328 and RO388 was approximately 650 bp. These isolated fragments were filled-in using T4 DNA polymerase, and the filled-in fragments were cloned into the PCR-Blunt vector (Invitrogen Corp., Carlsbad, CA). The clone of RO329/RO384 amplified fragment was designated as pRAE-7, and the clone of RO328/RO388 amplified fragment was designated as pRAE-8. Both ends of the clones were sequenced using ABI 373 DNA Sequencer (Applied Biosystems, Foster City, CA) and assembled using the Sequencer program (a sequence analysis program, Gene Codes Corporation, Ann Arbor, MI). This assembly of the sequences revealed that the two clones contained different sizes of the same gene (Figure 14). The complete sequence of the pRAE-7 gene was compiled (Figure 15) and searched against the known sequences in the public database.

The FastA algorithm is a Pearson and Lipman search for similarity between a query sequence and a group of sequences of the same type (nucleic acid or protein). The pRAE-7 gene sequence was translated in six reading frames, and using this method, the Swissprot database (Genetics Computer Group (GCG)

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(Madison, WI) was searched. The gene in pRAE-7 was identified as a putative human desaturase based on its homology to known desaturases. The Swissprot database search produced matches against the omega-3 fatty acid desaturase from mung bean (23.4% identity in 303 AA overlap), linoleoyl-CoA desaturase from *Synechocystis* sp. (24.3% identity in 280 AA overlap), omega-6 fatty acid desaturase from soybean (19.7% identity in 284 AA overlap), and acyl-CoA desaturase 1 from *Saccharomyces cerevisiae* (21.6% identity in 134 AA overlap). The FastA search against the *M. alpina* desaturases produced matches against the $\Delta 6$ - (31.9% identity in 285 AA overlap), the $\Delta 5$ - (28.4% identity in 292 AA overlap), and the $\Delta 12$ - (23.0% identity in 274 AA overlap) desaturases. The matched sequence alignment of the putative human desaturase gene in pRAE-7 against *M. alpina* $\Delta 5$ -desaturase (Ma29), *M. alpina* $\Delta 6$ -desaturase (Ma524) as well as to the contigs 2535 and 38 are displayed in Figures 16, 17, 18, and 19 respectively.

The contigs 2535, 38, and 253538a were generated based on assemblies of various sequences as well as their homologies against the known desaturases. However, upon examining Figures 18 and 19, it can be concluded that the contigs are merely indications as to what the sequences of the human desaturases might possibly be.

The 5' end of the gene, the ATG (Methionine), is necessary for expressing the human desaturase in yeast. Figures 16 and 17 show that pRAE-7 is probably just the last 2/3 of a desaturase gene. Several of the omega-3 and omega-6 fatty acid desaturases, as well as the linoleoyl-CoA desaturase mentioned above, are smaller than the *M. alpina* $\Delta 5$ - and $\Delta 6$ -desaturases, ranging in sizes of 359-380 amino acids. It was concluded from all of the sequences evaluated thus far that the isolated gene

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All of the clones have the same sequence downstream of the *Bam*HI site (see Figure 12, underlined). But each group represents a different 5' sequence, with a total of 10 clones being too short to be the full length gene. Group 1, represented by clone A-1, is comprised of 5 clones which have homology to cytochrome b5 gene (Figure 20). A translational

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start codon, ATG, is not present in clone A-1; however, as can be seen in Figure 21, there is an ATG (underlined) present in the ac004228 sequence 17 bp upstream of the strong area of homology between A-1 and ac004228. Starting from the strong area of homology, A-1 has an open reading frame of 1318 bp. However, starting from the ATG, the open reading frame is 1335 bp. Group 2, represented by clone 3-5, is comprised of 3 clones which have an ATG within an *NcoI* site, but four translational stop codons between the ATG and the *BamHI* site (Figure 22, the *NcoI*, *BamHI* sites are in bold, and the four termination codons are underlined). Group 3 is comprised of one clone, A-10, which has an ATG 135 bp upstream of the *BamHI* site, giving an open reading frame of 1267 bp (Figure 23). Group 4 is comprised of 2 clones, represented by clone A-16, which does not have an ATG; however, upstream of where the sequence aligns with ac004228, there is an ATG (Figure 24, underlined). The open reading frame of this group is 1347 bp. Group 5 is comprised of one clone which does not have an ATG. However, this clone matches the ac004228 sequence even upstream of the *BamHI* site (Figure 25).

As illustrated in Figure 26, many of the clones from the five groups are represented in order with the ac004228 sequence. There appeared to be a high level of splicing, with the sequence downstream of the *BamHI* site (in bold) acting as the common anchor for the various 5' exons. All of the potential start sites are also in bold, and the sequences found within the clones have been underlined.

The A-1 sequence was used to search the LifeSeq database of Incyte Pharmaceuticals, Inc., Palo Alto, CA, to see if its latest version would also have sequences with homology to our desaturase gene sequence. Two contigs were generated in this search, contig 3381584 and contig 2153526. The human desaturase gene sequence was initially compiled based on sequences from

Group I clones and ac004228. However, Figure 12 represents the actual DNA sequence of the isolated gene. The Incyte contigs were used to confirm this sequence (see Figures 27 and 28). The human desaturase translated sequence, consisting of 445 amino acids (Figure 13), was also matched with the original contigs 253538a and 38. These alignments are shown in Figures 29 and 30, respectively.

The FastA search of the human desaturase gene against the Swissprot database produced matches against the omega-3 fatty acid desaturase from mung bean (22.4% identity in 381 AA overlap), linoleoyl-CoA desaturase from *Synechocystis* Sp. (24.5% identity in 335 AA overlap), omega-6 fatty acid desaturase from soybean (20.3% identity in 290 AA overlap), and acyl-CoA desaturase 1 from *Saccharomyces cerevisiae* (21.4% identity in 168 AA overlap). The FastA search against *M. alpina* desaturases produced matches against the Δ6-(30.5% identity in 455 AA overlap), Δ5-(27.5% identity in 455 AA overlap), and Δ12-desaturases (22.5% identity in 382 AA overlap). The FastA match of the human desaturase translated sequence against the ma524 (*M. alpina* Δ6-desaturase) and ma29 (*M. alpina* Δ5-desaturase) sequences are shown in Figures 31 and 32, respectively.

EXAMPLE II

CONSTRUCTION OF CLONES

New clones were generated based on clones from three of the Groups mentioned above, clones A-1, A-10, and A-16. Two primers which were modified with 5' phosphate, R0526 (5'-CAT GGC CCC CGA CCC GGT GG-3') and R0527 (5'-GCG GCC ACC GGG TCG GGG GC-3'), were annealed together to form an adaptor. This adaptor which has *Nco*I and *Bsa*I overhangs, were ligated with the A-1 clone, which had been cut with *Bsa*I/*Hind*III and gel purified, for 15 min at room temperature. The pYX242(*Nco*I/*Hind*III) vector

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EXAMPLE IIIEXPRESSION OF HUMAN $\Delta 5$ -DESATURASE

The constructs pRAE-26-1, pRAE-28-5, pRAE-33, and pRAE-35 were transformed into *S. cerevisiae* 334 and screened for desaturase activity. The substrates DGLA (20:3n-6), OA(18:1n-9), AA(20:4n-6), and LA(18:2n-6) were used to determine the activity of the expressed gene from constructs pRAE-26-1 and pRAE-28-5. Only the substrate DGLA was used to determine the activity of the expressed gene from all of the constructs. The negative control strain was *S. cerevisiae* 334 containing the unaltered pYX242 vector. The cultures were grown for 48 hours at 30°C, in selective media (Ausubel et al., Short Protocols in Molecular Biology, Ch. 13, P. 3-5 (1992)), in the presence of a particular substrate. Lipid fractions of each culture were extracted for analysis. The desaturase activity results are provided in Figures 33 and 34.

All of the values in Figure 33 are the average of two separate samples per strain, tested in the same run. The substrate, as well as the fatty acid it was converted to, is shown in bold. The expressed gene in the strain 334 (pRAE-28-5) is a $\Delta 5$ -desaturase. It converted the substrate DGLA to a higher percent of AA than the control strain 334(pYX242), 0.127% vs. 0.062%, respectively. The percent of AA present in the cultures of strains 334(pRAE-26-1), 334(pRAE-33), and 334(pRAE-35) are comparable to that of the control strain (0.075%, 0.062%, and 0.063%, respectively). Therefore, it can be concluded that the cyt b5 sequence containing gene in the construct pRAE-28-5 expresses an active human $\Delta 5$ -desaturase; whereas, the other variations of the gene do not.

The activity of the human $\Delta 5$ -desaturase was further confirmed in the experiment outlined in Figure 34. Included in

this figure are the fatty acid profiles of the strains 334(pRAE-28-5), 334(pRAE-26-1), and the control strain 334(pYX242) when DGLA(20:3n-6), OA(18:1n-9), AA(20:4n-6), or LA(18:2n-6) was used as the substrate, as well as when no substrate was added.

- 5 Again, the strain 334(pRAE-28-5) expressed an active human $\Delta 5$ -desaturase, converting DGLA to AA at a higher percent than the control strain, 0.106% vs. 0.065%, respectively. The strain 334(pRAE-26-1) had about the same amount of AA (0.06%) as the control. The conversion of the substrate OA to LA was not
- 10 detected, confirming that the strains do not have a $\Delta 12$ -desaturase activity. The conversion of the substrate AA to eicosapentaenoic acid (EPA, 20:5n-3) was detected, but at a very low level equal to that of the control strain, confirming that the strains do not have a $\Delta 17$ -desaturase activity. The
- 15 conversion of the substrate LA to GLA was detected, but again at a very low level equal to the control strain, confirming that the strains do not have a $\Delta 6$ -desaturase activity.

The present sequence (Figure 12) differs from the Genbank sequence g3169158 of the LifeSeq database with respect to two positions. In particular, with respect to the nucleotide sequence of sequence g3169158, position 1082 is an adenosine; however, in the present sequence position 1082 is a thymine (see Figure 12). Furthermore, position 1229 of sequence g3169158 is an adenine whereas in the present sequence position 1229 is a

25 guanine. In terms of an amino acid sequence comparison, position 361 of the present sequence is a leucine (see Figure 13), and position 361 of sequence g3169158 is a glutamine. Furthermore, position 410 of the present sequence is an arginine, whereas position 410 of sequence g3169158 is a

30 histidine. Additionally, sequence g3169158 is described, in the database, as a "hypothetical protein" which "exhibits similarity to motifs found in delta 6 desaturase, a hypothetical cytochrome

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b5 containing fusion protein." However, as demonstrated in the above example, the protein encoded by the sequence in Figure 12 is a human $\Delta 5$ -desaturase, not a $\Delta 6$ -desaturase.

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Nutritional Compositions

The PUFAs described in the Detailed Description may be utilized in various nutritional supplements, infant formulations, nutritional substitutes and other nutritional solutions.

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I. INFANT FORMULATIONS

A. Isomil® Soy Formula with Iron:

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cows milk. A feeding for patients with disorders for which lactose should be avoided: lactase deficiency, lactose intolerance and galactosemia.

Features:

- Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.
- Lactose-free formulation to avoid lactose-associated diarrhea.
- Low osmolality (240 mOs/kg water) to reduce risk of osmotic diarrhea.
- 25 -Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.
- 1.8 mg of Iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- 30 -Recommended levels of vitamins and minerals.
- Vegetable oils to provide recommended levels of essential fatty acids.

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-Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6% sugar (sucrose), 2.1 % soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0.11 % calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

B. Isomil® DF Soy Formula For Diarrhea:

Usage: As a short-term feeding for the dietary management of diarrhea in infants and toddlers.

Features:

-First infant formula to contain added dietary fiber from soy fiber specifically for diarrhea management.

-Clinically shown to reduce the duration of loose, watery stools during mild to severe diarrhea in infants.

-Nutritionally complete to meet the nutritional needs of the infant.

-Soy protein isolate with added L-methionine meets or exceeds an infant's requirement for all essential amino acids.

-Lactose-free formulation to avoid lactose-associated diarrhea.

-Low osmolality (240 mOsm/kg water) to reduce the risk of osmotic diarrhea.

-Dual carbohydrates (corn syrup and sucrose) designed to enhance carbohydrate absorption and reduce the risk of exceeding the absorptive capacity of the damaged gut.

-Meets or exceeds the vitamin and mineral levels recommended by the Committee on Nutrition of the American Academy of Pediatrics and required by the Infant Formula Act.

-1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.

-Vegetable oils to provide recommended levels of essential fatty acids.

Ingredients: (Pareve) 86% water, 4.8% corn syrup, 2.5% sugar (sucrose), 2.1% soy oil, 2.0% soy protein isolate, 1.4% coconut oil, 0.77% soy fiber, 0.12% calcium citrate, 0.11% calcium phosphate tribasic, 0.10% potassium citrate, potassium chloride, potassium phosphate monobasic, mono and diglycerides, soy lecithin, carrageenan, magnesium chloride, ascorbic acid, L-methionine, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

C. Isomil® SF Sucrose-Free Soy Formula With Iron:

Usage: As a beverage for infants, children and adults with an allergy or sensitivity to cow's-milk protein or an intolerance to sucrose. A feeding for patients with disorders for which lactose and sucrose should be avoided.

Features:

- Soy protein isolate to avoid symptoms of cow's-milk-protein allergy or sensitivity.
- Lactose-free formulation to avoid lactose-associated diarrhea (carbohydrate source is Polycose® Glucose Polymers).
- Sucrose free for the patient who cannot tolerate sucrose.
- Low osmolality (180 mOsm/kg water) to reduce risk of osmotic diarrhea.
- 1.8 mg of iron (as ferrous sulfate) per 100 Calories to help prevent iron deficiency.
- Recommended levels of vitamins and minerals.
- Vegetable oils to provide recommended levels of essential fatty acids.
- Milk-white color, milk-like consistency and pleasant aroma.

Ingredients: (Pareve) 75% water, 11.8% hydrolized cornstarch, 4.1% soy oil, 4.1 % soy protein isolate, 2.8% coconut oil, 1.0% modified cornstarch, 0.38% calcium phosphate tribasic, 0.17% potassium citrate, 0.13% potassium chloride, mono- and diglycerides, soy lecithin, magnesium chloride, ascorbic acid, L-methionine, calcium carbonate, sodium chloride, choline chloride,

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carrageenan, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

D. Isomil® 20 Soy Formula With Iron Ready To Feed,
20 Cal/fl oz.:

Usage: When a soy feeding is desired.

Ingredients: (Pareve) 85% water, 4.9% corn syrup, 2.6% sugar(sucrose), 2.1 % soy oil, 1.9% soy protein isolate, 1.4% coconut oil, 0.15% calcium citrate, 0.11% calcium phosphate tribasic, potassium citrate, potassium phosphate monobasic, potassium chloride, mono- and diglycerides, soy lecithin, carrageenan, ascorbic acid, L-methionine, magnesium chloride, potassium phosphate dibasic, sodium chloride, choline chloride, taurine, ferrous sulfate, m-inositol, alpha-tocopheryl acetate, zinc sulfate, L-carnitine, niacinamide, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, potassium iodide, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

E. Similac® Infant Formula:

Usage: When an infant formula is needed: if the decision is made to discontinue breastfeeding before age 1 year, if a

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- Protein of appropriate quality and quantity for good growth; heat-denatured, which reduces the risk of milk-associated enteric blood loss.
- Fat from a blend of vegetable oils (doubly homogenized), providing essential linoleic acid that is easily absorbed.
- Carbohydrate as lactose in proportion similar to that of human milk.
- Low renal solute load to minimize stress on developing organs.
- Powder, Concentrated Liquid and Ready To Feed forms.

Ingredients: (-D) Water, nonfat milk, lactose, soy oil, coconut oil, mono- and diglycerides, soy lecithin, ascorbic acid, carrageenan, choline chloride, taurine, m-inositol, alpha-tocopheryl acetate, zinc sulfate, niacinamide, ferrous sulfate, calcium pantothenate, cupric sulfate, vitamin A palmitate, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

F. Similac® NeoCare Premature Infant Formula With Iron:

Usage: For premature infants' special nutritional needs after hospital discharge. Similac NeoCare is a nutritionally complete formula developed to

provide premature infants with extra calories, protein, vitamins and minerals needed to promote catch-up growth and support development.

Features:

- Reduces the need for caloric and vitamin supplementation. More calories (22 Cal/fl oz) than standard term formulas (20 Cal/fl oz).
- Highly absorbed fat blend, with medium-chain triglycerides (MCT oil) to help meet the special digestive needs of premature infants.
- Higher levels of protein, vitamins and minerals per 100 calories to extend the nutritional support initiated in-hospital.
- More calcium and phosphorus for improved bone mineralization.

Ingredients: -D Corn syrup solids, nonfat milk, lactose, whey protein concentrate, soy oil, high-oleic safflower oil, fractionated coconut oil (medium chain triglycerides), coconut oil, potassium citrate, calcium phosphate tribasic, calcium carbonate, ascorbic acid, magnesium chloride, potassium chloride, sodium chloride, taurine, ferrous sulfate, m-inositol, choline chloride, ascorbyl palmitate, L-carnitine, alpha-tocopheryl acetate, zinc sulfate, niacinamide, mixed tocopherols, sodium citrate, calcium pantothenate, cupric sulfate, thiamine chloride hydrochloride, vitamin A palmitate, beta carotene, riboflavin, pyridoxine hydrochloride, folic acid, manganese sulfate, phylloquinone, biotin, sodium selenite, vitamin D3 and cyanocobalamin.

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G. Similac Natural Care Low-Iron Human Milk Fortifier
Ready To Use, 24 Cal/fl oz.:

Usage: Designed to be mixed with human milk or to be fed alternatively with human milk to low-birth-weight infants.

Ingredients: -D Water, nonfat milk, hydrolyzed cornstarch, lactose, fractionated coconut oil (medium-chain triglycerides), whey protein concentrate, soy oil, coconut oil, calcium phosphate tribasic, potassium citrate, magnesium chloride, sodium citrate, ascorbic acid, calcium carbonate, mono and diglycerides, soy lecithin, carrageenan, choline chloride, m-inositol, taurine, niacinamide, L-carnitine, alpha tocopheryl acetate, zinc sulfate, potassium chloride, calcium pantothenate, ferrous sulfate, cupric sulfate, riboflavin, vitamin A palmitate, thiamine chloride hydrochloride, pyridoxine hydrochloride, biotin, folic acid, manganese sulfate, phylloquinone, vitamin D3, sodium selenite and cyanocobalamin.

Various PUFAs of this invention can be substituted and/or added to the infant formulae described above and to other infant formulae known to those in the art.

II. NUTRITIONAL FORMULATIONS

A. ENSURE®

Usage: ENSURE is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE is lactose- and gluten-free, and is suitable for use in modified diets, including low-

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- For patients on modified diets
- For elderly patients at nutrition risk
- For patients with involuntary weight loss
- For patients recovering from illness or surgery
- For patients who need a low-residue diet

Ingredients: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower Oil, Soy Protein Isolate, Soy Oil, Canola Oil, Potassium Citrate, Calcium Phosphate Tribasic, Sodium Citrate, Magnesium Chloride, Magnesium Phosphate Dibasic, Artificial Flavor, Sodium Chloride, Soy Lecithin, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Gellan Gum, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Sodium Molybdate, Chromium Chloride, Biotin, Potassium Iodide, Sodium Selenate.

B. ENSURE® BARS:

Usage: ENSURE BARS are complete, balanced nutrition for supplemental use between or with meals. They provide a delicious, nutrient-rich alternative to other snacks.

ENSURE BARS contain <1 g lactose/bar, and Chocolate Fudge Brownie flavor is gluten-free. (Honey Graham Crunch flavor contains gluten.)

Patient Conditions:

- For patients who need extra calories, protein, vitamins and minerals.
- Especially useful for people who do not take in enough calories and nutrients.
- For people who have the ability to chew and swallow
- Not to be used by anyone with a peanut allergy or any type of allergy to nuts.

Ingredients: Honey Graham Crunch -- High-Fructose Corn Syrup, Soy Protein Isolate, Brown Sugar, Honey, Maltodextrin (Corn), Crisp Rice (Milled Rice, Sugar [Sucrose], Salt [Sodium Chloride] and Malt), Oat Bran, Partially Hydrogenated Cottonseed and Soy Oils, Soy Polysaccharide, Glycerine, Whey Protein Concentrate, Polydextrose, Fructose, Calcium Caseinate, Cocoa Powder, Artificial Flavors, Canola Oil, High-Oleic Safflower Oil, Nonfat Dry Milk, Whey Powder, Soy Lecithin and Corn Oil. Manufactured in a facility that processes nuts.

Vitamins and Minerals: Calcium Phosphate Tribasic, Potassium Phosphate Dibasic, Magnesium Oxide, Salt (Sodium Chloride), Potassium Chloride, Ascorbic Acid, Ferric Orthophosphate, Alpha-Tocopheryl Acetate, Niacinamide, Zinc Oxide, Calcium Pantothenate, Copper Gluconate, Manganese Sulfate, Riboflavin, Beta Carotene, Pyridoxine Hydrochloride, Thiamine Mononitrate, Folic Acid, Biotin, Chromium Chloride, Potassium Iodide, Sodium Selenate, Sodium Molybdate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

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Protein: Honey Graham Crunch - The protein source is a blend of soy protein isolate and milk proteins.

Soy protein isolate	74%
Milk proteins	26%

Fat: Honey Graham Crunch - The fat source is a blend of partially hydrogenated cottonseed and soybean, canola, high oleic safflower, oils, and soy lecithin.

Partially hydrogenated cottonseed and soybean oil	76%
Canola oil	8%
High-oleic safflower oil	8%
Corn oil	4%
Soy lecithin	4%

Carbohydrate: Honey Graham Crunch - The carbohydrate source is a combination of high-fructose corn syrup, brown sugar, maltodextrin, honey, crisp rice, glycerine, soy polysaccharide, and oat bran.

High-fructose corn syrup	24%
Brown sugar	21%
Maltodextrin	12%
Honey	11%
Crisp rice	9%
Glycerine	9%
Soy Polysaccharide	7%
Oat bran	7%

C. ENSURE® HIGH PROTEIN:

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Usage: ENSURE HIGH PROTEIN is a concentrated, high-protein liquid food designed for people who require additional calories, protein, vitamins, and minerals in their diets. It can be used as an oral nutritional supplement with or between meals or, in appropriate amounts, as a meal replacement. ENSURE HIGH PROTEIN is lactose- and gluten-free, and is suitable for use by people recovering from general surgery or hip fractures and by patients at risk for pressure ulcers.

Patient Conditions:

-For patients who require additional calories, protein, vitamins, and minerals, such as patients recovering from general surgery or hip fractures, patients at risk for pressure ulcers, and patients on low-cholesterol diets

Features:

- Low in saturated fat
- Contains 6 g of total fat and < 5 mg of cholesterol per serving
- Rich, creamy taste
- Excellent source of protein, calcium, and other essential vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

Ingredients:

Vanilla Supreme: -D Water, Sugar (Sucrose), Maltodextrin (Corn), Calcium and Sodium Caseinates, High-Oleic Safflower

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The protein source is a blend of two high-biologic-value proteins: casein and soy.

Fat :

High-oleic safflower oil	40%
Canola oil	30%
Soy oil	30%

The level of fat in ENSURE HIGH PROTEIN meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE HIGH PROTEIN represent 24% of the total calories,

with 2.6% of the fat being from saturated fatty acids and 7.9% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

Carbohydrate:

ENSURE HIGH PROTEIN contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla supreme, chocolate royal, wild berry, and banana), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors:

Sucrose	60%
Maltodextrin	40%

Chocolate:

Sucrose	70%
Maltodextrin	30%

D. ENSURE® LIGHT

Usage: ENSURE LIGHT is a low-fat liquid food designed for use as an oral nutritional supplement with or between meals. ENSURE LIGHT is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

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Patient Conditions:

- For normal-weight or overweight patients who need extra nutrition in a supplement that contains 50% less fat and 20% fewer calories than ENSURE.
- For healthy adults who don't eat right and need extra nutrition.

Features:

- Low in fat and saturated fat
- Contains 3 g of total fat per serving and < 5 mg cholesterol
- Rich, creamy taste
- Excellent source of calcium and other essential vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

Ingredients:

French Vanilla: -D Water, Maltodextrin (Corn), Sugar (Sucrose), Calcium Caseinate, High-Oleic Safflower Oil, Canola Oil, Magnesium Chloride, Sodium Citrate, Potassium Citrate, Potassium Phosphate Dibasic, Magnesium Phosphate Dibasic, Natural and Artificial Flavor, Calcium Phosphate Tribasic, Cellulose Gel, Choline Chloride, Soy Lecithin, Carrageenan, Salt (Sodium Chloride), Ascorbic Acid, Cellulose Gum, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Vitamin A Palmitate, Pyridoxine

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Hydrochloride, Riboflavin, Chromium Chloride, Folic Acid, Sodium Molybdate, Biotin, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is calcium caseinate.

Calcium caseinate	100%
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Fat:

The fat source is a blend of two oils: high-oleic safflower and canola.

High-oleic safflower oil	70%
Canola oil	30%

The level of fat in ENSURE LIGHT meets American Heart Association (AHA) guidelines. The 3 grams of fat in ENSURE LIGHT represent 13.5% of the total calories, with 1.4% of the fat being from saturated fatty acids and 2.6% from polyunsaturated fatty acids. These values are within the AHA guidelines of < 30% of total calories from fat, < 10% of the, calories from saturated fatty acids, and < 10% of total calories from polyunsaturated fatty acids.

Carbohydrate:

ENSURE LIGHT contains a combination of maltodextrin and sucrose. The chocolate flavor contains corn syrup as well. The mild sweetness and flavor variety (French vanilla, chocolate supreme, strawberry swirl), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and

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orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors:

Sucrose	51%
Maltodextrin	49%

Chocolate:

Sucrose	47.0%
Corn Syrup	26.5%
Maltodextrin	26.5%

Vitamins and Minerals:

An 8-fl-oz serving of ENSURE LIGHT provides at least 25% of the RDIs for 24 key vitamins and minerals.

Caffeine:

Chocolate flavor contains 2.1 mg caffeine/8 fl oz.

E. ENSURE PLUS®

Usage: ENSURE PLUS is a high-calorie, low-residue liquid food for use when extra calories and nutrients, but a normal concentration of protein, are needed. It is designed primarily as an oral nutritional supplement to be used with or between meals or, in appropriate amounts, as a meal replacement. ENSURE PLUS is lactose- and gluten-free. Although it is primarily an oral nutritional supplement, it can be fed by tube.

Patient Conditions:

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- For patients who require extra calories and nutrients, but a normal concentration of protein, in a limited volume
- For patients who need to gain or maintain healthy weight

Features:

- Rich, creamy taste
- Good source of essential vitamins and minerals

Ingredients:

Vanilla: -D Water, Corn Syrup, Maltodextrin (Corn), Corn Oil, Sodium and Calcium Caseinates, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Potassium Chloride, Choline Chloride, Ascorbic Acid, Carrageenan, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D3.

Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	84%
Soy protein isolate	16%

Fat:

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Carbohydrate:

ENSURE PLUS contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, strawberry, coffee, butter pecan, and eggnog), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla, strawberry, butter pecan, and coffee flavors:

Corn Syrup	39%
Maltodextrin	38%
Sucrose	23%

Chocolate and eggnog flavors:

Corn Syrup	36%
Maltodextrin	34%
Sucrose	30%

Vitamins and Minerals:

An 8-fl-oz serving of ENSURE PLUS provides at least 15% of the RDIs for 25 key Vitamins and minerals.

Caffeine:

Chocolate flavor contains 3.1 mg Caffeine/8 fl oz.
Coffee flavor contains a trace amount of caffeine.

F. ENSURE PLUS® HN

Usage: ENSURE PLUS HN is a nutritionally complete high-calorie, high-nitrogen liquid food designed for people with higher calorie and protein needs or limited volume tolerance. It may be used for oral supplementation or for total nutritional support by tube. ENSURE PLUS HN is lactose- and gluten-free.

Patient Conditions:

- For patients with increased calorie and protein needs, such as following surgery or injury.
- For patients with limited volume tolerance and early satiety.

Features:

- For supplemental or total nutrition
- For oral or tube feeding
- 1.5 CaV/mL,
- High nitrogen
- Calorically dense

Ingredients:

Vanilla: -D Water, Maltodextrin (Corn), Sodium and Calcium Caseinates, Corn Oil, Sugar (Sucrose), Soy Protein Isolate, Magnesium Chloride, Potassium Citrate, Calcium Phosphate Tribasic, Soy Lecithin, Natural and Artificial Flavor, Sodium Citrate, Choline Chloride, Ascorbic Acid, Taurine, L-Carnitine, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Carrageenan, Calcium Pantothenate, Manganese Sulfate, Cupric Sulfate,

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Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Chromium Chloride, Sodium Molybdate, Potassium Iodide, Sodium Selenite, Phylloquinone, Cyanocobalamin and Vitamin D3.

G. ENSURE® POWDER:

Usage: ENSURE POWDER (reconstituted with water) is a low-residue liquid food designed primarily as an oral nutritional supplement to be used with or between meals. ENSURE POWDER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:

- For patients on modified diets
- For elderly patients at nutrition risk
- For patients recovering from illness/surgery
- For patients who need a low-residue diet

Features:

- Convenient, easy to mix
- Low in saturated fat
- Contains 9 g of total fat and < 5 mg of cholesterol per serving
- High in vitamins and minerals
- For low-cholesterol diets
- Lactose-free, easily digested

Ingredients: -D Corn Syrup, Maltodextrin (Corn), Sugar (Sucrose), Corn Oil, Sodium and Calcium Caseinates, Soy

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Protein Isolate, Artificial Flavor, Potassium Citrate, Magnesium Chloride, Sodium Citrate, Calcium Phosphate Tribasic, Potassium Chloride, Soy Lecithin, Ascorbic Acid, Choline Chloride, Zinc Sulfate, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Niacinamide, Calcium Pantothenate, Manganese Sulfate, Thiamine Chloride Hydrochloride, Cupric Sulfate, Pyridoxine Hydrochloride, Riboflavin, Vitamin A Palmitate, Folic Acid, Biotin, Sodium Molybdate, Chromium Chloride, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is a blend of two high-biologic-value proteins: casein and soy.

Sodium and calcium caseinates	84%
Soy protein isolate	16%

Fat:

The fat source is corn oil.

Corn oil	100%
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Carbohydrate:

ENSURE POWDER contains a combination of corn syrup, maltodextrin, and sucrose. The mild sweetness of ENSURE POWDER, plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, helps to prevent flavor fatigue and aid in patient compliance.

Vanilla:

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Corn Syrup	35%
Maltodextrin	35%
Sucrose	30%

H. ENSURE® PUDDING

Usage: ENSURE PUDDING is a nutrient-dense supplement providing balanced nutrition in a nonliquid form to be used with or between meals. It is appropriate for consistency-modified diets (e.g., soft, pureed, or full liquid) or for people with swallowing impairments. ENSURE PUDDING is gluten-free.

Patient Conditions:

- For patients on consistency-modified diets (e.g., soft, pureed, or full liquid)
- For patients with swallowing impairments

Features:

- Rich and creamy, good taste
- Good source of essential vitamins and minerals
- Convenient-needs no refrigeration
- Gluten-free

Nutrient Profile per 5 oz: Calories 250, Protein 10.9%, Total Fat 34.9%, Carbohydrate 54.2%

Ingredients:

Vanilla: -D Nonfat Milk, Water, Sugar (Sucrose), Partially Hydrogenated Soybean Oil, Modified Food Starch, Magnesium Sulfate, Sodium Stearoyl Lactylate, Sodium Phosphate Dibasic, Artificial Flavor, Ascorbic Acid, Zinc Sulfate,

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Ferrous Sulfate, Alpha-Tocopheryl Acetate, Choline Chloride, Niacinamide, Manganese Sulfate, Calcium Pantothenate, FD&C Yellow #5, Potassium Citrate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, FD&C Yellow #6, Folic Acid, Biotin, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is nonfat milk.

Nonfat milk 100%

Fat:

The fat source is hydrogenated soybean oil.

Hydrogenated soybean oil 100%

Carbohydrate:

ENSURE PUDDING contains a combination of sucrose and modified food starch. The mild sweetness and flavor variety (vanilla, chocolate, butterscotch, and tapioca) help prevent flavor fatigue. The product contains 9.2 grams of lactose per serving.

Vanilla and other nonchocolate flavors:

Sucrose 56%
Lactose 27%
Modified food starch 17%

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Chocolate:

Sucrose	58%
Lactose	26%
Modified food starch	16%

I. ENSURE® WITH FIBER:

Usage: ENSURE WITH FIBER is a fiber-containing, nutritionally complete liquid food designed for people who can benefit from increased dietary fiber and nutrients. ENSURE WITH FIBER is suitable for people who do not require a low-residue diet. It can be fed orally or by tube, and can be used as a nutritional supplement to a regular diet or, in appropriate amounts, as a meal replacement. ENSURE WITH FIBER is lactose- and gluten-free, and is suitable for use in modified diets, including low-cholesterol diets.

Patient Conditions:

-For patients who can benefit from increased dietary fiber and nutrients

Features:

- New advanced formula-low in saturated fat, higher in vitamins and minerals
- Contains 6 g of total fat and < 5 mg of cholesterol per serving
- Rich, creamy taste
- Good source of fiber
- Excellent source of essential vitamins and minerals
- For low-cholesterol diets
- Lactose- and gluten-free

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Ingredients:

Vanilla: -D Water; Maltodextrin (Corn), Sugar (Sucrose), Sodium and Calcium Caseinates, Oat Fiber, High-Oleic Safflower Oil, Canola Oil, Soy Protein Isolate, Corn Oil, Soy Fiber, Calcium Phosphate Tribasic, Magnesium Chloride, Potassium Citrate, Cellulose Gel, Soy Lecithin, Potassium Phosphate Dibasic, Sodium Citrate, Natural and Artificial Flavors, Choline Chloride, Magnesium Phosphate, Ascorbic Acid, Cellulose Gum, Potassium Chloride, Carrageenan, Ferrous Sulfate, Alpha-Tocopheryl Acetate, Zinc Sulfate, Niacinamide, Manganese Sulfate, Calcium Pantothenate, Cupric Sulfate, Vitamin A Palmitate, Thiamine Chloride Hydrochloride, Pyridoxine Hydrochloride, Riboflavin, Folic Acid, Chromium Chloride, Biotin, Sodium Molybdate, Potassium Iodide, Sodium Selenate, Phylloquinone, Vitamin D3 and Cyanocobalamin.

Protein:

The protein source is a blend of two high-biologic-value proteins-casein and soy.

Sodium and calcium caseinates	80%
Soy protein isolate	20%

Fat:

The fat source is a blend of three oils: high-oleic safflower, canola, and corn.

High-oleic safflower oil	40%
Canola oil	40%

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Corn oil

20%

The level of fat in ENSURE WITH FIBER meets American Heart Association (AHA) guidelines. The 6 grams of fat in ENSURE WITH FIBER represent 22% of the total calories, with 2.01 % of the fat being from saturated fatty acids and 6.7% from polyunsaturated fatty acids. These values are within the AHA guidelines of $\leq 30\%$ of total calories from fat, $< 10\%$ of the calories from saturated fatty acids, and $\leq 10\%$ of total calories from polyunsaturated fatty acids.

Carbohydrate:

ENSURE WITH FIBER contains a combination of maltodextrin and sucrose. The mild sweetness and flavor variety (vanilla, chocolate, and butter pecan), plus VARI-FLAVORS® Flavor Pacs in pecan, cherry, strawberry, lemon, and orange, help to prevent flavor fatigue and aid in patient compliance.

Vanilla and other nonchocolate flavors:

Maltodextrin	66%
Sucrose	25%
Oat Fiber	7%
Soy Fiber	2%

Chocolate:

Maltodextrin	55%
Sucrose	36%
Oat Fiber	7%
Soy Fiber	2%

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The various nutritional supplements described above and known to others of skill in the art can be substituted and/or supplemented with the PUFAs produced in accordance with the present invention.

Oxepa is a low-carbohydrate, calorically dense, enteral nutritional product designed for the dietary management of patients with or at risk for ARDS. It has a unique combination of ingredients, including a patented oil blend containing eicosapentaenoic acid (EPA from fish oil), γ -linolenic acid (GLA from borage oil), and elevated antioxidant levels.

Caloric density is high at 1.5 Cal/mL (355 Cal/8 fl oz), to minimize the volume required to meet energy needs. The distribution of Calories in Oxepa is shown in Table IV.

	per 8 fl oz.	per liter	% of Cal
Calories	355	1,500	---
Fat (g)	22.2	93.7	55.2
Carbohydrate (g)	25	105.5	28.1
Protein (g)	14.8	62.5	16.7

Water (g)	186	785	---
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Fat:

-Oxepa contains 22.2 g of fat per 8-fl oz serving (93.7 g/L).

-The fat source is an oil blend of 31.8% canola oil, 25% medium-chain triglycerides (MCTs), 20% borage oil, 20% fish oil, and 3.2 % soy lecithin. The typical fatty acid profile of Oxepa is shown in Table V.

-Oxepa provides a balanced amount of polyunsaturated, monounsaturated, and saturated fatty acids, as shown in Table VI.

-Medium-chain triglycerides (MCTs) -- 25% of the fat blend -- aid gastric emptying because they are absorbed by the intestinal tract without emulsification by bile acids.

The various fatty acid components of Oxepa™ nutritional product can be substituted and/or supplemented with the PUFAs produced in accordance with this invention.

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Fatty acids equal approximately 95% of total fat.

Fatty acids equal approximately 95% of total fat.

Table VI. Fat Profile of Oxepa.

% of total calories from fat	55.2
Polyunsaturated fatty acids	31.44 g/L
Monounsaturated fatty acids	25.53 g/L
Saturated fatty acids	32.38 g/L
n-6 to n-3 ratio	1.75:1
Cholesterol	9.49 mg/8 fl oz
	40.1 mg/L

Carbohydrate:

-The carbohydrate content is 25.0 g per 8-fl-oz serving (105.5 g/L).

-The carbohydrate sources are 45% maltodextrin (a complex carbohydrate) and 55% sucrose (a simple sugar), both of which are readily digested and absorbed.

-The high-fat and low-carbohydrate content of Oxepa is designed to minimize carbon dioxide (CO₂) production. High CO₂ levels can complicate weaning in ventilator-dependent patients. The low level of carbohydrate also may be useful for those patients who have developed stress-induced hyperglycemia.

-Oxepa is lactose-free.

Dietary carbohydrate, the amino acids from protein, and the glycerol moiety of fats can be converted to glucose within the body. Throughout this process, the carbohydrate requirements of glucose-dependent tissues (such as the central nervous system and red blood cells) are met. However, a diet free of carbohydrates can lead to ketosis, excessive catabolism of tissue protein, and loss of fluid and electrolytes. These effects can be prevented by daily ingestion of 50 to 100 g of digestible carbohydrate, if caloric intake is adequate. The carbohydrate level in Oxepa

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* Oxepa is gluten-free.

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266CLAIMS:

1. An isolated nucleotide sequence corresponding to or complementary to at least about 50% of the nucleotide sequence represented by SEQ ID NO:1 (Figure 12).
2. The isolated nucleotide sequence of claim 1 wherein said sequence is represented by SEQ ID NO:1.
3. The isolated nucleotide sequence of claims 1 or 2 wherein said sequence encodes a functionally active desaturase which utilizes a polyunsaturated fatty acid as a substrate.
4. The nucleotide sequence of claim 1 wherein said sequence is derived from a mammal.
5. The nucleotide sequence of claim 4 wherein said Sequence is derived from a human.
6. A purified protein encoded by said nucleotide sequence of claims 1 or 2.
7. A purified polypeptide which desaturates polyunsaturated fatty acids at carbon 5 and has at least about 50% amino acid similarity to the amino acid sequence of said purified protein of claim 6.

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8. A method of producing a human $\Delta 5$ -desaturase comprising the steps of:

- a) isolating said nucleotide sequence represented by SEQ ID NO:1 (Figure 12);
- b) constructing a vector comprising: i) said isolated nucleotide sequence operably linked to ii) a promoter;
- c) introducing said vector into a host cell under time and conditions sufficient for expression of said human $\Delta 5$ -desaturase.

9. The method of claim 8 wherein said host cell is a eukaryotic cell or a prokaryotic cell.

10. The method of claim 9 wherein said prokaryotic cell is selected from the group consisting of E. coli, cyanobacteria, and B. subtilis.

11. The method of claim 9 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

12. The method of claim 11 wherein said fungal cell is a yeast cell.

13. The method of claim 12 wherein said yeast cell is selected from the group consisting of Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Candida spp., Lipomyces starkey, Yarrowia lipolytica, Kluyveromyces spp., Hansenula spp., Trichoderma spp. and Pichia spp.

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14. The method of claim 13 wherein said yeast cell is Saccharomyces cerevisiae.

15. A vector comprising: a) a nucleotide sequence as represented by SEQ ID NO:1 (Figure 12) operably linked to b) a promoter.

16. A host cell comprising said vector of claim 15.

17. The host cell of claim 16, wherein said host cell is a eukaryotic cell or a prokaryotic cell.

18. The host cell of claim 17 wherein said prokaryotic cell is selected from the group consisting of E. coli, Cyanobacteria, and B. subtilis.

19. The host cell of claim 17 wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, an insect cell, a plant cell and a fungal cell.

20. The host cell of claim 19 wherein said fungal cell is a yeast cell.

21. The host cell of claim 20 wherein said yeast cell is selected from the group consisting of Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Candida spp., Lipomyces starkey, Yarrowia lipolytica, Kluyveromyces spp., Hansenula spp., Trichoderma spp. and Pichia spp.

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24. The plant cell, plant or plant tissue of claim 23 wherein said polyunsaturated fatty acid is AA or EPA.

25. One or more plant oils or acids expressed by said plant cell, plant or plant tissue of claim 23.

26. A transgenic plant comprising said vector of claim 15, wherein expression of said nucleotide sequence of said vector results in production of a polyunsaturated fatty acid in seeds of said transgenic plant.

27. A mammalian cell comprising said vector of claim 15, wherein expression of said nucleotide sequence of said vector results in production of altered levels of AA or EPA when said cell is grown in a culture media comprising a fatty acid selected from the group consisting of an essential fatty acid, LA and ALA.

28. A transgenic, non-human mammal whose genome comprises a DNA sequence encoding a human $\Delta 5$ -desaturase operably linked to a promoter.

29. The transgenic, non-human mammal of claim 28, wherein said DNA sequence is represented by SEQ ID NO:1 (Figure 12).

30. A fluid produced by said transgenic, non-human mammal of claim 29 wherein said fluid comprises a detectable level of at least human $\Delta 5$ -desaturase.

~~31.~~ A method for producing a polyunsaturated fatty acid comprising the steps of:

- a) isolating said nucleotide sequence represented by SEQ ID NO:1 (Figure 12);
- b) constructing a vector comprising said isolated nucleotide sequence;
- c) introducing said vector into a host cell under time and conditions sufficient for expression of said human $\Delta 5$ -desaturase enzyme; and
- d) exposing said expressed human $\Delta 5$ -desaturase enzyme to a substrate polyunsaturated fatty acid in order to convert said substrate to a product polyunsaturated fatty acid.

32. The method according to claim 31, wherein said substrate polyunsaturated fatty acid is DGLA or 20:4n-3 and said product polyunsaturated fatty acid is AA or EPA, respectively.

33. The method according to claim 31 further comprising the step of exposing said product polyunsaturated fatty acid to an elongase in order to convert said product polyunsaturated fatty acid to another polyunsaturated fatty acid.

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34. The method according to claim 33 wherein said product polyunsaturated fatty acid is AA or EPA and said another polyunsaturated fatty acid is adrenic acid or (n-3)-docosapentaenoic acid, respectively.

35. The method of claim 33 further comprising the steps of exposing said another polyunsaturated fatty acid to an additional desaturase in order to convert said another polyunsaturated fatty acid to a final polyunsaturated fatty acid.

36. The method of claim 35 wherein said final polyunsaturated fatty acid is (n-6)-docosapentaenoic acid or docosahexaenoic (DHA) acid.

37. A nutritional composition comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 31, said another polyunsaturated fatty acid produced according to the method of claim 33, and said final polyunsaturated fatty acid produced according to the method of claim 35.

38. The nutritional composition of claim 37 wherein said product polyunsaturated fatty acid is AA or EPA.

39. The nutritional composition of claim 37 wherein said another polyunsaturated fatty acid is adrenic acid or (n-3)-docosapentaenoic acid.

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40. The nutritional composition of claim 37 wherein said final polyunsaturated fatty acid is DHA.

41. The nutritional composition of claim 37 wherein said nutritional composition is selected from the group consisting of an infant formula, a dietary supplement and a dietary substitute.

42. The nutritional composition of claim 41 wherein said nutritional composition is administered to a human or an animal.

43. The nutritional composition of claim 42 wherein said nutritional composition is administered enterally or parenterally.

44. The nutritional composition of claim 41 wherein said nutritional composition further comprises at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, monoglycerides, diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and protein hydrolysates.

45. The nutritional composition of claim 44 wherein said nutritional composition further comprises at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex and at least one mineral selected from the group consisting of calcium magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium and iron.

658070-CT9/2250

46. A pharmaceutical composition comprising 1) at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 31, said another polyunsaturated fatty acid produced according to the method of claim 33, and said final polyunsaturated fatty acid produced according to the method of claim 35 and 2) a pharmaceutically acceptable carrier.

47. The pharmaceutical composition of claim 46 wherein said pharmaceutical composition is administered to a human or an animal.

48. The pharmaceutical composition of claim 46 wherein said pharmaceutical composition further comprises an element selected from the group consisting of a vitamin, a mineral, a carbohydrate, an amino acid, a free fatty acid, a phospholipid, an antioxidant, and a phenolic compound.

49. An animal feed comprising at least one polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 31, said another polyunsaturated fatty acid produced according to the method of claim 33 and said final polyunsaturated fatty acid produced according to the method of claim 35.

50. The animal feed of claim 49 wherein said

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product polyunsaturated fatty acid is AA or EPA.

51. The animal feed of claim 49 wherein said another polyunsaturated fatty acid is adrenic acid or (n-3)-docosapentaenoic acid.

52. The animal feed of claim 49 wherein said final polyunsaturated fatty acid is (n-6)-docosapentaenoic acid or DHA.

53. A cosmetic comprising a polyunsaturated fatty acid selected from the group consisting of said product polyunsaturated fatty acid produced according to the method of claim 31, said another polyunsaturated fatty acid produced according to the method of claim 33 and said final polyunsaturated fatty acid produced according to the method of claim 35.

54. A method of preventing or treating a condition caused by insufficient intake of polyunsaturated fatty acids comprising administering to said patient said nutritional composition of claim 37 in an amount sufficient to effect said treatment.

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25ABSTRACT OF THE DISCLOSURE

The subject invention relates to the identification of a gene involved in the desaturation of polyunsaturated fatty acids at carbon 5 (i.e., "human $\Delta 5$ -desaturase") and to uses thereof. In particular, human $\Delta 5$ -desaturase may be utilized, for example, in the conversion of dihomo- γ - linolenic acid (DGLA) to arachidonic acid (AA) and in the conversion of 20:4n-3 to eicosapentaenoic acid (EPA). AA or polyunsaturated fatty acids produced therefrom may be added to pharmaceutical compositions, nutritional compositions, animal feeds, as well as other products such as cosmetics.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT

278
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Applicant(s): Pradip Mukerji et al.

Serial No.:

Filed: January 8, 1999

For: HUMAN DESATURASE GENE AND
USES THEREOF

Case No.: 6295.US.P1

Examiner: Not Yet Assigned

Group Art Unit:

Date: January 8, 1999

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Kathleen T. Litz 1/8/99
Kathleen T. Litz DATE

**Declaration and Power of Attorney
for a United States Patent Application**

As a below-named inventor, I hereby declare:

My residence, post office address and citizenship are as stated below next to my name. I believe I am an original and first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled **HUMAN DESATURASE GENE AND USES THEREOF** the specification of which is enclosed herewith.

I hereby state that I have reviewed and understand the contents of the above-mentioned specification, including the claims.

I acknowledge a duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

Claim to benefit of foreign application(s) as follows:

I hereby claim foreign priority benefits under 35 U.S.C. §119 for the following foreign application(s) for patent or inventor's certificate:

International Application PCT/US98/07422

The following foreign applications for patent or inventor's certificate have a filing date earlier than the filing date of the application(s) identified above:

Claim to benefit of earlier U.S. application(s) as follows:

I hereby claim the benefit under 35 U.S.C. §120 of the following earlier-filed United States patent application(s). Insofar as the subject matter of each of the claims of this/these application(s) is not disclosed in the prior U.S. applications in the manner required by 35 U.S.C. §112, first paragraph, I acknowledge a duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. which came into existence between the filing date(s) of the prior applications and the national or PCT filing date of this application.

U.S. Serial No., 08/833,610 Filed: April 11, 1997

I hereby claim the benefit under Title 35, United States Code § 119 (e) of any United States provisional application(s) listed below.

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I hereby appoint the following Attorneys and/or agents to prosecute this application and any continuation or divisional applications based hereon, and to transact all business in the Patent and Trademark Office connected therewith:

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Citizenship:	United States of America
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Residence:	
Citizenship:	Canada and Taiwan

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that all statements made herein were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Pradip Mukerji _____ Date _____

Amanda E. Leonard _____ Date _____

Yung-Sheng Huang _____ Date _____

0922613-010800

668070" ET922260

Sections of the Desaturases	Clone ID from Incyte LifeSeq Database	Keyword
151-300 delta 5	3808675	fatty acid desaturase
301-446 delta 5	354535	delta 6
151-300 delta 6	3448789	delta 6
151-300 delta 6	1362863	delta 6
151-300 delta 6	2394760	delta 6
301-457 delta 6	3350263	delta 6

Figure 1

CBL

668070" ETS2260

Edited Contig 2692004

GCACGCCGACCGCGCCGGGAGATCCTGGCAAAGTATCCAGAGATAAAGTCCTTGATGAAACCTGATCCCAATTTGATATGGATTATAATTA
TGATGGTTCTCACCCAGTTGGGTGCATTTTACATAGTAAAAGACTTGGACTGGAAATGGGTCATATTTGGGGCCTATGCGTTTGGCAGTTGC
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GTTTGCTAATCTTCCTATTGGGATTCCATATTCAATTTCTTTAAGAGGTATCACATGGATCATCATCGGTACCTTGGAGCTGATGGCGTCG
ATGTAGATATTCCCTACCGATTTTGAGGGCTGGTTCTTCTGTACCGCTTTCAGAAAGTTTATATGGGTTATTCTTCAGCCTCTCTTTTATGCC
TTTCGACCTCTGTTCATCAACCCCAAACCAATTACGTATCTGGAAGTTATCAATACCGTGGCACAGGTCACTTTTGACATTTTAATTTATTA
CTTTTTGGGAATTAAATCCTTAGTCTACATGTTGGCAGCATCTTTACTTGGCCTGGGTTTGCACCCAATTTCTGGACATTTTATAGCTGAGC
ATTACATGTTCTTAAAGGGTCATGAAACTTACTCATATTATGGGCCTCTGAATTTACTTACCTTCAATGTGGGTTATCATAATGAACATCAT
GATTTCCCCAACATTCCTGGAAAAAGTCTTCCACTGGTGAGGAAAATAGCAGCTGAATACTATGACAACCTCCCTCACTACAATTCCTGGAT
AAAAGTACTGTATGATTTTGTGATGGATGATACAATAAGTCCCTACTCAAGAATGAAGAGGCACCAAAAAGGAGAGATGGTGCTGGAGTAAA
TATCATTAGTGCCAAAGGGATTCTTCTCCAAAACCTTAGATGATAAAATGGAATTTTGCATTATTAACTTGAGACCAGTGATGCTCAGAA
GCTCCCCTGGCACAATTTAGAGTAAGAGCTCGGTGATACCAAGAAGTGAATCTGGCTTTTAAACAGTCAGCCTGACTCTGTACTGCTCAGT
TTCACTCACAGGAACTTGTGACTTGTGTATTATCGTCATTGAGGATGTTTCACTCATGTCTGTCAITTTTATAAGCATATCATTTAAAAAGC
TTCTAAAAAGCTATTTTCGCCAGG

Figure 2

187

668010" ET942260

Edited Contig 2153526

TTACCTTCTACGTCCGCTTCTTCCTCACTTATGTGCCACTATTGGGGCTGAAAGCTTCCTGGGCCTTTTCTTCATAGTCAGGTTCCCTGGAAA
GCAACTGGTTTGTGTGGGTGACACAGATGAACCATATTCCTATGCACATTGATCATGACCGGAACATGGACTGGGT'TTCCACCCAGCTCCAG
GCCACATGCAATGTCCACAAGTCTGCCTTCAATGACTGGTTCAGTGGACACCTCAACTTCCAGATTGAGCACCATCTTTTCCCACGATGCC
TCGACACAATTACCACAAAGTGGCTCCCTGGTGCAGTCCTTGTGTGCCAAGCATGGCATAGAGTACCAGTCCAAGCCCCTGCTGTCAGCCT
TCGCCGACATCATCCACTCACTAAAGGAGTCAGGGCAGCTCTGGCTAGATGCCTATCTTCACCAATAACAACAGCCACCCTGCCCAGTCTGG
AAGAAGAGGAGGAAGACTCTGGAGCCAAGGCAGAGGGGAGCTTGAGGGACAATGCCACTATAGTTTAATACTCAGAGGGGGTTGGG'TTTGGG
GACATAAAGCCTCTGACTCAAACCTCCCTTTTATCTTCTAGCCACAGTTCTAAGACCCAAAGTGGGGGGTGGACACAGAAGTCCCTAGGA
GGGAAGGAGCT

Figure 3

668070" ET92260

Edited Contig 3506132

GTCTTTTACTTTGGCAATGGCTGGATTCTACCTCATCACGGCCTTTGTCCTTGCTACCTCTCAGGCCCAAGCTGGATGGCTGCAACATGA
TTATGGCCACCTGTCTGTCTACAGAAAACCCAAGTGGAACCACTTGTCCACAAATTCGTCATTGGCCACTTAAAGGGTGCCTCTGCCAACT
GGTGGAATCATCGCCACTTCCAGCACCACGCCAAGCCTAACATCTTCCACAAGGATCCCGATGTGAACATGCTGCACGTGTTTGTTCTGGGC
GAATGGCAGCCCATCGAGTACGGCAAGA

Figure 4

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668070" ET922260

Edited Contig 3854933

CAGGGACCTACCCCGCGCTACTTCACCTGGGACGAGGTGGCCCAGCGCTCAGGGTGCGAGGAGCGGTGGCTAGTGATCGACCGTAAGGTGTA
CAACATCAGCGAGTTCACCCGCCGGCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGGCAGGATGCCACGGATCCCTTTGTGGCCT
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AATAAAGAGCTGACAGATGAGTTCCGGGAGCTGCGGGCCACAGTGGAGCGGATGGGGCTCATGAAGGCCAACCATGTCTTCTTCCTGCTGTA
CCTGCTGCACATCTTGCTGCTGGATGGTGCAGCCTGGCTCACCCCTTGGGTCTTTGGGACGTCCTTTTTGCCCTTCCTCCTCTGTGCGGTGC
TGCTCAGTGCAGTTCAGGCCCAGGCTGGCTGGCTGCAGCATGACTTTGGGCACCTGTCGGTCTTCAGCACCTCAAAGTGGAACCATCTGCTA
CATCATTTTTGTGATTGGCCACCTGAAGGGGGCCCCGCCAGTTGGTGGAACCACATGCACTTCCAGCACCATGCCAAGCCCCAACTGCTTCCG
CAAAGACCCAGACATCAACATGCATCCCTTCTTCTTTGCCTTGGGGAAGATCCTCTCTGTGGAGCTTGGGAAACAGAAGAAAAAATATATGC
CGTACAACCACCAGCACARATACTTCTTCCTAATTGGGCCCCCAGCCTTGCTGCCTCTCTACTTCCAGTGGTATATTTTCTATTTTGTATC
CAGCGAAAGAAGTGGGTGGACTTGGCCTGGATCAGCAAACAGGAATACGATGAAGCCGGGCTTCATTGTCCACCGCAAATGCTTCTAAA

Figure 5

282

Edited Contig 2511785

GCCACTTAAAGGGTGCCTCTGCCAACTGGTGAATCATCGCCACTTCCAGCACCACGCCAAGCCTAACATCTTCCACAAGGATCCCGATGTG
AACATGCTGCACGTGTTTGTCTGGGCGAATGGCAGCCCATCGAGTACGGCAAGAAGAAGCTGAAATACCTGCCCTACAATCACCAGCACGA
ATACTTCTTCTGATTGGGCCGCCGCTGCTCATCCCCATGTATTTCCAGTACCAGATCATCATGACCATGATCGTCCATAAGAACTGGGTGG
ACCTGGCCTGGGCCGTCAGCTACTACATCCGGTTCTTCATCACCTACATCCCTTTCTACGGCATCCTGGGAGCCCTCCTTTTCCTCAACTTC
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CAGTAGCCAGCTGACAGCCACCTGCAACGTGGAGCAGTCCTTCTTCAACGACTGGTTCAGTGGACACCTTAACTTCCAGATTGAGCACCACC
TCTTCCCCACCATGCCCCGGCACAACTTACACAAGATCGCCCCGCTGGTGAAGTCTCTATGTGCCAAGCATGGCATTGAATACCAGGAGAAG
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GACCTGCCCTCCCTCAGCCGTCAGCCATCAGCCATGGCCCTCCCAGTGCCTCCTAGCCCCCTTCTTCCAAGGAGCAGAGAGGTGGCCACCGGG
GGTGGCTCTGTCCTACCTCCACTCTCTGCCCCCTAAAGATGGGAGGAGACCAGCGGTCCATGGGTCTGGCCTGTGAGTCTCCCCCTTGCAGCCT
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TCGGTTAAGTACCCGAGGCCTCTCTTAAGATGTCCAGGGCCCCAGGGCCGCGGGCACAGCCAGCCAAACCTTGGGGCCCTGGAAGAGTCCTC
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GGAGTCCCTCGTCTCTTGTGACTCAGCAGAGGCAGTGCCACGTTACGGGAGGGGCCGGCTGGCCTGGAGGCTCAGCCACCTTCCAGCTTT
TCCTCAGGGTGTCTGAGGTCCAAGATTCTGGAGCAATCTGACCCTTCTCCAAAGGCTCTGTTATCAGCTGGGCAGTGCCAGCCAATCCCTG
GCCATTTGGCCCCAGGGGACGTGGGCCCTG

Figure 6

5/2

668070" E7922260

Contig 2535

GTCTTTTACTTTGGCAATGGCTGGATTCTACCCCTCATCACGGCCTTTGTCCTTGCTACCTCTCAGGCCCAAGCTGGATGGC
TGCAACATGATTATGGCCACCTGTCTGTCTACAGAAAACCAAGTGGAAACCACCTTGTCACAAATTCGTCAATTGGCCACT
TAAAGGGTGCCTCTGCCAACTGGTGAATCATCGCCACTTCCAGCACCACGCCAAGCCTAACATCTTCCACAAGGATCCC
GATGTGAACATGCTGCACGTGTTTGTCTGGGCGAATGGCAGCCCATCGAGTACGGCAAGAAGAAGCTGAAATACCTGCC
CTACAATCACCAGCACGAATACTTCTTCTGATTGGGCCGCCGCTGCTCATCCCCATGTATTTCCAGTACCAGATCATCATG
ACCATGATCGTCCATAAGAACTGGGTGGACCTGGCCTGGGCCGTCAGCTACTACATCCGGTTCTTCATCACCTACATCCCT
TTCTACGGCATCCTGGGAGCCCTCCTTTTCTCAACTTCATCAGGTTCTCTGGAGAGCCACCTGGTTTGTGTGGGTACACAG
ATGAATCACATCGTCATGGAGATTGACCAGGAGGCCTACCGTGACTGGTTCAGTAGCCAGCTGACAGCCACCTGCAACGT
GGAGCAGTCCCTTCTTCAACGACTGGTTCAGTGGACACCTTAACTTCCAGATTGAGCACCACCTCTTCCCCACCATGCCCG
GCACAACCTTACACAAGATCGCCCCGCTGGTGAAGTCTCTATGTGCCAAGCATGGCATTGAATACCAGGAGAAGCCGCTAC
TGAGGGCCCTGCTGGACATCATCAGGTCCCTGAAGAAGTCTGGGAAGCTGTGGCTGGACGCCTACCTTCACAAATGAAGC
CACAGCCCCCGGGACACCGTGGGGAAGGGGTGCAGGTGGGGTGTATGGCCAGAGGAATGATGGGCTTTTGTCTGAGGG
GTGTCCGAGAGGGCTGGTGTATGCACTGCTCACGGACCCCATGTTGGATCTTTCTCCCTTTCTCCTCTCCTTTTCTCTTAC
ATCTCCCCCATAGCACCTGCCCTCATGGGACCTGCCCTCCCTCAGCCGTCAGCCATCAGCCATGGCCCTCCCAGTGCCTC
CTAGCCCCCTTCTCCAAGGAGCAGAGAGGTGGCCACCGGGGGTGGCTCTGTCTACCTCCACTCTCTGCCCCCTAAAGATG
GGAGGAGACCAGCGGTCCATGGGTCTGGCCTGTGAGTCTCCCTTGCAGCCCTGGTCACTTACCTTACCCCCGCTTTGGT
TCTTCAGATGCTCTTGGGGTTCATAGGGGCAGGTCTAGTCGGGCAGGGCCCCCTGACCCTCCCGGCCTGGCTTCACTCTC
CCTGACGGCTGCCATTGGTCCACCCTTTCATAGAGAGGCCTGCTTTGTTACAAAGCTCGGGTCTCCCTCCTGCAGCTCGGT
TAAGTACCCGAGGCCTCTCTTAAGATGTCCAGGGCCCCAGGCCGCGGGCACAGCCAGCCCAACCTTGGGCCCTGGAA
GAGTCCTCCACCCCATCACTAGAGTGCTCTGACCCTGGGCTTTACGGGGCCCCATTCCACCGCCTCCCCAACTTGAGCCTG
TGACCTTGGGACCAAGGGGGAGTCCCTCGTCTCTTGTGACTCAGCAGAGGCAGTGGCCACGTTAGGGAGGGGGCCGGC
TGGCCTGGAGGCTCAGCCCACCCTCCAGCTTTTCTCAGGGTGTCTGAGGTCCAAGATTCTGGAGCAATCTGACCCTTCT
CCAAAGGCTCTGTTATCAGCTGGGCAGTGCCAGCCAATCCCTGGCCAATTTGGCCCCAGGGGACGTGGGCCCTG

Figure 7

284

Edited Contig 253538a

CA GGGACCTACCCCGCGCTACTTTCACCTGGGACGAGGTGGCCACAGCGCTCAGGGTGCGAGGAGCGGTGGCTAGTGATC
GACCGTAAGGTGTACAACATCAGCGAGTTCACCCGCCGGCATCCAGGGGGCTCCCGGGTTCATCAGCCACTACGCCGGGC
AGGATGCCACGGATCCCTTTGTGGCCTTCCACATCAACAAGGGCCGTGTGAAGAAGTATGAACTCTCTCTGAITGGAG
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CAGCCTGGCTCACCCCTTTGGGTCTTTGGGACGTCCTTTTTGGCCCTTCCTCCTCTGTGCGGTGCTGCTCAGTGCAGTTCAGC
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TGACAGCCACCTGCAACGTGGAGCAGTCCCTTCTTCAACGACTGGTTCAGTGGACACCTTAACITCCAGATTGAGCACCACC
TCITCCCCACCATGCCCCGGGCACAACCTTACACAAGATCGCCCCGCTGGTGAAGTCTCTATGTGCCAAGCATGGCATIGAAT
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CCTCTCCTTTTTCTTTCACATCTCCCCATAGCACCCCTGCCCTCATGGGACCTGCCCTCCCTCAGCCGTCAGCCATCAGCC
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GCATCACCCCCGCTTTGGTTCTTCAGATGCTCTTGGGGTTCATAGGGGCAGGTCCCTAGTCCGGCAGGGCCCTGACCTTC
CCGGCTGGCTTCACTCTCCCTGACGGCTGCCATTGGTCCACCCTTTCATAGAGAGGCCTGCTTTGTACAAAGCTCGGGT
CTCCCTCCTGCAGCTCGGTTAAGTACCCGAGGCCTCTCTTAAGATGTCCAGGGCCCCAGGCCCGCGGGCACAGCCAGCCC
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CTCCCCAACTTGAGCCTGTGACCTTGGGACCAAAGGGGGAGTCCCTCGTCTCTTGTGACTCAGCAGAGGCAGTGGCCAC
GTTCAGGGAGGGGCCGGCTGGCCTGGAGGCTCAGCCACCCTCCAGCTTTTCTCAGGGTGTCTGAGGTCCAAGATTCT
GGAGCAATCTGACCTTCTCCAAGGCTCTGTTATCAGCTGGGCAGTGCCAGCCAATCCCTGGCCAATTGGCCCCAGGGG
ACGTGGGCCCTG

Figure 8

~~20~~

288
286

FastA Match of Ma29 and contig 253538a

SCORES Init1: 117 Initn: 225 Opt: 256
Smith-Waterman score: 408; 27.0% identity in 441 aa overlap

Ma29.pep	10	20	30	40	50	
	MGTDQGKT---FTWEELAAHNTKDDLLLAIRGRVYDVTKFLSRHPGGVDTL LLGAGRDVT					
253538a	: : :: :: : : : : : :: : :					
	10	20	30	40	50	
	QGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPGGSRVISHYAGQDAT					
Ma29.pep	60	70	80	90	100	110
	PVFEMYHAF-GAADAIMKKYVGTLSNELPIFPEPTVFHKTIKTRVEGYFTDRNIDPKN					
253538a	: : : : : :: : : :					
	60	70	80	90	100	110
	DPFVAFHINKGLVKKYMNSLLIGEL-SPEQPSF-EPTKNKELTDEFRELRAIVERMGLMK					
Ma29.pep	120	130	140	150	160	170
	RPEIWGRYALIFGSLIASYYAQLFVPFVVERTWLQVVF-AIIMGFACAQVGLNPLHDASH					
253538a	::: : : :: ::: :: :: : : : : : :					
	120	130	140	150	160	170
	ANHVF--FLLYLLHILLLDGAAWLTWVFGTSFLPFLCAVLLSAVQAQAGWLQ-HDYGH					
Ma29.pep	180	190	200	210	220	
	FSVTHNPTVWKILGATHDF---FNGASYLVVMYQHMLGHHPTNLAGADPDVSTSE---					
253538a	: :: : : :: : : : :					
	180	190	200	210	220	
	LSVYRKPK-WNHL--VHKFVIGHLKGASANWNHRRH-FQHHAKPNIFHKDPDVNMLHVFV					
Ma29.pep	230	240	250	260	270	280
	----PDVRRIPKPNQKWF-VNHINQHMV--PFLYGLLAFKVRIQDINILYFVKINDAIRV					
253538a	:: : : :::: : : : : : : : :::					
	230	240	250	260	270	280
	LGEWQPIEYGGKKLKYLPYNHQHEYFFLIGPPLLIPMYFYQYQI----IMTMIVHKNWVDL					
Ma29.pep	290	300	310	320	330	340
	NPISWHTVMFWGGKAFFVWYRLVPLQYLPLGKVLLFTVADMVSSYWLALTFQANHVV					
253538a	: : :: : : : : : : :: : : : :					
	290	300	310	320	330	340
	----AWAVSYI---RFFITY---IPF-YGILG-ALLFLNFIRFLESHWFVWVTQMNHIV					
Ma29.pep	350	360	370	380	390	
	EEVQWPLPDENGIIQKDWAAQVETT---QDYAHDShLWTSITGSLNYQAVHHLFPNVS					
253538a	: : : : : : : :::					
	340	350	360	370		
	MEI-----DQEAY--RDWFSQLTATCNVEQSFEND--WFS--GHLNFQIEHHLFPTMP					
Ma29.pep	400	410	420	430	440	
	QHHPDILAIKNTCSEYKVPYLVDITFWQAFASHLEHLRVLGLRPKEEX					
253538a	: : : : : : : : :					
	380	390	400	410	420	430
	RHNHLKIAPLVKSLCAKHGIEYQEKPLLRALLDIIRSLKSGKLWLDAYLHKX					

Figure 9

02261-01059

289
287

FastA Match of Ma524 and contig 253538a

SCORES Initl: 231 Initn: 499 Opt: 401
Smith-Waterman score: 620; 27.3% identity in 455 aa overlap

	10	20	30	40	50	59
Ma524.pep	MAAAPSVRTFTTAEVLNAEALNEGKKDAEAPFLMIIDNKVYDVREFVPDHPGGSVILTH-					
	:	:	:	:	:	:
253538a	QGTPRYFTWDEV-----AQRSGCEERWLVIDRKVYNISEFTRRHPGGSRVISHY					
	10	20	30	40	50	

	60	70	80	90	100	110
Ma524.pep	VGKDGTDVFDTFHPEAAW--ETLANFYVGDIDE---SDRDIKNDDFAAEVRKLRTLQSL					
	: :	:	:	:	:	:
253538a	AGQDATDPFVAFHINKGLVKKYMNSLLIGELSPEQPSFEPTKNKELTDEFRELRAIVERM					
	60	70	80	90	100	110

	120	130	140	150	160	170
Ma524.pep	GYYDSSKAYYAFKVSFNLCTIWLSTVIVAKWGQTSTLANVLSAALLGLFWQCGWLAHDF					
	: : :	:	:	:	:	:
253538a	GLMKANHVFFLLYLLHILLDGAAWLTLWVFG-TSFLPFLLCVLLSAVQAQAGWLQHDY					
	120	130	140	150	160	

	180	190	200	210	220	230
Ma524.pep	LHHQVFQDRFWGDLFGAFLGGVCGQFSSSWWKDKHNTHHAAPNVHGEDPDIDTHPLLTWS					
	: :	:	:	:	:	:
253538a	GHLVYRKPKWNHLVHKFVIGHLKGASANWNNHRHFQHHAKPNIFHKDPDVN---ML---					
	170	180	190	200	210	220

	240	250	260	270	280	290
Ma524.pep	EHALEMFSVDPDEELTRMWSRFMVLNQTFWYFPILS---FARLSWCLQSILFVLPNGQAH					
	: : :	:	:	:	:	:
253538a	-HVF-VLGEWQPIEYGGKKLKYLPYNHQHEYFFLIGPPLLIPMYQYQIIMTMI---VH					
	230	240	250	260	270	

	300	310	320	330	340	349
Ma524.pep	KPSGARVPISLVEQLSLAMHWIWLATMFLFIK--DPVNMLVYFLVSQAVCGNLLAIVFS					
		:	:	:	:	:
253538a	K-----NWVDLAWAVSYIRFFITYIPFYGILGALLFLNFIREFLESHWFVWVTQ					
	280	290	300	310	320	

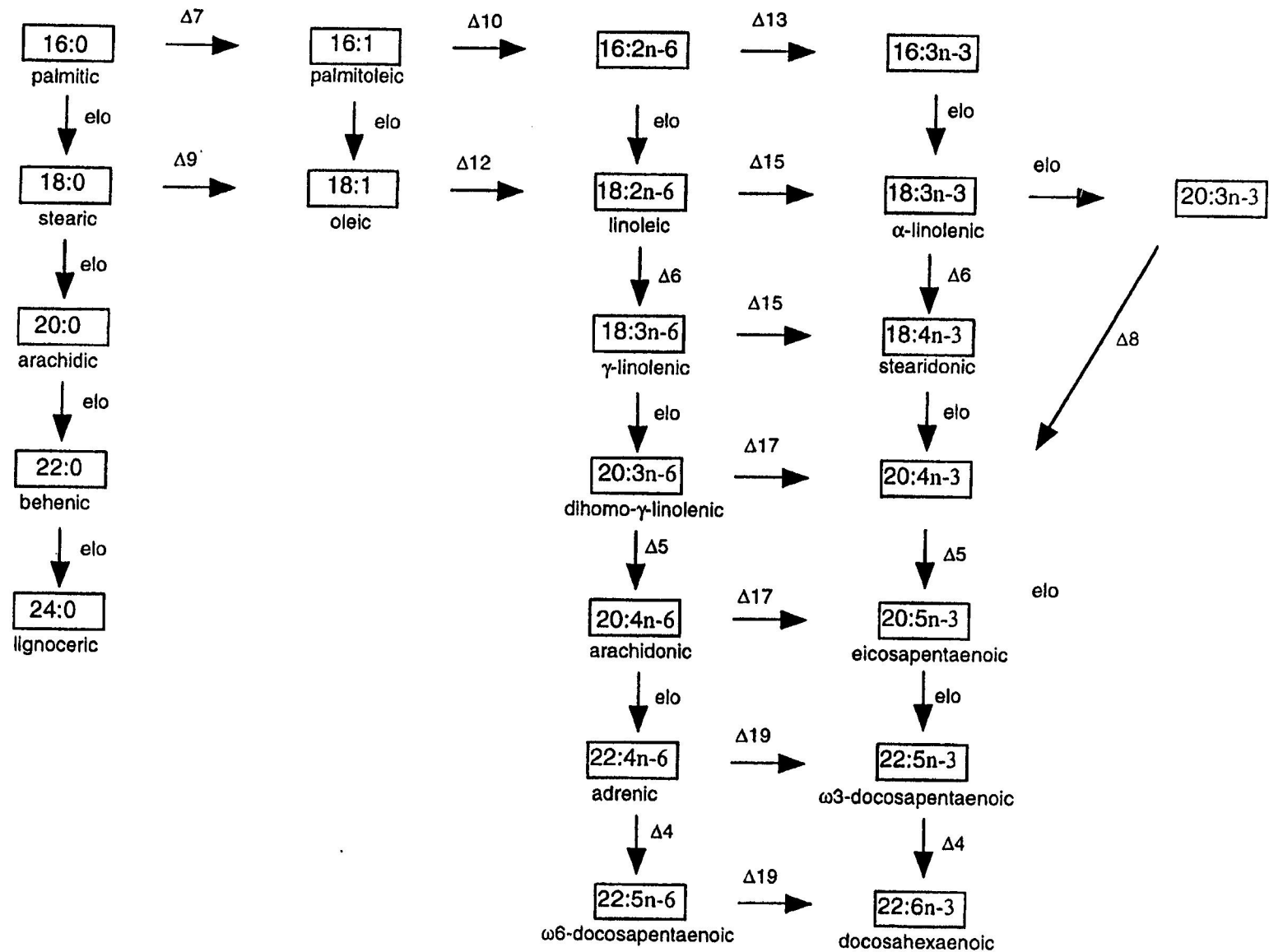
	350	360	370	380	390	400	409
Ma524.pep	LNHNGMPVISKEEAVDMDFFTKQIITGRDVHPGLFANWFTGGLNYQIEHHLFSPMPRHNF						
	:	:	:	:	:	:	:
253538a	MNHIVMEI--DQEAYR-DWFSSQLTATCNVEQSFFNDWFSGHLNFQIEHHLFPTMPRHNL						
	330	340	350	360	370	380	

	410	420	430	440	450
Ma524.pep	SKIQPAVETLCKKYNVRYHTTGMIEGTAEVFSRLNEVSKAASKMGKAQX				
		:	:	:	:
253538a	HKIAPLVKSLCAKHGIEYQEKPLLRLLDIIRSLKKGKGLWLDAYLHKX				
	390	400	410	420	430

668070"ET92260

Figure 10

Fatty Acid Biosynthesis Pathways



282 2/12

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Human $\Delta 5$ -desaturase

ATGGCCCCGACCCGGTGGCCGCCGAGACCGCGGCTCAGGGACCTACCCCGCGCTACTTCACCTGG
GACGAGGTGGCCCAGCGCTCAGGGTGGGAGGAGCGGTGGCTAGTGATCGACCGTAAGGTGTACAAC
ATCAGCGAGTTCACCCGCCGGCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGGCAGGAT
GCCACGGATCCCTTTGTGGCCTTCCACATCAACAAGGGCCTTGTGAAGAAGTATATGAACTCTCTC
CTGATTGGAGAACTGTCTCCAGAGCAGCCCAGCTTTGAGCCCAAGAATAAAGAGCTGACAGAT
GAGTTCCGGGAGCTGCGGGCCACAGTGGAGCGGATGGGGCTCATGAAGGCCAACCATGTCTTCTTC
CTGCTGTACCTGCTGCACATCTTGCTGCTGGATGGTGCAGCCTGGCTCACCTTTGGGTCTTTGGG
ACGTCCTTTTTTGCCCTTCTCCTCTGTGCGGTGCTGCTCAGTGCAGTTCAGGCCCAGGCTGGCTGG
CTGCAGCATGACTTTGGGCACCTGTGCGTCTTCAGCACCTCAAAGTGGAAACCATCTGCTACATCAT
TTTGTGATTGGCCACCTGAAGGGGGCCCCCGCCAGTTGGTGGAACCACATGCACTTCCAGCACCAT
GCCAAGCCCAACTGCTTCCGCAAAGACCCAGACATCAACATGCATCCCTTCTTCTTTGCCTTGGGG
AAGATCCTCTCTGTGGAGCTTGGGAAACAGAAGAAAAAATATATGCCGTACAACCACCAGCACAAA
TACTTCTTCTAATTGGGCCCCCAGCCTTGCTGCCTCTCTACTTCCAGTGGTATATTTTCTATTTT
GTTATCCAGCGAAAAGAAGTGGGTGGACTTGGCCTGGATGATTACCTTCTACGTCCGCTTCTTCCTC
ACTTATGTGCCACTATTGGGGCTGAAAGCCTTCCTGGGCCTTTTCTTCATAGTCAGGTTCTTGGA
AGCAACTGGTTTGTGTGGGTGACACAGATGAACCATATTCCCATGCACATTGATCATGACCGGAAC
ATGGACTGGGTTTCCACCCAGCTCCTGGCCACATGCAATGTCCACAAGTCTGCCTTCAATGACTGG
TTCAGTGGACACCTCAACTTCCAGATTGAGCACCATCTTTTCCACGATGCCTCGACACAATTAC
CACAAAGTGGCTCCCTGGTGCAGTCCTTGTGTGCCAAGCGTGGCATAGAGTACCAGTCCAAGCCC
CTGCTGTCAGCCTTCGCCGACATCATCCACTCACTAAAGGAGTCAGGGCAGCTCTGGCTAGATGCC
TATCTTCACCAATAA

0927613-010399

Figure 12

Contig[0023]
Sequencher™ "pRAE-7 sequence"

293
291

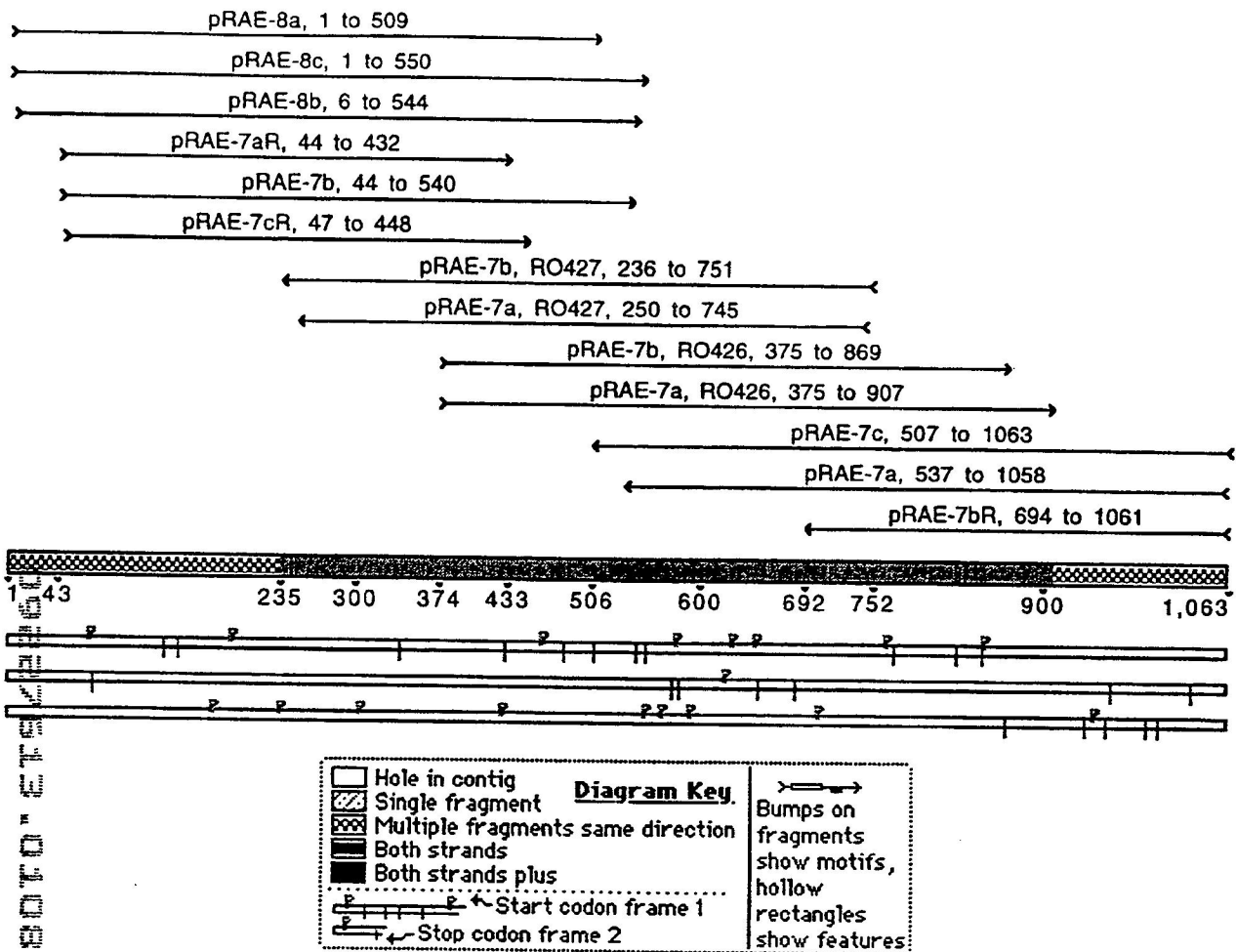


Figure 14


```
*          *           *            *              *                *                  *                    *
TTC TAC GTC CGC TTC TTC CTC ACT TAT GTG CCA CTA TTG GGG CTG AAA
Phe Tyr Val Arg Phe Phe Leu Thr Tyr Val Pro Leu Leu Gly Leu Lys>
__a__a__a__a__TRANSLATION OF PRAE-7 MV [ A ] __a__a__a__a__>

      490               500                 510                   520
    *         *           *             *              *                *                  *
GCC TTC CTG GGC CTT TTC TTC ATA GTG AGG TTC CTG GAA AGC AAC TGG
Ala Phe Leu Gly Leu Phe Phe Ile Val Arg Phe Leu Glu Ser Asn Trp>
__a__a__a__a__TRANSLATION OF PRAE-7 MV [ A ] __a__a__a__a__>

530               540               550                 560                   570
*         *           *             *              *                *                  *
TTT GTG TGG GTG ACA CAG ATG AAC CAT ATT CCC ATG CAC ATT GAT CAT
Phe Val Trp Val Thr Gln Met Asn His Ile Pro Met His Ile Asp His>
__a__a__a__a__TRANSLATION OF PRAE-7 MV [ A ] __a__a__a__a__>

     580               590               600                 610                   620
    *         *           *             *              *                *                  *
GAC CGG AAC ATG GAC TGG GTT TCC ACC CAG CTC CAG GCC ACA TGC AAT
Asp Arg Asn Met Asp Trp Val Ser Thr Gln Leu Gln Ala Thr Cys Asn>
__a__a__a__a__TRANSLATION OF PRAE-7 MV [ A ] __a__a__a__a__>

     630               640               650                 660                   670
*         *           *             *              *                *                  *
GTC CAC AAG TCT GCC TTC AAT GAC TGG TTC AGT GGA CAC CTC AAC TTC
Val His Lys Ser Ala Phe Asn Asp Trp Phe Ser Gly His Leu Asn Phe>
__a__a__a__a__TRANSLATION OF PRAE-7 MV [ A ] __a__a__a__a__>

     680               690               700                 710                   720
    *         *           *             *              *                *                  *
CAG ATT GAG CAC CAT CTT TTT CCC ACG ATG CCT CGA CAC AAT TAC CAC
Gln Ile Glu His His Leu Phe Pro Thr Met Pro Arg His Asn Tyr His>
__a__a__a__a__TRANSLATION OF PRAE-7 MV [ A ] __a__a__a__a__>

     730               740               750                 760
    *         *           *             *              *                *                  *
AAA GTG GCT CCC CTG GTG CAG TCC TTG TGT GCC AAG CAT GGC ATA GAG
Lys Val Ala Pro Leu Val Gln Ser Leu Cys Ala Lys His Gly Ile Glu>
__a__a__a__a__TRANSLATION OF PRAE-7 MV [ A ] __a__a__a__a__>

770               780               790                 800                   810
*         *           *             *              *                *                  *
TAC CAG TCC AAG CCC CTG CTG TCA GCC TTC GCC GAC ATC ATC CAC TCA
Tyr Gln Ser Lys Pro Leu Leu Ser Ala Phe Ala Asp Ile Ile His Ser>
__a__a__a__a__TRANSLATION OF PRAE-7 MV [ A ] __a__a__a__a__>

     820               830               840                 850                   860
    *         *           *             *              *                *                  *
CTA AAG GAG TCA GGG CAG CTC TGG CTA GAT GCC TAT CTT CAC CAA TAA
Leu Lys Glu Ser Gly Gln Leu Trp Leu Asp Ala Tyr Leu His Gln ***>
__a__a__a__a__TRANSLATION OF PRAE-7 MV [ A ] a a a a >
```

Figure 15b

296
294

FastA Match of the Gene in pRAE-7 and the *M. alpina* $\Delta 5$ -desaturase (Ma29) Translated Sequences

SCORES Init1: 62 Initn: 105 Opt: 245
Smith-Waterman score: 271; 28.4% identity in 292 aa overlap

10 20 30 40
pRAE-7.pep LLEPVSI GGIPAVQAQAGWLQ-HDFGHL SV-FSTSKWNHL--LH
Ma29.pep ASYYAQLFVPFVVERTWLQVVFALIMGFACAQVGLNPLHDASHFSVTHNPTVWKILGATH
140 150 160 170 180 190

50 60 70 80 90
pRAE-7.pep HFVIGH LKGAPASWNNHMH-FQHHAKPNCFRKDPDINM-HPFFFALGKILSVELGKQKKK
Ma29.pep DF----FNGASYLVVMYQHMLGHHPTNLAGADPDVSTSEP-----DVRRIKPNQK
200 210 220 230

100 110 120 130 140 149
pRAE-7.pep YMPYNH--QHKYF-FLIGPPALLPLYFQWYIFYFVIQ----RKKWVDLAWMITFY--VRF
Ma29.pep WF-VNHINQHMFVPFLYGLLAFKVRIQDINILYFVKTNDAIRVNPISTWHTVMFWGGKAF
240 250 260 270 280 290

150 160 170 180 190
pRAE-7.pep FLTY---VPL--LGLKAFLGLFFIVRFLESNWFVWVTQMNHHPMHID---HDRN---MD
Ma29.pep FVWYRLIVPLQYLPLGKVLLLFVADMVSSYWLALTFQANHVVVEEVQWPLPDENGIIQKD
300 310 320 330 340 350

200 210 220 230 240 250
pRAE-7.pep WVSTQLQATCN-VHKSAFNDWFSGLNLFQIEHHLFPTMPRHNYHKVAPLVQSLCAKHGIE
Ma29.pep WAAMQVETTDYAHDSHLWTSITGSLNYQAVHHLFPNVSQHHYPDILAIKNTCSEYKVP
360 370 380 390 400 410

260 270 280
pRAE-7.pep YQSKPLL-SAFADI IHS LKESGQLWLDAYLHQX
Ma29.pep YLVKDTFWQAFASHLEHLRVLGLRPKEEX
420 430 440

002261010899

Figure 16

299
295

FastA Match of the Gene in pRAE-7 and *M. alpina* $\Delta 6$ -desaturase (Ma524) Translated Sequences

SCORES Init1: 278 Initn: 483 Opt: 301
Smith-Waterman score: 498; 31.9% identity in 285 aa overlap

pRAE-7.pep
LLEPVSI GGIPAVQAQAGWLQHDFGHLSVFSTSKWNHLLHHFVIG
Ma524.pep
GLSTVIVAKWGQTSTLANVLSAALLGLFWQCCGWL AHDFLHHQVFQDRFWGDLFGAFLGG
140 150 160 170 180 190

pRAE-7.pep
HLKGAPASWNNHMHFQHHAKPNCFRKOPDINMHPFF---FALGKILSV---ELGKQKKK
Ma524.pep
VCQGFSSSWWKDKHNTTHHAAPNVHGEDPDIDTHPLL TWSEHALEMFSDVPDEELTRMWSR
200 210 220 230 240 250

pRAE-7.pep
YMPYNHQHKYFFLLIGPPALLPLYFQWYIFVFV-----IQRKKWVDLAWMITF
Ma524.pep
FMVLN-QTWFFYPILSFARLSWCLQSILFVLPNGQAHKPSGARVPISLVEQLSLAMHWTW
260 270 280 290 300 310

pRAE-7.pep
YVRFFLTIVV--PLLGLKAFLGLFFIVRFLESNWFVWVTQMNHIPMHI---DHDRNMDWVS
Ma524.pep
YLATMFLFIKDPV----NMLVYFLVSQAVCGNLLAIVFSLNHNGMPVISKEEAVDMDFFT
320 330 340 350 360 370

pRAE-7.pep
TQLQATCNVHKSAFNDWFSGHLNFQIEHHLFPTMPRHNYHKVAPLVQSLCAKHGIEYQSK
Ma524.pep
KQIITGRDVHPGLFANWFTGGLNYQIEHHLFPSMPRHNFSKIQPAVETLCKKYNVRYHTT
380 390 400 410 420 430

pRAE-7.pep
PLLSAFADI IHS LKESGQLWLDAYLHQX
Ma524.pep
GMIEGTAEVFSRLNEVSKAASKMGKAQX
440 450

05227613.010830

Figure 17

298
296

FastA Match of the Gene in pRAE-7 and contig 2535

SCORES Initl: 1028 Initn: 1424 Opt: 1430
Smith-Waterman score: 1430; 71.0% identity in 276 aa overlap

	10	20	30	40	50
pRAE-7.pep	LLEPVSI GGIPAVQAQAGWLQHD FGHLSV FSTSKWNHLLHHFVIGHLKGAPA				
2535	VFYFGNGWIPTLITAFVLATSQAQAGWLQHDYGHLSVYRKPKWNHLVHKFVIGHLKGASA				
	10	20	30	40	50
	60	70	80	90	100
pRAE-7.pep	SWWNHMHFQHHAKPNCFRKDPDINM-HPFFFALGKILSVELGKQKKKYPYNHQHKYFFL				
2535	NWNHHRHFQHHAKPNIFHKDPDVNMLH--VFVLGEWQPIEYGGKKLKYLPYNHQHEYFFL				
	70	80	90	100	110
	120	130	140	150	160
pRAE-7.pep	IGPPALLPLYFQWYIFYFVIQRKKWVDLAWMITFYVRFFLTYPVLLG-LKAFLGLFFIVR				
2535	IGPPLLIPMYFQYQIIMIMIVHKWVDLAWAVSYIRFFITYIPFYGILGALLFLNFI-R				
	120	130	140	150	160
	180	190	200	210	220
pRAE-7.pep	FLESNWFVWVTQMNHHPMHIDHNRMDWVSTQLQATCNVHKSAFNDWFSGHLNFQIEHHL				
2535	FLESHWFVWVTQMNHIVMEIDQEAYRDWFSQLTATCNVEQSFFNDWFSGHLNFQIEHHL				
	180	190	200	210	220
	240	250	260	270	280
pRAE-7.pep	FPTMPRHNHYHKVAPLVQSLCAKHGIEYQSKPLL SAFADI IHS LKESGQLWLDAYLHQX				
2535	FPTMPRHNHLHKIAPLVKSLCAKHGIEYQEKPLL RALLDI IRS LKKSGLWLDAYLHKXSH				
	240	250	260	270	280
	300	310	320	330	340
2535	SPRDTVGKGCRCWGDGQRNDGLFXGVSERLVYALLTDPMLDLSPFLLSFFSSHLP HSTLP				
	300	310	320	330	340

09227513-010899

Figure 18

FastA Match of the Gene in pRAE-7 and contig 38

SCORES Initl: 965 Initn: 965 Opt: 968
Smith-Waterman score: 968; 97.0% identity in 133 aa overlap

```

pRAE-7.pep      10      20      30      39
                  LLEPVSI GGI PAVQAQAGWLQHD FGHLSVFSTSKWNHLL
                  : |||||
38      LHILLLDGAAWLT LWVFGTSFLPFLLCVLLSAVQAQAGWLQHD FGHLSVFSTSKWNHLL
          130      140      150      160      170      180

pRAE-7.pep      40      50      60      70      80      90      99
                  HHFVIGHLKGAPASWWNHMHFQHHAKPNCFRKDPDINMHPFFALGKILSVELGKQKKKY
                  |||||
38      HHFVIGHLKGAPASWWNHMHFQHHAKPNCFRKDPDINMHPFFALGKILSVELGKQKKKY
          190      200      210      220      230      240

pRAE-7.pep      100     110     120     130     140     150     159
                  MPYNHQHKYFFLIGPPALLPLYFQWYIFYFVIQRKKWVDLAWMITFYVRFFLTIVPLLGL
                  |||||
38      MPYNHQHKYFFLIGPPALLPLYFQWYIFYFVIQRKKWVDLAWISKQEYDEAGLPLSTANA
          250     260     270     280     290     300

pRAE-7.pep      160     170     180     190     200     210     219
                  KAFLGLFFIVRFLESNWFVWVTQMNHIFMHIDHNRNMDWVSTQLQATCNVHKSAFNDWFS
38      SK
```

00227613-010899

Figure 19

~~310~~
293

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          650      660      670      680      690      700
A-1.pdt      ELTDEFRELRAIVEQRFFVXFLLTCTGAHGFFSLEVPGLPDSNKXFSWTSRPIDVWNGKRP
CYB5_HUMAN   PPETLITTTIDSSSSWITNWIPAISSAVAVALMYRLYMAED
          100      110      120      130

```

Figure 20

FastA Match of 5' Sequence of Clone A-1 and ac004228

LOCUS AC004228 170743 bp DNA HTG 26-FEB-1998
DEFINITION *** SEQUENCING IN PROGRESS *** Homo sapiens Chromosome 11q12 pac
pDJ519o3; HTGS phase 1, 18 unordered pieces.
ACCESSION AC004228
NID g2911733
KEYWORDS HTG; HTGS_PHASE1. . . .

SCORES Init1: 913 Initn: 1123 Opt: 916
94.6% identity in 203 bp overlap

668810"ET22260

A-1	389	379	369	359	349	339	330
	CCCCACCAATATGATGGAATAAAGGAAAGCGGCCGCTGAATTATAGGCCGCCGAGACCGC						
ac004228	CCCCGCGCGCGGCGTCGCCAGGCCAGCTATGGCCCCGACCCGGTGGCCGCCGAGACCGC						
	60090	60100	60110	60120	60130	60140	
A-1	329	319	309	299	289	279	270
	GGCTCAGGGACCTACCCCGCGTTACTTCACATGGGACGAGGTGGCCCAGCGCTCAGGGTG						
ac004228	GGCTCAGGGACCTACCCCGCGCTACTTCACCTGGGACGAGGTGGCCCAGCGCTCAGGGTG						
	60150	60160	60170	60180	60190	60200	
A-1	269	259	249	239	229	219	210
	CGAGGAGCGGTGGCTTGTGATCGACCGTAAGGTGTACAACATCAGCGAGTTACCCGCCG						
ac004228	CGAGGAGCGGTGGCTAGTGATCGACCGTAAGGTGTACAACATCAGCGAGTTACCCGCCG						
	60210	60220	60230	60240	60250	60260	
A-1	209	199	189	179	169	159	150
	GCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGCAGGATGCCACGGATCCCTT						
ac004228	GCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGCAGGATGCCACGGTGAGCGC						
	60270	60280	60290	60300	60310	60320	
A-1	149	139	129	119	109	99	90
	CGTGGCCTTCCACATCAACAAGGGCCTTGTGAAGAAGTATATGAACTCTCTCCTGATTGG						
ac004228	AGCCAGGCGGGGGCACAGGAGAGGGCGGGACCGGAGGCTGAGTGCCAGGGGAGACAGAGTT						
	60330	60340	60350	60360	60370	60380	

Figure 21

302
300

FastA Match of 5' Sequence of Clone 3-5 and ac004228

SCORES Initl: 1365 Initn: 2510 Opt: 1377
98.6% identity in 285 bp overlap

	20	30	40	50	60	70
3-5	AATACGACTCACTATAGGGCTCGAGCGGCCCGGGCAGGTCCGGACCTGCCAACGTGA					
ac004228	CCCCGCCCCACAGCCGCATCACTTACAGGGCCCGGGGCTG-CCGGACCTGCCAACGTGA					
	61710	61720	61730	61740	61750	61760
	80	90	100	110	120	130
3-5	ATCTTATCGCCATGGACCTTACCTTGCACAACCCAAAGTAGCTGCCTTGGGGCAGGGGGT					
ac004228	ATCTTATCGCCATGGACCTTACCTTGCACAACCCAAAGTAGCTGCCTTGGGGCAGGGGGT					
	61770	61780	61790	61800	61810	61820
	140	150	160	170	180	190
3-5	GGCCAGAGTGCTTAGGGAAATGTGGAGCCCTACCCAGAACAACGGTGGAGGGAAAGGGAA					
ac004228	GGCCAGAGTGCTTAGGGAAATGTGGAGCCCTACCCAGAACAACGGTGGAGGGAAAGGGAA					
	61830	61840	61850	61860	61870	61880
	200	210	220	230	240	250
3-5	GAAACGCAGAAAGTGCCCCAGTTCGGACGTAGGGAAGTCTTCTCTTCGTGGTTTTTGGAG					
ac004228	GAAACGCAGAAAGTGCCCCAGTTCGGACGTAGGGAAGTCTTCTCTTCGTGGTTTTTGGAG					
	61890	61900	61910	61920	61930	61940
	260	270	280	290	300	310
3-5	AACCCTAGCTAAGAGAGGAAAGGGACTTATTGAAAGACCCGCAAGAAGGGACGGAAGTCT					
ac004228	AACCCTAGCTAAGAGAGGAAAGGGACTTATTGAAAGACCCGCAAGAAGGGACGGAAGTCT					
	61950	61960	61970	61980	61990	62000
	320	330	340	350	360	370
3-5	CATAGCCCTGAGAGGATCCCTTTGTGGCCTTCCACATCAACAAGGGCCTTGTGAAGAAGT					
ac004228	CATAGCCCTGAGAGGTGAAGCCAGCTGGAGTTGATGGGTGCAATGGGGACCTAGAGAAGT					
	62010	62020	62030	62040	62050	62060

Figure 22

0027613.010399

303
301

FastA Match of 5' Sequence of Clone A-10 and ac004228

SCORES Initl: 931 Initn: 1309 Opt: 934
97.0% identity in 200 bp overlap

	30	40	50	60	70	80	89
A-10	TATAGGGCTCGAGCGGCCCGCCCGGCAGGTGCCCGGAGGCGCCTGATCATACCTGTTGCC						
ac004228	CGAGCCAAACACCGACTAATTCGAGGAAAGCCCGGAGGCGCCTGATCATACCTGTTGCC						
	60400	60410	60420	60430	60440	60450	
	90	100	110	120	130	140	149
A-10	CGGTGATTGGGTGTCCTGCGGATGCGGGATGAAAAGGCGGGAGAGAGGCCTGGAAAAGTG						
ac004228	CGGTGATTGGGTGTCCTGCGGATGCGGGATGAAAAGGCGGGAGAGAGGCCTGGAGAAGTG						
	60460	60470	60480	60490	60500	60510	
	150	160	170	180	190	200	209
A-10	GAGTCTGGGGAGTGGGGATGGAGGCCAACACACGACACACAAACAAAGGTCCCGCCT						
ac004228	GAGTCTGGGGAGTGGGGATGGAGGCCAACACACGACACACAAACAAAGGTCCCGCCT						
	60520	60530	60540	60550	60560	60570	
	210	220	230	240	250	260	269
A-10	CCCTGCCGTGCATTCCATCTGCAGCCCCGAGCCTCAGGATCCCTTTGTGGCCTTCCACAT						
ac004228	CCCTGCCGTGCATTCCATCTGCAGCCCCGAGCCTCAGG-TCTCTGGGCGGGACAGAACC						
	60580	60590	60600	60610	60620	60630	

09227513.010895

Figure 23

304
302

FastA Match of 5' Sequence of Clone A-16 and ac004228

SCORES Init1: 985 Initn: 1488 Opt: 997
98.1% identity in 209 bp overlap

	40	50	60	70	80	90
A-16	CGAGCGGCCCGCCCGGCAGGTCTAGAATTCAGCGGCCGCTGAAGCCGCGTCTGGACCTAG					
ac004228	AGGGAGTCACATCCTGTCTCGATGGCTAGGAGAGGCAGC-GCAGCCGCGTCTGGACCTAG					
	60720	60730	60740	60750	60760	
	100	110	120	130	140	150
A-16	GTGCCGGTCTCCACTCGCCAGCAGGAGCGGAGAGGGAGCAGGAAAGGAGCCCATTTCTCGA					
ac004228	GTGCCGGTCTCCACTCGCCAGCAGGAGCGGAGAGGGAGCAGGAAAGGAGCCCATTTCTCGA					
	60770	60780	60790	60800	60810	60820
	160	170	180	190	200	210
A-16	GGATGGGGCTGAAACGGGAAGCTTGGGGAGACCGCTGCCTTGGGGACCCCTGCGTCTGTGT					
ac004228	GGATGGGGCTGAAACGGGAAGCTTGGGGAGACCGCTGCCTTGGGGACCCCTGCGTCTGTGT					
	60830	60840	60850	60860	60870	60880
	220	230	240	250	260	270
A-16	GAAGACTGGAGGACGCGGAAGGGACAGCGCTGGCCGGGGAGGGCAAGCGGCCGCTGGCGA					
ac004228	GAAGACTGGAGGACGCGGAAGGGACAGCGCTGGCCGGGGAGGGCAAGCGGCCGCTGGCGT					
	60890	60900	60910	60920	60930	60940
	280	290	300	310	320	330
A-16	TCCCTTTGTGGCCTTCCACATCAACAAGGGCCTTGTGAAGAAGTATATGAAGTCTCTCCT					
ac004228	ACATAAGGGATTGGGAATGGCATACATTAGCGAGGACCCCCAGAGCTGTTCTCGAATCG					
	60950	60960	60970	60980	60990	61000

0927613.010899

Figure 24

305
303

FastA Match of 5' Sequence of Clone A-19 and ac004228

SCORES Initl: 1227 Initn: 1409 Opt: 1532
94.0% identity in 349 bp overlap

	60	70	80	90	100	110
A-19	TTATTCCTTATTTGTCCTGCCCATGTCCTGCTGATTGGTCCATTTTACCTCTAGCTAG					
ac004228	ATAGAGCACTGATTGGTCCATTTTACAGGGTGCTGATTGGTCCATTTTACCTCTAGCTAG					
	63250	63260	63270	63280	63290	63300
	120	130	140	150	160	170
A-19	CTAAAGAGCACGGATTGGTGCATTTTGCAAACCTCTGGCTACAGAGGGTTCTCCAGGTC					
ac004228	CTAAAGAGCACGGATTGGTGCATTTTACAAACCTCTAGCTACAGAAAAGTTCTCCAAGTC					
	63310	63320	63330	63340	63350	63360
	180	190	200	210	220	230
A-19	TGCACTCGACCCAGGAAGTCCATCTGGCTTCACCTCTCACTTCAACTTGGGTACAGCCTT					
ac004228	TGCACTCGACCCAGGAAGTCCATCTGGCTTCACCTCTCACTTCAACTTGGGTACAGCCTT					
	63370	63380	63390	63400	63410	63420
	240	250	260	270	280	290
A-19	CTGGCGGGCAGGAAGATGGCCTTTGGTGCGAACACTGCCGGAGTCCAGGGGGCTGGCTCC					
ac004228	CTGGCGGGCAGGAGGATGGCCTTTGGTGCGAACACTGCCGGAGTCCAGGGGGCTGGCTCC					
	63430	63440	63450	63460	63470	63480
	300	310	320	330	340	
A-19	CTCACCTTTTCATCTTCTCCCGGCACCTTGCAAGATCCCTTTGTGGCC					
ac004228	CTCACCTTTTCATCTTCTCCCGGCACCTTGCAAGATCCCTTTGTGGCC					
	63490	63500	63510	63520	63530	

09227613-010899

Figure 25

Partial Sequence of ac004228

59751 ACTAGAACCG CTGTTCTTAC CGCGGCGCCC CCTGGGAGCC AACGCCGCGA
59801 TGCCCGCCTG ACGTCAGGAA GTCGAATCCG GCGGCGACGC TTTTAGGGAG
59851 CCCGCGAGGG GCGCGTGTGTT GGCAGCCCAG CTGTGAGTTG CCAAGACCC
59901 ACCGGGGGAC GGGATCTCGC TCCCCGCGCC ACGAGGCTCG GCCAATGGGA Possible start
59951 ACGCGCGCTG CGAGGCCCGC CGGTCTGCCC TCGGTGCTG AAAACCCGGC
60001 GCGCAGGCGG CTGGCTCTGG GCGCGCGCCA GCAAATCCAC TCCTGGAGCC
60051 CGCGGACCCC GAGCACGCGC CTGACAGCCC CTGCTGGCCC GCGCGCGGGC
60101 GTCGCCAGGC CAGCTATGGC CCCCAGCCCG GTGGCCGCGG AGACCGCGGC
60151 TCAGGGACCT ACCCCGCGCT ACTTCACCTG GGACGAGGTG GCCCAGCGCT Clone A-1
60201 CAGGGTGCGA GGAGCGGTGG CTAGTGATCG ACCGTAAGGT GTACAACATC
60251 AGCGAGTTCA CCCGCCGGCA TCCAGGGGGC TCCCGGGTCA TCAGCCACTA
60301 CGCCGGGCAG GATGCCACGG TGAGCGCAGC CAGGCGGGGG CACAGGAGAG
60351 GGCGGGACCG GAGGCTGAGT GCAGGGGAGA CAGAGTTACG CACTCCGAGC
60401 CAAACACCGA CTAATTCGGA GGAAAGCCCG GAGGCGCCTG ATCATACTG
60451 TTGCCCCGTG ATTGGGTGTC CTGCGGATGC GGGATGAAAA GGCGGGAGAG Clone A-10
60501 AGGCCTGGAG AAGTGGAGTC TGGGGAGTGG GGATGGAGGC CAACAACACG
60551 CACACACAAA CAAAGGGTCC CGCTCCCTG CCGTGCATTG CATCTGCAGC
60601 CCCGAGCCTC AGGTCTCTGG GCGGGGACAG AACCCCGAGC TGGGTAGGCT
60651 AGGAGGGAGG AGAGCAAGGA TGCAGGCCGC CTGGGGAGGG AGGGGGTCAG
60701 TGGCCAGGGG AGGGAGTCAC ATCCTGTCTC GATGGCTAGG AGAGGCAGCG
60751 CAGCCGCGTC TGGACCTAGG TGCCGGTCTC CACTCGCCAG CAGGAGCGGA Clone B-17
60801 GAGGGAGCAG GAAAGGAGCC CATCTCGAG GATGGGGCTG AAACGGGAAG
60851 CTTGGGGAGA CCGCTGCCTT GGGGACCCCT GCGTCGTGTG AAGACTGGAG
60901 GACGCGGAAG GGACAGCGCT GGCCGGGGAG GGCAAGCGGC CGCTGGCGTA
60951 CATAAGGGAT TGGGAATGGC ATACACTTAG CGAGGACCCC CAGAGCTGTT
61001 CTCGAATCGC CGGGGAGGCC ACTGAGCCGC AGGCCAGCGA GGTCTTCAGC

Figure 26a

307
305

09622613-010359

61051 TATTCGCGG AGCGGACCGC TGTTCACGCT CTGGGGCGGT AGGCCCTTCG
 61101 CGGGGTCTCTG TCCCTTCTTC CCTTGGTCTC ACTGCGGGGT CGGCGCGCGC
 61151 CCCAGCCCCA GGCTGCTGC TTCCCTTTCT AGACCACAGC CCTCAGAGCT
 61201 AAGCCCCCGG CGCTCTCTG CTGGGTGGA GTCCTGGGA CTCAGTCTTA
 61251 GGGACTCGAA AGTCGGGGCG TTCCCTTCAC CGCGTTTCCC CCTTGGCGGC
 61301 CAGAATGGCG TCCCTTCCCC TTGCATCCCC CTCTGATCCC GTGCCCTGCA
 61351 GCGTGATGCC CTCCACTGTC CCTATCCACT ACCCTGGCGT CCCAGAGTGT
 61401 GCCGCGGGTC ACCAGGTTCC CATAACGTCG CAGCAGAGCT TAGACGCTGC
 61451 GGGGCGAAGA CCCGCCCCAC CCTCTGACGC GACCAGCCTA GTGGGCGAGG
 61501 CCAGAGCTTG CGCGGGTCAA CCAGAGTGAC CACTCGGGAG CCCTGACTGC
 61551 GGCCAAGGGC GCAGGCGTGT CCCGGCGCAT GCGCAGACGA AACAGGCACC
 61601 AACGCTGGAG CTTCCTCGCAG TGTGATTTGG GGCCGGGGAT GCCGCGGCGG
 61651 GGACGGCGAT TGGTCCGTAT GTGTGGTGCC ACCGGCCGCC GCTCCGCCCC
 61701 GGCCCCCGCC CCACACGCCG CATCACTTAC AGGGCCCGGG GCTGCCGGAC
 61751 CTGCCAACGT GAATCTTATC GCCATGGACC TTACCTTGCA CAACCCAAAG
 61801 TAGCTGCCTT GGGGCAGGGG GTGGCCAGAG TGCTTAGGA AATGTGGAGC Clone 3-5
 61851 CCTACCCAGA ACAACGGTGG AGGGAAAGGG AAGAAACGCA GAAGTGCCCC
 61901 AGTTCGGACG TAGGAAGTC TTCCTCTTCG TGTMTTGG AGAACCTAG
 61951 CTAAGAGAGG AAAGGGACTT ATTGAAAGAC CCGCAAGAAG GGACGGAAGT
 62001 CTCATAGCCC TGAGAGGTGA AGCCAGCTGG AGTTGATGGG TCGAATGGGG
 62051 ACCTAGAGAA CTTTCTGTGA TCTAGAGGTT TGTAAAATGC ACCAATCAGT
 62101 GCTCTGTAAA AACGCACCAA TTGGCGCTCT GTAGCTAGCT AGAGGTTTGT
 62151 AAAATGAGCC AATCAGCAGG ACGTGGGCAG GGACAACTAA GACAATAAAA
 62201 GCTGGCCACC CCAGCCAGCT GCTGCAACCC GCTCCAGTTC CCTTACAGGC
 62251 TGTGGAAGCA TTGTTCTTTT GCTCGTCACA CTAAACCTTG CTGCTGCTCA
 62301 TTCTTTGGGT CTGCAAAGAG TGTATTCTT TTAAGAGCTA TAACAGCGGG
 62351 AAGGTCCACG GCTCCATTCT TGAAGTCAGT GAGACCATAC CCGCCGGAAG
 62401 GAACCAACGC CCGACACAGC CCCACCCATC TCTCTGTTT CTCACCTATA

Figure 26b

62451 CTGAAATTCT TGGGCAAAAG CTGTCTGTGG ACACACCCAG GGGAAAGGCC
62501 AGCCCAGGCA GGTGTTTCTT AGTGGTTCCC CTCAGCCAAT GCTTCCCATT
62551 CCTTGATGCA TCCTTCTAAC TAGAGCAGAT GCTCGGTGAT CTTAAACTGT
62601 GGACACCTGG GAGCACCCCTC AAAAGGCAGC TGGGCCTAGG GAGATGGCCT
62651 GTGCTTCTGT GTCAGGAGTT GGTTCCTTCA GGTGGGCTTG TGGTCTCGCT
62701 GACGTCAAGA ATGAAGCCAT GAACCTTCGC GGTGAGTGT ACAGCTCTTA
62751 CAGGTGGCGT GGACCCAAAG AGTGAGCAGC AGCAAGATTT ATTGTGAAGA
62801 GCAAAGAACA AAGCTTCAC AGCGTGAAG GGTACCCGAG CAGGTGCGCG
62851 CTGCTGGACG TTGGGGGGTG TGAGGGGGAG CAGCCTTTTT TTTTCTTTTT
62901 TTTTGTAGAC GGAGTCTCCC TGTCGCCCAG GCTGGAGTGC AGTGGCGCGA
62951 TCTCGGCTCA CTGCAGGCTC CGCCCCCCCC CCGGGGTTCG CGCCATTCTC
63001 CTGCCTCAGC CTCCCGAGTA GCTGGGACTA CAGGCGCCCG CTACCTCGCC
63051 CGGCTAATTT TTTGTATTT TAGTAGAGAC GGGGTTTCAC TGTGTTAGCC
63101 AGGATGGTCT CGATCTCCTG ACCTCGTGAT CCACCCGCCT TGGCCTCCCA
63151 AAGTGCTGGG ATTACAGCG TGAGCCACCG CGCCCGGCCG GGAGCAGCTT
63201 TTATTCCCTT ATTTGTCCCT GCCCATGTCC TGCTGATTG TCCATTTTAT
63251 AGAGCACTGA TTGGTCCATT TTACAGGGTG CTGATTGGTC CATTTTACCT
63301 CTAGCTAGCT AAAGAGCAG GATTGGTGCA TTTTACAAAC CTCTAGCTAC
63351 AGAAAAGTTC TCCAAGTCTG CACTCGACCC AGGAAGTCCA TCTGGCTTCA
63401 CCTCTCACTT CAACTTGGGT ACAGCCTTCT GCGGGGAGG AGGATGGCCT
63451 TTGGTGCGAA CACTGCCGGA GTCCAGGGGG CTGGCTCCCT CACCTTTCAT Clone B-19
63501 CTTCTCCCGG CACTTGCAGG ATCCCTTTGT GGCTTTCAC ATCAACAAGG
63551 GCCTTGTGAA GAAGTATATG AACTCTCTCC TGATTGGAGA ACTGTCTCCA
63601 GAGCAGCCCA GCTTTGAGCC CACCAAGAAT GTAAGACCTT GTGTTTGCTA
63651 TGTCGCAACT ATTGGTTGTT GAGGGGGACA GAGAGGGGGT GGAAGGAGAG
63701 TCTAGATGGA ATCACAGTCA TAGTAATCAC AGTCAGTAGT AGCTCTGGGG
63751 AGTCTTGAGG TCCCTGCTTC TCTTGCATAG TCATGAGGTC ACAGGCCCAA

Figure 26c

309
307

63801 GGGAGCATGG CTTTGCAACC TATGGCTCCC CCAAGGCTGC CACTACCATG
63851 GCTGCCATCA TTGTTATCAT CATTGTTATC ATATGAGCAC TTACTATGCA
63901 CCAAGCATAA ACTCATAACT CTTACACATT TACAGATGAG ATAACAGGCT
63951 CAGGGAGGTT AAGCAACACA GCCAAGGATC ACACAGTTAG TAAATGGCAG
64001 AGCAAGGACT TAGTCCCCTG AACTCTTAGG CACTATCCCA TGGCACCTCC
64051 TCCTGTCATC CTCATTGTCG TGGTATCTTT GCCTAGGACT GTGGACTTCC
64101 CACAGCTACC TCAGTGGGAG GTCCTTGAGC CTGAGAGGGC CCTTGTCTCC
64151 AGTAGCATTG GGGTGCAGAT GAGAAGAATA ACAGCTCCTC TTCCTCTTCT
64201 GCAGAAAGAG CTGACAGATG AGTTCCGGGA GCTGCGGGCC ACAGTGGAGC
64251 GGATGGGGCT CATGAAGGCC AACCATGTCT TCTTCTGCT GTACCTGCTG
64301 CACATCTTGC TGCTGGATGG TGCAGCCTGG CTCACCCTTT GGGTCTTTGG
64351 GACGTCCTTT TIGCCCTTCC TCCTCTGTGC GGTGCTGCTC AGTGCAGTTC
64401 AGGTGAGAGC CTTTGGCTTG TCAAGTGCAC AGCAATGCTC AGCATCCCTG

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Figure 26d

310
308

FastA Match of Human $\Delta 5$ -desaturase and Contig 3381584

SCORES Initl: 4480 Initn: 4480 Opt: 4481
99.9% identity in 898 bp overlap

human $\Delta 5$ ATGGCCCCCGACCCGGTGGCCGCCGAGACC
3381584 GGCCCCGGCGCGCGCTCGCCAGGCCAGCTATGGCCCCCGACCCGGTGGCCGCCGAGACC
80 90 100 110 120 130

human $\Delta 5$ GCGGCTCAGGGACCTACCCCGCGCTACTTCACCTGGGACGAGGTGGCCCGAGCGCTCAGGG
3381584 GCGGCTCAGGGACCTACCCCGCGCTACTTCACCTGGGACGAGGTGGCCCGAGCGCTCAGGG
140 150 160 170 180 190

human $\Delta 5$ TGCAGGAGCGGTGGCTAGTGATCGACCGTAAGGTGTACAACATCAGCGAGTTACCCCGC
3381584 TGCAGGAGCGGTGGCTAGTGATCGACCGTAAGGTGTACAACATCAGCGAGTTACCCCGC
200 210 220 230 240 250

human $\Delta 5$ CGGCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGGCAGGATGCCACGGATCCC
3381584 CGGCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGGCAGGATGCCACGGATCCC
260 270 280 290 300 310

human $\Delta 5$ TTGTGGCCTTCCACATCAACAAGGGCCTTGTAAGAAGTATATGAAGTCTCTCTGATT
3381584 TTGTGGCCTTCCACATCAACAAGGGCCTTGTAAGAAGTATATGAAGTCTCTCTGATT
320 330 340 350 360 370

human $\Delta 5$ GGAGAACTGTCTCCAGAGCAGCCAGCTTTGAGCCACCAAGAATAAAGAGCTGACAGAT
3381584 GGAGAACTGTCTCCAGAGCAGCCAGCTTTGAGCCACCAAGAATAAAGAGCTGACAGAT
380 390 400 410 420 430

human $\Delta 5$ GAGTTCGGGAGCTGCGGGCCACAGTGGAGCGGATGGGGCTCATGAAGGCCAACCATGTC
3381584 GAGTTCGGGAGCTGCGGGCCACAGTGGAGCGGATGGGGCTCATGAAGGCCAACCATGTC
440 450 460 470 480 490

human $\Delta 5$ TTCTTCCTGCTGTACCTGCTGCACATCTTGCTGCTGGATGGTGCAGCCTGGCTCACCCCTT
3381584 TTCTTCCTGCTGTACCTGCTGCACATCTTGCTGCTGGATGGTGCAGCCTGGCTCACCCCTT
500 510 520 530 540 550

Figure 27a

668010" ET92260

371
304

human Δ5 460 470 480 490 500 510
TGGGTCTTTGGGACGTCCTTTTGGCCCTTCCTCCTCTGTGCGGTGCTGCTCAGTGCAGTT
3381584 TGGGTCTTTGGGACGTCCTTTTGGCCCTTCCTCCTCTGTGCGGTGCTGCTCAGTGCAGTT
560 570 580 590 600 610

human Δ5 520 530 540 550 560 570
CAGGCCACGGCTGGCTGGCTGCAGCATGACTTTGGGCACCTGTCGGTCTTCAGCACCTCA
3381584 CAGGCCACGGCTGGCTGGCTGCAGCATGACTTTGGGCACCTGTCGGTCTTCAGCACCTCA
620 630 640 650 660 670

human Δ5 580 590 600 610 620 630
AAGTGAACCATCTGCTACATCATTGTTGATTGGCCACCTGAAGGGGGCCCCGCCAGT
3381584 AAGTGAACCATCTGCTACATCATTGTTGATTGGCCACCTGAAGGGGGCCCCGCCAGT
680 690 700 710 720 730

human Δ5 640 650 660 670 680 690
TGGTGAACCATGCACTTCCAGCACCATGCCAAGCCCAACTGCTTCCGCAAAGACCCA
3381584 TGGTGAACCATGCACTTCCAGCACCATGCCAAGCCCAACTGCTTCCGCAAAGACCCA
740 750 760 770 780 790

human Δ5 700 710 720 730 740 750
GACATCAACATGCATCCCTTCTTCTTGGCCTTGGGGAAGATCCTCTCTGTGGAGCTTGGG
3381584 GACATCAACATGCATCCCTTCTTCTTGGCCTTGGGGAAGATCCTCTCTGTGGAGCTTGGG
800 810 820 830 840 850

human Δ5 760 770 780 790 800 810
AAACAGAAGAAAAATATATGCCGTACAACCACCAGCACAAATACTTCTTCCTAATTGGG
3381584 AAACAGAAGAAAAATATATGCCGTACAACCACCAGCACAAATACTTCTTCCTAATTGGG
860 870 880 890 900 910

human Δ5 820 830 840 850 860 870
CCCCCAGCCTTGCTGCCTCTCTACTTCCAGTGGTATATTTCTATTTTGTATCCAGCGA
3381584 CCCCCAGCCTTGCTGCCTCTCTACTTCCAGTGGTATATTTCTATTTTGTATCCAGCGA
920 930 940 950 960 970

human Δ5 880 890 900 910 920 930
AAGAAGTGGGTGGACTTGGCCTGGATGATTACCTTCTACGTCCGCTTCTTCCTCACTTAT
3381584 AAGAAGTGGGTGGACTTGGCCTGGATGATTACCTTCTACGTCCGCTTCTTCCTCACTTAT
980 990 1000 1010 1020 1030

0922513.010899

Figure 27b

Figure 28

313
311

FastA Match of Human $\Delta 5$ -desaturase and Contig 253538a

SCORES Init1: 1479 Initn: 2483 Opt: 2489
Smith-Waterman score: 2489; 81.3% identity in 434 aa overlap

human $\Delta 5$ MAPDPVAAETAAQGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPPGGSRVIS
253538a QGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPPGGSRVIS

human $\Delta 5$ HYAGQDATDPFVAFHINKGLVKYMNSSLIGELSPEQPSFEPTKNKELTDEFRELRAIVE
253538a HYAGQDATDPFVAFHINKGLVKYMNSSLIGELSPEQPSFEPTKNKELTDEFRELRAIVE

human $\Delta 5$ RMGLMKANHVFFLLYLLHILLDGAAWLTWVFGTSFLPFLCAVLLSAVQAQAGWLQHD
253538a RMGLMKANHVFFLLYLLHILLDGAAWLTWVFGTSFLPFLCAVLLSAVQAQAGWLQHD

human $\Delta 5$ FGHLVSFSTSKWNHLLHHFVIGHLKGAPASWNNHMHFQHHAKPNCFRKDPDINM-HPFFF
253538a YGHLVSVYRKPKWNHLVHKFVIGHLKGASANWNNHRHFQHHAKPNIFHKDPDVNMLH--VF

human $\Delta 5$ ALGKILSVELGKQKKKYPYNHQHYFFLIGPPALLPLYFQWYIFYFVIQKKWVDLAWM
253538a VLGEWQPIEYKKKLYLPYNHQHEYFFLIGPPALLIPMYFQYQIIMTMIVHKNWVDLAWA

human $\Delta 5$ ITFYVRFFLTIVPLLGL-LKAFLGLFFIVRFLESNWFVWVTQMNHHPMHIDHRNMDWVST
253538a VSYYIRFFITYIPFYGILGALLFLNFI-RFLESHWFVWVTQMNHIVMEIDQEAYRDWFS

human $\Delta 5$ QLLATCNVHKSANFNDWFSGLNFQIEHHLFPTMPRHNYHKVAPLVQSLCAKRGIEYQSKP
253538a QLTATCNVEQSFFNDWFSGLNFQIEHHLFPTMPRHNLHKIAPLVKSLCAKHGIEYQEK

human $\Delta 5$ LLSAFADIHSLKESGQLWLDAYLHGX
253538a LLRALLDIIRSLKKSGLWLDAYLHKXSHSPRDTVGKGCWGDGQRNDGLLFXGVSERLV

Figure 29

314
312

FastA Match of Human Δ5-desaturase and Contig 38

SCORES Init1: 2024 Initn: 2024 Opt: 2026
Smith-Waterman score: 2026; 99.3% identity in 287 aa overlap

human Δ5 10 20 30 40 50 60
MAPDPVAAETAAQGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPPGGSRVIS
38 QGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPPGGSRVIS
10 20 30 40

human Δ5 70 80 90 100 110 120
HYAGQDATDPFVAFHINKGLVKKYMNSLLIGELSPEQPSFEPTKNKELTDEFRELRAIVE
38 HYAGQDATDPFVAFHINKGLVKKYMNSLLIGELSPEQPSFEPTKNKELTDEFRELRAIVE
50 60 70 80 90 100

human Δ5 130 140 150 160 170 180
RMGLMKANHVFLLYLLHILLDGAAWLTLWVFGTSFLPFLCAVLLSAVQAQAGWLQHD
38 RMGLMKANHVFLLYLLHILLDGAAWLTLWVFGTSFLPFLCAVLLSAVQAQAGWLQHD
110 120 130 140 150 160

human Δ5 190 200 210 220 230 240
FGHLSVSTSKWNHLLHHFVIGHLKGAPASWNNMHFQHHAKPNCFRKDPDINMHPFFFA
38 FGHLSVSTSKWNHLLHHFVIGHLKGAPASWNNMHFQHHAKPNCFRKDPDINMHPFFFA
170 180 190 200 210 220

human Δ5 250 260 270 280 290 300
LGKILSVELGKQKKKYPYNHQQHYFFLIGPPALLPLYFQWYIFYFVIQRKKWDLAWMI
38 LGKILSVELGKQKKKYPYNHQQHYFFLIGPPALLPLYFQWYIFYFVIQRKKWDLAWIS
230 240 250 260 270 280

human Δ5 310 320 330 340 350 360
TFYVRFFLTYPVLLGLKAFGLGFFIVRFLESNWFVWVTQMNHHPMHIDHNRNMDWVSTQL
38 KQEYDEAGLPLSTANASKRDLPRATSPGTRWPSAQGARSGGXSTVRCCTTSASSPAGIQG
290 300 310 320 330 340

09227613.010899

Figure 30

315
313

56863

human Δ5
Ma524.pep

Figure 31

3/6
314

FastA Match of Δ5-Desaturase (Ma29) and Human Δ5-Desaturase

SCORES Init1: 145 Initn: 236 Opt: 266
Smith-Waterman score: 400; 27.5% identity in 455 aa overlap

	10	20	30	40	50	60
human Δ5	MAPDPVAAETAAQGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPGGSRVIS					
Ma29.pep	MGTDQGKT---FTWEELAAHNTKDDLLLAIRGRVYDVTKFLSRHPGGVDTL					
	10	20	30	40		
	70	80	90	100	110	
human Δ5	HYAGQDATDPFVAFHINKGLVKKYMNSLLIGEL-SPEQPSF-EPTKNKELTDEFRELRAT					
Ma29.pep	LGAGRDVTPVFEMYHAF-GAADAIMKKYVGTLSNELPIFPEPTVFHKTIKTRVEGYFT					
	50	60	70	80	90	100
	120	130	140	150	160	170
human Δ5	VERMGLMKANHVF--FLLYLLHILLDGAAWLTLWVFGTSFLPFLLCVLLSAVQAQAGW					
Ma29.pep	DRNIDPKNRPEIWGRYALIFGSLIASYYAQLFVPFVVERIWLQVVF-AIIMGFACAQVGL					
	110	120	130	140	150	160
	180	190	200	210	220	230
human Δ5	LQ-HDFGHLV-FSTSKWNHL--LHHFVIGHLKGAPASWWNHMH-FQHHAKPNCFRKDPD					
Ma29.pep	NPLHDASHFSVTHNPTVWKILGATHDF---FNGASYLVWVYQHMLGHHPTNIAGADPD					
	170	180	190	200	210	220
	240	250	260	270	280	
human Δ5	INM-HPFFALGKILSVELGKQKKKYPYNH--QHKYF-FLIGPPALLPLYFQWYIFYFV					
Ma29.pep	VSTSEP-----DVRRIKPNQKWF-VNHINQHMVFVFLYGLLAFKVRIQDINILYFV					
	230	240	250	260	270	
	290	300	310	320	330	
human Δ5	IQ----RKKWVDLAWMITFY--VRFFLTYY--VPL--LGLKAFLGLFFIVRFLESNWFW					
Ma29.pep	KTNDAIRVNPISITWHTVMFWGGAFFVWYRLIVPLQYLPGLKVLLEFTVADMVSSYWLAL					
	280	290	300	310	320	330
	340	350	360	370	380	
human Δ5	VTQMNHHPMHID---HDRN---MDWVSTQLLATCN-VHKSANFNDWFSGHLNFQIEHHLF					
Ma29.pep	TFQANHVVVEEVQWPLPDENGIIQKDWAAQVETTDQYAHDSHLWTSTIGSLNYQAVVHHLF					
	340	350	360	370	380	390
	390	400	410	420	430	440
human Δ5	PTMPRHNHYHKVAPLVQSLCAKRGIEYQSKPLL-SAFADIHSLKESGQLWLDAYLHQX					
Ma29.pep	PNVSQHHPDILAIKNTCSEYKVPYLVKDTFWQAFASHLEHLRVILGLRPKEEX					
	400	410	420	430	440	

Figure 32

0922613-010899

668070" ET9/2260
Figure 33

Host(plasmid)	334(pRAE-28-5)	334(pRAE-26-1)	334(pRAE-33)	334(pRAE-35)	334(pYX242)
Added substrate	25 μ M DGLA	25 μ M DGLA	25 μ M DGLA	25 μ M DGLA	25 μ M DGLA
			30°C/48 hrs		
Fatty acid	(g fatty acid/100 g fatty acid)		lipid (μ g)		
C16:0	151.580	202.175	285.291	281.298	304.229
C16:1	406.279	485.631	552.951	569.298	608.123
C18:0	16.494	25.995	32.162	27.479	30.093
C18:1n-9	100.031	133.349	173.772	184.740	187.780
C18:2n-6		0.180			0.946
C18:3n-6		0.058	0.074		0.074
C20:0	3.844	4.205	7.118	7.285	6.288
C20:3n-6	96.576	118.657	134.859	139.292	125.448
C20:4n-6	(0.127%) 1.204	(0.075%) 0.878	(0.062%) 0.902	(0.063%) 0.927	(0.062%) 0.958
C22:0		0.150	0.119		0.125
C22:1n-9	0.162	0.139	0.299	0.275	0.392
Total Lipid	949.0	1169.0	1445.5	1468.0	1538.5

6/7/03

668070 "FTIR" 660
Figure 34a

Host(plasmid)	334(pRAE-28-5)	334(pRAE-26-1)	334(pYX242)	334(pRAE-28-5)	334(pRAE-26-1)	334(pYX242)	334(pRAE-28-5)	334(pRAE-26-1)	334(pYX242)
Added substrate	25 μ M DGLA	25 μ M DGLA	25 μ M DGLA	25 μ M OA	25 μ M OA	25 μ M OA	25 μ M AA	25 μ M AA	25 μ M AA
			30°C/48 hrs						30°C/48 hrs
Fatty acid	(g fatty acid/100 g fatty acid)		lipid (μ g)						
C16:0	49.332	106.358	93.225	84.327	37.013	51.018	78.471	53.685	74.099
C16:1	141.178	256.622	277.028	269.009	107.066	172.485	230.45	141.526	181.298
C18:0	9.301	14.819	12.908	11.871	8.3	9.047	11.283	9.97	10.969
C18:1n-9	39.876	87.564	72.842	106.416	52.634	71.453	61.754	42.289	46.873
C18:2n-6				ND	ND	ND			
C18:3n-6									
C20:0	2.154	7.339			3.729			2.685	
C20:3n-6	45.395	56.346	55.306						
C20:4n-6	(0.106%) 0.412	(0.060%) 0.396	(0.065%) 0.402				63.584	68.442	60.89
C20:5n-3							(0.026%) 0.139	(0.019%) 0.077	(0.027%) 0.126
Total Lipid	387	665	620	562	284	363	535	404	466

34a

668070-FT9/2260
Figure 34b

Host(plasmid)	334(pRAE-28-5)	334(pRAE-26-1)	334(pYX242)	334(pRAE-28-5)	334(pRAE-26-1)	334(pYX242)
Added substrate	25 μ M LA	25 μ M LA	25 μ M LA 30°C/48 hrs	no substrate	no substrate	no substrate
Fatty acid	(g fatty acid/100 g fatty acid)		lipid (μ g)			
C16:0	56.631	45.393	74.247	174.138	25.574	33.44
C16:1	181.311	117.045	208.029	277.122	43.193	47.189
C18:0	9.549	9.251	11.45	22.547	5.119	8.432
C18:1n-9	48.256	46.496	51.342	134.822	21.89	32.618
C18:2n-6	31.91	23.221	36.821			
C18:3n-6	(0.02%) 0.082	ND	(0.012%) 0.056			
C20:0		0.339		0.702		
C20:3n-6						
C20:4n-6						
C20:5n-3	0.121					
Total Lipid	407	279	460	746	127	168

3A

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SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
E. _____ S. _____ D. _____

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Fecha (AMD): 2000-04-10 16:36:44
Tramite : C02 PATENTES D 1 REGISTRO/ 444 REPUESTA
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

Folios: 93

Re: P1.-ABBOTT LABORATORIES.-Solicitud de Patente de Invención para
"GEN DE LA DESATURASA HUMANA" C12N.-Expediente número 00-
000.434.

1. Me refiero a la solicitud arriba nombrada de fecha 5 de Enero de 2000 y al auto dictado por ese Despacho notificado por Estado el 2 de Marzo del mismo año.

Por medio del presente atentamente manifiesto a usted que, debidamente autorizado por mi poderdante, **excluyo** de la presente solicitud la reivindicación **25**.

3. De la misma manera, muy comedidamente manifiesto a usted que, debidamente autorizado por mi poderdante, modifíco el título de la solicitud con el fin de dar cumplimiento a los requerimientos del señor examinador, para que quede como sigue:

"GEN DE LA DESATURASA HUMANA"

4. Con respecto a la objeción del señor Examinador de acuerdo con la cual **"Las reivindicaciones 1 y 7 no se encuentran totalmente definidas ya que contienen palabras subjetivas como "aproximadamente" las cuales no delimitan de manea precisa la invención. Además decir que una secuencia de nucleótidos es 50% complementaria con otra, no permite establecer cual es la secuencia que se desea proteger. Se erecoeminda que omita el término aproximadamente y se elimine el segmento que se refiere a una secuencia de nucleótidos que es 50% complementaria a otra pues eso le resta exactitud a la reivindicación"**, me permito manifestar que se han modificado las reivindicaciones 1 y 7 con el fin de eliminar la expresión "aprox."

De otra parte, en relación con la expresión "...es complementaria a por lo menos 50% de la secuencia...", atentamente llamo la atención del señor Examinador al hecho de que dicha expresión es necesaria para proteger de manera precisa el alcance de la presente invención. Dicha expresión hace referencia al porcentaje de identidad de la secuencia de nucleótidos de la reivindicación 1 y al porcentaje de similitud del polipéptido de la reivindicación 7 e intenta establecer de manera explícita que el alcance de la presente invención **no se limita** a la secuencia precisa de nucleótidos (SEC NO:1) ilustrada en la figura 12 y a la respectiva proteína codificada por dicha secuencia SEC NO:1, sino que además comprende aquellas secuencias que presentan un grado de complementariedad de al menos el 50%.

El señor Examinador debe comprender que el objeto de emplear dichas expresiones es precisamente evitar una situación en la cual, al obtenerse protección únicamente para la secuencia específica de la figura 12, un competidor pueda cambiar un nucleótido o un aminoácido en las secuencias protegidas, **sin infringir los derechos de la patente concedida y perjudicando al solicitante de la presente invención**. Por consiguiente, este tipo de expresiones son necesarias y muy empleadas en el campo de la propiedad intelectual, toda vez que el objetivo de la concesión de una patente es proteger en su totalidad el trabajo investigativo desarrollado por el solicitante.

De acuerdo con los argumentos anteriormente expuestos, se solicita al señor Examinador que acepte las reivindicaciones 1 y 7 como han sido modificadas.

Sobre este particular, atentamente me permito manifestar los siguientes hechos:

- (a) No obstante el hecho de que el solicitante discrepa de las objeciones formuladas por el señor Examinador, tal y como se manifestó mediante los argumentos aquí expuestos, estos argumentos fueron **formulados de buena fe**.

- (b) En virtud de la discrepancia del solicitante con las objeciones del señor Examinador de conformidad con los argumentos debidamente presentados, es deseo del solicitante insistir en que estos argumentos sean aceptados como **respuesta** a las objeciones formuladas.
- (c) En caso de que el señor Examinador considere que los argumentos desarrollados para estas objeciones no son satisfactorios, expresamente manifiesto a usted que es **deseo del solicitante continuar con el trámite de la presente solicitud de patente.**
- (d) En consecuencia, si el señor Examinador considera que no son satisfactorias las respuestas dadas por el solicitante, ruego muy comedidamente que los puntos respondidos y objeto de discrepancia por parte del señor Examinador sean notificados con ocasión del examen consagrado en el Artículo 27 de la Decisión 344 del Acuerdo de Cartagena, con el fin de que el solicitante sea requerido para hacer las modificaciones o rectificaciones a que halla lugar.
- (e) Finalmente, tratándose de aspectos relativos a las reivindicaciones, solicito de la manera más atenta, que éstas sean examinadas de acuerdo con las disposiciones del Artículo 29 de la Decisión 344. Es decir, que después de ser estudiadas nuevamente, se otorgue el privilegio de patente para las reivindicaciones aceptadas, pero que en ningún momento la discrepancia que pueda hallarse entre la respuesta del solicitante y la objeción del señor Examinador conlleve el abandono de la solicitud, por considerar, de manera impropia que no se ha dado respuesta a las objeciones técnicas formuladas.

5. Anexos me permito presentar:

- (1) Nuevo Resumen, Objeto y Finalidad de la Invención **el cual reemplaza el originalmente presentado**, en el cual se incluye el nuevo título de la invención;
- (2) En treinta y nueve (39) hojas de papel, copia formal de los dibujos de la solicitud;

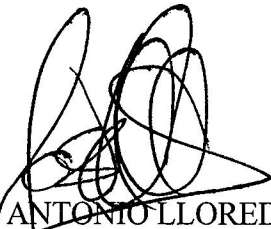
- (3) Nuevas páginas 1, 79, 80, 81, 82 y 83 del texto de la descripción y reivindicaciones **que reemplazan las originalmente presentadas, bajo los mismos números**, en las cuales:
- (i) Se ha modificado la página 1, con el objeto de incluir el nuevo título de la solicitud;
 - (ii) Se han modificado las reivindicaciones 1 y 2, con el fin de eliminar el término "aprox.";
 - (iii) se han modificado las reivindicaciones 8 y 29, con el fin de eliminar la expresión objetada **"bajo las condiciones y durante un tiempo suficientes"**;
 - (iv) Se ha modificado la reivindicación 23 con el fin de dividirla en dos nuevas reivindicaciones 23 y 24, las cuales protegen una célula de planta y un tejido de planta, respectivamente, de conformidad con los requerimientos del señor examinador.
 - (v) De acuerdo con la modificación a la reivindicación 23, anteriormente expuesta, se ha reenumerado la reivindicación 24 original como nueva reivindicación 25 y adicionalmente, se ha modificado dicha reivindicación con el fin de hacerla dependiente de la célula de planta de la reivindicación 23 o del tejido de planta de la reivindicación 24.
 - (vi) Se ha eliminado la reivindicación 25 original.
- (4) Nuevo extracto, en sus dos formas, **que reemplaza el originalmente presentado**, el cual incluye el nuevo título de la invención, las reivindicaciones 1, 8 y 29 modificadas y la reivindicación 15 como fue originalmente presentada.

6. Llamo su atención al hecho de que la copia debidamente certificada de la primera solicitud presentada para el invento arriba nombrado **respecto de la cual reclamo prioridad**, a saber la solicitud estadounidense número 09/227.613 de 8 de Enero de 1999, junto con la traducción oficial correspondiente a la totalidad del documento y la acreditación de traductor oficial, fueron presentadas a ese Despacho mediante memorial de 28 de Marzo de 2000.

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7. Ruego a usted tomar atenta nota de lo anterior y proceder de conformidad.

Señor Ministro,

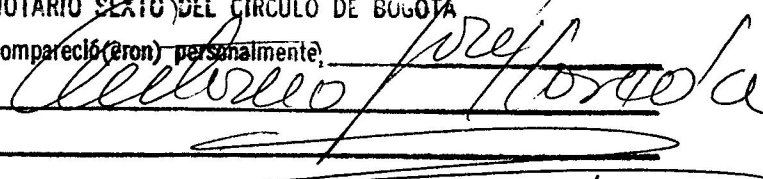

JOSE ANTONIO LLOREDA
T.P. 10.784
Código 231

NOTARIA SEXTA DE BOGOTA
RECONOCIMIENTO Y PRESENTACION PERSONAL

Santafé de Bogotá D. C. 10 ABR 2000

Ante Mí JUAN MANUEL BOTERO MEDINA
NOTARIO SEXTO DEL CIRCULO DE BOGOTA

Compareció(eron) personalmente:

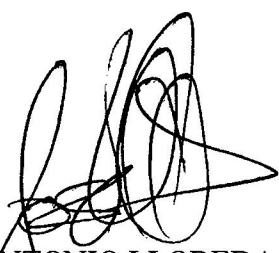


quien(es) exhibió(eron) la(s) C. C.

CC 17159681
10784

y la(s) Libreta(s) Militar(es):

domiciliado(s) en Bogotá, y declaró(aron) que la(s) firma(s) que aparece(n) en el presente documento es(son) la(s) suya(s) y que el contenido del mismo es cierto.
En constancia se firma esta diligencia.


JOSE ANTONIO LLOREDA
C.C. 17.159.681 de Bogotá



NOTARIA SEXTA
SANTAFE DE BOGOTA

RESUMEN OBJETO Y FINALIDAD DE LA INVENCION

GEN DE LA DESATURASA HUMANA

La presente invención se refiere a la identificación de un gen involucrado en la des-saturación de ácidos grasos poliinsaturados en el carbono 5 (v.g., " $\Delta 5$ -desaturasa humana") y a sus usos. En particular, la $\Delta 5$ -desaturasa humana puede utilizarse, por ejemplo, en la conversión del ácido dihomo- γ -linolénico (ADGL) en ácido araquidónico (AA) y en la conversión del ácido 20:4n-3 en el ácido eicosapentaenoico (AEP). El ácido araquidónico (AA), y los ácidos grasos poliinsaturados producidos a partir de estas conversiones pueden agregarse a composiciones farmacéuticas, composiciones nutricionales, alimentos para animales, así como también a otros productos como por ejemplo los cosméticos.

GEN DE LA DESATURASA HUMANA

La presente invención es una Continuación en Parte de la Solicitud pendiente de Patente de los Estados Unidos No. de Serie 09/227 613 radicada el 8 de enero de 1999, la cual es una Continuación en Parte de la Solicitud pendiente de Patente Internacional PCT/US98/07422 radicada el 11 de abril de 1998 (la cual designó a los Estados Unidos), esta última una Continuación en Parte de la Solicitud pendiente de Patente de los Estados Unidos No. de Serie 08/833 610 radicada el 11 de abril de 1997, todas citadas en el presente documento en toda su integridad con propósitos de solo referencia.

ANTECEDENTES DE LA INVENCION

Campo Técnico

La presente invención se refiere a la identificación y aislamiento de un gen que codifica una enzima (v.g., la $\Delta 5$ -desaturasa humana) involucrada en la síntesis de ácidos grasos poliinsaturados y a sus usos. En particular, la enzima $\Delta 5$ -desaturasa cataliza la conversión de, por ejemplo, el ácido dihomo- γ -linolénico (ADGL) en ácido araquidónico (AA) y la conversión del ácido (n-3)-eicosatetraenoico ($20:4n-3$) en el ácido eicosapentaenoico ($20:5n-3$). El producto convertido puede emplearse a manera de sustrato en la producción de otros ácidos grasos poliinsaturados (AGPIs). El producto u otros ácidos grasos poliinsaturados pueden agregarse a composiciones farmacéuticas, composiciones nutricionales, a alimentos para animales, así como también a otros productos tales como los cosméticos.

Información Sobre el Estado Previo de los Conocimientos

Las desaturasas son críticas en la producción de ácidos grasos

REIVINDICACIONES

1. Una secuencia de nucleótidos (aislada) que corresponde ó es complementaria a por lo menos 50% la secuencia de nucleótidos representada por la ID SEC NO:1 (Figura 12).
2. La secuencia de nucleótidos aislada acorde con la Reivindicación 1, en donde dicha secuencia se representa por la ID SEC NO:1.
3. La secuencia de nucleótidos aislada acorde con las Reivindicaciones 1 ó 2, en donde dicha secuencia codifica una desaturasa funcionalmente activa la cual utiliza un ácido graso poliinsaturado como sustrato.
4. La secuencia de nucleótidos aislada acorde con la Reivindicación 1, en donde dicha secuencia se obtiene a partir de un mamífero.
5. La secuencia de nucleótidos aislada acorde con la Reivindicación 4, en donde dicha secuencia se obtiene de un humano.
6. Una proteína purificada codificada por dicha secuencia de nucleótidos acorde con las Reivindicaciones 1 ó 2.
7. Un polipéptido purificado el cual desatura ácidos grasos poliinsaturados en el carbono 5 y tiene por lo menos 50% de similaridad en cuanto a aminoácidos respecto de la secuencia de aminoácidos de dicha proteína purificada codificada acorde con la Reivindicación 6.
8. Un método de producción de una $\Delta 5$ -desaturasa humana que comprende los pasos de:

- a) aislamiento de dicha secuencia de nucleótidos representada por la ID SEC NO:1 (Figura 12);
- b) construcción de un vector que comprende: i) dicha secuencia de nucleótidos aislada operablemente ligado a ii) un promotor;
- c) introducción de dicho vector dentro de una célula huésped para la expresión de dicha enzima $\Delta 5$ -desaturasa humana.

9. El método de la Reivindicación 8, en donde dicha célula huésped es una célula eucariótica ó una célula procariótica.

10. El método de la Reivindicación 9, en donde dicha célula procariótica se selecciona del grupo conformado por E. Coli, Cianobacterias, y B. subtilis.

11. El método de la Reivindicación 9, en donde dicha célula eucariótica se selecciona del grupo conformado por una célula de mamífero, una célula de insecto, una célula de planta y una célula micótica.

12. El método de la Reivindicación 11, en donde dicha célula micótica es una célula de levadura.

13. El método de la Reivindicación 12, en donde dicha célula de levadura se selecciona del grupo conformado por Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Candida spp., Lipomyces starkey, Yarrowia lipolytica, Kluyveromyces spp., Hansenula spp., Trichoderma spp. y Pichia spp..

14. El método de la Reivindicación 13, en donde dicha célula de levadura es de Saccharomyces cerevisiae.

15. Un vector que comprende a) una secuencia de nucleótidos como la

representada por la ID SEC NO:1 (Figura 12) operablemente ligado a b) un promotor.

16. Una célula huésped que comprende dicho vector de la Reivindicación 15.

17. La célula huésped acorde con la Reivindicación 16, en donde dicha célula huésped es una célula eucariótica ó una célula procariótica.

18. La célula huésped acorde con la Reivindicación 17, en donde dicha célula procariótica se selecciona del grupo conformado por E. Coli, Cianobacterias, y B. subtilis.

19. La célula huésped acorde con la Reivindicación 17, en donde dicha célula eucariótica se selecciona del grupo conformado por una célula de mamífero, una célula de insecto, una célula de planta y una célula micótica.

20. La célula huésped acorde con la Reivindicación 19, en donde dicha célula micótica es una célula de levadura.

21. La célula huésped acorde con la Reivindicación 20, en donde dicha célula de levadura se selecciona del grupo conformado por Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Candida spp., Lipomyces starkey, Yarrowia lipolytica, Kluyveromyces spp., Hansenula spp., Trichoderma spp. ó Pichia spp..

22. La célula huésped acorde con la Reivindicación 21, en donde dicha célula de levadura es de Saccharomyces cerevisiae,

23. Una célula de planta que comprende dicho vector acorde con la Reivindicación 15, en donde la expresión de dicha secuencia de nucleótidos de dicho vector da como resultado la producción de un ácido graso poliinsaturado por parte de dicha célula de planta.

24. Un tejido de planta que comprende dicho vector acorde con la Reivindicación 15, en donde la expresión de dicha secuencia de nucleótidos de dicho vector da como resultado la producción de un ácido graso poliinsaturado por parte de dicho tejido de planta.

25. La célula de planta de la Reivindicación 23 ó el tejido de planta de la Reivindicación 24, en donde dicho ácido graso poliinsaturado es ácido araquidónico (AA) ó ácido eicosapentaenoico (AEP).

26. Una planta transgénica que comprende dicho vector acorde con la Reivindicación 15, en donde la expresión de dicha secuencia de nucleótidos de dicho vector da como resultado la producción de un ácido graso poliinsaturado en las semillas de dicha planta transgénica.

27. Una célula de mamífero que comprende dicho vector acorde con la Reivindicación 15, en donde la expresión de dicha secuencia de nucleótidos de dicho vector da como resultado la producción de niveles modificados de AA ó de AEP cuando dicha célula se hace crecer en un medio de cultivo que comprenda un ácido graso seleccionado del grupo conformado por un ácido graso esencial, AL (ácido linoleico) y AAL (ácido α -linolénico).

28. Un fluido producido por un mamífero transgénico no humano cuyo genoma comprende una secuencia de ADN que se representa por la ID SEC NO: 1 (Figura 12) que codifica una $\Delta 5$ -desaturasa humana operablemente ligada a un promotor, en donde dicho fluido comprende un nivel detectable de por lo menos una $\Delta 5$ -desaturasa humana.

29. Un método para la producción de un ácido graso poliinsaturado que comprende los pasos de:

- a) aislamiento de dicha secuencia de nucleótidos representada por la ID SEC NO:1 (Figura 12);

- b) construcción de un vector que comprende dicha secuencia de nucleótidos aislada;
- c) introducción de dicho vector dentro de una célula huésped para la expresión de dicha enzima $\Delta 5$ -desaturasa humana; y
- d) exposición de dicha enzima $\Delta 5$ -desaturasa humana expresada a un substrato ácido graso poliinsaturado con el fin de convertir dicho substrato en un producto ácido graso poliinsaturado.

30. El método acorde con la Reivindicación 29, en donde dicho substrato ácido graso poliinsaturado es el ADGL ó el ácido 20:4n-3 y dicho producto ácido graso poliinsaturado es AA ó AEP, respectivamente.

31. El método acorde con la Reivindicación 29, el cual comprende adicionalmente el paso de exposición de dicho producto ácido graso poliinsaturado a una elongasa con el fin de convertir dicho producto ácido graso poliinsaturado en un segundo ácido graso poliinsaturado.

32. El método acorde con la Reivindicación 31, en dicho producto ácido graso poliinsaturado es AA ó AEP y dicho segundo ácido graso poliinsaturado es el ácido adrenico ó el ácido (n-3)-docosapentaenoico, respectivamente.

33. El método acorde con la Reivindicación 31, el cual comprende el paso adicional de exposición de dicho segundo ácido graso poliinsaturado a una desaturasa adicional con el fin de convertir dicho segundo ácido graso poliinsaturado a un ácido graso poliinsaturado final.

34. El método acorde con la Reivindicación 33, en dicho ácido graso poliinsaturado final es el ácido (n-6)-docosapentaenoico ó el ácido docosahexaenoico (ADH).

151-300	5	3808675	
301-446	5	354535	6
151-300	6	3448789	6
151-300	6	1362863	6
151-300	6	2394760	6
301-457	6	3350263	6

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FIG.1

GCACGCCGACCGGCCCGGAGATCCTGGCAAAGTATCCAGAGATAAAGTCCTTGATGAAACCTGATCCCAATTTGATATGGATTATAATTA
 TGATGGTTCTCACCCAGTTGGGTGCATTTACATAGTAAAAGACTTGGACTGGAAATGGGTGCATTTGGGGCCTATGCGTTTGGCAGTTGC
 ATTAACCACTCAATGACTCTGGCTATTCATGAGATTGCCACAATGCTGCCTTTGGCAACTGCAAAGCAATGTGGAATCGCTGGTTTGAAT
 GTTTGCTAATCTTCCTATTGGGATTCCATATTCAATTTCTTTAAGAGGTATCACATGGATCATCATCGGTACCTTGGAGCTGATGGCGTCG
 ATGTAGATATTCCTACCGATTTTGAGGGCTGGTTCTTCTGTACCGCTTTCAGAAAGTTTATATGGGTATTCTTCAGCCTCTCTTTTATGCC
 TTTTCGACCTCTGTTTCATCAACCCCAAACCAATTACGTATCTGGAAGTTATCAATACCGTGGCACAGGTCACTTTTGACATTTTAATTTATTA
 CTTTTTGGGAATTAATCCTTAGTCTACATGTTGGCAGCATCTTTACTTGGCCTGGGTTTGCACCCAATTTCTGGACATTTTATAGCTGAGC
 ATTACATGTTCTTAAAGGGTCATGAACTTACTCATATTATGGGCCTCTGAATTTACTTACCTTCAATGTGGGTATCATAATGAACATCAT
 GATTTCCCAACATTCCTGGAAAAAGTCTTCCACTGGTGAGGAAAATAGCAGCTGAATACTATGACAACCTCCCTCACTACAATTCCTGGAT
 AAAAGTACTGTATGATTTTGTGATGGATGATACAATAAGTCCCTACTCAAGAATGAAGAGGCACCAAAAAGGAGAGATGGTGCTGGAGTAA
 TATCATTAGTCCCAAAGGGATTCTTCTCCAAAACCTTTAGATGATAAAATGGAATTTTTGCATTATTAACCTTGAGACCAGTGATGCTCAGAA
 GCTCCCTGGCACAATTTAGAGTAAGAGCTCGGTGATACCAAGAAGTGAATCTGGCTTTTAAACAGTCAGCCTGACTCTGTACTGCTCAGT
 TTCCTCACAGGAACTTGTGACTTGTGATTATCGTCATTGAGGATGTTTCACTCATGTCTGTCAATTTATAAGCATATCATTTAAAAAGC
 TTCTAAAAAGCTATTTGCCCAGG

FIG.2

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TTACCTTCTACGTCCGCTTCTTCTCACTTATGTGCCACTATTGGGGCTGAAAGCTTCCTGGGCCTTTTCTTCATAGTCAGGTTCTTGAAA
GCAACTGGTTTGTGTGGGTGACACAGATGAACCATATTCATGCACATTGATCATGACCGGAACATGGACTGGGTTTCCACCCAGCTCCAG
GCCACATGCAATGTCCACAAGTCTGCCTTCAATGACTGGTTTCAGTGGACACCTCAACTTCCAGATTGAGCACCATCTTTTTCCACGATGCC
TCGACACAATTACCACAAAGTGGCTCCCTGGTGCAGTCTTGTGTGCCAAGCATGGCATAGAGTACCAGTCCAAGCCCCCTGCTGTCAGCCT
TCGCCGACATCATCCACTCACTAAAGGAGTCAGGGCAGCTCTGGCTAGATGCCTATCTTCACCAATAACAACAGCCACCCTGCCAGTCTGG
AAGAAGAGGAGGAGACTCTGGAGCCAAGGCAGAGGGGAGCTTGAGGGACAATGCCACTATAGTTTAATACTCAGAGGGGTTGGGTTTGGG
GACATAAAGCCTCTGACTCAAACCTCTCCCTTTTATCTTCTAGCCACAGTTCTAAGACCCAAAGTGGGGGTGGACACAGAAGTCCCTAGGA
 GGAAGGAGCT

FIG.3

B/E

GCCACTTAAGGGTGCCTCTGCCAACTGGTGAATCATCGCCACTTCCAGCACCAGCCCAAGCCTAACATCTTCCACAAGGATCCCGATGTG
AACATGCTGCACGTGTTTGTTCGGGCGAATGGCAGCCCATCGAGTACGGCAAGAAGAAGCTGAAATACCTGCCCTACAATCACCAGCACGA
ATACTTCTTCTGATTGGGCGCGCTGCTCATCCCCATGTATTTCCAGTACCAGATCATCATGACCATGATCGTCCATAAGAACTGGGTGG
ACCTGGCCTGGGCGCTCAGCTACTACATCCGGTTCTTCATCACCTACATCCCTTTCTACGGCATCCTGGGAGCCCTCCTTTTCTCAACTTC
ATCAGGTTCTGGAGAGCCACTGGTTTGTGTGGGTACACAGATGAATCACATCGTCATGGAGATTGACCAGGAGGCCTACCGTGACTGGTT
CAGTAGCCAGCTGACAGCCACCTGCAACGTGGAGCAGTCCTTCTTCAACGACTGGTTCAGTGGACACCTTAACTTCCAGATTGAGCACCACC
TCTTCCCCACCATGCCCCGGCACAACTTACACAAGATCGCCCCGCTGGTGAAGTCTCTATGTGCCAAGCATGGCATTGAATACCAGGAGAAG
CCGCTACTGAGGGCCCTGCTGGACATCATCAGGTCCTGAAGAAGTCTGGGAAGCTGTGGCTGGACGCCTACCTTCACAAATGAAGCCACAG
CCCCCGGACACCGTGGGAAGGGGTGCAGGTGGGTGATGGCCAGAGGAATGATGGGCTTTTGTTCGAGGGGTGTCCGAGAGGCTGGTGT
ATGCACTGCTCACGGACCCCATGTTGGATCTTTCTCCCTTTCTCCTCTCCTTTTTCTCTTCACATCTCCCCCATAGCACCTGCCCTCATGG
GACCTGCCCTCCCTCAGCCGTAGCCATCAGCCATGGCCCTCCCAGTGCCTCCTAGCCCCCTTCTTCCAAGGAGCAGAGAGGTGGCCACCGGG
GGTGGCTCTGTCTACCTCCACTCTCTGCCCTAAAGATGGGAGGAGACCAGCGGTCCATGGGTCTGGCCTGTGAGTCTCCCCTTGACGCCT
GGTCACTAGGCATACCCCCGCTTTGGTTCTTCAGATGCTCTTGGGGTTCATAGGGGCACCTCCTAGTCGGGCACCCCCCTGACCTCCCC
GCCTGGCTTCACTCTCCCTGACGGCTGCCATTGGTCCACCTTTCTATAGAGAGGCCTGCTTTGTTACAAAGCTCGGGTCTCCCTCCTGCAGC
TCGGTTAAGTACCCGAGGCCTCTCTTAAGATGTCCAGGGCCCCAGGCCCCGGGCACAGCCAGCCCAAACCTTGGGCCCTGGAAGAGTCCTC
CACCCCATCACTAGAGTGCTCTGACCTGGGCTTTACGGGGCCCATTCACCGCCTCCCCAACTTGAGCCTTGTGACCTTGGGACCAAAGGG
GGAGTCCCTCGTCTCTTGTGACTCAGCAGAGGCAGTGGCCACGTTACGGGAGGGGCGGGCTGGCCTGGAGGCTCAGCCACCTCCAGCTTT
TCCTCAGGGTGTCTGAGGTCCAAGATTCTGGAGCAATCTGACCTTCTCCAAAGGCTCTGTTATCAGCTGGGCAGTGGCAGCCAATCCCTG
GCCATTTGGCCCCAGGGACGTGGGCCCTG

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FIG.6

GTCTTTTACTTTGGCAATGGCTGGATTCTACCTCATCACGGCCTTTGTCTTGCTACCTCTCAGGCCCAAGCTGGATGGC
TGCAACATGATTATGGCCACCTGTCTGTCTACAGAAAACCCAAGTGAACCACCTTGTCCACAAATTCGTATTGGCCACT
TAAAGGGTGCTCTGCCAACTGGTGAATCATCGCCACTTCCAGCACCACGCCAAGCCTAACATCTTCCACAAGGATCCC
GATGTGAACATGCTGCACGTGTTTGTCTGGGCGAATGGCAGCCCATCGAGTACGGCAAGAAGAAGCTGAAATACCTGCC
CTACAATCACCAGCACGAATACTTCTTCCTGATTGGGCCGCCGTGCTCATCCCCATGTATTCCAGTACCAGATCATCATG
ACCATGATCGTCCATAAGAAGTGGGTGGACCTGGCCTGGGCCGTCAGCTACTACATCCGGTTCTTCATCACCTACATCCCT
ATGAATCACATCGTCATGGAGATTGACCAGGAGGCCTACCGTGACTGGTTTCACTAGCCAGCTGACAGCCACCTGCAACGT
GGAGCAGTCCTTCTTCAACGACTGGTTTCACTGGACACCTTAACCTCCAGATTGAGCACCACCTCTTCCCCACCATGCCCCG
GCACAACTTACACAAGATCGCCCCGCTGGTGAAGTCTCTATGTGCCAAGCATGGCATTGAATACCAGGAGAAGCCGCTAC
TGAGGGCCCTGCTGGACATCATCAGTCCCTGAAGAAGTCTGGGAAGCTGTGGCTGGACGCCTACCTTCACAAATGAAGC
CACAGCCCCCGGGACACCGTGGGAAGGGGTGCAGGTGGGTGATGCCAGAGGAATGATGGGCTTTTGTCTGAGGG
GTGTCCGAGAGGCTGGTGTATGCACTGCTCACGGACCCCATGTTGGATCTTTCTCCCTTTCTCCTCTCCTTTTCTCTTCAC
ATCTCCCCCATAGCACCTGCCCTCATGGGACCTGCCCTCCCTCAGCCGTCAGCCATCAGCCATGGCCCTCCCAGTGCCTC
CTAGCCCCCTTCTTCCAAGGAGCAGAGAGGTGGCCACCGGGGGTGGCTCTGTCTTACCTCCACTCTCTGCCCCCTAAAGATG
GGAGGAGACCAGCGGTCCAAGGTCTGGCCTGTGAGTCTCCCTTGCAGCCTGGTCACTAGGCATCACCCCCGCTTTGGT
TCTTCAGATGCTCTTGGGGTTCATAGGGGCAGGTCTAGTCGGGCAGGGCCCCGACCTCCCGGCCTGGCTTCACTCTC
CCTGACCGCTGCCATTGGTCCACCCTTTCATAGAGAGGCCTGCTTTGTTACAAAGCTCGGGTCTCCCTCCTGCAGCTCGGT
TAAGTACCCGAGGCCTCTCTTAAGATGTCCAGGGCCCCAGGCCCGGGGCACAGCCAGCCCAAACCTTGGGCCCTGGAA
GAGTCTCCACCCCATCACTAGAGTGCTCTGACCCTGGGCTTTCACGGGCCCCATTCCACCGCCTCCCCAACTTGAGCCTG
TGACCTTGGGACCAAAGGGGGAGTCCCTCGTCTCTTGACTCAGCAGAGGCAGTGGCCACGTTACGGGAGGGGCGGGC
TGGCCTGGAGGCTCAGCCACCTCCAGCTTTTCTCAGGTGTCTGAGGTCCAAGATTCTGGAGCAATCTGACCCTTCT
CCAAAGGCTCTGTTATCAGCTGGGCAGTGCCAGCCAATCCCTGGCCATTGGCCCCAGGGGACGTGGGCCCTG

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FIG.7

CAGGGACCTACCCCGGCTACTTCACCTGGGACGAGGTGGCCAGCGCTCAGGGTGGAGGAGCGGTGGCTAGTGATC
GACCGTAAGGTGTACAACATCAGCGAGTTCACCCGCCGGCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGGC
AGGATGCCACGGATCCCTTTGTGGCCTTCCACATCAACAAGGGCCTTGTGAAGAAGTATATGAACTCTCTCTGATTGGAG
AACTGTCTCCAGAGCAGCCAGCTTTGAGCCACCAAGAATAAAGAGCTGACAGATGAGTCCGGGAGCTGCGGGCCAC
AGTGGAGCGGATGGGGCTCATGAAGGCCAACCATGTCTTCTTCTGCTGTACCTGCTGCACATCTTGCTGCTGGATGGTG
CAGCCTGGCTCACCTTTGGGTCTTTGGGACGTCTTTTGGCCCTTCTCTCTGTGCGGTGCTGCTCAGTGCAGTTCAGC
AGGCCAAGCTGGATGGCTGCAACATGATTATGGCCACCTGTCTGTCTACAGAAAACCAAGTGAACCACCTTGTTCCAC
AAATTGCTCATTGGCCACTTAAAGGGTGCCTCTGCCAACTGGTGAATCATCGCCACTTCCAGCACCAGCCAAGCCTAAC
ATCTTCCACAAGGATCCCGATGTGAACATGCTGCAGGTGTTGTTCTGGGCGAATGGCAGCCCATCGAGTACGGCAAGAA
GAAGCTGAAATACCTGCCCTACAATCACCAGCAGCAATACTTCTTCTGATTGGGCCGCCGTGCTCATCCCCATGTATTT
CCAGTACCAGATCATCATGACCATGATCGTCCATAAGAAGTGGGTGGACCTGGCTGGGCCGTGAGTACTACATCCGGT
TCTTCATCACCTACATCCCTTTCTACGGCATCTGGGAGCCCTCCTTTCTCAACTTCATCAGTTTCTGGAGAGCCACTG
GTTTGTTGGTCSACACAGATGAATCACATCGTATGGAGATTGACCAGGAGGCCTACCGTGACTGGTTCAGTAGCCAGC
TGACAGCCACCTGCAAGCTGGAGCAGTCTTCTTCAACGACTGGTTTCACTGGACACCTTAACITCCAGATTGAGCACCACC
TCTTCCCCACCATGCCCGGCACAACCTACACAAGATCGCCCCGTGGTGAAGTCTCTATGTGCCAAGCATGGCATTGAAT
ACCAGGAGAAGCCGCTACTGAGGGCCCTGCTGGACATCATCAGGTCCCTGAAGAAGTCTGGGAAGCTGTGGCTGGACGC
CTACCTTCACAAATGAAGCCACAGCCCCGGGACACCGTGGGGAAGGGGTGCAGGTGGGGTGATGGCCAGAGGAATGA
TGGGCTTTTGTCTGAGGGGTGTCCGAGAGGCTGGTGTATGCACTGCTCACGGACCCCATGTGGATCTTTCTCCCTTTCT
CCTCTCCTTTTCTCTTACATCTCCCCATAGCACCTGCCCTCATGGGACCTGCCCTCCCTCAGCCGTGAGCCATCAGCC
ATGGCCCTCCAGTGCCTCCTAGCCCTTCTTCCAAGGAGCAGAGAGGTGGCCACCGGGGTGGCTCTGTCTACCTCCA
CTCTCTGCCCCATAAAGATGGGAGGAGACCAGCGTCCATGGGTCTGGCCTGTGAGTCTCCCTTGCAGCCTGGTCACTAG
GCATCACCCCCGCTTTGGTTCTTCAATGCTCTTGGGGTTCATAGGGGCAGGTCTAGTCGGGCAGGGCCCTGACCCTC
CGGCCTGGCTTCACTCTCCCTGACGGCTGCCATTGGTCCACCCTTTCATAGAGAGGCTGCTTTGTTACAAAGCTCGGGT
CTCCCTCTGCAGCTCGGTTAAGTACCCGAGGCCTCTCTTAAGATGTCCAGGGCCCCAGGCCCGGGCACAGCCAGCCC
AAACCTTGGGCCCTGGAAGAGTCTTCCACCCCATCACTAGAGTGCTCTGACCCTGGGCTTTCAGGGCCCCATTCCACCG
CCTCCCCAACTTGAGCCTGTGACCTTGGGACCAAAGGGGAGTCCCTCGTCTCTTGTAAGTCACTCAGCAGAGGCAGTGGCCAC
GTTTCAAGGAGGGCGGGCTGGCCTGGAGGCTCAGCCACCCTCCAGCTTTTCTCAGGGTGTCTGAGGTCCAAGATTCT
GGAGCAATCTGACCCTTCTCCAAGGCTCTGTTATCAGCTGGGCAGTGCCAGCCAATCCCTGGCCATTGGCCCCAGGGG
ACGTGGGCCCTG

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FIG.8

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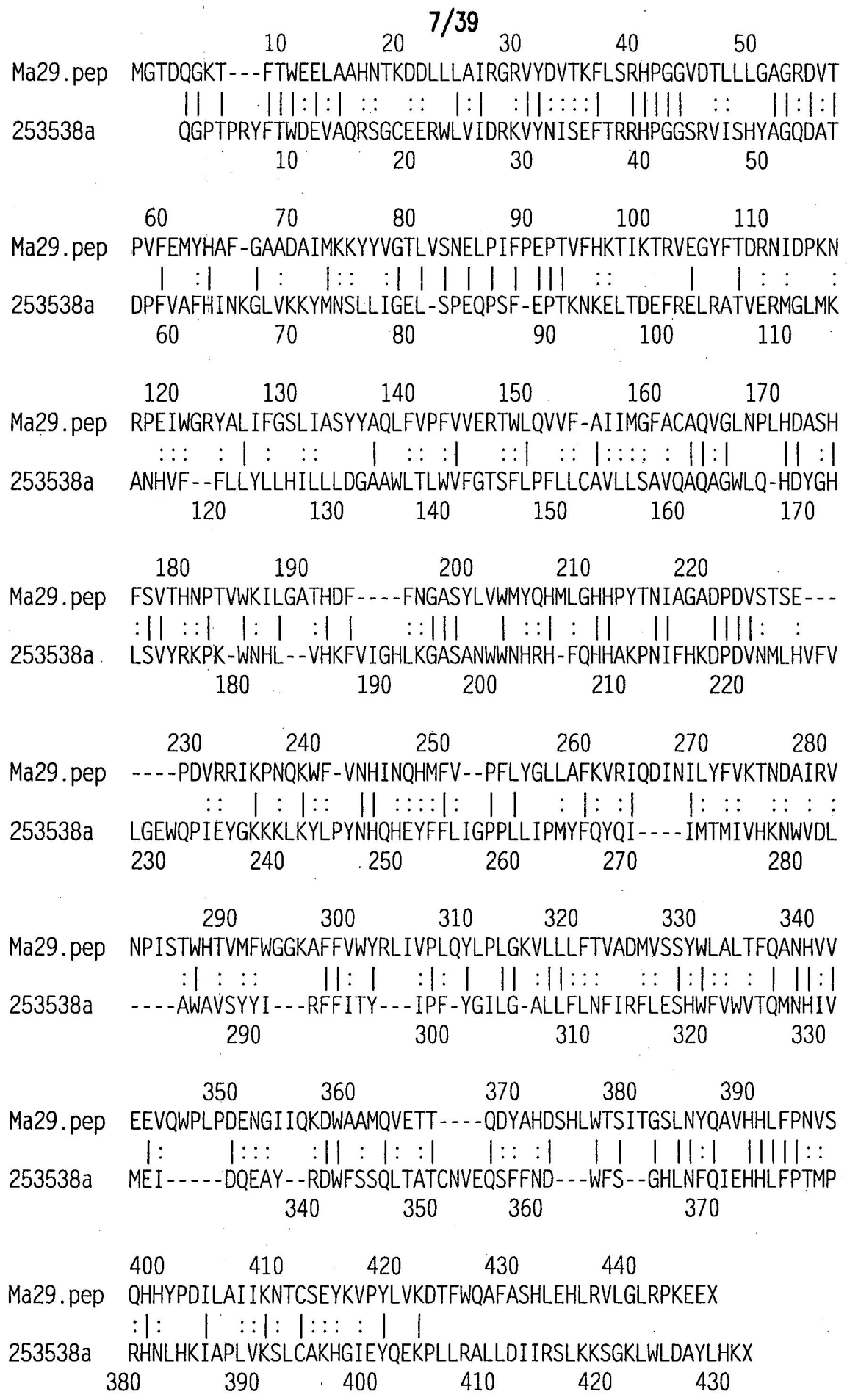


FIG.9

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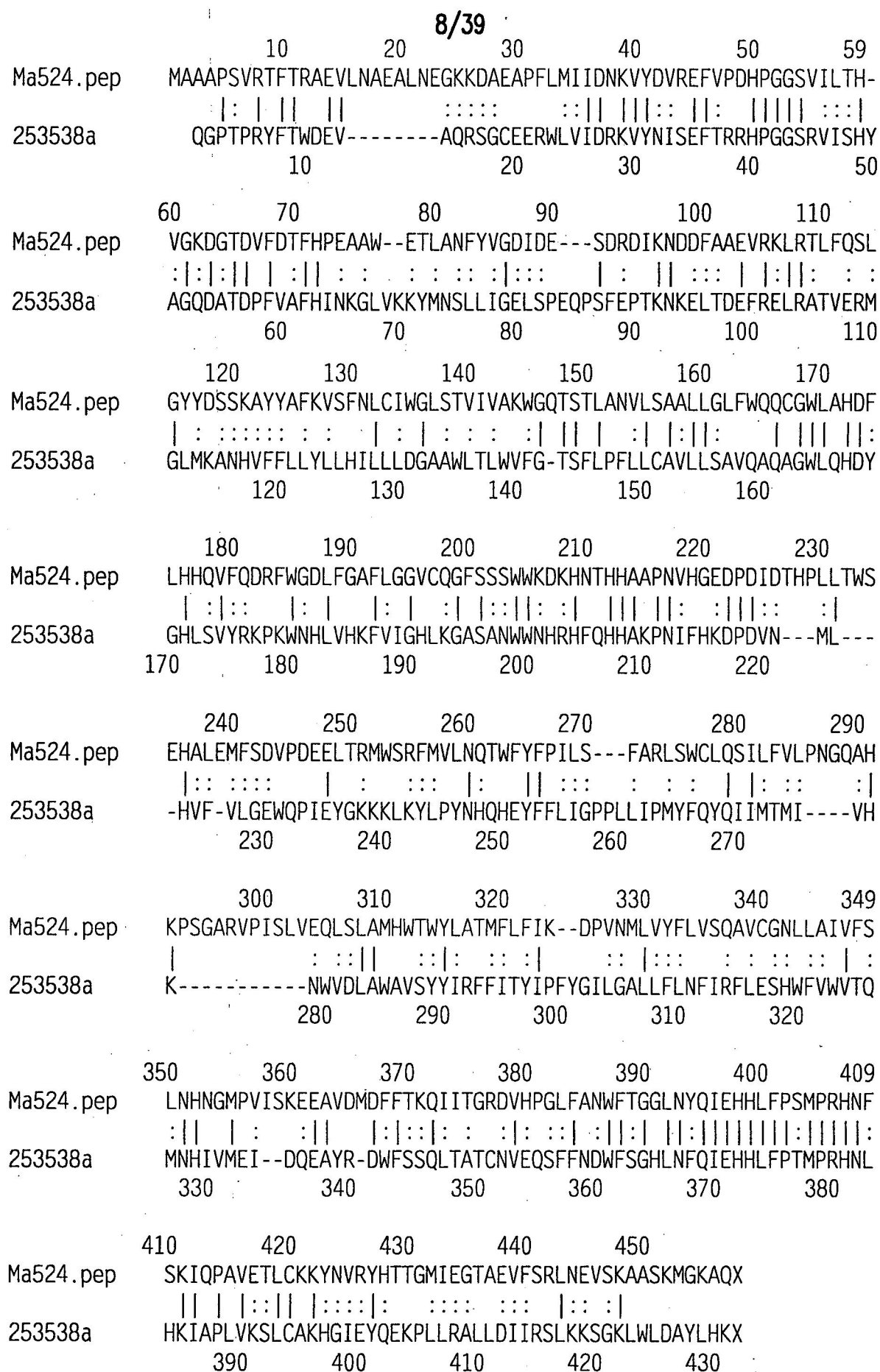
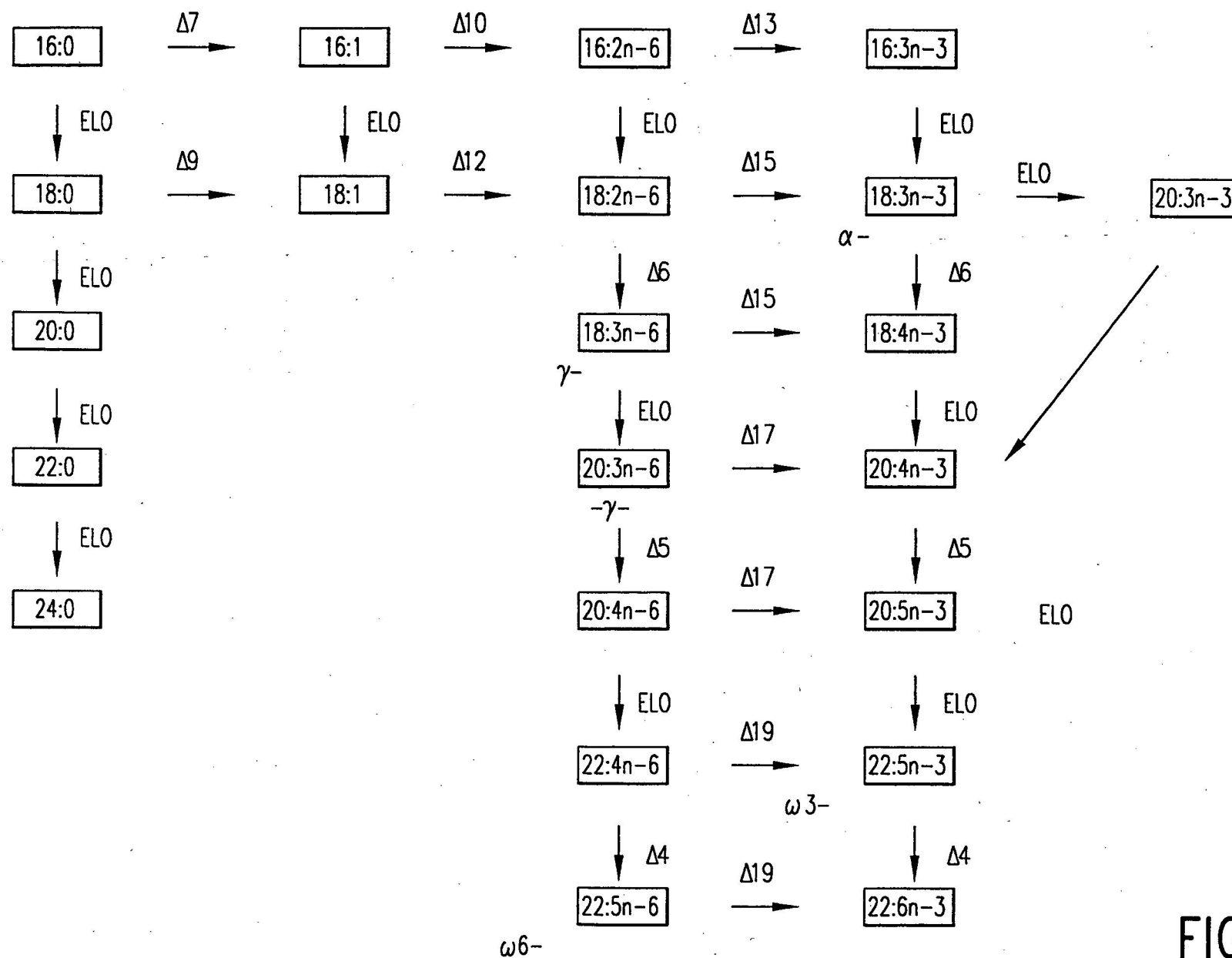


FIG.10



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FIG.11

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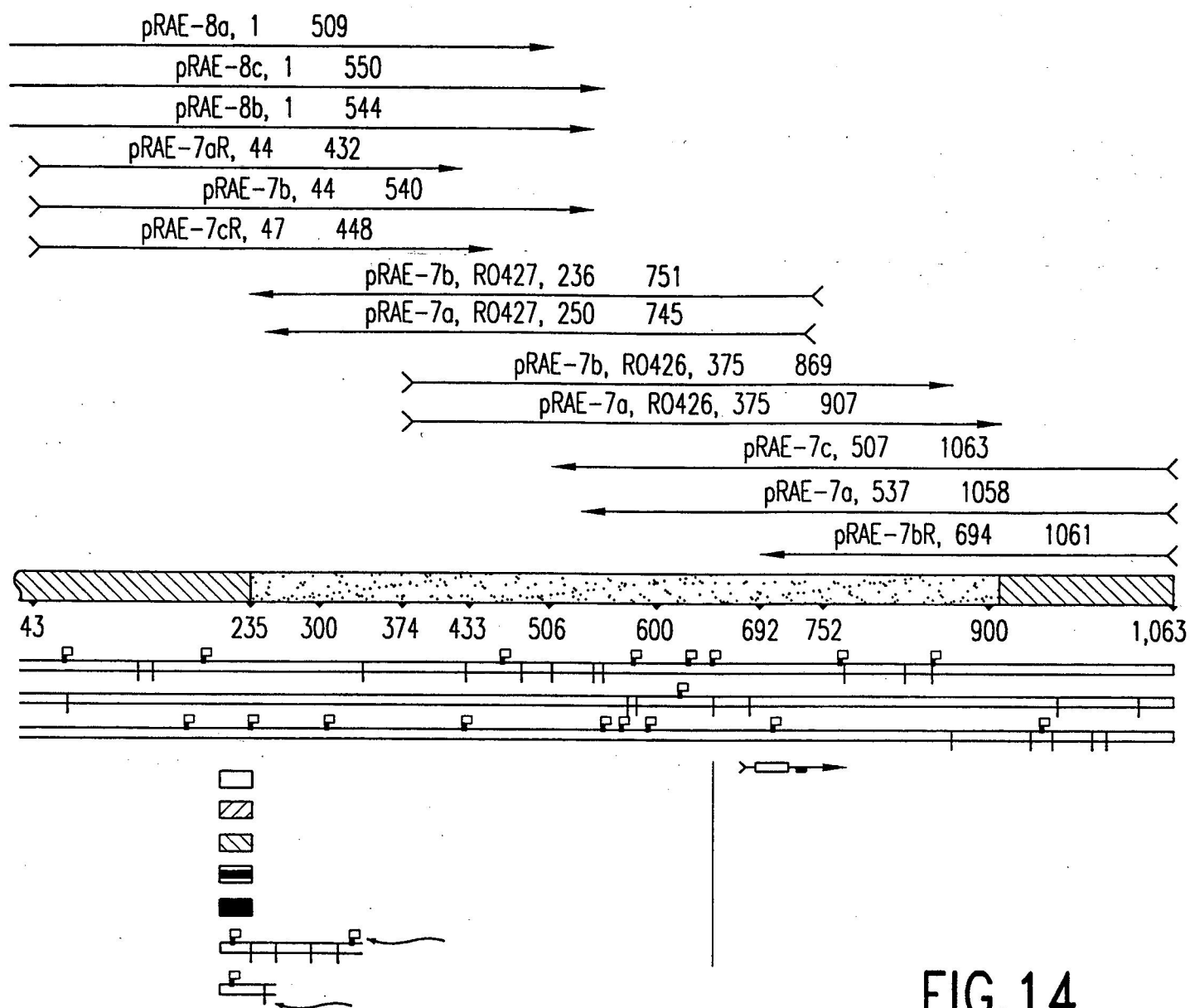
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ATGCCCCCGACCCGGTGGCCGCCGAGACCGCGGCTCAGGGACCTACCCCGCGCTACTTCACCTGG
GACGAGGTGGCCCAGCGCTCAGGGTGGAGGAGCGGTGGCTAGTGATCGACCGTAAGGTGTACAAC
ATCAGCGAGTTCACCCGCCGGCATCCAGGGGGCTCCCGGTCATCAGCCACTACGCCGGCAGGAT
GCCACGGATCCCTTTGTGGCCTTCCACATCAACAAGGGCCTTGTAAGAAGTATATGAACTCTCTC
CTGATTGGAGAACTGTCTCCAGAGCAGCCCAGCTTTGAGCCACCAAGAATAAAGAGCTGACAGAT
GAGTTCGGGAGCTGCGGGCCACAGTGGAGCGGATGGGGTCATGAAGGCCAACCATGTCTTCTTC
CTGCTGTACCTGCTGCACATCTTGCTGCTGGATGGTGCAGCCTGGCTCACCCTTTGGGTCTTTGGG
ACGTCCTTTTTGCCCTTCTCTCTGTGCGGTGCTGCTCAGTGCAGTTCAGGCCCAGGCTGGCTGG
CTGCAGCATGACTTTGGGCACCTGTGGTCTTCAGCACCTCAAAGTGAACCATCTGCTACATCAT
TTGTGATTGGCCACCTGAAGGGGGCCCCCGCCAGTTGGTGAACCACATGCACTTCCAGCACCAT
GCCAAGCCCAACTGCTTCCGCAAAGACCCAGACATCAACATGCATCCCTTCTTCTTTGCCCTGGGG
AAGATCCTCTCTGTGGAGCTTGGGAAACAGAAGAAAAAATATATGCCGTACAACCACCAGCACAAA
TACTTCTTCCTAATTGGGCCCCCAGCCTTGCTGCCTCTCTACTTCCAGTGGTATATTTTCTATTTT
GTTATCCAGCGAAAGAAGTGGGTGGACTTGGCCTGGATGATTACCTTCTACGTCCGCTTCTTCCTC
ACTTATGTGCCACTATTGGGGCTGAAAGCCTTCTGGGCCTTTTCTTCATAGTCAGGTTCCTGGAA
AGCAACTGGTTTGTGTGGTGACACAGATGAACCATATTCCCATGCACATTGATCATGACCGGAAC
ATGGAAGTGGTTTCCACCCAGCTCCTGGCCACATGCAATGTCCACAAGTCTGCCTTCAATGACTGG
TTCAGTGGACACCTCACTTCCAGATTGAGCACCATCTTTTCCACGATGCCTCGACACAATTAC
CACAAAGTGGCTCCCTGGTGCAGTCCTTGTTGCCAAGCGTGGCATAGAGTACCAGTCCAAGCCC
CTGCTGTACGCTTCGCCGACATCATCCACTCACTAAAGGAGTCAGGGCAGCTCTGGCTAGATGCC
TATCTTCACCAATAA

FIG.12

1 MAPDPVAAET AAQGTPRYF TWDEVAQRSG CEERWLVIDR KVYNISEFTR
51 RHPGGSRVIS HYAGQDATDP FVAFHINKGL VKKYMNSLLI GELSPEQPSF
101 EPTKNKELTD EFRELRAIVE RMGLMKANHV FFLLYLLHIL LLDGAAWLTL
151 WVGTSFLPF LLCVLLSAV QAQAGWLQHD FGHLSVFSTS KWNHLLHHFV
201 IGHKLGAPAS WNNHMHFQHH AKPNCFRKDP DINMHPFFFA LGKILSVELG
251 KQKKKYPYN HQHKYFFLIG PPALLPLYFQ WYIFYFVIQR KKWVDLAWMI
301 TFYVRFFLT YVPLLGLKAFGLFFIVRFLE SNWFVWVTQM NHIPMHIDHD
351 RNMDWVSTQL LATCNVHKSA FNDWFSGHLN FQIEHHLFPT MPRHNYHKVA
401 PLVQSLCAKR GIEYQSKPLL SAFADIHSL KESGQLWLDA YLHQ*

FIG.13



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FIG.14

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340	350	360	370	380
* * * * *				
GGG CCC CCA GCC TTG CTG CCT CTC TAC TTC CAG TGG TAT ATT TTC TAT				
Gly Pro Pro Ala Leu Leu Pro Leu Tyr Phe Gln Trp Tyr Ile Phe Tyr>				
__a__a__a__a__				PRAE-7 MV [A]__a__a__a__a__>
390	400	410	420	430
* * * * *				
TTT GTT ATC CAG CGA AAG AAG TGG GTG GAC TTG GCC TGG ATG ATT ACC				
Phe Val Ile Gln Arg Lys Lys Trp Val Asp Leu Ala Trp Met Ile Thr>				
__a__a__a__a__				PRAE-7 MV [A]__a__a__a__a__>
440	450	460	470	480
* * * * *				
TTC TAC GTC CGC TTC TTC CTC ACT TAT GTG CCA CTA TTG GGG CTG AAA				
Phe Tyr Val Arg Phe Phe Leu Thr Tyr Val Pro Leu Leu Gly Leu Lys>				
__a__a__a__a__				PRAE-7 MV [A]__a__a__a__a__>
490	500	510	520	
* * * * *				
GCC TTC CTG GGC CTT TTC TTC ATA GTC AGG TTC CTG GAA AGC AAC TGG				
Ala Phe Leu Gly Leu Phe Phe Ile Val Arg Phe Leu Glu Ser Asn Trp>				
__a__a__a__a__				PRAE-7 MV [A]__a__a__a__a__>
530	540	550	560	570
* * * * *				
TTT GTG TGG GTG ACA CAG ATG AAC CAT ATT CCC ATG CAC ATT GAT CAT				
Phe Val Trp Val Thr Gln Met Asn His Ile Pro Met His Ile Asp His>				
__a__a__a__a__				PRAE-7 MV [A]__a__a__a__a__>
580	590	600	610	620
* * * * *				
GAC CGG AAC ATG GAC TGG GTT TCC ACC CAG CTC CAG GCC ACA TGC AAT				
Asp Arg Asn Met Asp Trp Val Ser Thr Gln Leu Gln Ala Thr Cys Asn>				
__a__a__a__a__				PRAE-7 MV [A]__a__a__a__a__>
630	640	650	660	670
* * * * *				
GTC CAC AAG TCT GCC TTC AAT GAC TGG TTC AGT GGA CAC CTC AAC TTC				
Val His Lys Ser Ala Phe Asn Asp Trp Phe Ser Gly His Leu Asn Phe>				
__a__a__a__a__				PRAE-7 MV [A]__a__a__a__a__>

FIG.15B

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680	690	700	710	720
* * * *	* *	* *	* *	* *
CAG ATT GAG CAC CAT CTT TTT CCC	ACG ATG CCT CGA CAC AAT TAC CAC			
Gln Ile Glu His His Leu Phe Pro Thr Met Pro Arg His Asn Tyr His>				
__a__a__a__a__		PRAE-7 MV [A]	__a__a__a__a__>	
730	740	750	760	
* * *	* *	* *	* *	
AAA GTG GCT CCC CTG GTG CAG TCC TTG TGT GCC AAG CAT GGC ATA GAG				
Lys Val Ala Pro Leu Val Gln Ser Leu Cys Ala Lys His Gly Ile Glu>				
__a__a__a__a__		PRAE-7 MV [A]	__a__a__a__a__>	
770	780	790	800	810
* * *	* *	* *	* *	* *
TAC CAG TCC AAG CCC CTG CTG TCA GCC TTC GCC GAC ATC ATC CAC TCA				
Tyr Gln Ser Lys Pro Leu Leu Ser Ala Phe Ala Asp Ile Ile His Ser>				
__a__a__a__a__		PRAE-7 MV [A]	__a__a__a__a__>	
820	830	840	850	860
* * *	* *	* *	* *	* *
CTA AAG GAG TCA GGG CAG CTC TGG CTA GAT GCC TAT CTT CAC CAA TAA				
Leu Lys Glu Ser Gly Gln Leu Trp Leu Asp Ala Tyr Leu His Gln ***>				
__a__a__a__a__		PRAE-7 MV [A]	__a__a__a__a__>	

FIG.15C

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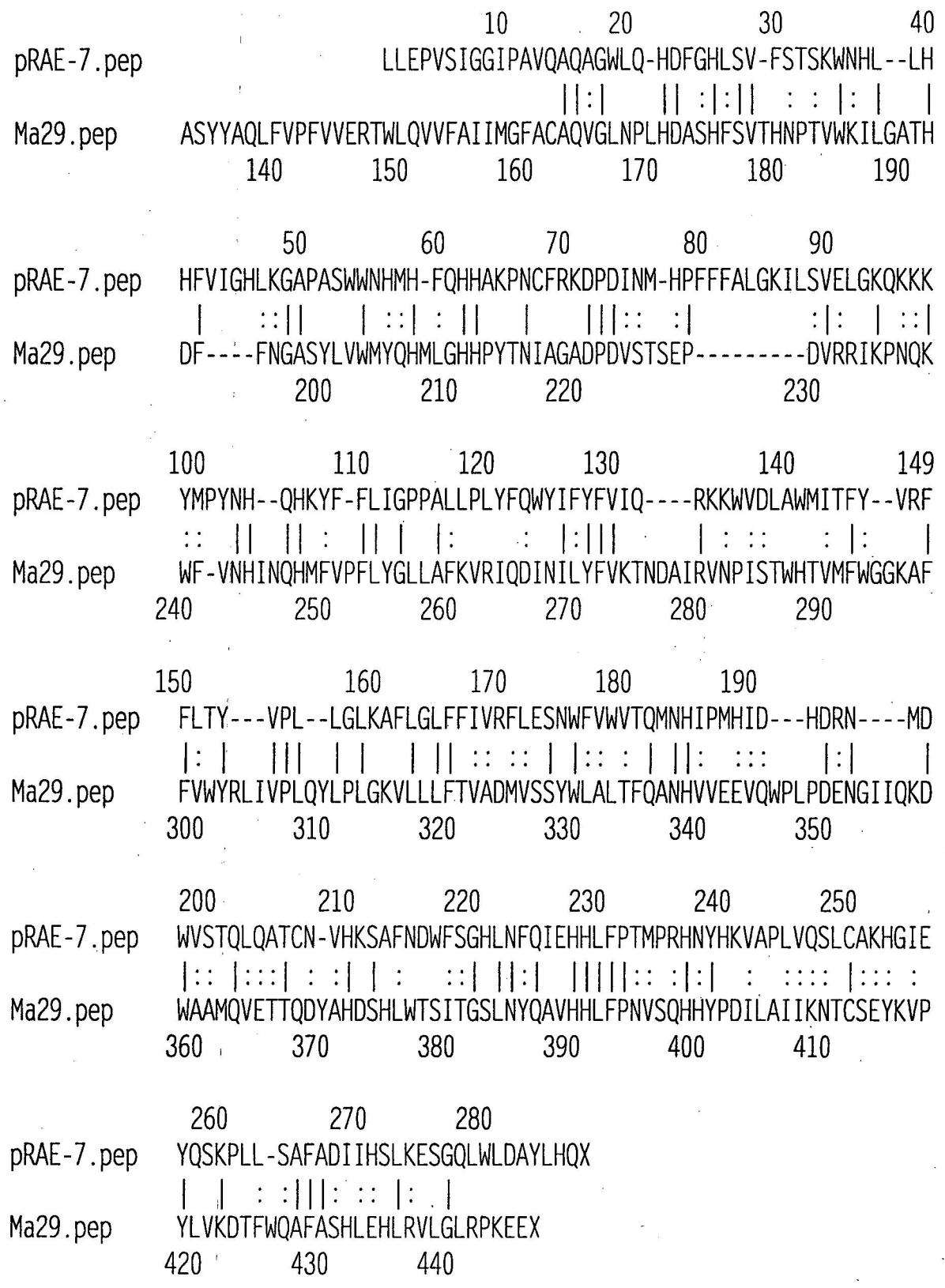


FIG.16

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	10	20	30	40
pRAE-7.pep	LLEPVSIGGIPAVQAQAGWLQHDFGHLVSFSTSKWNHLLHHFVIG			
		: : : :		
Ma524.pep	GLSTVIVAKWGQTSTLANVLSAALLGLFWQQCGWLAHDFLHHQVFQDRFWGDLFGAFLGG			
	140	150	160	170 180 190
	50	60	70	80 90
pRAE-7.pep	HLKGAPASWWNHMHFQHHAKPNCFRKDPDINMHPFF----FALGKILSV---ELGKQKKK			
	: : : : : :: : : :: ::			
Ma524.pep	VCQGFSSSWKDKHNTHHAAPNVHGEDPDIDTHPLLTWSEHALEMFSDDVPDEELTRMWSR			
	200	210	220	230 240 250
	100	110	120	130 140
pRAE-7.pep	YMPYNHQHKYFFLIGPPALLPLYFQWYIFYFV-----IQRKKWVDLAWMITF			
	: : : : : : : : :			
Ma524.pep	FMVLN-QTWFYFPILSFARLSWCLOSLFVLPNGQAHKPSGARVPISLVEQLSLAMHWTW			
	260	270	280	290 300 310
	150	160	170	180 190 200
pRAE-7.pep	YVRFFLTIV--PLLGLKAFLGLFFIVRFLESNWVFWVTQMNHIPMHI---DHDRNMDWVS			
	: : : : : : : : : : : : : : :			
Ma524.pep	YLATMFLFIKDPV----NMLVYFLVSQAVCGNLLAIVFSLNHNGMPVISKEEAVDMDFFT			
	320	330	340	350 360 370
	210	220	230	240 250 260
pRAE-7.pep	TQLQATCNVHKSAFNDWFSGHLNFQIEHHLFPTMPRHNYHKVAPLVQSLCAKHGIEYQSK			
	: : : : : : : : : : : :			
Ma524.pep	KQIITGRDVHPGLFANWFTGGLNYQIEHHLFSPMPRHNFSKIQPAVETLCKKYNVRYHTT			
	380	390	400	410 420 430
	270	280		
pRAE-7.pep	PLLSAFADIIHSLKESGQLWLDAYLHQX			
	: : : : :			
Ma524.pep	GMIEGTAEVFSRLNEVSKAASKMGKAQX			
	440	450		

FIG.17

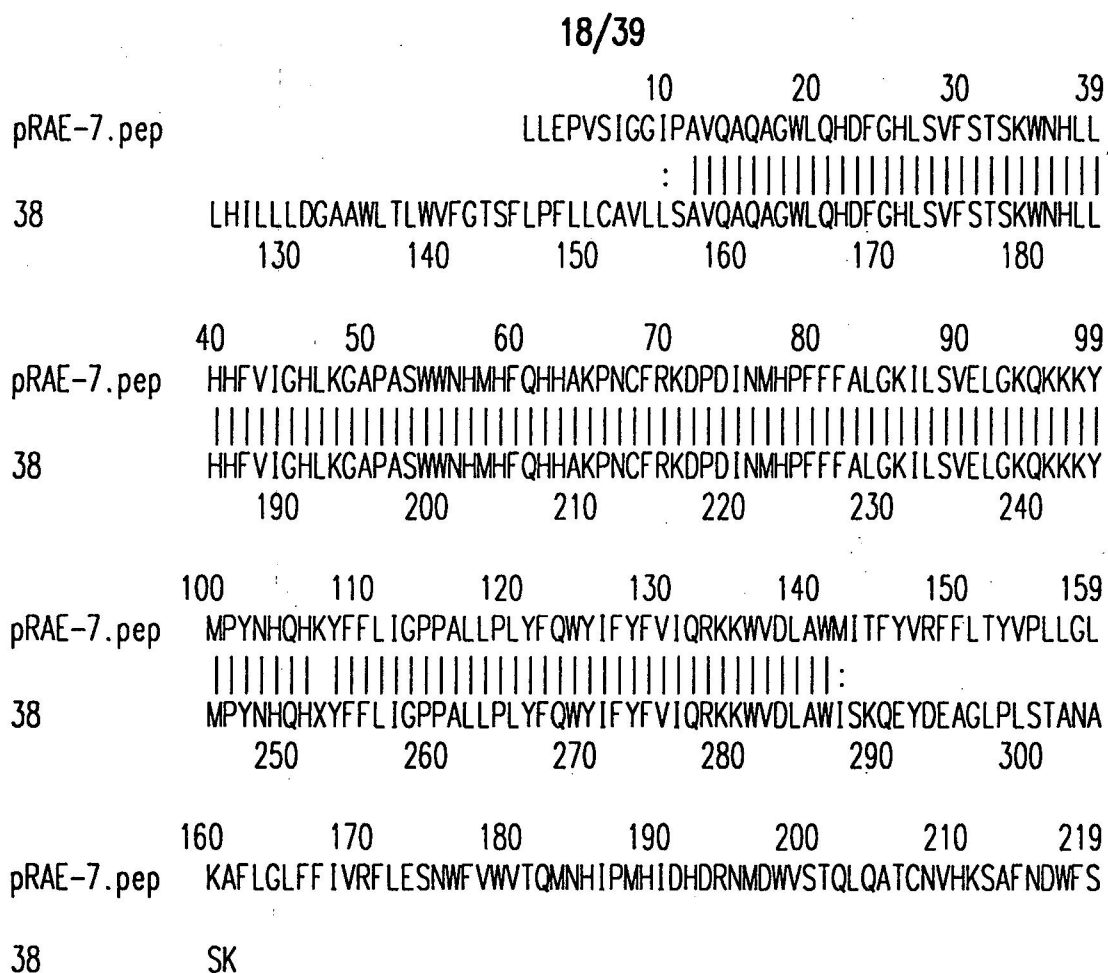


FIG.19

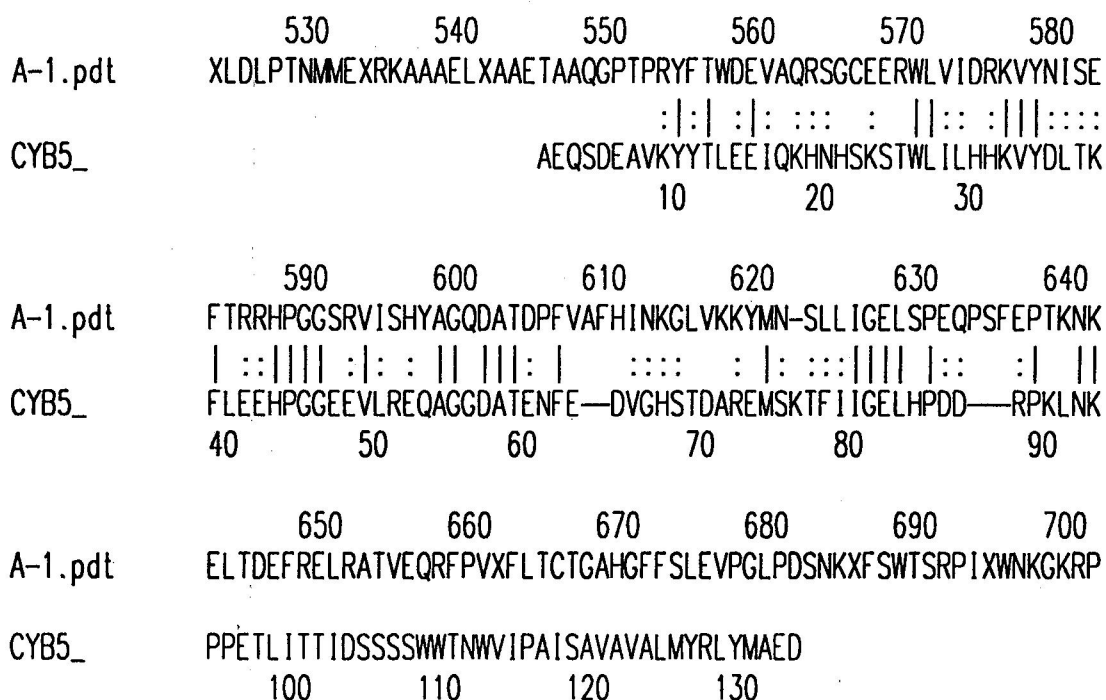


FIG.20

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	389	379	369	359	349	339	330
A-1	CCCGACCAATATGATGGAATAAAGGAAAGCGGCCGCTGAATTATAGGCCGCCGAGACCGC						
ac004228	CCCGGCCGCCGCGCTCGCCAGGCCAGCTATGGCCCCGACCCGGTGGCCGCCGAGACCGC						
	60090	60100	60110	60120	60130	60140	
	329	319	309	299	289	279	270
A-1	GGCTCAGGGACCTACCCCGCGTTACTTCACATGGGACGAGGTGGCCCAGCGCTCAGGGTG						
ac004228	GGCTCAGGGACCTACCCCGCGTACTTCACCTGGGACGAGGTGGCCCAGCGCTCAGGGTG						
	60150	60160	60170	60180	60190	60200	
	269	259	249	239	229	219	210
A-1	CGAGGAGCGGTGGCTTGTGATCGACCGTAAGGTGTACAACATCAGCGAGTTCACCCGCCG						
ac004228	CGAGGAGCGGTGGCTAGTGATCGACCGTAAGGTGTACAACATCAGCGAGTTCACCCGCCG						
	60210	60220	60230	60240	60250	60260	
	209	199	189	179	169	159	150
A-1	GCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGGCAGGATGCCACGGATCCCTT						
ac004228	GCATCCAGGGGGCTCCCGGGTCATCAGCCACTACGCCGGGCAGGATGCCACGGTGAGCGC						
	60270	60280	60290	60300	60310	60320	
	149	139	129	119	109	99	90
A-1	CGTGGCCTTCCACATCAACAAGGCCTTGTGAAGAAGTATATGAAGTCTCTCTGATTGG						
ac004228	AGCCAGGCGGGGCACAGGAGAGGGCGGACCGGAGGCTGAGTGAGGGGAGACAGAGTT						
	60330	60340	60350	60360	60370	60380	

FIG.21

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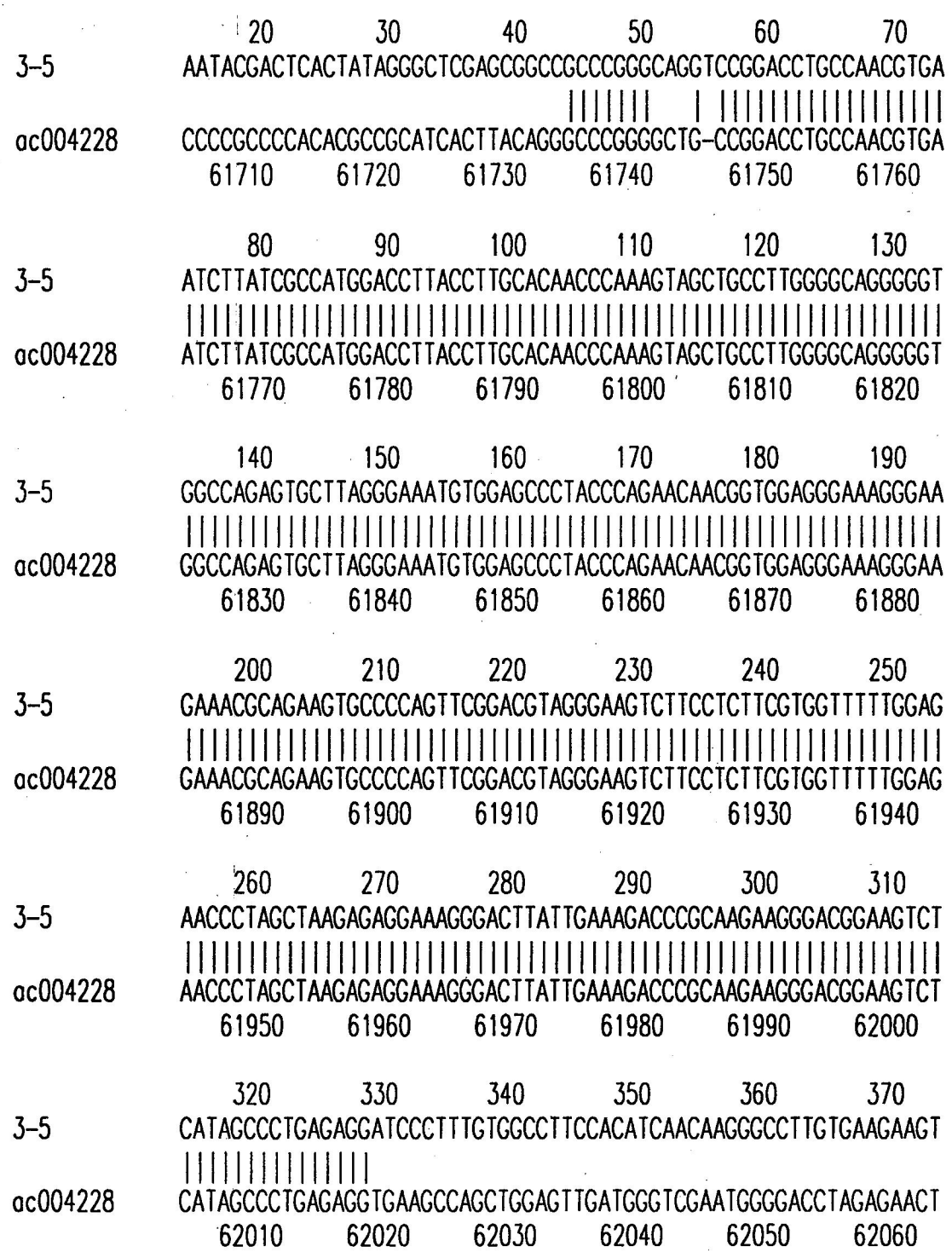


FIG.22

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	30	40	50	60	70	80	89
A-10	TATAGGGCTCGAGCGGCCCGCCGGGCAGGTGCCCGGAGGCGCCTGATCATACCTGTTGCC						
ac004228	CGAGCCAAACACCGACTAATTCGGAGGAAAGCCCGGAGGCGCCTGATCATACCTGTTGCC						
	60400	60410	60420	60430	60440	60450	
	90	100	110	120	130	140	149
A-10	CGGTGATTGGGTGTCCTGCGGATGCGGGATGAAAAGGCGGGAGAGAGGCCTGGAAAAGTG						
ac004228	CGGTGATTGGGTGTCCTGCGGATGCGGGATGAAAAGGCGGGAGAGAGGCCTGGAGAAGTG						
	60460	60470	60480	60490	60500	60510	
	150	160	170	180	190	200	209
A-10	GAGTCTGGGGAGTGGGGATGGAGGCCAACACGCACACACAAACAAAGGTCCCCGCCT						
ac004228	GAGTCTGGGGAGTGGGGATGGAGGCCAACACGCACACACAAACAAAGGTCCCCGCCT						
	60520	60530	60540	60550	60560	60570	
	210	220	230	240	250	260	269
A-10	CCCTGCCGTGCATTCCATCTGCAGCCCCGAGCCTCAGGATCCCTTTGTGGCCTTCCACAT						
ac004228	CCCTGCCGTGCATTCCATCTGCAGCCCCGAGCCTCAGG-TCTCTGGGCGGGACAGAACC						
	60580	60590	60600	60610	60620	60630	

FIG.23

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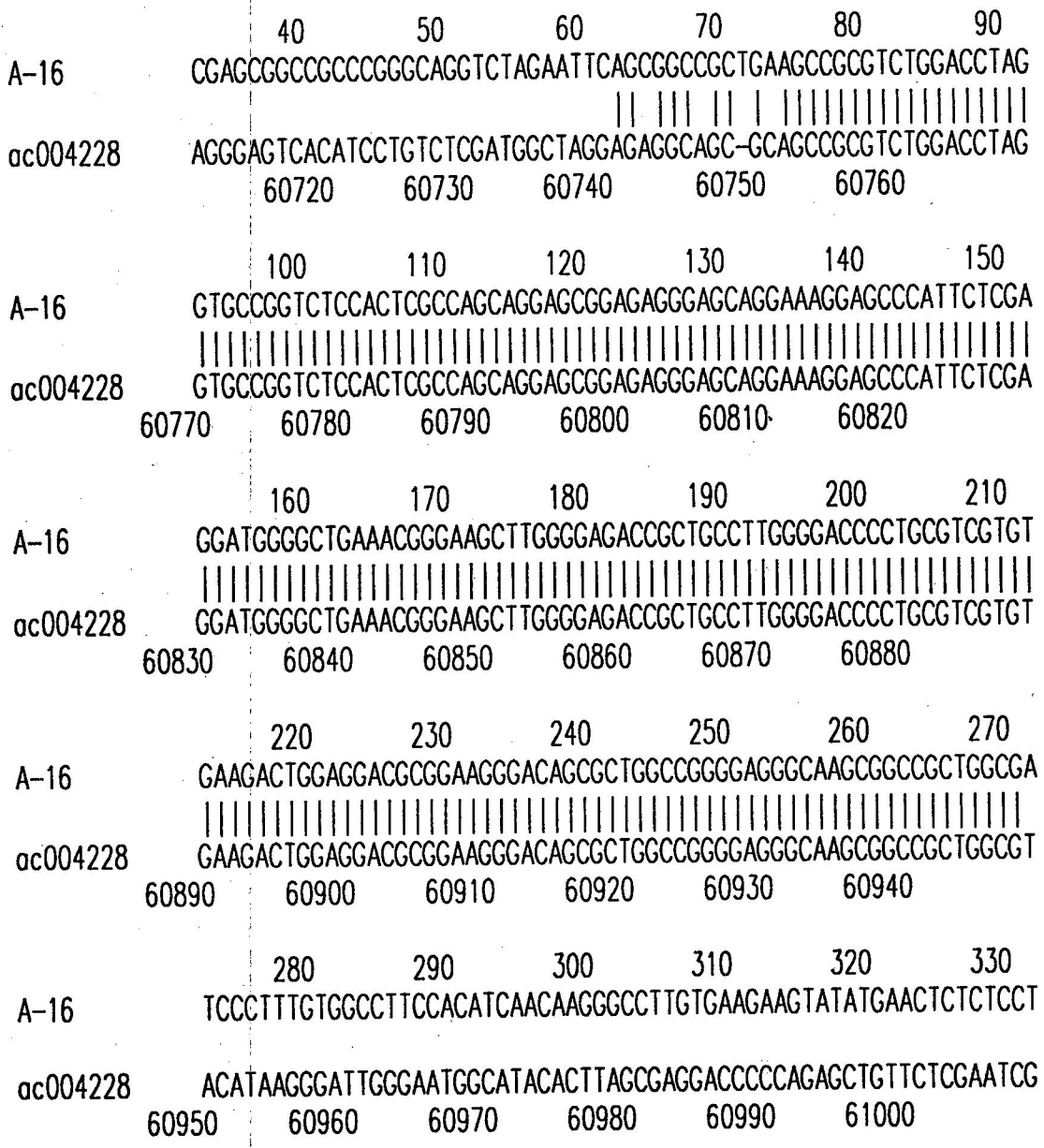


FIG.24

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59751 ACTAGAACCG CTGTTCTAC CGCGGCGCCC CCTGGGAGCC AACGCCGCGA
59801 TCCCCGCTG ACGTCAGGAA GTCGAATCCG GCGGCGACGC TTTTAGGGAG
59851 CCCCGAGGG GGGCGTGTT GGCAGCCCAG CTGTGAGTTG CCCAAGACCC
59901 ACCGGGGGAC GGGATCTCGC TCCCCGCGCC ACGAGGCTCG GCCAATGGGA
59951 ACGCGCGCTG CGAGGCCCGC CGGTCTGCCC TCGGTGCTG AAAACCCGGC
60001 GCGCAGGCGG CTGGCTCTGG GCGCGGCCA GCAAATCCAC TCCTGGAGCC
60051 CGCGGACCCC GAGCAGCGC CTGACAGCCC CTGCTGGCCC GCGCGCGGC
60101 GTCGCCAGGC CAGCTATGGC CCCCAGCCG GTGGCCGCG AGACCGCGC
60151 TCAGGGACCT ACCCGCGCT ACTTCACCTG GGACGAGGTG GCCCAGCGT
60201 CAGGGTGCGA GGAGCGGTGG CTAGTGATCG ACCGTAAGGT GTACAACATC
60251 AGCGAGTTCA CCCGCCGCA TCCAGGGGGC TCCCGGTCA TCAGCCACTA
60301 CGCCGGGCAG GATGCCACGG TGAGCGCAGC CAGGCGGGG CACAGGAGAG
60351 GCGGGACCG GAGGCTGAGT GCAGGGGAGA CAGAGTTACG CACTCCGAGC
60401 CAAACACCGA CTAATTGGA GAAAGCCCC GAGGCCCTG ATCATACCTG
60451 TTGCCCGGTG ATTGGGTGTC CTGCGGATGC GGGATGAAAA GCGGGGAGAG
60501 AGGCCTGGAG AAGTGGAGTC TCGGGAGTGG GGATGGAGGC CAACAACAGC
60551 CACACACAAA CAAAGGTCC CGCCTCCCTG CCGTGCATTG CATCTGCAGC
60601 CCCGAGCCTC AGGTCTCTGG GCGGGACAG AACCCGAGC TGGGTAGGCT
60651 AGGAGGGAGG AGAGCAAGGA TGCAGGCCG CTGGGGAGG AGGGGGTCAG
60701 TGGCCAGGGG AGGGAGTCAC ATCCTGTCTC GATGGCTAGG AGAGGCAGCG

A-1

A-10

FIG.26A

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60751 CAGCCCGGTC TGGACCTAGG TGCCGGTCTC CACTCGCCAG CAGGAGCGGA
60801 GAGGGAGCAG GAAAGGAGCC CATTCTCGAG GATGGGGCTG AAACGGGAAG
60851 CTTGGGGAGA CCGCTGCCTT GGGGACCCCT GCGTCGTGTG AAGACTGGAG
60901 GACCGGAAG GGACAGCGCT GGCCGGGGAG GGCAAGCGGC CGCTGGCGTA
60951 CATAAGGGAT TGGGAATGGC ATACACTTAG CGAGGACCCC CAGAGCTGTT
61001 CTGGAATCGC CGGGGAGGCC ACTGAGCCGC AGGCCAGCGA GGTCTTCAGC
61051 TATTCCGGCG AGCGGACCGC TGTTTACGCT CTGGGGCGGT AGGCCCTTCG
61101 CGGGGTCTG TCCCTTCTTC CCTTGGTCTC ACTGCGGGGT CGGCGGCGC
61151 CCCAGCCCCA GGCCTGCTGC TTCCCTTTCT AGACCACAGC CCTCAGAGCT
61201 AAGCCCCCGG CGCCTCTCTG CTGGGTTGGA GTCCTGGGGA CTCAGTCCTA
61251 GGGACTCGAA AGTCGGGGCG TTCCCTTCAC CGCGTTTCCC CCTTGGCGGC
61301 CAGAATGGCG TCCCCTCCCC TTGCATCCCC CTCTGATCCC GTGCCCTGCA
61351 GCGTGATGCC CTCCACTGTC CCTATCCACT ACCCTGGCGT CCCAGAGTGT
61401 GCCGCGGTC ACCAGGTTCC CATAACGTCG CAGCAGAGCT TAGACGCTGC
61451 GGGCGAAGA CCCGCCCCAC CCTCTGACGC GACCAGCCTA GTGGCGAGG
61501 CCAGAGCTTG CGCGGTCAA CCAGAGTGAC CACTCGGGAG CCCTGACTGC
61551 GGCCAAGGGC GCAGGCGTGT CCCGGCGCAT GCGCAGACGA AACAGGCACC
61601 AACGCTGGAG CTTCCCGCAG TGTGATTGG GGCCGGGGAT GCCGCGCGG
61651 GGACGGCGAT TGGTCCGTAT GTGTGGTGCC ACCGGCCGCC GCTCGCCCC
61701 GGGCCCCGCC CCACAGCCG CATCACTTAC AGGGCCCCGG GCTGCCGGAC
61751 CTGCCAACGT GAATCTTATC GCCATGGACC TTACCTTGCA CAACCCAAAG
61801 TAGCTGCCTT GGGGCAGGGG GTGGCCAGAG TGCTTAGGGA AATGTGGAG

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FIG.26B

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61851 CCTACCCAGA ACAACGGTGG AGGGAAGGG AAGAAACGCA GAAGTGCCCC
61901 AGTTCCGACG TAGGGAAGTC TTCCTCTTCG TGGTTTTTGG AGAACCTAG
61951 CTAAGAGAGG AAAGGGACTT ATTGAAAGAC CCGCAAGAAG GGACGGAAGT
62001 CTCATAGCCC TGAGAGGTGA AGCCAGCTGG AGTTGATGGG TCGAATGGGG
62051 ACCTAGAGAA CTTTTCTGTA TCTAGAGGTT TGTAATATGC ACCAATCAGT
62101 GCTCTGTAAA AACGCACCAA TTGGCGCTCT GTAGCTAGCT AGAGGTTTGT
62151 AAAATGAGCC AATCAGCAGG ACGTGGGCAG GGACAATAA GACAATAAAA
62201 GCTGGCCACC CCAGCCAGCT GCTGCAACCC GCTCCAGTCC CTTACAGGC
62251 TGTGAAGCA TTGTTCTTTT GCTCGTCACA CTAAACCTTG CTGCTGCTCA
62301 TTCTTTGGT CTGCAAAGAG TGTTATTCCT TTAAGAGCTA TAACAGCGGG
62351 AAGGTCCACG GCTCCATTCT TGAAGTCAGT GAGACCATAC CCGCCGGAAG
62401 GAACCAACGC CCGACACAGC CCCACCCATC TCTCCTGTTT CTCACCTATA
62451 CTGAAATTCT TGGGCAAAAG CTGTCTGTGG ACACACCCAG GGGAAAGGCC
62501 AGCCCAGGCA GGTGTTTCTT AGTGTTTCCC CTCAGCCAAT GCTTCCATT
62551 CCTTGATGCA TCCTTCTAAC TAGAGCAGAT GCTCGGTGAT CTTAAACTGT
62601 GGACACCTGG GAGCACCTC AAAAGGCAGC TGGGCCTAGG GAGATGGCCT
62651 GTGCTTCTGT GTCAGGAGTT GGTTCCTTCA GGTGGGCTTG TGGTCTCGCT
62701 GACGTCAAGA ATGAAGCCAT GAACCTTCGC GGTGAGTGTT ACAGCTCTTA
62751 CAGGTGGCGT GGACCCAAAG AGTGAGCAGC AGCAAGATTT ATTGTGAAGA
62801 GCAAAGAACA AAGCTTCCAC AGCGTGAAG GGTACCCGAG CAGGTTGCCG

FIG.26C

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62851 CTGCTGGACG TTGGGGGGTG TGAGGGGGAG CAGCCTTTTT TTTCTTTTT
62901 TTTTGAGAC GGAGTCTCCC TGTCGGCCAG GCTGGAGTGC AGTGGCGGA
62951 TCTCGGCTCA CTGCAGGCTC CGCCCCCCCC CCGGGTTCA CGCCATTCTC
63001 CTGCCTCAGC CTCCCGAGTA GCTGGGACTA CAGGCGCCCG CTACCTCGCC
63051 CGGCTAATTT TTTGTATTT TAGTAGAGAC GGGTTTCAC TGTGTTAGCC
63101 AGGATGGTCT CGATCTCCTG AGGTCGTGAT CCACCCGCCT TGGCTCCCA
63151 AAGTGCTGGG ATTACAGCGG TGAGCCACCG CGCCCGGCCG GGAGCAGCTT
63201 TTATTCCCTT ATTTGTCCCT GCCCATGTCC TGCTGATTG TCCATTTAT
63251 AGAGCACTGA TTGGTCCATT TTACAGGGTG CTGATTGGTC CATTITACCT
63301 CTAGCTAGCT AAAGAGCAGG GATTGGTGCA TTTTACAAAC CTCTAGCTAC
63351 AGAAAAGTTC TCCAAGTCTG CACTCGACCC AGGAAGTCCA TCTGGCTTCA
63401 CCTCTCACTT CAACTTGGGT ACAGCCTTCT GCGGGGCAGG AGGATGGCCT
63451 TTGGTGCGAA CACTGCCGGA GTCCAGGGGG CTGGCTCCCT CACCTTTCAT
63501 CTTCTCCCGG CACTGCAGG ATCCCTTTGT GGCCTTCCAC ATCAACAAGG
63551 GCCTTGTA GAAGTATATG AACTCTCTCC TGATTGGAGA ACTGTCTCCA
63601 GAGCAGCCCA GCTTTGAGCC CACCAAGAAT GTAAGACCCT GTGTTTGCTA
63651 TGTCGCAACT ATTGGTTGTT GAGGGGGACA GAGAGGGGGT GGAAGGAGAG
63701 TCTAGATGGA ATCACAGTCA TAGTAATCAC AGTCAGTAGT AGCTCTGGGG
63751 AGTCTTGAGG TCCCTGCTTC TCTTGCATAG TCATGAGGTC ACAGGCCCAA
63801 GGGAGCATGG CTTTGCAACC TATGGCTCCC CCAAGGCTGC CACTACCATG
63851 GCTGCCATCA TTGTTATCAT CATTGTTATC ATATGAGCAC TTACTATGCA

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FIG.26D

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63901 CCAAGCATAA ACTCATAACT CTTACACATT TACAGATGAG ATAACAGGCT
63951 CAGGGAGGTT AAGCAACACA GCCAAGGATC ACACAGTTAG TAAATGGCAG
64001 AGCAAGGACT TAGTCCCCTG AACTCTTAGG CACTATCCCA TGGCACCTCC
64051 TCCTGTCATC CTCATTGTCG TGGTATCTTT GCCTAGGACT GTGGACTTCC
64101 CACAGCTACC TCAGTGGGAG GTCCTTGAGC CTGAGAGGGC CCTTGTCTCC
64151 AGTAGCATTG GGGTGCAGAT GAGAAGAATA ACAGCTCCTC TTCCTCTTCT
64201 GCAGAAAGAG CTGACAGATG AGTTCCGGGA GCTGCCGGCC ACAGTGGAGC
64251 GGATGGGGCT CATGAAGGCC AACCATGTCT TCTTCCTGCT GTACCTGCTG
64301 CACATCTTGC TGCTGGATGG TGCAGCCTGG CTCACCCCTT GGGTCTTTGG
64351 GACGTCCCTT TTGCCCTTCC TCCTCTGTGC GGTGCTGCTC AGTGCAGTTC
64401 AGGTGAGAGC CTTTGGCTTG TCAAGTGCAC AGCAATGCTC AGCATCCCTG

FIG.26E

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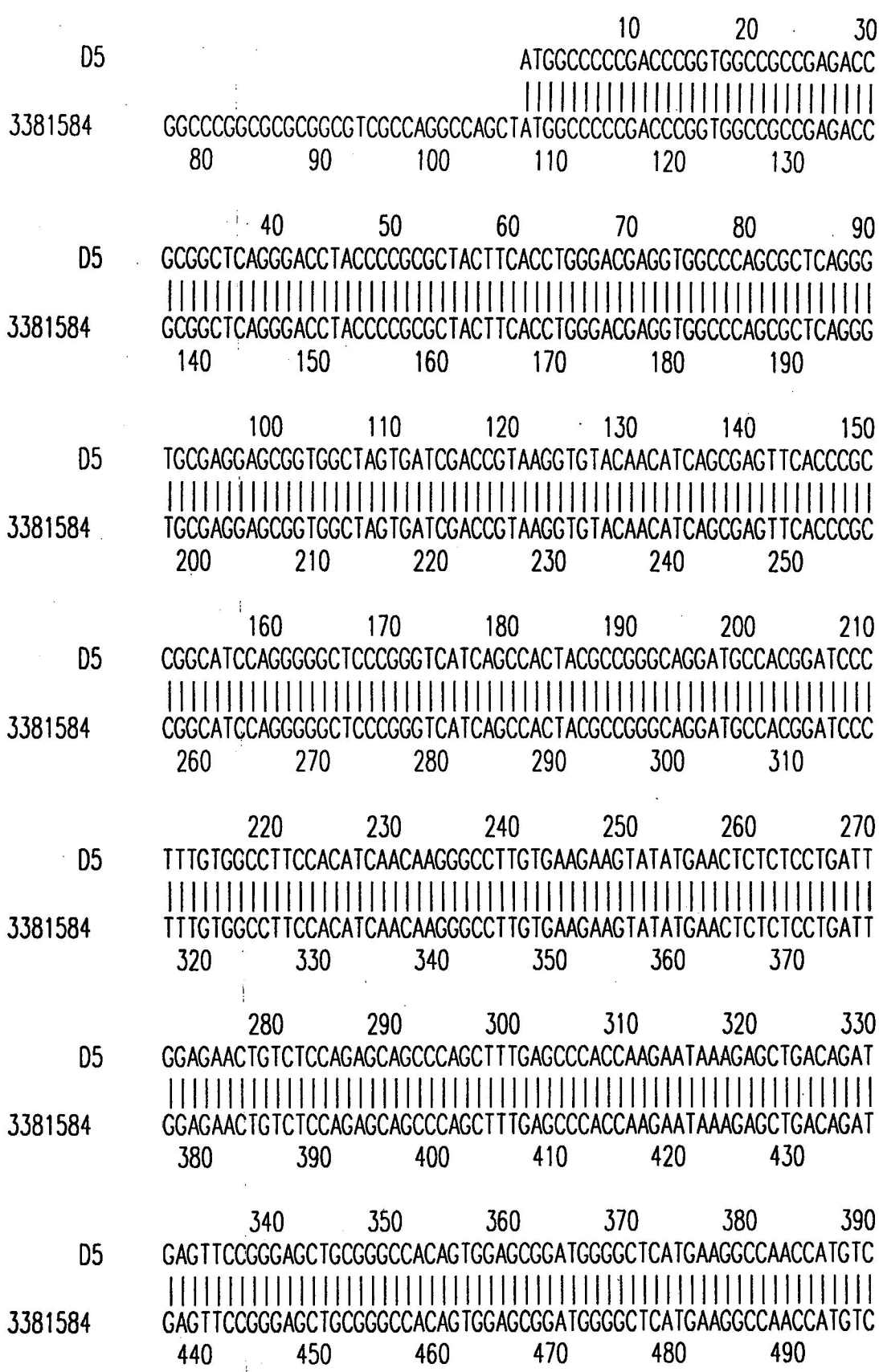


FIG.27A

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		400	410	420	430	440	450
D5		TTCTTCCTGCTGTACCTGCTGCACATCTTGCTGCTGGATGGTGCAGCCTGGCTCACCTT					
3381584		TTCTTCCTGCTGTACCTGCTGCACATCTTGCTGCTGGATGGTGCAGCCTGGCTCACCTT					
		500	510	520	530	540	550
		460	470	480	490	500	510
D5		TGGGTCTTTGGGACGTCCTTTTGGCCCTTCCTCCTCTGTGCGGTGCTGCTCAGTGCAGTT					
3381584		TGGGTCTTTGGGACGTCCTTTTGGCCCTTCCTCCTCTGTGCGGTGCTGCTCAGTGCAGTT					
		560	570	580	590	600	610
		520	530	540	550	560	570
D5		CAGGCCCAGGCTGGCTGGCTGCAGCATGACTTTGGGCACCTGTCGGTCTTCAGCACCTCA					
3381584		CAGGCCCAGGCTGGCTGGCTGCAGCATGACTTTGGGCACCTGTCGGTCTTCAGCACCTCA					
		620	630	640	650	660	670
		580	590	600	610	620	630
D5		AAGTGAACCATCTGCTACATCATTTTGTGATTGGCCACCTGAAGGGGGCCCCCGCCAGT					
3381584		AAGTGAACCATCTGCTACATCATTTTGTGATTGGCCACCTGAAGGGGGCCCCCGCCAGT					
		680	690	700	710	720	730
		640	650	660	670	680	690
D5		TGGTGAACCACATGCACTTCCAGCACCATGCCAAGCCCAACTGCTTCCGCAAAGACCCA					
3381584		TGGTGAACCACATGCACTTCCAGCACCATGCCAAGCCCAACTGCTTCCGCAAAGACCCA					
		740	750	760	770	780	790
		700	710	720	730	740	750
D5		GACATCAACATGCATCCCTTCTTCTTTCCTTGGGGAAGATCCTCTCTGTGGAGCTTGGG					
3381584		GACATCAACATGCATCCCTTCTTCTTTCCTTGGGGAAGATCCTCTCTGTGGAGCTTGGG					
		800	810	820	830	840	850
		760	770	780	790	800	810
D5		AAACAGAAGAAAAATATATGCCGTACAACCACCAGCACAAATACTTCTTCCTAATTGGG					
3381584		AAACAGAAGAAAAATATATGCCGTACAACCACCAGCACAAATACTTCTTCCTAATTGGG					
		860	870	880	890	900	910

FIG.27B

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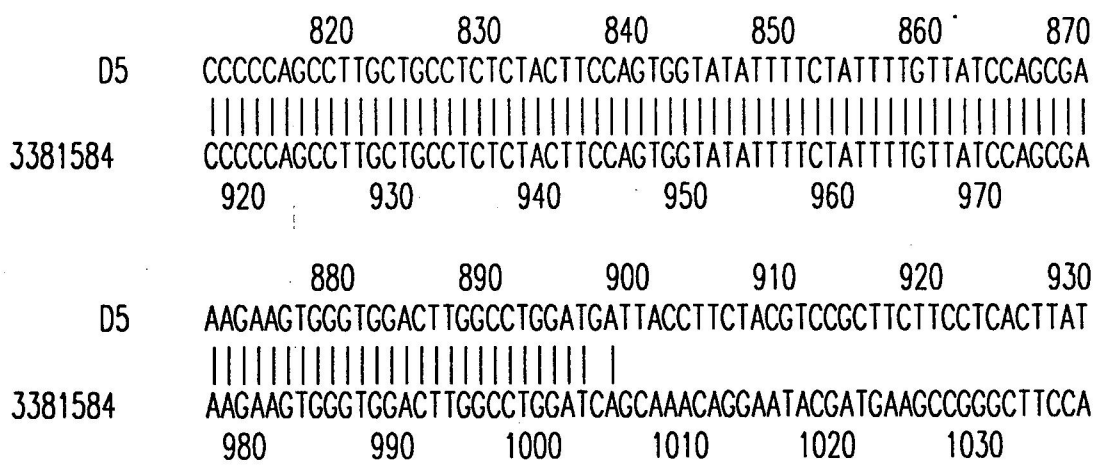


FIG.27C

FIG. 28

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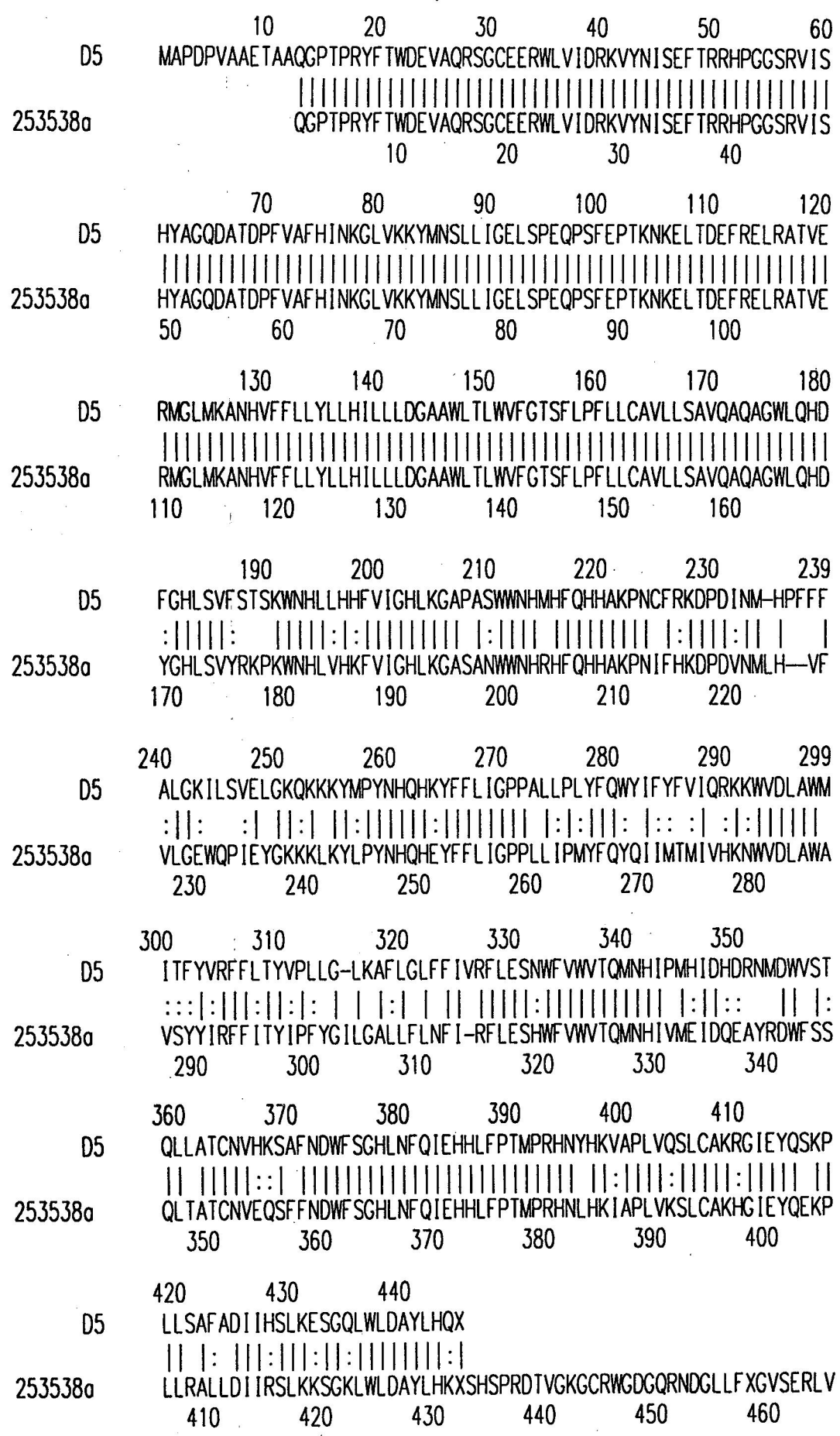


FIG.29

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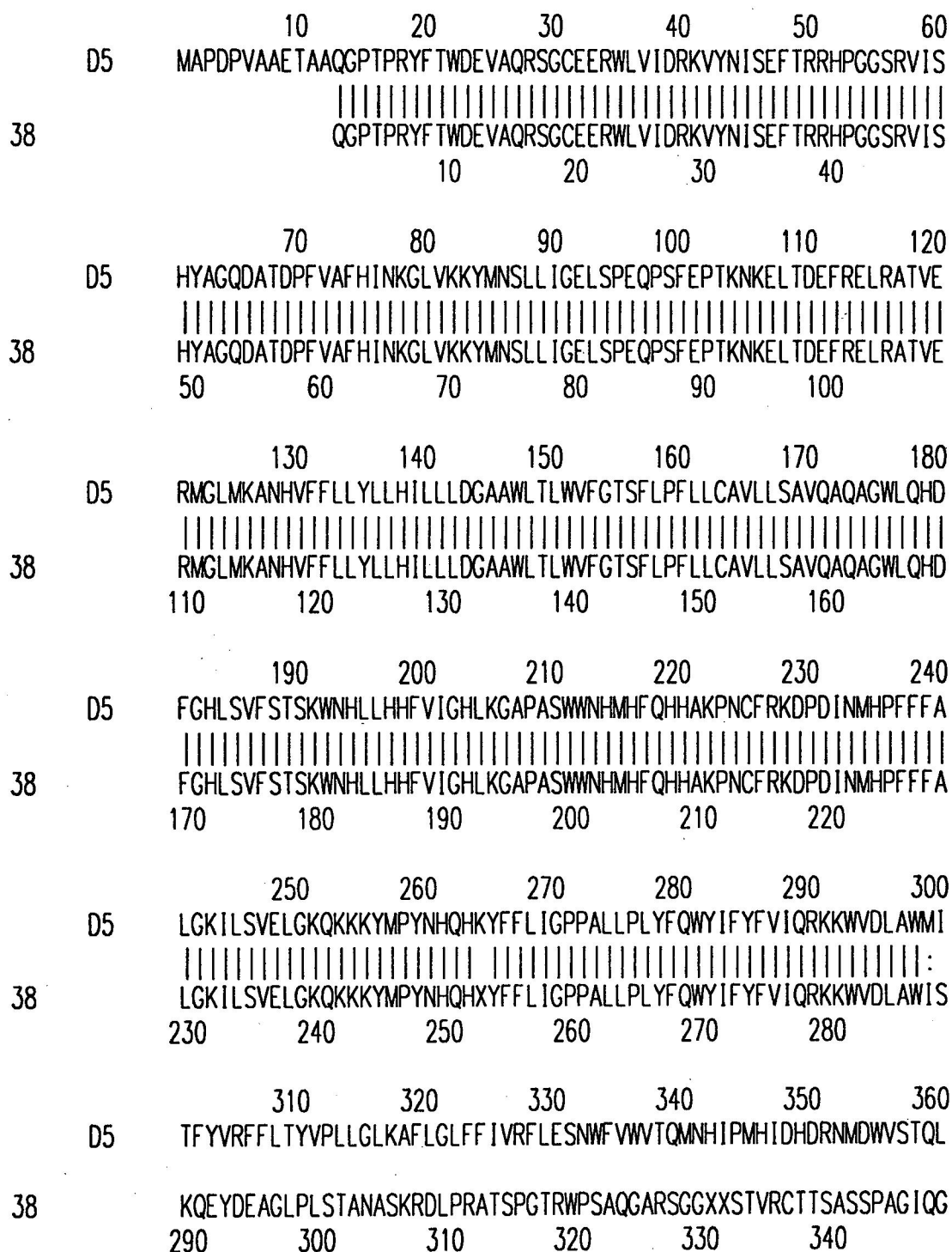


FIG.30

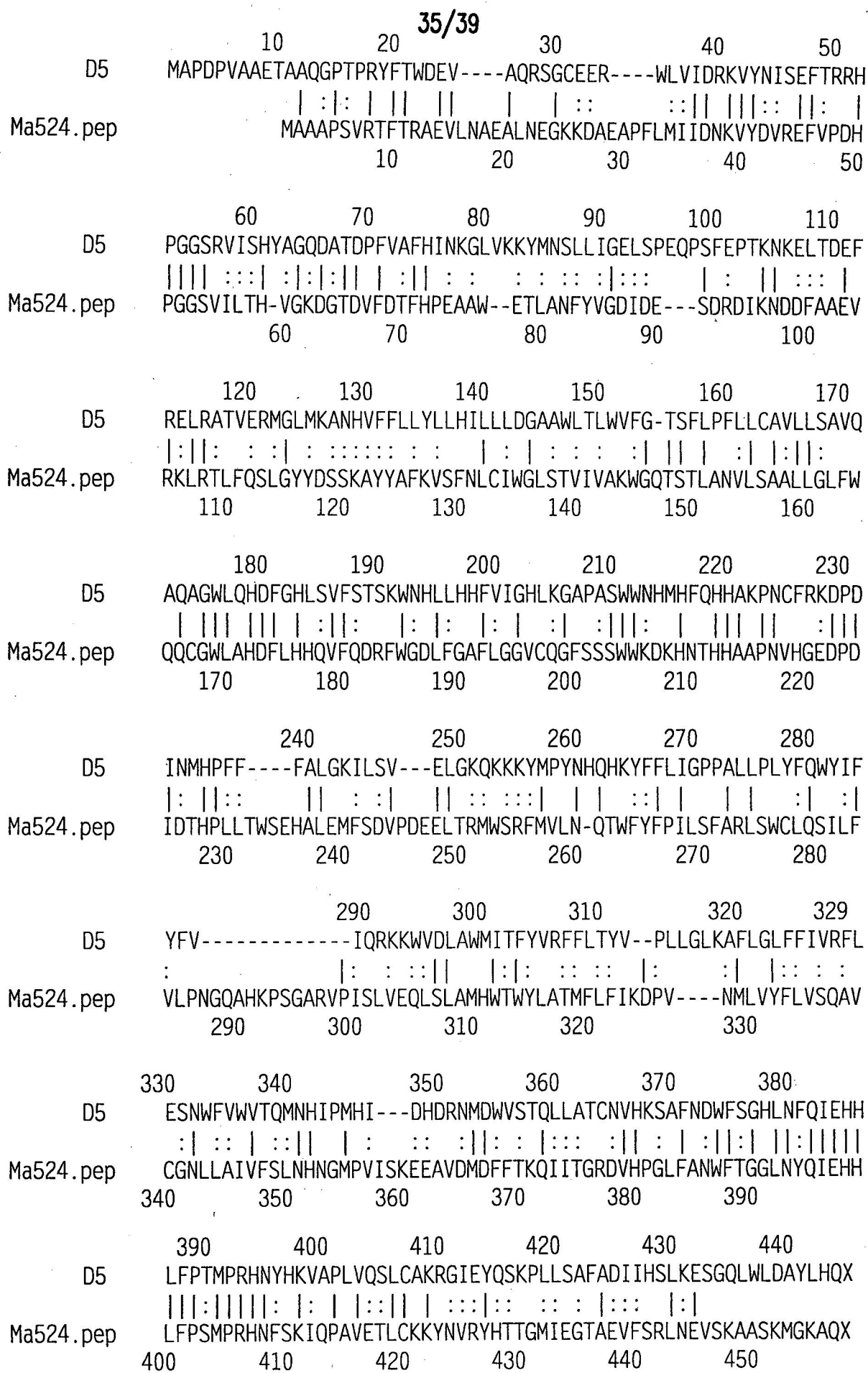


FIG.31

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D5	10	20	30	40	50	60
	MAPDPVAAETA AQGPTPRYFTWDEVAQRSGCEERWLVIDRKVYNISEFTRRHPPGGSRVIS					
Ma29.pep	: : :: :: : : ::: :: MGTDQGKT---FTWEELAAHNTKDDL LLAIRGRVYDVTKFLSRHPGGVDTL					
	10	20	30	40		
D5	70	80	90	100	110	
	HYAGQDATDPFVAFHINKGLVKKYMNSLLIGEL-SPEQPSF-EPTKNKELTDEFREL RAT					
Ma29.pep	: : : : :: : :: LGAGRDVTPVFEMYHAF-GAADAIMKKYYVGTLSNELPIFPEPTVFHKTIKTRVEGYFT					
	50	60	70	80	90	100
D5	120	130	140	150	160	170
	VERMGLMKANHVF--FLLYLLHILL LDGAAWLTWVFGTSFLPFLLCAVLLSAVQAQAGW					
Ma29.pep	: : : :: : : : : : : : : : : : : : DRNIDPKNRPEIWGRYALIFGSLIASYYAQLFVPFVERTWLQVVF-AIIMGFACAQVGL					
	110	120	130	140	150	160
D5	180	190	200	210	220	230
	LQ-HDFGHL SV-FSTSKWNHL--LHHFVIGH LKGAPASWWNHMH-FQHHAKPNCFRKDPD					
Ma29.pep	: : : : : : : : : : NPLHDASHFSVTHNPTVWKILGATHDF----FNGASYLVWMYQHMLGHHPYTNIAGADPD					
	170	180	190	200	210	220
D5	240	250	260	270	280	
	INM-HPFFFALGKILSVELGKQKKKYMPYNH--QHKEY-FLIGPPALLPLYFQWYIFYFV					
Ma29.pep	:: : : : : : : : : : VSTSEP-----DVRRIKPNQKWF-VNHINQHMFPFLYGLLAFKVR IQDINILYFV					
	230	240	250	260	270	
D5	290	300	310	320	330	
	IQ----RKKWVDLAWMITFY--VRFFLT Y---VPL--LGLKAFLGLFFIVRFLESNWFVW					
Ma29.pep	: :: : : : : : : :: KTNDAIRVNP ISTWHTVMFWGGKAFFVWYRLIVPLQYLPLGKVLL LFTVADMVSSYWLAL					
	280	290	300	310	320	330
D5	340	350	360	370	380	
	VTQMNHIPMHID---HDRN---MDWVSTQLLATCN-VHKSAFNDWFSGHLNFQIEHHLF					
Ma29.pep	: : :: : : :: : : : : : : : TFQANHVV EEVQWPLPDENGIIQKDWAA MQVETTQDYAHD SHLWTSITGSLNYQAVHHLF					
	340	350	360	370	380	390
D5	390	400	410	420	430	440
	PTMPRHNYHKVAPLVQSLCAKRGIEYQSKPLL-SAFADIHSLKESGQLWLDAYLHQX					
Ma29.pep	:: : : : :::: :: : : : : :: : PNVSQHHYPDILAI IKNTCSEYKVPYLVKDTFWQAFASHLEHLRVLGLRPKEEX					
	400	410	420	430	440	

FIG. 32

	334(pRAE-28-5) 25 μ M DGLA	334(pRAE-26-1) 25 μ M DGLA (g /100 g)	334(pRAE-33) 25 μ M DGLA 30°C/48 HRS (μ g)	334(pRAE-35) 25 μ M DGLA (μ g)	334(pYX242) 25 μ M DGLA
C16:0	151.580	202.175	285.291	281.298	304.229
C16:1	406.279	485.631	552.951	569.298	608.123
C18:0	16.494	25.995	32.162	27.479	30.093
C18:1n-9	100.031	133.349	173.772	184.740	187.780
C18:2n-6		0.180			0.946
C18:3n-6		0.058	0.074		0.074
C20:0	3.844	4.205	7.118	7.285	6.288
C20:3n-6	96.576	118.657	134.859	139.292	125.448
C20:4n-6	(0.127%) 1.204	(0.075%) 0.878	(0.062%) 0.902	(0.063%) 0.927	(0.062%) 0.958
C22:0		0.150	0.119		0.125
C22:1n-9	0.162	0.139	0.299	0.275	0.392
	949.0	1169.0	1445.5	1468.0	1538.5

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FIG.33

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	334(pRAE-28-5) 25 μ M DGLA	334(pRAE-26-1) 25 μ M DGLA	334(pYX242) 25 μ M DGLA 30°C/48 HRS	334(pRAE-28-5) 25 μ M OA	334(pRAE-26-1) 25 μ M OA	334(pYX242) 25 μ M OA	334(pRAE-28-5) 25 μ M AA	334(pRAE-26-1) 25 μ M AA	334(pYX242) 25 μ M AA 30°C/48 HRS
	(g / 100 g)		(μg)						
C16:0	49.332	106.358	93.225	84.327	37.013	51.018	78.471	53.685	74.099
C16:1	141.178	256.622	277.028	269.009	107.066	172.485	230.45	141.526	181.298
C18:0	9.301	14.819	12.908	11.871	8.3	9.047	11.283	9.97	10.969
C18:1n-9	39.875	87.564	72.842	106.416	52.634	71.453	61.754	42.289	46.873
C18:2n-6				ND	ND	ND			
C18:3n-6									
C20:0	2.154	7.339			3.729			2.685	
C20:3n-6	45.395	56.346	55.306						
C20:4n-6	(0.106%) 0.412	(0.060%) 0.396	(0.065%) 0.402				63.584	68.442	60.89
C20:5n-3							(0.026%) 0.139	(0.019%) 0.077	(0.027%) 0.126
	387	665	620	562	284	363	535	404	466

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FIG.34A

	334(pRAE-28-5) 25 μ M LA	334(pRAE-26-1) 25 μ M LA (g /100 g)	334(pYX242) 25 μ M LA 30°C/48 HRS (μ g)	334(pRAE-28-5)	334(pRAE-26-1)	334(pYX242)
C16:0	56.631	45.393	74.247	174.138	25.574	33.44
C16:1	181.311	117.045	208.029	277.122	43.193	47.189
C18:0	9.549	9.251	11.45	22.547	5.119	8.432
C18:1n-9	48.256	46.496	51.342	134.822	21.89	32.618
C18:2n-6	31.91	23.221	36.821			
C18:3n-6	(0.02%) 0.082	ND	(0.012%) 0.056			
C20:0		0.339		0.702		
C20:3n-6						
C20:4n-6						
C20:5n-3	0.121					
	407	279	460	746	127	168

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FIG.34B

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