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Murphy et al.

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(45) **Date of Patent: *Jun. 11, 2013**

(54) **CHARACTERIZATION OF GRANULOCYTIC EHRLICHIA AND METHODS OF USE**

(75) Inventors: **Cheryl Murphy**, Hopkinton, MA (US); **James Storey**, Lynwood, MA (US); **Gerald A. Beltz**, Lexington, MA (US); **Richard T. Coughlin**, Portland, ME (US)

(73) Assignee: **Antigenics, Inc.**, Lexington, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

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(22) Filed: **Jan. 9, 2012**

(65) **Prior Publication Data**

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Related U.S. Application Data

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(51) **Int. Cl.**

C07H 21/04	(2006.01)
A61K 39/02	(2006.01)
G01N 33/53	(2006.01)

(52) **U.S. Cl.**

USPC **536/23.7**; 424/184.1; 424/185.1; 424/190.1; 424/234.1; 435/7.2; 435/243; 530/300; 530/350

(58) **Field of Classification Search**

USPC 424/184.1, 185.1, 190.1, 234.1; 435/7.2, 435/243; 530/300, 350; 536/23.7

See application file for complete search history.

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Primary Examiner — Rodney P. Swartz

(74) *Attorney, Agent, or Firm* — Wolf, Greenfield & Sacks, P.C.

(57) **ABSTRACT**

The present invention relates, in general, to polypeptides having antigenic epitopes from granulocytic ehrlichia (GE) proteins and methods of use thereof.

21 Claims, 72 Drawing Sheets

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901   AGCGAACGAGGAGTCAAAGACCCTGGAGTCTATGCCTTCTGATAGCCTAGAAGACAATGT
        A  N  E  E  S  K  T  L  E  S  M  P  S  D  S  L  E  D  N  V
961   TCAGGAACTAGGTACATTGCCTTTACAAGAAGGTGAACAGTTTCTGAGGGCAACACACG
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1141  CATTTCACAAGACTCAGTTGGTGTAAGTACAGATCTTGAAGCTCATTCTAAAGGAGTTGA
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1201  AATAGTTTCTGAGGGCGGCACACAAGATTCACTATCCGCTGATTTTCCAATAAACACAGT
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1261  TGAAAGTGAAAGTACAGATCTTGAAGCTCATTCTCAAGAAGTTGAAACTGTTTCTGAATT
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        T  Q  D  S  L  S  T  N  I  S  Q  D  S  V  G  V  S  T  D  L
1381  TGAAGTTCATTCTCAAGAAGTTGAAATAGTTTCTGAGGGCGGCACACAAGATTCACTATC
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1441  CACTAACATTTCACAAGACTCAGTTGGTGTAAGTACAGATCTTGAAGCTCATTCTCAAGA
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1621  CGGCACACAAGATTCACTATCCACTAACATTTCACAAGACTCAGTTGGTGTAAGTACAGA
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FIG. 5B-2

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Ser Pro Tyr Asn Asn Asp Arg Met Glu Thr Ala Ala Phe Ser Leu Thr
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Pro Asp Ile Ile Glu Pro Ser Ala Leu Leu Gln Glu Ser His Ser Thr
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Gly Val Ser Thr Asp Leu Glu Ala His Ser Gln Glu Val Glu Thr Val
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Ser Glu Val Ser Thr Gln Asp Ser Leu Ser Thr Asn Ile Ser Gln Asp
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Glu Val Glu Thr Val Ser Glu Phe Thr Gln Asp Ser Leu Ser Thr Asn
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Gln Glu Val Glu Ile Val Ser Glu Gly Gly Thr Gln Asp Ser Leu Ser
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Thr Asn Ile Ser Gln Asp Ser Val Gly Val Ser Thr Asp Leu Glu Ala
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His Ser Gln Glu Val Glu Thr Val Ser Glu Phe Thr Gln Asp Ser Leu
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Ser Thr Asp Leu Glu Ala His Ser Pro Glu Gly Glu Ile Val Ser Glu
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cctgctgaaa gggctttttt gttatgaaac tatcctcgct cgattttctt atctttggat 4680

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<210> SEQ ID NO 4
<211> LENGTH: 748
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: S22 of Granulocytic Ehrlichia

<400> SEQUENCE: 4

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Met Leu Arg Cys Asn Ser Met Asp Val Asp Ala Arg Ser His Asp Gly
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Arg Thr Ile Ile His Tyr Ala Ala Lys Asp Gly Asn Leu Glu Ile Leu
            20                  25                  30

Gln Gln Ala Leu Gly Arg Lys Ser Ser Tyr Ser Lys Phe Pro Val Lys
        35                  40                  45

Asp Gly Val Pro Thr Pro Gly Val Tyr Ala Ile Arg Glu Ala Ser Gly
    50                  55                  60

Gly Lys Val Ser Leu Gln Ala Leu Asp Met Leu Met Arg Tyr Glu Pro
65                  70                  75                  80

His Pro Gln His Val Ala Val Glu Ala Val Arg Thr Gly Ala Val Gly
                85                  90                  95

Val Leu Glu His Leu Ile Thr Thr Glu Val Ile Ser Val Asn Glu Glu
            100                 105                 110

Ile Thr Thr Pro Glu Gly Lys Lys Thr Thr Leu Thr Ala Glu Ala Leu
        115                 120                 125

Thr Ser Gly Lys Tyr Gly Val Val Lys Ala Leu Ile Lys Asn Ser Ala
    130                 135                 140

Asp Val Asn Ala Ser Pro Glu Pro Ala Ile Thr Leu Gly Ile Gln Gly
145                 150                 155                 160

Arg Cys Phe Gln Gly Ser Lys Ala Ile Lys His Leu Lys Arg Val Val
                165                 170                 175

Glu Ala Gly Ala His Ile Asn Thr Pro Thr Gly Ser Met Ser Pro Leu
            180                 185                 190

Ala Ala Ala Val Gln Ala Ala Asn Glu Ala Ser Asn Leu Lys Glu Ala
        195                 200                 205

Asn Lys Ile Val Asn Phe Leu Leu His Arg Gly Ala Asp Leu Ser Ser
    210                 215                 220

Thr Glu His Thr Gly Thr Pro Ala Leu His Leu Ala Thr Ala Ala Gly
225                 230                 235                 240

Asn His Arg Thr Ala Met Leu Leu Leu Asp Lys Gly Ala Pro Ala Thr
                245                 250                 255

Gln Arg Asp Ala Arg Gly Arg Thr Ala Leu His Ile Ala Ala Ala Asn
            260                 265                 270

Gly Asp Gly Lys Leu Tyr Arg Met Ile Ala Lys Lys Cys Pro Asp Ser
        275                 280                 285

Cys Gln Pro Leu Cys Ser Asp Met Gly Asp Thr Ala Leu His Glu Ala
    290                 295                 300

Leu Tyr Ser Asp Asn Val Thr Glu Lys Cys Phe Leu Lys Met Leu Lys
305                 310                 315                 320

Glu Ser Arg Lys His Leu Ser Asn Ser Ser Phe Phe Gly Asp Leu Leu
                325                 330                 335

Asn Thr Pro Gln Glu Ala Asn Gly Asp Thr Leu Leu His Leu Ala Ala
            340                 345                 350

Ser Arg Gly Phe Gly Lys Ala Cys Lys Ile Leu Leu Lys Ala Gly Ala
        355                 360                 365

Ser Val Ser Val Val Asn Val Glu Gly Lys Thr Pro Val Asp Val Ala
    370                 375                 380

Asp Pro Ser Leu Lys Thr Arg Pro Trp Phe Phe Gly Lys Ser Val Val
385                 390                 395                 400

Thr Met Met Ala Glu Arg Val Gln Val Pro Glu Gly Gly Phe Pro Pro
                405                 410                 415

Tyr Leu Pro Pro Glu Ser Pro Thr Pro Ser Leu Gly Ser Ile Ser Ser
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            420                 425                 430

Phe Glu Ser Val Ser Ala Leu Ser Ser Leu Gly Ser Gly Leu Asp Thr
        435                 440                 445

Ala Gly Ala Glu Glu Ser Ile Tyr Glu Glu Ile Lys Asp Thr Ala Lys
    450                 455                 460

Gly Thr Thr Glu Val Glu Ser Thr Tyr Thr Thr Val Gly Ala Glu Glu
465                 470                 475                 480

Ser Ile Tyr Glu Glu Ile Lys Asp Thr Ala Lys Gly Thr Thr Glu Val
                485                 490                 495

Glu Ser Thr Tyr Thr Thr Val Gly Ala Glu Gly Pro Arg Thr Pro Glu
            500                 505                 510

Gly Glu Asp Leu Tyr Ala Thr Val Gly Ala Ala Ile Thr Ser Glu Ala
        515                 520                 525

Gln Ala Ser Asp Ala Ala Ser Ser Lys Gly Glu Arg Pro Glu Ser Ile
    530                 535                 540

Tyr Ala Asp Pro Phe Asp Ile Val Lys Pro Arg Gln Glu Arg Pro Glu
545                 550                 555                 560

Ser Ile Tyr Ala Asp Pro Phe Ala Ala Glu Arg Thr Ser Ser Gly Val
                565                 570                 575

Thr Thr Phe Gly Pro Lys Glu Glu Pro Ile Tyr Ala Thr Val Lys Lys
            580                 585                 590

Gly Pro Lys Lys Ser Asp Thr Ser Gln Lys Glu Gly Thr Ala Ser Glu
        595                 600                 605

Lys Val Cys Ser Thr Ile Thr Val Ile Lys Lys Lys Val Lys Pro Gln
    610                 615                 620

Val Pro Ala Arg Thr Ser Ser Leu Pro Thr Lys Glu Gly Ile Gly Ser
625                 630                 635                 640

Asp Lys Asp Leu Ser Ser Gly Thr Ser Ser Ser Phe Ala Ala Glu Leu
                645                 650                 655

Gln Ala Gln Arg Gly Lys Leu Arg Pro Val Lys Gly Gly Ala Pro Asp
            660                 665                 670

Ser Thr Lys Asp Lys Thr Ala Thr Ser Ile Phe Ser Ser Lys Glu Phe
        675                 680                 685

Lys Lys Glu Leu Thr Lys Ala Ala Glu Gly Leu Gln Gly Ala Val Glu
    690                 695                 700

Glu Ala Gln Lys Gly Asp Gly Gly Ala Ala Lys Ala Lys Gln Asp Leu
705                 710                 715                 720

Gly Met Glu Ser Gly Ala Pro Gly Ser Gln Pro Glu Ala Pro Gln Ser
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Glu Gly Pro Lys Ser Val Lys Gly Gly Arg Gly Arg
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<210> SEQ ID NO 5
<211> LENGTH: 3998
<212> TYPE: DNA
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: S7 of Granulocytic Ehrlichia

<400> SEQUENCE: 5

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aaatatgcat attgtatgta taggtgtgca agatatcatc tctttaggtg tatcgtgtag    180

cacttaaaca aatgctggtg aacgtagagg gattaaagga ggatttgcgt atatgtatgg    240
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caagtgtagg gtagtacggg gaacgtggag accgagcgaa ctggttttat atgttggtga 420

tgagcatgtg gcatgtagag atgttgcttc gggtatgcat catggtaatt tgccagggaa 480

ggtgtatttt atagaggcag aagcgggcag agctgctact gctgaaggtg gtgtttatac 540

taccgttgtg gaggcattat cgctggtgca agaggaagag ggtacaggta tgtacttgat 600

aaacgcacca gaaaaagcgg tcgtaaggtt tttcaagata gaaaagagtg cagcagagga 660

acctcaaaca gtagatccta gtgtagttga gtcagcaaca gggtcgggtg tagatacgca 720

agaagaacaa gaaatagatc aagaagcacc agcaattgaa gaagttgaga cagaagagca 780

agaagttatt ctggaagaag gtactttgat agatcttgag caacctgtag cgcaagtacc 840

tgtagtagct gaagcagaat tacctggtgt tgaagctgca gaagcgattg taccatcact 900

agaagaaaat aagcttcaag aagtggtagt tgctccagaa gcgcaacaac tagaatcagc 960

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<210> SEQ ID NO 6
<211> LENGTH: 578
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: S7 of Granulocytic Ehrlichia

<400> SEQUENCE: 6

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Thr Ile Ser Ser Gly Asp Asp Gly Tyr Tyr Glu Gly Cys Ala Cys Asp
            20 25 30

Lys Asp Ala Ser Thr Asn Ala Tyr Ser Tyr Asp Lys Cys Arg Val Val
        35 40 45

Arg Gly Thr Trp Arg Pro Ser Glu Leu Val Leu Tyr Val Gly Asp Glu
    50 55 60

His Val Ala Cys Arg Asp Val Ala Ser Gly Met His His Gly Asn Leu
65 70 75 80

Pro Gly Lys Val Tyr Phe Ile Glu Ala Glu Ala Gly Arg Ala Ala Thr
                85 90 95

Ala Glu Gly Gly Val Tyr Thr Thr Val Val Glu Ala Leu Ser Leu Val
            100 105 110

Gln Glu Glu Glu Gly Thr Gly Met Tyr Leu Ile Asn Ala Pro Glu Lys
        115 120 125

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Ala Val Val Arg Phe Phe Lys Ile Glu Lys Ser Ala Ala Glu Glu Pro
    130                 135                 140

Gln Thr Val Asp Pro Ser Val Val Glu Ser Ala Thr Gly Ser Gly Val
145                 150                 155                 160

Asp Thr Gln Glu Glu Gln Glu Ile Asp Gln Glu Ala Pro Ala Ile Glu
                165                 170                 175

Glu Val Glu Thr Glu Glu Gln Glu Val Ile Leu Glu Glu Gly Thr Leu
            180                 185                 190

Ile Asp Leu Glu Gln Pro Val Ala Gln Val Pro Val Val Ala Glu Ala
        195                 200                 205

Glu Leu Pro Gly Val Glu Ala Ala Glu Ala Ile Val Pro Ser Leu Glu
    210                 215                 220

Glu Asn Lys Leu Gln Glu Val Val Val Ala Pro Glu Ala Gln Gln Leu
225                 230                 235                 240

Glu Ser Ala Pro Glu Val Ser Ala Pro Ala Gln Pro Glu Ser Thr Val
                245                 250                 255

Leu Gly Val Ala Glu Gly Asp Leu Lys Ser Glu Val Ser Val Glu Ala
            260                 265                 270

Asn Ala Asp Val Pro Gln Lys Glu Val Ile Ser Gly Gln Gln Glu Gln
        275                 280                 285

Glu Ile Ala Glu Ala Leu Glu Gly Thr Glu Ala Pro Val Glu Val Lys
    290                 295                 300

Glu Glu Thr Glu Val Leu Leu Lys Glu Asp Thr Leu Ile Asp Leu Glu
305                 310                 315                 320

Gln Pro Val Ala Gln Val Pro Val Val Ala Glu Ala Glu Leu Pro Gly
                325                 330                 335

Val Glu Ala Ala Glu Ala Ile Val Pro Ser Leu Glu Glu Asn Lys Leu
            340                 345                 350

Gln Glu Val Val Val Ala Pro Glu Ala Gln Gln Leu Glu Ser Ala Pro
        355                 360                 365

Glu Val Ser Ala Pro Ala Gln Pro Glu Ser Thr Val Leu Gly Val Thr
    370                 375                 380

Glu Gly Asp Leu Lys Ser Glu Val Ser Val Glu Ala Asp Ala Gly Met
385                 390                 395                 400

Gln Gln Glu Ala Gly Ile Ser Asp Gln Glu Thr Gln Ala Thr Glu Glu
                405                 410                 415

Val Glu Lys Val Glu Val Ser Val Glu Thr Lys Thr Glu Glu Pro Glu
            420                 425                 430

Val Ile Leu Glu Glu Gly Thr Leu Ile Asp Leu Glu Gln Pro Val Ala
        435                 440                 445

Gln Val Pro Val Val Ala Glu Ala Glu Leu Pro Gly Val Glu Ala Ala
    450                 455                 460

Glu Ala Ile Val Pro Ser Leu Glu Glu Asn Lys Leu Gln Glu Val Val
465                 470                 475                 480

Val Ala Pro Glu Ala Gln Gln Leu Glu Ser Ala Pro Glu Val Ser Ala
                485                 490                 495

Pro Val Gln Pro Glu Ser Thr Val Leu Gly Val Thr Glu Gly Asp Leu
            500                 505                 510

Lys Ser Glu Val Ser Val Glu Ala Asp Ala Gly Met Gln Gln Glu Ala
        515                 520                 525

Gly Ile Ser Asp Gln Glu Thr Gln Ala Thr Glu Glu Val Glu Lys Val
    530                 535                 540

Glu Val Ser Val Glu Ala Asp Ala Gly Met Gln Gln Glu Leu Val Asp
545                 550                 555                 560
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Val Pro Thr Ala Leu Pro Leu Lys Asp Pro Asp Asp Glu Asp Val Leu
565 570 575

Ser Tyr

<210> SEQ ID NO 7
<211> LENGTH: 5570
<212> TYPE: DNA
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: S23 of Granulocytic Ehrlichia

<400> SEQUENCE: 7

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cttatggaaa agcgttaggg agttgcttcg cttgtcacgc gtgcgttagg ttttacgtat 180

acgtgtctgg gacttcacga aaactcgacg caggcggatt ttgtactatg tttcacttaa 240

caaggtatta taattgtttg aacacaatat tcctgataca tacacaggaa caactgcaga 300

aggttctcct ggcttagcag gcggggattt tagtttaagt tctattgact ttacaaggga 360

ctttacaatt gaatcacata gaggaagctc agctgatgac ccaggttaca tcagctttag 420

ggatcaagac ggaaacgtca tgtcacgttt tcttgatgtg tacgtagcta atttcagctt 480

gcgatgcaag cattctccct ataacaacga cagaatggaa acagctgcgt tctctctaac 540

tcccgacata atagagcctt ctgctttatt gcaagaatca catagtacac aaaacaatgt 600

agaagaggca gtacaagtta cagctcttga gtgccctcca tgtaatccag tccctgccga 660

ggaagtagct cctcaaccgt cttttctaag cagaataatt caggcgttct tgtggttatt 720

cacgccttct tctactaccg acactgctga agacagcaag tgtaatagta gcgatacttc 780

aaaatgtacc tctgctagca gtgagtcatt agagcagcaa caagaatcag tggaagtgca 840

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ccaagtaaac actactgcag tacaagtaga atcatccatt attgtgccag aatcgcaaca 960

cactgacgtt accgtgctcg aagatactac tgagacgata actgttgatg gggaatatgg 1020

acattttagt gacattgctt caggtgaaca caataacgat ctgcctgcca tgttgttaga 1080

tgaagcagac ttcactatgt tattagcgaa cgaggagtca aagaccctgg agtctatgcc 1140

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aacagtttct gagggcaaca cacgagagtc actacccact gacgtttcac aagactcagt 1260

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cacacaagat tcactatcca ctaacatttc acaagactca gttggtgtaa gtacagatct 1380

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agttgaaata gtttctgagg gcggcacaca aaattcacta tccgctgatt ttccaataaa 1560

cacagttgaa agtgaaagta cagatcttga agctcattcc ccagaaggtg aaatagtttc 1620

tgaggtcagc acacaagatg cgccatccac tggagtagag atcagattta tggatcgtga 1680

ttttgatgat gacgtgctcg cgttgtgaag tgatcatggt aggggaaaca gttatggcgt 1740

aaagacatct ttgatgactt gtcttgcgtg aataagtagt gcaagttttt tatgcattga 1800

tgtgcatgat cattgcccct aaggaaagca gtactaatgg tagtctaaga tcttatacag 1860

ggtttcggac taccactttt ggtgttttaa aacgtcttat tcgcgttggg tgcttgctta 1920

```
1681  TCTTGAAGCTCATTCTAAAGGAGTTGAAATAGTTTCTGAGGGCGGCACACAAGATTCACT
        L  E  A  H  S  K  G  V  E  I  V  S  E  G  G  T  Q  D  S  L
1741  ATCCGCTGATTTTTCCAATAAACACAGTTGAAAGTGAAAGTACAGATCTTGAAGCTCATTC
        S  A  D  F  P  I  N  T  V  E  S  E  S  T  D  L  E  A  H  S
1801  CCCAGAAGGTGAAATAGTTTCTGAGGTCAGCACACAAGATGCGCCATCCACTGGAGTAGA
        P  E  G  E  I  V  S  E  V  S  T  Q  D  A  P  S  T  G  V  E
1861  GATCAGATTTATGGATCGTGATTCTGATGATGACGTGCTCGCGTTGTGAAGTGATCATGG
        I  R  F  M  D  R  D  S  D  D  D  D  V  L  A  L  L
1921  TAGGGGAAA
```

FIG. 5B-3

-continued

caatgtacct gtacgtgccc aacactaaaa atggtcagta ttacttaggg gagttcgtag 1980

acgaggcatc tcgatttact cttggtaagc tacaaataac tcagtcatat caaggtagtt 2040

caagatgaaa gcagtcctat gcttatcatg gagaattcct gcggttctct tcaaaattct 2100

cttttcccgc aagggcagac tcttatttgt taaaataaca aaatttctct acaggaagcg 2160

acatttcata tcaaagctga ttgtgaaata atggcattga gtatttttct cgccctagaa 2220

gataatcatt tcggcactat caaagcattt acgatattct ccattatctt gtaatcagat 2280

ggctatcttg aaagcaacca aggatatccg tacatggtag cttacatact gctatcaatc 2340

tcctatacga ccttcaatga aacggtaact gttgctgaca gcttgcacat gctgtgattc 2400

aattcctggt tcctagatgt tctactacgt ttatccggta ctaatattat tctttggcgc 2460

tctattatct agcaactcag agtccattat tggatctcta ataccaaggg tataagggaa 2520

agtggaagag tattattaga gagaagaagc aaatacagta tatctactag ctaaggagtt 2580

agcttatgat gttgttactg gacagactga taagcttgct gctgctcttg ccaagacctc 2640

cgggaaagat atcgttcagt ttgctaaggc agttgagatt tcggctccta agatcgataa 2700

gcaagtttgt gtgactaata agaatgggga tagcggaaca agatatgcta agtacctcga 2760

agaagctgga acgtctagca atgctggcac gtcgttgtgt ggtggtaaaa acctaaagac 2820

gactgactcc aacacaggag tagagaaagg acaggtgtta catgactttg tttctggaac 2880

gttgagtggg ggtactaaga actggccgac atctagtgaa agtactaaag aaaataacga 2940

caacgcaggg aaggtagcta aagacctgac aaaactaacc cctgaggaaa aaaccatagt 3000

agcagggtta ctagctaaga ctattgaagg gggtgaagtt gttgagatca gggcggtttc 3060

ttctacttct gtgatggtta atgcttgtta tgatcttctt agtgaaggtt taggtgtcgt 3120

tccttacgct tgtgttggtc ttgggggtaa cttcgtgggg ggttgttgat ggcacggcgc 3180

agcgttacac aatccgtcct tgacctgaat actctagtta agcactaggc aaaattagtg 3240

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gtggctttat actcttaccc agcatgaaat tacttgttat ctaagaatct ccacagctga 3360

ccttagaaag gttatctgtc cttcgagaga aagctaatct gtgtcttatg cggatggcgt 3420

tgaacgtatt acaggtccca agctgtcttg caagtttcta aggatattat aagggcacac 3480

ctataaaact gcgcaatata tcacctgcaa tacggtcccg attcgaaaac actgggaagt 3540

gcgctcatta tctatgaatc gctagctagg cataaataag agtatacgca ataacgctta 3600

ttattaaaaa caagaccaag ggtattagag atagtggtag taaggaagat gaagctgata 3660

cagtatatct actagctaag gagttagctt atgatgttgt tactgggcag actgataacc 3720

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atatttctca ctctaatatc gatgggaagg tttgtaggag ggaaaagcat gggagtcaag 3840

gtttgactgg aaccaaagca ggttcgtgtg atagtcagcc acaaacggcg ggtttcgatt 3900

ccatgaaaca aggtttgatg gcagctttag gcgaacaagg cgctgaaaag tggcccaaaa 3960

ttaacaatgg tggccacgca acaatttata gtagtagcgc aggtccagga aatgcgtatg 4020

ctagagatgc atctactacg gtagctacag acctaacaaa gctcactact gaagaaaaaa 4080

ccatagtagc agggttacta gctagaacta ttgaaggggg tgaagttgtt gagattaggg 4140

cagtttcttc tacttctgtg atggttaatg cttgttatga tcttctgaag ggaaacgcat 4200

cacattattt atctggttgc tgtaatctga tcttcccgtt gctatgatcg catctccccc 4260

tcacttctct cgcaaactct ggattaacct ctggatgcga ataatgttta tcagctttga 4320

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gaaaaacata ttagagtttt atacagcacc aatgataagc gtgggcactt aaataaaggt 4380

tcatatccct agaaatttat cccactagct aaaattattg aaggggggtga ggtcgttgag 4440

ataagggcag tttcttctac ttctgtgatg gttaatgctt gttatgatct tcttagtgaa 4500

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gatgggcata tcacaaacca ctccatctct gaccctgtat gcactagcaa gtaactaggc 4620

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tggtgtctag aatatcactc ctaagcttgc ttataggtta aaggctgggt tgagttatca 4860

gctttctcct gaaatctctg cttttgtagg tggtttctat cattggtttg ttggttatgg 4920

tgtttatgat gatcttccgg ctcaacgtct tgtagatgat actagtccgg cgggtcgtac 4980

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aaatgagtgc atacgtgacg gcaagcgcta aaacgctaaa gctgatgcat cacattaaaa 5280

tgtgttgacg ttagaccttc caagcactgt aaagtgtggc gattcccccg catacgttct 5340

tatgcgtgat tttgaaaaac ccggcgtctg caacttcgga agagaattgt ttttgtgtgg 5400

gaaaagctct tatgctttca actaagtagg aatatgcatc aatatccccg gctactattt 5460

tgccaatttt cggtataaca aagaaagaat acaggtcata agctgttttg aatgcactgt 5520

cttcgagtat aggagaaaac tccaagcata aaaacctacc acatggtttc 5570

<210> SEQ ID NO 8
<211> LENGTH: 484
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: S23 of Granulocytic Ehrlichia

<400> SEQUENCE: 8

Met Phe Glu His Asn Ile Pro Asp Thr Tyr Thr Gly Thr Thr Ala Glu
 1   5   10   15

Gly Ser Pro Gly Leu Ala Gly Gly Asp Phe Ser Leu Ser Ser Ile Asp
            20   25   30

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

Asp Pro Gly Tyr Ile Ser Phe Arg Asp Gln Asp Gly Asn Val Met Ser
    50   55   60

Arg Phe Leu Asp Val Tyr Val Ala Asn Phe Ser Leu Arg Cys Lys His
65   70   75   80

Ser Pro Tyr Asn Asn Asp Arg Met Glu Thr Ala Ala Phe Ser Leu Thr
                85   90   95

Pro Asp Ile Ile Glu Pro Ser Ala Leu Leu Gln Glu Ser His Ser Thr
            100   105   110

Gln Asn Asn Val Glu Glu Ala Val Gln Val Thr Ala Leu Glu Cys Pro
        115   120   125

Pro Cys Asn Pro Val Pro Ala Glu Glu Val Ala Pro Gln Pro Ser Phe
    130   135   140

Leu Ser Arg Ile Ile Gln Ala Phe Leu Trp Leu Phe Thr Pro Ser Ser

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145                 150                 155                 160

Thr Thr Asp Thr Ala Glu Asp Ser Lys Cys Asn Ser Ser Asp Thr Ser
                165                 170                 175

Lys Cys Thr Ser Ala Ser Ser Glu Ser Leu Glu Gln Gln Gln Glu Ser
            180                 185                 190

Val Glu Val Gln Pro Ser Val Leu Met Ser Thr Ala Pro Ile Ala Thr
        195                 200                 205

Glu Pro Gln Asn Ala Val Val Asn Gln Val Asn Thr Thr Ala Val Gln
    210                 215                 220

Val Glu Ser Ser Ile Ile Val Pro Glu Ser Gln His Thr Asp Val Thr
225                 230                 235                 240

Val Leu Glu Asp Thr Thr Glu Thr Ile Thr Val Asp Gly Glu Tyr Gly
                245                 250                 255

His Phe Ser Asp Ile Ala Ser Gly Glu His Asn Asn Asp Leu Pro Ala
            260                 265                 270

Met Leu Leu Asp Glu Ala Asp Phe Thr Met Leu Leu Ala Asn Glu Glu
        275                 280                 285

Ser Lys Thr Leu Glu Ser Met Pro Ser Asp Ser Leu Glu Asp Asn Val
    290                 295                 300

Gln Glu Leu Gly Thr Leu Pro Leu Gln Glu Gly Glu Thr Val Ser Glu
305                 310                 315                 320

Gly Asn Thr Arg Glu Ser Leu Pro Thr Asp Val Ser Gln Asp Ser Val
                325                 330                 335

Gly Val Ser Thr Asp Leu Glu Ala His Ser Gln Glu Val Glu Thr Val
            340                 345                 350

Ser Glu Val Ser Thr Gln Asp Ser Leu Ser Thr Asn Ile Ser Gln Asp
        355                 360                 365

Ser Val Gly Val Ser Thr Asp Leu Glu Val His Ser Gln Glu Val Glu
    370                 375                 380

Ile Val Ser Glu Gly Gly Thr Gln Asp Ser Leu Ser Thr Asn Ile Ser
385                 390                 395                 400

Gln Asp Ser Val Gly Val Ser Thr Asp Leu Glu Ala His Ser Lys Gly
                405                 410                 415

Val Glu Ile Val Ser Glu Gly Gly Thr Gln Asp Ser Leu Ser Ala Asp
            420                 425                 430

Phe Pro Ile Asn Thr Val Glu Ser Glu Ser Thr Asp Leu Glu Ala His
        435                 440                 445

Ser Pro Glu Gly Glu Ile Val Ser Glu Val Ser Thr Gln Asp Ala Pro
    450                 455                 460

Ser Thr Gly Val Glu Ile Arg Phe Met Asp Arg Asp Ser Asp Asp Asp
465                 470                 475                 480

Val Leu Ala Leu


<210> SEQ ID NO 9
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: proposed ankyrin repeat

<400> SEQUENCE: 9

Asp Gly Arg Thr Ile Ile His Tyr Ala Ala Lys Asp Gly Asn Leu Glu
 1               5                  10                  15

Ile Leu Gln Gln Ala Leu Gly Arg Lys Ser Ser Tyr Ser Lys Phe Pro
            20                  25                  30
```

Val

<210> SEQ ID NO 10
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: proposed ankyrin repeat

<400> SEQUENCE: 10

Lys Lys Thr Thr Leu Thr Ala Glu Ala Leu Thr Ser Gly Lys Tyr Gly
1 5 10 15

Val Val Lys Ala Leu Ile Lys Asn Ser Ala Asp Val Asn Ala Ser Pro
20 25 30

Glu

<210> SEQ ID NO 11
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: proposed ankyrin repeat

<400> SEQUENCE: 11

Ala Val Gln Ala Ala Asn Glu Ala Ser Asn Leu Lys Glu Ala Asn Lys
1 5 10 15

Ile Val Asn Phe Leu Leu His Arg Gly Ala Asp Leu Ser Ser Thr Glu
20 25 30

His

<210> SEQ ID NO 12
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: proposed ankyrin repeat

<400> SEQUENCE: 12

Thr Gly Thr Pro Ala Leu His Leu Ala Thr Ala Ala Gly Asn His Arg
1 5 10 15

Thr Ala Met Leu Leu Leu Asp Lys Gly Ala Pro Ala Thr Gln Arg Asp
20 25 30

Ala

<210> SEQ ID NO 13
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: proposed ankyrin repeat

<400> SEQUENCE: 13

Arg Gly Arg Thr Ala Leu His Ile Ala Ala Ala Asn Gly Asp Gly Lys
1 5 10 15

Leu Tyr Arg Met Ile Ala Lys Lys Cys Pro Asp Ser Cys Gln Pro Leu
20 25 30

Cys

<210> SEQ ID NO 14
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:

<223> OTHER INFORMATION: proposed ankyrin repeat

<400> SEQUENCE: 14

Met Gly Asp Thr Ala Leu His Glu Ala Leu Tyr Ser Asp Asn Val Thr
1 5 10 15

Glu Lys Cys Phe Leu Lys Met Leu Lys Glu Ser Arg Lys Arg Leu Ser
20 25 30

Asn

<210> SEQ ID NO 15
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: proposed ankyrin repeat

<400> SEQUENCE: 15

Asn Gly Asp Thr Leu Leu His Leu Ala Ala Ser Arg Gly Phe Gly Lys
1 5 10 15

Ala Cys Lys Ile Leu Leu Lys Ala Gly Ala Ser Val Ser Val Val Ala
20 25 30

Ser Asn Val
35

<210> SEQ ID NO 16
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: proposed ankyrin repeat

<400> SEQUENCE: 16

Glu Gly Lys Thr Pro Val Asp Val Ala Asp Pro Ser Leu Lys Thr Arg
1 5 10 15

Pro Trp Phe Phe Gly Lys Ser Val Val Thr Met Met Ala Glu Arg Val
20 25 30

Gln

<210> SEQ ID NO 17
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: 1, 3, 9, 12, 13, 16, 21, 25, 27, 29, 30, 31, 32, 33
<223> OTHER INFORMATION: Xaa = Any Amino Acid
<220> FEATURE:
<223> OTHER INFORMATION: Consensus sequence of ankyrin repeats

<400> SEQUENCE: 17

Xaa Thr Xaa Ser Thr Pro Leu His Xaa Ala His Xaa Xaa Thr Thr Xaa
1 5 10 15

Thr His His Thr Xaa Leu Leu Thr Xaa Thr Xaa Thr Xaa Xaa Xaa Xaa
20 25 30

Xaa

<210> SEQ ID NO 18
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: E. chaffeensis

<400> SEQUENCE: 18

Ser Gly Thr Asp Leu Thr Leu Glu Ser Ala Val His Ser Gln Lys Gln

-continued

```
 1               5                  10                  15

Pro Glu Gly Val Asp Gly Glu Ile Thr Val Ser Glu Gln
            20                  25


<210> SEQ ID NO 19
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia

<400> SEQUENCE: 19

Gln Pro Ser Val Ser Ala Leu Ser Gln Glu Val Pro Phe Val Val Ala
 1               5                  10                  15

Glu Ser Ala Glu Val Leu Ser Pro Lys Gly Val Glu
            20                  25


<210> SEQ ID NO 20
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: E. chaffeensis

<400> SEQUENCE: 20

Glu Ser Ala Gly Val Ile Ser Asp Gln Pro Glu Ala Thr Gln Val Ala
 1               5                  10                  15

Val Thr Thr Glu Glu Arg Val Glu
            20


<210> SEQ ID NO 21
<211> LENGTH: 406
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: C6.1 of Granulocytic Ehrlichia

<400> SEQUENCE: 21

Met Gly Asp Ala Val Glu Val Arg Ala Glu Asn Leu Gly Gly Glu Ser
 1               5                  10                  15

Ile Leu Glu Ala Pro Ile Arg Val Met Lys Lys Val Gly Asp Thr Val
            20                  25                  30

Ser Ala Glu Asp Val Leu Phe Ile Val Glu Thr Asp Lys Thr Ser Leu
        35                  40                  45

Glu Ile Ser Ala Pro Val Ala Gly Val Leu Thr Glu Leu Arg Val Ala
    50                  55                  60

Asp Glu Glu Val Ile Thr Lys Gly Gln Val Leu Ala Ile Ile Arg Pro
65                  70                  75                  80

Gln Gly Glu Ala Thr Ala Glu Gly Val Asn Lys Glu Pro Glu Ser Lys
                85                  90                  95

Glu Glu Val Pro Ala Gln Pro Val Val Ala Gln Ala Val Ser Thr Gln
            100                 105                 110

Lys Pro Gln Glu Lys Thr Ile Ile Glu Gly Lys Gly Leu Val Thr Pro
        115                 120                 125

Thr Val Glu Asp Phe Val Ala Gly Ile Asn Thr Thr Pro Thr Ser Arg
    130                 135                 140

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

Ser Gln Pro Ser Lys Asp Leu Met Ser Cys His Gly Asp Val Val Gly
                165                 170                 175

Glu Arg Arg Val Lys Met Ser Lys Ile Arg Gln Val Ile Ala Ala Arg
            180                 185                 190

Leu Lys Glu Ser Gln Asn Thr Ser Ala Thr Leu Ser Thr Phe Asn Glu
```

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        195                 200                 205

Val Asp Met Ser Lys Val Met Glu Leu Arg Ala Lys Tyr Lys Asp Ala
    210                 215                 220

Phe Val Lys Arg Tyr Asp Val Lys Leu Gly Phe Met Ser Phe Phe Ile
225                 230                 235                 240

Arg Ala Val Val Leu Val Leu Ser Glu Ile Pro Val Leu Asn Ala Glu
                245                 250                 255

Ile Ser Gly Asp Asp Ile Val Tyr Arg Asp Tyr Cys Asn Ile Gly Val
            260                 265                 270

Ala Val Gly Thr Asp Lys Gly Leu Val Val Pro Val Ile Arg Arg Ala
        275                 280                 285

Glu Thr Met Ser Leu Ala Glu Met Glu Gln Ala Leu Val Asp Leu Ser
    290                 295                 300

Thr Lys Ala Arg Ser Gly Lys Leu Ser Val Ser Asp Met Ser Gly Ala
305                 310                 315                 320

Thr Phe Thr Ile Thr Asn Gly Gly Val Tyr Gly Ser Leu Leu Ser Thr
                325                 330                 335

Pro Ile Ile Asn Pro Pro Gln Ser Gly Ile Leu Gly Met His Ala Ile
            340                 345                 350

Gln Gln Arg Pro Val Ala Val Asp Gly Lys Val Glu Ile Arg Pro Met
        355                 360                 365

Met Tyr Leu Ala Leu Ser Tyr Asp His Arg Ile Val Asp Gly Gln Gly
    370                 375                 380

Ala Val Thr Phe Leu Val Arg Val Lys Gln Tyr Ile Glu Asp Pro Asn
385                 390                 395                 400

Arg Leu Ala Leu Gly Ile
                405


<210> SEQ ID NO 22
<211> LENGTH: 264
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: C6.2 of Granulocytic Ehrlichia

<400> SEQUENCE: 22

Met Gly Arg Gly Thr Ile Thr Ile His Ser Lys Glu Asp Phe Ala Cys
 1               5                  10                  15

Met Arg Arg Ala Gly Met Leu Ala Ala Lys Val Leu Asp Phe Ile Thr
            20                  25                  30

Pro His Val Val Pro Gly Val Thr Thr Asn Ala Leu Asn Asp Leu Cys
        35                  40                  45

His Asp Phe Ile Ile Ser Ala Gly Ala Ile Pro Ala Pro Leu Gly Tyr
    50                  55                  60

Arg Gly Tyr Pro Lys Ser Ile Cys Thr Ser Lys Asn Phe Val Val Cys
65                  70                  75                  80

His Gly Ile Pro Asp Asp Ile Ala Leu Lys Asn Gly Asp Ile Val Asn
                85                  90                  95

Ile Asp Val Thr Val Ile Leu Asp Gly Trp His Gly Asp Thr Ser Arg
            100                 105                 110

Met Tyr Trp Val Gly Asp Asn Val Ser Ile Lys Ala Lys Arg Ile Cys
        115                 120                 125

Glu Ala Ser Tyr Lys Ala Leu Met Ala Ala Ile Gly Val Ile Gln Pro
    130                 135                 140

Gly Lys Lys Leu Asn Ser Ile Gly Leu Ala Ile Glu Glu Glu Ile Arg
145                 150                 155                 160
```

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```
Gly Tyr Gly Tyr Ser Ile Val Arg Asp Tyr Cys Gly His Gly Ile Gly
                165                 170                 175

Arg Glu Phe His Ala Ala Pro Asn Ile Val His Tyr Tyr Asp Glu Glu
            180                 185                 190

Asp Asp Val Thr Ile Gln Glu Gly Met Phe Phe Thr Val Glu Pro Met
        195                 200                 205

Ile Asn Ala Gly Lys Tyr His Thr Val Leu Asp Lys Lys Asp Gly Trp
    210                 215                 220

Thr Val Thr Thr Arg Asp Phe Ser Leu Ser Ala Gln Phe Glu His Thr
225                 230                 235                 240

Leu Gly Val Thr Glu Thr Gly Val Glu Ile Phe Thr Met Ser Pro Lys
                245                 250                 255

Asn Trp His Cys Pro Pro Tyr Leu
            260
```

<210> SEQ ID NO 23
<211> LENGTH: 2706
<212> TYPE: DNA
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: C6 of Granulocytic Ehrlichia

<400> SEQUENCE: 23

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gaattccgga attccggaat tcctatggat cgtgcagtga tggaagaggg cagcatgtta     60

gctgcaggtt cactgctcac taggggtaag attgtaaaat ctggagagtt atgggcaggt    120

aggcctgcaa aatttctacg tatgatgact gaagaggaga ttttatacct acaaaaatct    180

gctgaaaatt acatagcgtt atcgcgtgga tacttataac aaggtattca tctatggttt    240

gacattagtg tcttttggtg attacactgc ctttcaatct gtgttttttg ttttagttct    300

ggtttgtatt tatgggtgat gctgtagaag ttagggctga gaatcttggt ggcgaatcca    360

ttctagaagc tccgattcgg gtaatgaaaa aggtgggaga tactgtatct gcagaagatg    420

tgctcttcat tgttgaaaca gacaagactt ctcttgaaat atcagcccct gttgctggtg    480

ttctcacaga gttgagagtt gcagatgaag aagtgattac caaggggcag gtcttggcta    540

tcatacggcc acagggtgag gctactgcag agggtgttaa taaggagcca gagagcaagg    600

aggaggtgcc tgctcaaccc gttgttgcac aggcagtgag cactcaaaaa ccgcaggaaa    660

agacaattat tgaaggcaaa ggtctagtaa ctcctactgt agaagatttt gttgcaggaa    720

tcaacacaac tcctacttct agagctttgg gtatgagtgc taagagtgaa caagacaaga    780

agatagttgc tagccagccg tctaaggatc tgatgagttg ccatggcgac gtggtgggtg    840

aaagacgcgt gaagatgagc aaaatccgcc aagttatagc tgctaggctt aaggagtcac    900

aaaatacctc tgctacactc agcaccttta atgaagttga tatgagcaaa gtgatggagc    960

tcagagctaa gtacaaagat gcctttgtga agaggtatga tgttaagctt gggtttatgt   1020

ccttctttat cagagcggtt gtgctagtcc tttccgaaat tcctgtgctg aatgcggaga   1080

tttcaggcga tgatatagtc tacagggact attgtaacat tggagtcgcg gtaggtaccg   1140

ataaggggtt agtggtgcct gttatcagaa gagcggaaac tatgtcactt gctgaaatgg   1200

agcaagcact tgttgactta agtacaaaag caagaagtgg caagctctct gtttctgata   1260

tgtctggtgc aacctttact attaccaatg gtggtgtgta tgggtcgcta ttgtctaccc   1320

ctataatcaa ccctcctcaa tctggaatct tgggtatgca tgctatacag cagcgtcctg   1380

tggcagtaga tggtaaggta gagataaggc ctatgatgta tttggcgcta tcatatgatc   1440
```

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atagaatagt tgacgggcaa ggtgctgtga cgtttttggt aagagtgaag cagtacatag 1500

aagatcctaa cagattggct ctaggaattt agggggtttt tatggggcgg ggtacaataa 1560

ccatccactc caaagaggat tttgcctgta tgagaagggc tgggatgctt gcagctaagg 1620

tgcttgattt tataacgccg catgttgttc ctggtgtgac tactaatgct ctgaatgatc 1680

tatgtcacga tttcatcatt tctgccgggg ctattccagc gcctttgggc tatagagggt 1740

atcctaagtc tatttgtact tcgaagaatt ttgtggtttg ccatggcatt ccagatgata 1800

ttgcattaaa aaacggcgat atagttaaca tagacgttac tgtgatcctc gatggttggc 1860

acggggatac tagtaggatg tattgggttg gtgataacgt ctctattaag gctaagcgca 1920

tttgtgaggc aagttataag gcattgatgg cggcgattgg tgtaatacag ccaggtaaga 1980

agctcaatag catagggtta gctatagagg aagaaatcag aggttatgga tactccattg 2040

ttagagatta ctgcggacat gggataggtc gcgaatttca tgctgctcct aacatagttc 2100

actactatga cgaagaggat gatgttacga ttcaggaggg aatgtttttc actgttgagc 2160

caatgatcaa tgctggaaag tatcatactg tgctagataa gaaagacgga tggacagtta 2220

caacgagaga cttttccctt tcagcgcagt ttgaacatac cttgggtgta actgaaactg 2280

gcgttgagat ttttactatg tcgccaaaaa attggcattg tccgccatac ctttaagtag 2340

gatatttttg ttatgtgtaa agcgtgtggc agggtaatgt taggtgcatg ttctgttgac 2400

gatgtgtgct gataagaaat tgtacaatca tactgcgttg gaagttagga atatgtactt 2460

atgagtgcta ataagcttgc tgtgttatta agcgaagccg cttcagtttt gaaaagagta 2520

ggaatagata caccggggtt agacgctcga ctaattgcgg gacatgtttt gggtttaagt 2580

gagcatgagg tgctaataaa tccagattta gttgttactg ctgctaaaac aaaagaattt 2640

tttgaagtta ttgcaagacg tttagccggg gtaccagttt cgcatatttt acgcagacga 2700

gaattc 2706

<210> SEQ ID NO 24
<211> LENGTH: 176
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence encoded by 550 bp PCR product

<400> SEQUENCE: 24

Thr Gly Gly Ala Gly Tyr Phe Tyr Val Gly Leu Asp Tyr Ser Pro Ala
1 5 10 15

Phe Ser Lys Ile Arg Asp Phe Ser Ile Arg Glu Ser Asn Gly Glu Thr
20 25 30

Lys Ala Val Tyr Pro Tyr Leu Lys Asp Gly Lys Ser Val Lys Leu Glu
35 40 45

Ser His Lys Phe Asp Trp Asn Thr Pro Asp Pro Arg Ile Gly Phe Lys
50 55 60

Asp Asn Met Leu Val Ala Met Glu Gly Ser Val Gly Tyr Gly Ile Gly
65 70 75 80

Gly Ala Arg Val Glu Leu Glu Ile Gly Tyr Glu Arg Phe Lys Thr Lys
85 90 95

Gly Ile Arg Asp Ser Gly Ser Lys Glu Asp Glu Ala Asp Thr Val Tyr
100 105 110

Leu Leu Ala Lys Glu Leu Ala Tyr Asp Val Val Thr Gly Gln Thr Asp
115 120 125

Asn Leu Ala Ala Ala Leu Ala Lys Thr Ser Gly Lys Asp Ile Val Gln

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    130                 135                 140

Phe Ala Lys Ala Val Gly Val Ser His Pro Gly Ile Asp Lys Lys Val
145                 150                 155                 160

Cys Asp Gly Gly His Ala Arg Gly Lys Lys Ser Gly Asp Asn Gly Ser
                165                 170                 175


<210> SEQ ID NO 25
<211> LENGTH: 181
<212> TYPE: PRT
<213> ORGANISM: A. marginale
<220> FEATURE:
<223> OTHER INFORMATION: Genbank accession number U07862

<400> SEQUENCE: 25

Gly Ala Gly Ala Gly Ser Phe Tyr Ile Gly Leu Asp Tyr Ser Pro Ala
 1               5                  10                  15

Phe Gly Ser Ile Lys Asp Phe Lys Val Gln Glu Ala Gly Gly Thr Thr
            20                  25                  30

Arg Gly Val Phe Pro Tyr Lys Arg Asp Ala Ala Gly Arg Val Asp Phe
        35                  40                  45

Lys Val His Asn Phe Asp Trp Ser Ala Pro Glu Pro Lys Ile Ser Phe
    50                  55                  60

Lys Asp Ser Met Leu Thr Ala Leu Glu Gly Ser Ile Gly Tyr Ser Ile
65                  70                  75                  80

Gly Gly Ala Arg Val Glu Val Glu Val Gly Tyr Glu Arg Phe Val Ile
                85                  90                  95

Lys Gly Gly Lys Lys Ser Asn Glu Asp Thr Ala Ser Val Phe Leu Leu
            100                 105                 110

Gly Lys Glu Leu Ala Tyr His Thr Ala Arg Gly Gln Val Asp Arg Leu
        115                 120                 125

Ala Thr Ala Leu Gly Lys Met Thr Lys Ser Glu Ala Lys Lys Trp Gly
    130                 135                 140

Asn Ala Ile Glu Ser Ala Thr Gly Thr Thr Ser Gly Asp Glu Leu Ser
145                 150                 155                 160

Lys Lys Val Cys Gly Lys Gly Thr Thr Ser Gly Ser Thr Asn Gln Cys
                165                 170                 175

Gly Thr Thr Asp Ser
            180


<210> SEQ ID NO 26
<211> LENGTH: 1840
<212> TYPE: DNA
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: E8 msp2 gene

<400> SEQUENCE: 26

gaggtcgacg gtatcgataa gcttgatatc gaattcctgg ctggagatgt tagaggggt     60

tagcccttga gtggaccggc tgaagtgagg agacgaagaa aaagaaggaa tttggagaag    120

ttgaaaagta tgagaaaagg aaagataatc ttaggaagcg taatgatgtc gatggctata    180

gtcatggctg ggaatgatgt cagggctcat gatgacgtta gcgctttgga gactggtggt    240

gcgggatatt tctatgttgg cttggattac agtccagcgt ttagcaagat aagagatttt    300

agtataaggg agagtaacgg agagactaag gcagtatatc catacttaaa ggatggaaag    360

agtgtaaagc tagagtcaca caagtttgac tggaacactc ctgatcctcg gattgggttt    420

aaggacaaca tgcttgtagc tatggaaggt agtgttggtt atggtattgg tggtgccagg    480
```

```
   1 GGATCCCCCG GGCTGCAGGA ATTCCTAAAA AGATCTGGCG CCTGAGCGTC TGCTACAGGC
  61 AGATTGTGCG CGCTAAGATA GGTTTAGTAA GACGTGTTTT TTATTTGAATA AAGGCCCCAA
 121 CAATGTTGAC AGAAGAAGAA AAGAAAAAGA GCGCCGGTGC TCTGCAAGCC ATTATCACAG
 181 GAGATTACGA GAGTGTTCAG GCGTCCGTTC AGGGAATTTC TTCCGAAGAC TTAATACTCC
 241 CGTTGATTAT GAGGGGAGAA CACTACTGCA CTATGCAGCT TCATCCCGTA ATGGTAATTT
 301 CTATGGCATT CTGGTTTGAAA GAGGATGTGT TACTAATATC AGAGATGCTT ATGGATTTAC
 361 TCCAGAACAA GCACGTGAGA AGGCAGGGTA TGCACGGCACA CAGTGGTATG GAGCAGATGT
 421 AAATGACCCT GGTGTATCTA GGCAGTTAAT GACGCAAGCT GTTCAGCAGT CTGCGAAAGG
 481 TAACATGTAT GCTGCTCTCG CTATATTAGA CCTTGTGCGT AATGACGATG CAAACATTC
 541 AGGTCAATGA GGAAAGGGGC ATAGTGTTTT GCATCTAGCA TGTATTGAAG GCAGTAATCC
 601 ATCTTTTCACT TCATCCCTCA TGCTAAAGGG TTGTTCTTTA AACATTAAGG ATGTAGATGG
 661 TAATACGCCA TTACATACAG CTGCGTTTTC AGTAGGCAAA AATGCTTTAG GCAATCTTGA
 721 TGTACTATGC GACAAGCTCT TATAGCAGAT GTTAATGCTA AGGGACCGGG TGGAAACACT
 781 CCGCTTCATA TTGCTACGGA GCGTATGGCT CACCAGAAAG TAGAGGCATCT TCTCTCAAGG
 841 TTAAGTGATA TTAGCGTTGC AAATCGATGC TGGTGAAACC GTTTGCCACA TTGTTGCAAA
 901 GCAATGGCCA AGGCGGGATG TTTTACCATA CATTGACAAG ATGCAAGAAG CGGTGTCGTC
 961 AAATATTGAG GGCAATCGCG AGTGTGCAGA GGCACTAATA TTCCCGGATA AAAAAGGGAT
1021 GAGTGCAGTA CAGTATGCTA TTAGAAGGCA TATACCGGAG CTGAGAAGAT CTTCGAGAAG
1081 GCCATTAACA TTGCAGATAA AGTGTATATGGC TTAGCTTCTT CAGAAGTAGA ATCTCTCTTT
1141 ACATGTCCTA ATCCAGAGGA CGCATCAACG CTGGTGCATT TTGTATCTTC TAATGGGACC
1201 CCAAATTTTG ATTCTCTTTGC GAAAAGGGTA TTGGAGGAAG CATATCATAG GTATGGAGAG
1261 AAACCTTTTA CTAATTTAGA TGTTGCAGGT AATGCACCTA TACATGCTGC AGCACAAAAA
1321 TCAACAGTGG GGGTTTTTGA GCAGGTGGTA AGATACACTC CTGAGTCTGT TGTAAACTCA
1381 ATTAGCACCG AATGGCAAAG CGCCTATTCA CATGATAGTT GAGGATGAGC CAAGCCATAA
1441 AAGCGTAAGC ATTAAATTGC AGATGTTGAT TGGGAATGTG CGTAATATTC CATCAATCAA
1501 TGTACCATCC CCAGTGACAG GTGAAACGCT GCGGTAGCTG CGTATAAAGG GGGCAACACT
```

FIG. 6A

-continued

gttgagcttg agattggtta cgagcgcttc aagaccaagg gtattagaga tagtggtagt 540

aaggaagatg aagctgatac agtatatcta ctagctaagg agttagctta tgatgttgtt 600

actgggcaga ctgataacct tgctgctgct cttgccaaga cctctggtaa agatattgtt 660

cagtttgcta aggcggttgg ggtttctcat cccggtattg ataagaaggt ttgtgatggg 720

ggtcatgcac ggggaaaaaa gagtggagat aatggctcgc tggccgacta tacggatggt 780

ggcgcgtcac agacgaataa gacggctcag tgtagtggta tgggaaccgg caaagccggc 840

aagagaggat tgggcttgac tgagtttgtt aacaaaacaa aggttggaga aggtaagaat 900

tggccaacgg ggtacgttaa tgatggcgac aacgttaatg tgctcggcga tacgaatggt 960

aacgccgaag ccgtagctaa agacctagta caggagctaa cccctgaaga aaaaaccata 1020

gtagcagggt tactagctaa gactattgaa gggggtgaag ttgttgagat cagggcggtt 1080

tcttctactt ccgtaatggt caatgcttgt tatgatcttc ttagtgaagg tttaggtgtt 1140

gttccttatg cttgtgttgg tcttggcggt aacttcgtgg gcgtggttga tggccatatc 1200

actcctaagc ttgcttatag attaaaggct gggttgagtt atcagctctc tcctgtaatc 1260

tccgcttttg cgggtggatt ctaccatcgc gttgtgggag atggcgttta tgatgatctg 1320

ccggctcaac gtcttgtaga tgatactagt ccggcgggtc gtactaagga tactgctatt 1380

gctaacttct ccatggctta tgtcggtggg gaatttggtg ttaggtttgc tttttaagct 1440

tgcttatcta aagaggggggg ctaagggctc ccctttttcta ctttaattct acttcctgcg 1500

gtacttcacc ctcttcctga cttcttctgg ttctgctacc attaattatt actccgtgac 1560

cgttcctatt atttattttc ttgctgctca ggttagaagg tttctatcag tgcttgatgg 1620

ggatttggcg tgtttttata gtgcaaatcg catcgctccc atttgtacaa atcttgacac 1680

ttttggcttc aatgtctatt gttgagcagt taaaattctt gtgatacccc atggttgaaa 1740

tgctattggt cagattgaac tgaaggactc cccctctgat aagcagcggg ctgttgatgc 1800

gcgcttttgg gcgtggatct attatgaggc tgcagaatag 1840

<210> SEQ ID NO 27
<211> LENGTH: 435
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: E8 msp2 gene

<400> SEQUENCE: 27

Met Arg Lys Gly Lys Ile Ile Leu Gly Ser Val Met Met Ser Met Ala
 1 5 10 15

Ile Val Met Ala Gly Asn Asp Val Arg Ala His Asp Asp Val Ser Ala
            20 25 30

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

Pro Ala Phe Ser Lys Ile Arg Asp Phe Ser Ile Arg Glu Ser Asn Gly
    50 55 60

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

Leu Glu Ser His Lys Phe Asp Trp Asn Thr Pro Asp Pro Arg Ile Gly
                85 90 95

Phe Lys Asp Asn Met Leu Val Ala Met Glu Gly Ser Val Gly Tyr Gly
            100 105 110

Ile Gly Gly Ala Arg Val Glu Leu Glu Ile Gly Tyr Glu Arg Phe Lys
        115 120 125

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```
Thr Lys Gly Ile Arg Asp Ser Gly Ser Lys Glu Asp Glu Ala Asp Thr
    130                 135                 140

Val Tyr Leu Leu Ala Lys Glu Leu Ala Tyr Asp Val Val Thr Gly Gln
145                 150                 155                 160

Thr Asp Asn Leu Ala Ala Ala Leu Ala Lys Thr Ser Gly Lys Asp Ile
                165                 170                 175

Val Gln Phe Ala Lys Ala Val Gly Val Ser His Pro Gly Ile Asp Lys
            180                 185                 190

Lys Val Cys Asp Gly Gly His Ala Arg Gly Lys Lys Ser Gly Asp Asn
        195                 200                 205

Gly Ser Leu Ala Asp Tyr Thr Asp Gly Gly Ala Ser Gln Thr Asn Lys
    210                 215                 220

Thr Ala Gln Cys Ser Gly Met Gly Thr Gly Lys Ala Gly Lys Arg Gly
225                 230                 235                 240

Leu Gly Leu Thr Glu Phe Val Asn Lys Thr Lys Val Gly Glu Gly Lys
                245                 250                 255

Asn Trp Pro Thr Gly Tyr Val Asn Asp Gly Asp Asn Val Asn Val Leu
            260                 265                 270

Gly Asp Thr Asn Gly Asn Ala Glu Ala Val Ala Lys Asp Leu Val Gln
        275                 280                 285

Glu Leu Thr Pro Glu Glu Lys Thr Ile Val Ala Gly Leu Leu Ala Lys
    290                 295                 300

Thr Ile Glu Gly Gly Glu Val Val Glu Ile Arg Ala Val Ser Ser Thr
305                 310                 315                 320

Ser Val Met Val Asn Ala Cys Tyr Asp Leu Leu Ser Glu Gly Leu Gly
                325                 330                 335

Val Val Pro Tyr Ala Cys Val Gly Leu Gly Gly Asn Phe Val Gly Val
            340                 345                 350

Val Asp Gly His Ile Thr Pro Lys Leu Ala Tyr Arg Leu Lys Ala Gly
        355                 360                 365

Leu Ser Tyr Gln Leu Ser Pro Val Ile Ser Ala Phe Ala Gly Gly Phe
    370                 375                 380

Tyr His Arg Val Val Gly Asp Gly Val Tyr Asp Asp Leu Pro Ala Gln
385                 390                 395                 400

Arg Leu Val Asp Asp Thr Ser Pro Ala Gly Arg Thr Lys Asp Thr Ala
                405                 410                 415

Ile Ala Asn Phe Ser Met Ala Tyr Val Gly Gly Glu Phe Gly Val Arg
            420                 425                 430

Phe Ala Phe
        435
```

<210> SEQ ID NO 28
<211> LENGTH: 3435
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: E46 gene inset in Lambda Zap II

<400> SEQUENCE: 28

```
tttttatatc tggagctctt gtactgtgtt taccacggga tttattattg ggtaggcttg     60

atattcaggc tctatcaacg cagctattca tggcattatt acagataaat ttggcatttt    120

ggagataggc gatctagggt tctattatta ggaatctatt atttagatat atagggatat    180

aagggagagt aacggagaga ctaaggcagt atatccatac ttaaaggatg gaaagagtgt    240

aaagctagag tcacacaagt ttgactggaa cactcctgat cctcggattg ggtttaagga    300
```

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caacatgctt gtagctatgg aaggcagtgt tggttatggt attggtggtg ccagggttga 360

gcttgagatt ggttacgagc gcttcaagac caagggtatt agagatagtg gtagtaagga 420

agatgaagca gatacagtat atctactagc taaggagtta gcttatgatg ttgttactgg 480

acagactgat aaccttgccg ctgctcttgc caaaacctcg gggaaggaca tcgttcagtt 540

tgccaatgct gtgaaaattt cttaccctaa aattgatgag caggtttgta ataaaaatca 600

tacagtgttg aatacgggga aagggacaac ctttaatcca gatcccaaga caaccgaaga 660

taatacagcg cagtgcagtg ggttgaacac gaagggaacg aataagttta gcgattttgc 720

tgaaggtgta ggtttgaaag ataataagaa ttggcctact ggtcaggctg ggaagagcag 780

tggtggtcct gtggtgggtg catctaatag taatgccaac gctatggcta gagacctagt 840

agatcttaat cgagacgaaa aaaccatagt agcagggtta ctagctaaaa ctattgaagg 900

tggtgaggtt gttgagatta gggcggtttc ttctacttct gtaatggtca atgcttgtta 960

tgatcttctt agtgaaggtc taggcgttgt tccttacgct tgtgtcggtc ttggaggtaa 1020

cttcgtgggc gttgttgatg ggcatatcac tcctaagctt gcttatagat taaaggctgg 1080

gttgagttat cagctctctc ctgaaatctc cgcttttgct gggggattct atcatcgcgt 1140

tgtgggagat ggtgtctatg atgatcttcc agctcaacgt cttgtagatg atactagtcc 1200

ggcgggtcgt actaaggata ctgctattgc taacttctcc atggcttatg tcggtgggga 1260

atttggtgtt aggtttgctt tttaaggtgg tttgttggaa gcggggtaag tcaaacttac 1320

cccgcttcta ttagggagtt agtatatgag atctagaagt aagctatttt taggaagcgt 1380

aatgatgtcg ttggctatag taatggctgg gaatgatgtc agggctcatg atgacgttag 1440

cgctttggat actggtggtg cgggatattt ctatgttggt ttggattaca gtccagcgtt 1500

tagcaagata agagatttta gtataaggga gagtaacgga gagactaagg cagtatatcc 1560

atacttaaag gatggaaaga gtgtaaagct agagtcacac aagtttgact ggaacactcc 1620

tgatcctcgg attgggttta aggacaacat gcttgtagct atggaaggta gtgttggtta 1680

tggtattggt ggtgccaggg ttgagcttga gattggttac gagcgcttca agaccaaggg 1740

tattagagat agtggtagta aggaagatga agctgataca gtatatctac tagctaagga 1800

gttggcttat gatgttgtta ctgggcagac tgataacctt gccgctgctc tggccaaaac 1860

ctccggtaaa gactttgtcc agtttgctaa ggcggttggg gtttctcatc ctagtattga 1920

tgggaaggtt tgtaagacga aggcggatag ctcgaagaaa tttccgttat atagtgacga 1980

aacgcacacg aaggggggcaa gtgaggggag aacgtctttg tgcggtgaca atggtagttc 2040

tacgataaca aacagtggtg cgaatgtaag tgaaactggg caggttttta gggattttat 2100

cagggcaacg ctgaaagagg atggtagtaa aaactggcca acttcaagcg gcacgggaac 2160

tccaaaacct gtcacgaacg acaacgccaa agccgtagct aaagacctag tacaggagct 2220

aacccctgaa gaaaaaacca tagtagcagg gttactagct aaaactattg aaggtggtga 2280

ggttattgaa atcagggcgg tttcttctac ttctgtgatg gtcaatgctt gttatgatct 2340

tcttagtgaa ggtttaggtg ttgtccctta tgcttgtgtt ggtctcggtg gtaacttcgt 2400

gggcgtggtt gatggaattc attacacaaa ccatctttaa ctctgaatac cctagttaag 2460

gtaagtgaag taactaggca aattagtgct gcaccactcg tgaaacaaac tacgatcagc 2520

gattcaccat acttagtaag tccgtacagt ggctttacgc tcttacccat catgaaaaat 2580

acttgctatc taggaatctc ctctaaaact ttacagaggt tatctgtact tcgagaggaa 2640

gctaatctgt ggctcatgag gatggtattt agcgtatcac aggttccagc tgtcttacag 2700

-continued

tctctggaga tgttataagg gtgcacatat aaaactatgc aatatttcgc tgcaatacga 2760

ttccgattcg aaaacactga aaagtattcc cattatctat gaatctctgt gtagatataa 2820

ataagggtat acgcagtaac tcttacttgt taaaaacaag accaatggta taaggaaaaa 2880

gcctcagtgt tgttcctcat gcttgcagct tacccgatgc actcttattt aataaggttg 2940

aatgttaatc agtgtttctg ggaagggaat atcttattgc aaaaccctca gcagctgctt 3000

agatattgaa acaaatgcga tcatgccgtc agcacaatta tgacatctct taaggctctg 3060

tagtgcgctt atttagtcta acatgtggta aagctttgcc agttctttac cacatgttca 3120

ccatcagtta attgaaagca aatcttgctc ctatgttgaa gccgtaacta gctatatttg 3180

cctttacctt ggctgcagca ccacctgcta tgtttacacg gttactagcg ggaatacctg 3240

catactgttc atcgaaaatt ccgtggtaaa aacctccagc tattaaagat atttcaggag 3300

taagcttgta acttacgcct acctttcctc tataagccaa cttacttgta acgtgatcgg 3360

cgatattaat aaagctcgcc cctaacccag cacacatgta aggagggaat tcgatatcaa 3420

gcttatcgat accgt 3435

<210> SEQ ID NO 29
<211> LENGTH: 326
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: E46#1

<400> SEQUENCE: 29

Met Leu Val Ala Met Glu Gly Ser Val Gly Tyr Gly Ile Gly Gly Ala
 1   5   10   15

Arg Val Glu Leu Glu Ile Gly Tyr Glu Arg Phe Lys Thr Lys Gly Ile
            20   25   30

Arg Asp Ser Gly Ser Lys Glu Asp Glu Ala Asp Thr Val Tyr Leu Leu
        35   40   45

Ala Lys Glu Leu Ala Tyr Asp Val Val Thr Gly Gln Thr Asp Asn Leu
    50   55   60

Ala Ala Ala Leu Ala Lys Thr Ser Gly Lys Asp Ile Val Gln Phe Ala
65   70   75   80

Asn Ala Val Lys Ile Ser Tyr Pro Lys Ile Asp Glu Gln Val Cys Asn
                85   90   95

Lys Asn His Thr Val Leu Asn Thr Gly Lys Gly Thr Thr Phe Asn Pro
            100   105   110

Asp Pro Lys Thr Thr Glu Asp Asn Thr Ala Gln Cys Ser Gly Leu Asn
        115   120   125

Thr Lys Gly Thr Asn Lys Phe Ser Asp Phe Ala Glu Gly Val Gly Leu
    130   135   140

Lys Asp Asn Lys Asn Trp Pro Thr Gly Gln Ala Gly Lys Ser Ser Gly
145   150   155   160

Gly Pro Val Val Gly Ala Ser Asn Ser Asn Ala Asn Ala Met Ala Arg
                165   170   175

Asp Leu Val Asp Leu Asn Arg Asp Glu Lys Thr Ile Val Ala Gly Leu
            180   185   190

Leu Ala Lys Thr Ile Glu Gly Gly Glu Val Val Glu Ile Arg Ala Val
        195   200   205

Ser Ser Thr Ser Val Met Val Asn Ala Cys Tyr Asp Leu Leu Ser Glu
    210   215   220

Gly Leu Gly Val Val Pro Tyr Ala Cys Val Gly Leu Gly Gly Asn Phe
225   230   235   240

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```
Val Gly Val Val Asp Gly His Ile Thr Pro Lys Leu Ala Tyr Arg Leu
                245                 250                 255

Lys Ala Gly Leu Ser Tyr Gln Leu Ser Pro Glu Ile Ser Ala Phe Ala
            260                 265                 270

Gly Gly Phe Tyr His Arg Val Val Gly Asp Gly Val Tyr Asp Asp Leu
        275                 280                 285

Pro Ala Gly Arg Leu Val Asp Asp Thr Ser Pro Ala Gly Arg Thr Lys
    290                 295                 300

Asp Thr Ala Ile Ala Asn Phe Ser Met Ala Tyr Val Gly Gly Glu Phe
305                 310                 315                 320

Gly Val Arg Phe Ala Phe
                325


<210> SEQ ID NO 30
<211> LENGTH: 364
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: E46#2

<400> SEQUENCE: 30

Met Arg Ser Arg Ser Lys Leu Phe Leu Gly Ser Val Met Met Ser Leu
 1               5                  10                  15

Ala Ile Val Met Ala Gly Asn Asp Val Arg Ala His Asp Asp Val Ser
            20                  25                  30

Ala Leu Asp Thr Gly Gly Ala Gly Tyr Phe Tyr Val Gly Leu Asp Tyr
        35                  40                  45

Ser Pro Ala Phe Ser Lys Ile Arg Asp Phe Ser Ile Arg Glu Ser Asn
    50                  55                  60

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

Lys Leu Glu Ser His Lys Phe Asp Trp Asn Thr Pro Asp Pro Arg Ile
                85                  90                  95

Gly Phe Lys Asp Asn Met Leu Val Ala Met Glu Gly Ser Val Gly Tyr
            100                 105                 110

Gly Ile Gly Gly Ala Arg Val Glu Leu Glu Ile Gly Tyr Glu Arg Phe
        115                 120                 125

Lys Thr Lys Gly Ile Arg Asp Ser Gly Ser Lys Glu Asp Glu Ala Asp
    130                 135                 140

Thr Val Tyr Leu Leu Ala Lys Glu Leu Ala Tyr Asp Val Val Thr Gly
145                 150                 155                 160

Gln Thr Asp Asn Leu Ala Ala Ala Leu Ala Lys Thr Ser Gly Lys Asp
                165                 170                 175

Phe Val Gln Phe Ala Lys Ala Val Gly Val Ser His Pro Ser Ile Asp
            180                 185                 190

Gly Lys Val Cys Lys Thr Lys Ala Asp Ser Ser Lys Lys Phe Pro Leu
        195                 200                 205

Tyr Ser Asp Glu Thr His Thr Lys Gly Ala Ser Glu Gly Arg Thr Ser
    210                 215                 220

Leu Cys Gly Asp Asn Gly Ser Ser Thr Ile Thr Asn Ser Gly Ala Asn
225                 230                 235                 240

Val Ser Glu Thr Gly Gln Val Phe Arg Asp Phe Ile Arg Ala Thr Leu
                245                 250                 255

Lys Glu Asp Gly Ser Lys Asn Trp Pro Thr Ser Ser Gly Thr Gly Thr
            260                 265                 270
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-continued

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Pro Lys Pro Val Thr Asn Asp Asn Ala Lys Ala Val Ala Lys Asp Leu
        275                 280                 285

Val Gln Glu Leu Thr Pro Glu Glu Lys Thr Ile Val Ala Gly Leu Leu
    290                 295                 300

Ala Lys Thr Ile Glu Gly Gly Glu Val Ile Glu Ile Arg Ala Val Ser
305                 310                 315                 320

Ser Thr Ser Val Met Val Asn Ala Cys Tyr Asp Leu Leu Ser Glu Gly
                325                 330                 335

Leu Gly Val Val Pro Tyr Ala Cys Val Gly Leu Gly Gly Asn Phe Val
            340                 345                 350

Gly Val Val Asp Gly Ile His Tyr Thr Asn His Leu
        355                 360


<210> SEQ ID NO 31
<211> LENGTH: 409
<212> TYPE: PRT
<213> ORGANISM: A. marginale
<220> FEATURE:
<223> OTHER INFORMATION: msp-2 gene

<400> SEQUENCE: 31

Met Ser Ala Val Ser Asn Arg Lys Leu Pro Leu Gly Gly Val Leu Met
 1               5                  10                  15

Ala Leu Ala Ala Ala Val Ala Pro Ile His Ser Leu Leu Ala Ala Pro
            20                  25                  30

Ala Ala Gly Ala Gly Ala Gly Gly Glu Gly Leu Phe Ser Gly Ala Gly
        35                  40                  45

Ala Gly Ser Phe Tyr Ile Gly Leu Asp Tyr Ser Pro Ala Phe Gly Ser
    50                  55                  60

Ile Lys Asp Phe Lys Val Gln Glu Ala Gly Gly Thr Thr Arg Gly Val
65                  70                  75                  80

Phe Pro Tyr Lys Arg Asp Ala Ala Gly Arg Val Asp Phe Lys Val His
                85                  90                  95

Asn Phe Asp Trp Ser Ala Pro Glu Pro Lys Ile Ser Phe Lys Asp Ser
            100                 105                 110

Met Leu Thr Ala Leu Glu Gly Ser Ile Gly Tyr Ser Ile Gly Gly Ala
        115                 120                 125

Arg Val Glu Val Glu Val Gly Tyr Glu Arg Phe Val Ile Lys Gly Gly
    130                 135                 140

Lys Lys Ser Asn Glu Asp Thr Ala Ser Val Phe Leu Leu Gly Lys Glu
145                 150                 155                 160

Leu Ala Tyr His Thr Ala Arg Gly Gln Val Asp Arg Leu Ala Thr Ala
                165                 170                 175

Leu Gly Lys Met Thr Lys Ser Glu Ala Lys Lys Trp Gly Asn Ala Ile
            180                 185                 190

Glu Ser Ala Thr Gly Thr Thr Ser Gly Asp Glu Leu Ser Lys Lys Val
        195                 200                 205

Cys Gly Lys Gly Thr Thr Ser Gly Ser Thr Asn Gln Cys Gly Thr Thr
    210                 215                 220

Asp Ser Thr Ala Thr Thr Lys Ile Ser Ala Val Phe Thr Glu Asp Ala
225                 230                 235                 240

Ala Ala Gln Leu Ser Thr Met Asp Asn Thr Thr Ile Asn Thr Thr Gly
                245                 250                 255

Met Ala Asn Asn Ile Asn Ser Leu Thr Lys Asp Glu Lys Ala Ile Val
            260                 265                 270

Ala Gly Ala Phe Ala Arg Ala Val Glu Gly Ala Glu Val Ile Glu Val
```

-continued

```
        275                 280                 285

Arg Ala Ile Gly Ser Thr Ser Val Met Leu Asn Ala Cys Tyr Asp Leu
    290                 295                 300

Leu Thr Asp Gly Ile Gly Val Val Pro Tyr Ala Cys Ala Gly Ile Gly
305                 310                 315                 320

Gly Asn Phe Val Ser Val Val Asp Gly His Ile Asn Pro Lys Phe Ala
                325                 330                 335

Tyr Arg Val Lys Ala Gly Leu Ser Tyr Ala Leu Thr Pro Glu Ile Ser
            340                 345                 350

Ala Phe Ala Gly Ala Phe Tyr His Lys Val Leu Gly Asp Gly Asp Tyr
        355                 360                 365

Asp Glu Leu Pro Leu Ser His Ile Ser Asp Tyr Thr Gly Thr Ala Gly
    370                 375                 380

Lys Asn Lys Asp Thr Gly Ile Ala Ser Phe Asn Phe Ala Tyr Phe Gly
385                 390                 395                 400

Gly Glu Leu Gly Val Arg Phe Ala Phe
                405


<210> SEQ ID NO 32
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 3, 18
<223> OTHER INFORMATION: n = A,T,C or G
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic nucleotide - degenerate primer

<400> SEQUENCE: 32

ccnttycaya tgtayccngg                                              20


<210> SEQ ID NO 33
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 3, 6, 15, 18
<223> OTHER INFORMATION: n = A,T,C or G
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic nucleotide - degenerate primer

<400> SEQUENCE: 33

ggnckngcrt aytcnccngc                                              20


<210> SEQ ID NO 34
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence of 64 kDa protein generated
      by using degenerate primers

<400> SEQUENCE: 34

Pro Phe His Met Tyr Pro Gly Ser Glu Asn Leu Phe Arg Ser Thr Arg
 1               5                  10                  15

Asp Leu Arg Gly Val Ser Gly Val
            20


<210> SEQ ID NO 35
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
```

-continued

<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence of 64 kDa protein generated by using degenerate primers

<400> SEQUENCE: 35

Phe Arg Leu Ser Leu Ala Gly Glu Tyr Ala Arg Pro Lys
1 5 10

<210> SEQ ID NO 36
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence of 64 kDa protein generated by using degenerate primers

<400> SEQUENCE: 36

Glu Leu Val Val Gly Glu Asn Thr Leu
1 5

<210> SEQ ID NO 37
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: amino acid sequence of 64 kDa protein generated by using degenerate primers

<400> SEQUENCE: 37

Glu Asp Thr Val Arg Asp Gly Ile Ala Gly Phe Asp Ser Leu
1 5 10

<210> SEQ ID NO 38
<211> LENGTH: 2097
<212> TYPE: DNA
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (338)...(1972)
<223> OTHER INFORMATION: S11 of Granulocytic Ehrlichia

<400> SEQUENCE: 38

gaattcctag caacaagggt ggatatttca cgcttgctag gctgagtgat ttaggactga 60

gggtgagcta tgagatgtat agggggggaga gtatgcgctg cgtgctttttt actcagcttc 120

ataagatagc ggcgagctac agctttgcta cggggttcgt agaaaagcgt tattgtcgct 180

ataacactcg tgatgtatat catcgtgatg tcggttataa ggatcatgga tgtgctatgg 240

ttaagccttt gaagtatgac tttggcttga tggctttagg tgtgaagctg gtcttctaag 300

aagagtgtgg gtgtttgtgg atttttgaag gttttgt atg aga ggt tct ctg gta 355
Met Arg Gly Ser Leu Val
1 5

gtt gtg agt atg gcg atg ctt ctc ctg ggg tcc tct ggt ggt gta gtt 403
Val Val Ser Met Ala Met Leu Leu Leu Gly Ser Ser Gly Gly Val Val
10 15 20

gct gca tct tct gga ggg ggg ttt gaa gga gag cgt gcg tcg gta acg 451
Ala Ala Ser Ser Gly Gly Gly Phe Glu Gly Glu Arg Ala Ser Val Thr
25 30 35

ggt aag gtg tta tct tat gcc tgg ttg ttg agt gat cgg gct gta aaa 499
Gly Lys Val Leu Ser Tyr Ala Trp Leu Leu Ser Asp Arg Ala Val Lys
40 45 50

ggg caa ggt aac agt gaa ggt cag aag ctc gcg ctg gaa atg tat ggc 547
Gly Gln Gly Asn Ser Glu Gly Gln Lys Leu Ala Leu Glu Met Tyr Gly
55 60 65 70

gca aag ttg ggc tat aag ggt tat ggt tat cca gga gtt gga gat gtc 595

-continued

```
Ala Lys Leu Gly Tyr Lys Gly Tyr Gly Tyr Pro Gly Val Gly Asp Val
                 75                  80                  85

ttt tct tcg ccg ttg gag cat ggt ctt gat tct tgg gga gct agc tat      643
Phe Ser Ser Pro Leu Glu His Gly Leu Asp Ser Trp Gly Ala Ser Tyr
             90                  95                 100

gat gcg atg tta tct ctt gga ttg cgt acg ggt cgt gat gtg cta ggt      691
Asp Ala Met Leu Ser Leu Gly Leu Arg Thr Gly Arg Asp Val Leu Gly
        105                 110                 115

acc caa tat ggg gca aat ttt tcc ctt atg gtt cct gcg ggt tct ggt      739
Thr Gln Tyr Gly Ala Asn Phe Ser Leu Met Val Pro Ala Gly Ser Gly
    120                 125                 130

gga tct atg gtg ttt cat ggt gcg cct ggt ata gag agc agg gtt ttt      787
Gly Ser Met Val Phe His Gly Ala Pro Gly Ile Glu Ser Arg Val Phe
135                 140                 145                 150

gct gat act tcc ttg gga aat ttt tct gtt ggt tac cag gaa ggt gtc      835
Ala Asp Thr Ser Leu Gly Asn Phe Ser Val Gly Tyr Gln Glu Gly Val
                155                 160                 165

gag tca aaa atg aag gtg gat gtc ttc ggt ggc tta tca ggt gaa aat      883
Glu Ser Lys Met Lys Val Asp Val Phe Gly Gly Leu Ser Gly Glu Asn
            170                 175                 180

gga agc gct tgg ggt cgg tac ttg cgt ggc ttt tta aag tat gcg aag      931
Gly Ser Ala Trp Gly Arg Tyr Leu Arg Gly Phe Leu Lys Tyr Ala Lys
        185                 190                 195

ggt gta cct ttt cac atg tat cca ggg ctt tac agt gag aat tta ttc      979
Gly Val Pro Phe His Met Tyr Pro Gly Leu Tyr Ser Glu Asn Leu Phe
    200                 205                 210

cgg tct aca aga gac tta cgg ggt gtt agt ggt gtt tct gcg aag aca     1027
Arg Ser Thr Arg Asp Leu Arg Gly Val Ser Gly Val Ser Ala Lys Thr
215                 220                 225                 230

aag gat gtc tta aat tct atg ccg ctg agg ttt tct ttt gag tct gct     1075
Lys Asp Val Leu Asn Ser Met Pro Leu Arg Phe Ser Phe Glu Ser Ala
                235                 240                 245

agg ttg ggt ggc ttg tct gtt ggt ttt agt tac tct cca acg gga tat     1123
Arg Leu Gly Gly Leu Ser Val Gly Phe Ser Tyr Ser Pro Thr Gly Tyr
            250                 255                 260

cgg gat gat atg tac aag ggt gga gag ttt act gta cgg gat ggt att     1171
Arg Asp Asp Met Tyr Lys Gly Gly Glu Phe Thr Val Arg Asp Gly Ile
        265                 270                 275

gct ggt ttc gat tcc ttg ggt aca gta aat tta ttc gcg aag acg ggg     1219
Ala Gly Phe Asp Ser Leu Gly Thr Val Asn Leu Phe Ala Lys Thr Gly
    280                 285                 290

gtt aag ttt ggc aaa atg att gcc gtg gtg cct cct cgt ttt gat tct     1267
Val Lys Phe Gly Lys Met Ile Ala Val Val Pro Pro Arg Phe Asp Ser
295                 300                 305                 310

ggt ccg gta tat aaa aac ata gta agc ggt gct gcg aat tac gag tac     1315
Gly Pro Val Tyr Lys Asn Ile Val Ser Gly Ala Ala Asn Tyr Glu Tyr
                315                 320                 325

gag tta gcc gat att gct aag ttt agg tta tcg ctt gct ggt gag tat     1363
Glu Leu Ala Asp Ile Ala Lys Phe Arg Leu Ser Leu Ala Gly Glu Tyr
            330                 335                 340

gca aga ccg aag aag gct agg gat ata gtg cca gaa gga aga aga aag     1411
Ala Arg Pro Lys Lys Ala Arg Asp Ile Val Pro Glu Gly Arg Arg Lys
        345                 350                 355

gaa gaa att tat gta gct gat tac aat gat ttg tcc gcg ttt tcc agt     1459
Glu Glu Ile Tyr Val Ala Asp Tyr Asn Asp Leu Ser Ala Phe Ser Ser
    360                 365                 370

ggc tta gaa ata gac ttg ggt agg ttg cgg ttt gct gtt ggc ggt gga     1507
Gly Leu Glu Ile Asp Leu Gly Arg Leu Arg Phe Ala Val Gly Gly Gly
375                 380                 385                 390

tac ctt ggg aag tct ggt agt cct aaa atg tac ata tta aag gat gta     1555
```

-continued

```
Tyr Leu Gly Lys Ser Gly Ser Pro Lys Met Tyr Ile Leu Lys Asp Val
                395                 400                 405

aga cat aag gta cct tat gtg aaa aag aag ggt ttg ccg tct cat tat     1603
Arg His Lys Val Pro Tyr Val Lys Lys Lys Gly Leu Pro Ser His Tyr
            410                 415                 420

gtg act tca gcg gtt tcc tat acg att ggt tct ttc tct gct aca gtt     1651
Val Thr Ser Ala Val Ser Tyr Thr Ile Gly Ser Phe Ser Ala Thr Val
        425                 430                 435

gct tac ttt atg agt agg tta acg cac att ccg cct gct acg gta tct     1699
Ala Tyr Phe Met Ser Arg Leu Thr His Ile Pro Pro Ala Thr Val Ser
    440                 445                 450

cat aag atc cca ggg aag tat gag ttg gat tcc gtt gtg gat ggg gag     1747
His Lys Ile Pro Gly Lys Tyr Glu Leu Asp Ser Val Val Asp Gly Glu
455                 460                 465                 470

aat acg ttg aag gat ttg gtt gta gga gtc ggt tat aac ctt ttt agt     1795
Asn Thr Leu Lys Asp Leu Val Val Gly Val Gly Tyr Asn Leu Phe Ser
                475                 480                 485

aag gga agt acg agc tta gaa gta ttt cta aat tgt cac atg ttc tct     1843
Lys Gly Ser Thr Ser Leu Glu Val Phe Leu Asn Cys His Met Phe Ser
            490                 495                 500

gtg caa cat aaa ttc aac atc cat gag tac aaa tct act gag agt agt     1891
Val Gln His Lys Phe Asn Ile His Glu Tyr Lys Ser Thr Glu Ser Ser
        505                 510                 515

ggg ttt gta ttg aaa gaa ggt gga gag cgt gca aat act aat aat ggc     1939
Gly Phe Val Leu Lys Glu Gly Gly Glu Arg Ala Asn Thr Asn Asn Gly
    520                 525                 530

gct gtg gcg tta tta gga atg aag ttt gcg ttt taataacaag gggttgttgc   1992
Ala Val Ala Leu Leu Gly Met Lys Phe Ala Phe
535                 540                 545

aagaatactc ttgtggttta tttagccaag tcttcttatt ggggcgtgta ctgaggtacg   2052

gcgccccttt ttttgtggag agtctaaggt ttgttatgtt gtaga                   2097


<210> SEQ ID NO 39
<211> LENGTH: 545
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: open reading frame of Granulocytic Ehrlichia
      S11

<400> SEQUENCE: 39

Met Arg Gly Ser Leu Val Val Val Ser Met Ala Met Leu Leu Leu Gly
 1               5                  10                  15

Ser Ser Gly Gly Val Val Ala Ala Ser Ser Gly Gly Gly Phe Glu Gly
            20                  25                  30

Glu Arg Ala Ser Val Thr Gly Lys Val Leu Ser Tyr Ala Trp Leu Leu
        35                  40                  45

Ser Asp Arg Ala Val Lys Gly Gln Gly Asn Ser Glu Gly Gln Lys Leu
    50                  55                  60

Ala Leu Glu Met Tyr Gly Ala Lys Leu Gly Tyr Lys Gly Tyr Gly Tyr
65                  70                  75                  80

Pro Gly Val Gly Asp Val Phe Ser Ser Pro Leu Glu His Gly Leu Asp
                85                  90                  95

Ser Trp Gly Ala Ser Tyr Asp Ala Met Leu Ser Leu Gly Leu Arg Thr
            100                 105                 110

Gly Arg Asp Val Leu Gly Thr Gln Tyr Gly Ala Asn Phe Ser Leu Met
        115                 120                 125

Val Pro Ala Gly Ser Gly Gly Ser Met Val Phe His Gly Ala Pro Gly
    130                 135                 140
```

```
1561 GAGGATGTTA AGACTATGTT ACGCTGTAAT AGCATGGACG TAGATGCTCG GTCACATGAT
1621 GGTAGAACTA TAATACATTA CGCAGCAAAG GATGGAAATT TAGAGATATT GCAGCAGGCT
1681 CTTGGAAGGA AGAGTAGTTA TTCTAAGTTT CCTGTAAAGG ATGGTGTTCC TACTCCAGGT
1741 GTATATGCGA TTCGTGAAGC AAGTGGTGGA AAAGTATCGC TACAAGCACT TGACATGTTA
1801 ATGAGATATG AGCCTCACCC GCAGCATGTT GCTGTCGAGG CAGTAAGAAC AGGTGCAGTA
1861 GGTGTATTGG AGCACCTTAT TACCACTGAA GTGATTAGTG TAAATGAAGA AATTACAACT
1921 CCTGAAGGAA AAAAGACAAC TTTGACCGCT GAAGCACTAA CTAGTGGTAA ATATGGTGTA
1981 GTGAAGGCGT TAATTAAAAA CAGTGCTGAT GTAAATGCGT CTCCAGAACC AGCTATTACT
2041 TTGGGTATAC AAGGAAGGTG CTTTCAGGGG AGTAAAGCTA TAAAGCATTT AAAGCGTGTT
2101 GTAGAAGCTG GGGCACATAT AAATACTCCT ACCGGATCTA TGAGCCCTTT AGCTGCTGCA
2161 GTTCAAGCGG CAAATGAGGC AAGTAACCTT AAAGAGGCTA ATAAGATTGT AAATTTCCTT
2221 TTACATAGGG GTGCAGATCT TTCGTCTACG GAACACACTG GAACTCCAGC CTTGCATTTA
2281 GCAACAGCTG CTGGCAACCA TAGGACTGCT ATGTTGCTCT TGGATAAAGG GGCTCCAGCA
2341 ACGCAGAGAG ATGCTAGGGG TAGGACGGCT TTACATATAG CAGCTGCTAA TGGTGACGGT
2401 AAGCTATATA GGATGATTGC GAAAAAATGC CCAGATAGCT GTCAACCACT CTGTTCTGAT
2461 ATGGGAGATA CAGCGTTACA TGAGGCTTTA TATTCTGATA ATGTTACAGA AAAATGCTTT
2521 TTAAAGATGC TTAAAGAGTC TCGAAAGCAT TTGTCAAACT CATCTTTTTT CGGAGACTTG
2581 CTTAATACTC CTCAAGAAGC AAATGGTGAC ACGTTACTGC ATCTGGCTGC ATCGCGTGGT
2641 TTTCGGTAAAG CATGTAAAAT ACTACTAAAG GCTGGGGCGT CAGTATCAGT CGTGAATGTA
2701 GAGGGAAAAA CACCGGTAGA TGTTGCGGAT CCATCATTGA AAACTCGTCC GTGGTTTTTT
2761 GGAAAGTCCG TTGTCACAAT GATGGCTGAA CGTGTTCAAG TTCCTGAAGG GGGATTCCCA
2821 CCATATCTGC CGCCTGAAAG TCCAACTCCT TCTTTAGGAT CTATTTCAAG TTTTGAGAGT
2881 GTCTCTGCGC TATCATCCTT GGGTAGTGGC CTAGATACTG CAGGAGCTGA GGAGTCTATC
2941 TACGAAGAAA TTAAGGATAC AGCAAAAGGT ACAACGGAAG TTGAAAGCAC ATATACAACT
3001 GTAGGAGCCTG AGGAGTCTAT CTACGAAGAA ATTAAGGATA CAGCAAAAGG TACAACGGAA
```

FIG. 6B

-continued

```
Ile Glu Ser Arg Val Phe Ala Asp Thr Ser Leu Gly Asn Phe Ser Val
145                 150                 155                 160

Gly Tyr Gln Glu Gly Val Glu Ser Lys Met Lys Val Asp Val Phe Gly
                165                 170                 175

Gly Leu Ser Gly Glu Asn Gly Ser Ala Trp Gly Arg Tyr Leu Arg Gly
            180                 185                 190

Phe Leu Lys Tyr Ala Lys Gly Val Pro Phe His Met Tyr Pro Gly Leu
        195                 200                 205

Tyr Ser Glu Asn Leu Phe Arg Ser Thr Arg Asp Leu Arg Gly Val Ser
    210                 215                 220

Gly Val Ser Ala Lys Thr Lys Asp Val Leu Asn Ser Met Pro Leu Arg
225                 230                 235                 240

Phe Ser Phe Glu Ser Ala Arg Leu Gly Gly Leu Ser Val Gly Phe Ser
                245                 250                 255

Tyr Ser Pro Thr Gly Tyr Arg Asp Asp Met Tyr Lys Gly Gly Glu Phe
            260                 265                 270

Thr Val Arg Asp Gly Ile Ala Gly Phe Asp Ser Leu Gly Thr Val Asn
        275                 280                 285

Leu Phe Ala Lys Thr Gly Val Lys Phe Gly Lys Met Ile Ala Val Val
    290                 295                 300

Pro Pro Arg Phe Asp Ser Gly Pro Val Tyr Lys Asn Ile Val Ser Gly
305                 310                 315                 320

Ala Ala Asn Tyr Glu Tyr Glu Leu Ala Asp Ile Ala Lys Phe Arg Leu
                325                 330                 335

Ser Leu Ala Gly Glu Tyr Ala Arg Pro Lys Lys Ala Arg Asp Ile Val
            340                 345                 350

Pro Glu Gly Arg Arg Lys Glu Glu Ile Tyr Val Ala Asp Tyr Asn Asp
        355                 360                 365

Leu Ser Ala Phe Ser Ser Gly Leu Glu Ile Asp Leu Gly Arg Leu Arg
    370                 375                 380

Phe Ala Val Gly Gly Gly Tyr Leu Gly Lys Ser Gly Ser Pro Lys Met
385                 390                 395                 400

Tyr Ile Leu Lys Asp Val Arg His Lys Val Pro Tyr Val Lys Lys Lys
                405                 410                 415

Gly Leu Pro Ser His Tyr Val Thr Ser Ala Val Ser Tyr Thr Ile Gly
            420                 425                 430

Ser Phe Ser Ala Thr Val Ala Tyr Phe Met Ser Arg Leu Thr His Ile
        435                 440                 445

Pro Pro Ala Thr Val Ser His Lys Ile Pro Gly Lys Tyr Glu Leu Asp
    450                 455                 460

Ser Val Val Asp Gly Glu Asn Thr Leu Lys Asp Leu Val Val Gly Val
465                 470                 475                 480

Gly Tyr Asn Leu Phe Ser Lys Gly Ser Thr Ser Leu Glu Val Phe Leu
                485                 490                 495

Asn Cys His Met Phe Ser Val Gln His Lys Phe Asn Ile His Glu Tyr
            500                 505                 510

Lys Ser Thr Glu Ser Ser Gly Phe Val Leu Lys Glu Gly Gly Glu Arg
        515                 520                 525

Ala Asn Thr Asn Asn Gly Ala Val Ala Leu Leu Gly Met Lys Phe Ala
    530                 535                 540

Phe
545
```

-continued

<210> SEQ ID NO 40
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 40

ctgcaggttt gatcctgg 18

<210> SEQ ID NO 41
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 41

ggatcctacc ttgttacgac tt 22

<210> SEQ ID NO 42
<211> LENGTH: 15
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 42

cacgccttct tctac 15

<210> SEQ ID NO 43
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 43

ctctgttgct ataggggc 18

<210> SEQ ID NO 44
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 44

gatgttgctt cgggtatgc 19

<210> SEQ ID NO 45
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 45

cagagattac ttctttttgc gg 22

<210> SEQ ID NO 46
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 46

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gcgtctccag aaccag 16

<210> SEQ ID NO 47
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 47

cctatatagc ttaccg 16

<210> SEQ ID NO 48
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 48

caggcagtga gcactcaaaa acc 23

<210> SEQ ID NO 49
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 49

gcgactccaa tgttacaata gtccc 25

<210> SEQ ID NO 50
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 50

tgtgatcctc gatggttggc 20

<210> SEQ ID NO 51
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 51

ccctcctgaa tcgtaacatc atcc 24

<210> SEQ ID NO 52
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 52

catgcttgta gctatg 16

<210> SEQ ID NO 53
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 53

gcaaactgaa caatatc 17

<210> SEQ ID NO 54
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 54

gacctagtac aggagc 16

<210> SEQ ID NO 55
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 55

ctataagcaa gcttag 16

<210> SEQ ID NO 56
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 56

gcgtcacaga cgaataagac gg 22

<210> SEQ ID NO 57
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 57

agcggagatt acaggagaga gctg 24

<210> SEQ ID NO 58
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 58

tgttgaatac ggggaaaggg ac 22

<210> SEQ ID NO 59
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 59

agcggagatt tcaggagaga gctg 24

-continued

<210> SEQ ID NO 60
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 60

tggtttggat tacagtccag cg 22

<210> SEQ ID NO 61
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 61

acctgcccag tttcacttac attc 24

<210> SEQ ID NO 62
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 62

ccggcatatg cttgtagcta tggaaggc 28

<210> SEQ ID NO 63
<211> LENGTH: 58
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 63

ccggctcgag ctagtggtgg tggtggtggt gaaaagcaaa cctaacacca aattcccc 58

<210> SEQ ID NO 64
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 64

ccccgggctt tacagt 16

<210> SEQ ID NO 65
<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 65

ccagcaagcg ataacc 16

<210> SEQ ID NO 66
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia

<400> SEQUENCE: 66

His Asp Asp Val Ser Ala Leu Glu Thr Gly Gly Ala Gly Tyr Phe

1 5 10 15

<210> SEQ ID NO 67
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia

<400> SEQUENCE: 67

Ser Gly Asp Asn Gly Ser Leu Ala Asp Tyr Thr Asp Gly Gly Ala Ser
1 5 10 15

Gln Thr Asn Lys
20

<210> SEQ ID NO 68
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia

<400> SEQUENCE: 68

Ala Val Gly Val Ser His Pro Gly Ile Asp Lys
1 5 10

<210> SEQ ID NO 69
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia

<400> SEQUENCE: 69

Phe Asp Trp Asn Thr Pro Asp Pro Arg
1 5

<210> SEQ ID NO 70
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia

<400> SEQUENCE: 70

Leu Ser Tyr Gln Leu Ser Pro Val Ile Ser Ala Phe Ala Gly Gly Phe
1 5 10 15

Tyr His

<210> SEQ ID NO 71
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 3, 6, 9, 15
<223> OTHER INFORMATION: n = A,T,C or G
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic nucleotide - degenerate primer

<400> SEQUENCE: 71

acnggnggng cwggntaytt y 21

<210> SEQ ID NO 72
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 3, 9, 18
<223> OTHER INFORMATION: n = A,T,C or G
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic nucleotide - degenerate primer

<400> SEQUENCE: 72

-continued

ccnccrtcng trtartcngc 20

<210> SEQ ID NO 73
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: E. chaffeensis

<400> SEQUENCE: 73

Ser Gly Ile Thr Glu Ser His Gly Lys Glu Asp Glu Ile Val Ser Gln
1 5 10 15

<210> SEQ ID NO 74
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia

<400> SEQUENCE: 74

Ser Thr Asp Leu Glu Ala His Ser Gln Glu Val Glu Thr Val Ser Glu
1 5 10 15

<210> SEQ ID NO 75
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia

<400> SEQUENCE: 75

Ser Thr Asp Leu Glu Ala His Ser Lys Gly Val Glu Ile Val Ser Glu
1 5 10 15

<210> SEQ ID NO 76
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia

<400> SEQUENCE: 76

Ser Thr Asp Leu Glu Val His Ser Gln Glu Val Glu Ile Val Ser Glu
1 5 10 15

<210> SEQ ID NO 77
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia

<400> SEQUENCE: 77

Ser Thr Asp Leu Glu Ala His Ser Pro Glu Gly Glu Ile Val Ser Glu
1 5 10 15

<210> SEQ ID NO 78
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: E. chaffeensis

<400> SEQUENCE: 78

Gln Pro Ser Ile Glu Pro Phe Val Ala Glu Ser Glu Val Ser Lys Val
1 5 10 15

Glu

<210> SEQ ID NO 79
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: E. chaffeensis

<400> SEQUENCE: 79

Gln Ser Ser Ser Glu Pro Phe Val Ala Glu Ser Glu Val Ser Lys Val

-continued

```
 1               5                  10                  15

Glu


<210> SEQ ID NO 80
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: E. chaffeensis

<400> SEQUENCE: 80

Gln Pro Ser Ser Glu Pro Phe Val Ala Glu Ser Glu Val Ser Lys Val
 1               5                  10                  15

Glu


<210> SEQ ID NO 81
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia

<400> SEQUENCE: 81

Gln Pro Val Ala Gln Val Pro Val Val Ala Glu Ala Glu Leu Pro Gly
 1               5                  10                  15

Val Glu


<210> SEQ ID NO 82
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: E. chaffeensis

<400> SEQUENCE: 82

Glu Ser Gly Val Ser Asp Gln Pro Ala Gln Val Val Thr Glu Arg Glu
 1               5                  10                  15


<210> SEQ ID NO 83
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia

<400> SEQUENCE: 83

Glu Ala Gly Ile Ser Asp Gln Glu Thr Gln Ala Thr Glu Glu Val Glu
 1               5                  10                  15


<210> SEQ ID NO 84
<211> LENGTH: 101
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia

<400> SEQUENCE: 84

Asn Phe Ala Phe Arg Ala Gly Ile Asn Phe Gly Tyr Ser Ala Ile Asn
 1               5                  10                  15

Ala Lys Val Lys Ala Ala Ala Gly Gly Ala Ile Asn Val Arg Asn Ser
            20                  25                  30

Ala Pro Ile Gly Ala Tyr Gln Glu Asp Phe Ile Gly His Tyr Phe Gly
        35                  40                  45

Gly Ala Ile Leu Ser Ile Glu Pro Thr Leu Lys Tyr Ser Val Gly Val
    50                  55                  60

Lys Gly Arg Tyr Ala Leu Lys Ser Thr Val His Asp Ala Ile Asn Ile
65                  70                  75                  80

Phe Ser Ala Gly Leu Gly Ala Cys Met Tyr Pro Pro Phe Glu Ile Asp
                85                  90                  95

Leu Lys Asp Ile Gly
            100
```

-continued

<210> SEQ ID NO 85
<211> LENGTH: 111
<212> TYPE: PRT
<213> ORGANISM: Granulocytic Ehrlichia

<400> SEQUENCE: 85

Gly Leu Ile Ser Pro Phe Thr Gln Leu Val Phe Ala Ser Leu Glu Phe
1 5 10 15

Phe Asn Gly Asn Tyr Leu Ser Ser Val Cys Val Phe Pro Ala Leu Ser
20 25 30

Pro Leu Val Asp Lys His Pro Ser Leu Pro Leu Glu Val Ile Val Phe
35 40 45

Leu Pro Ala Phe Thr Leu Ser Val Pro Cys Thr Lys Leu Ser Lys Ile
50 55 60

Leu Ala Val Ser Phe Ser Ser Pro Leu Leu Phe Gln Gly Val Glu Leu
65 70 75 80

Pro Val Pro Val Gly Phe Gly Thr Val Phe Ser Leu Ala Leu Ala Thr
85 90 95

Ala Leu Ser Arg Thr Cys Ser Ser Val Gly Ser Ser Phe Val Met
100 105 110

What is claimed is:

1. A polypeptide consisting essentially of an amino acid sequence encoded by a nucleotide sequence contained within nucleotides 232 to 760 of SEQ ID NO: 26, wherein the amino acid sequence encoded by the nucleotide sequence contained within nucleotides 232 to 760 of SEQ ID NO: 26 comprises an antigenic epitope of at least 7 amino acids in length.

2. The polypeptide of claim **1**, wherein the polypeptide consists of an amino acid sequence encoded by a nucleotide sequence contained within nucleotides 232 to 760 of SEQ ID NO:26.

3. The polypeptide of claim **1**, wherein the antigenic epitope comprises the amino acid sequence of SEQ ID NO: 69.

4. The polypeptide of claim **1**, wherein the amino acid sequence is at least 10 amino acids in length.

5. The polypeptide of claim **1**, wherein the amino acid sequence is at least 15 amino acids in length.

6. The polypeptide of claim **1**, wherein the amino acid sequence is at least 20 amino acids in length.

7. The polypeptide of claim **1**, wherein the amino acid sequence is at least 25 amino acids in length.

8. A composition comprising the polypeptide of any one of claims **1**-**3** and **7**.

9. A diagnostic kit comprising: a container that contains the polypeptide of any one of claims **1** to **3**.

10. A diagnostic kit comprising:

(i) a first container that contains the polypeptide of claim **7**; and

(ii) a second container that contains a detection reagent that detects an immunocomplex comprising the polypeptide bound to an antibody of a subject.

11. The diagnostic kit of claim **10**, wherein the first container further comprises a wash reagent.

12. The diagnostic kit of claim **10**, wherein the subject is a dog, horse, cow, pig, sheep, chicken, or human.

13. The diagnostic kit of claim **10**, wherein the subject is a dog.

14. The diagnostic kit of claim **10**, wherein the detection reagent comprises a radiolabel, an enzymatic label, or a fluorescent label.

15. A method of detecting an antibody to granulocytic Ehrlichia E8 protein in a sample obtained from a subject, comprising:

(a) contacting the sample with the polypeptide of any one of claims **1** to **3**, under conditions in which immunocomplexes form, wherein the immunocomplexes comprise the polypeptide bound to the antibody; and

(b) detecting the presence of the immunocomplexes, thereby detecting the antibody to granulocytic Ehrlichia E8 protein in the sample.

16. A method of detecting an antibody to granulocytic Ehrlichia E8 protein in a sample obtained from a subject, comprising:

(a) contacting the sample with the polypeptide of claim **7**, under conditions in which immunocomplexes form, wherein the immunocomplexes comprise the polypeptide bound to the antibody; and

(b) detecting the presence of the immunocomplexes, thereby detecting the antibody to granulocytic Ehrlichia E8 protein in the sample.

17. The method of claim **16**, wherein, in step (b), detecting the presence of the immunocomplexes comprises mixing the immunocomplexes with a detectably labeled antibody that binds specifically to the antibody in the immunocomplexes.

18. The method of claim **17**, wherein the detectably labeled antibody comprises a radiolabel, an enzymatic label, or a fluorescent label.

19. The method of claim **16**, wherein the sample is tissue, blood, serum, plasma, or urine.

20. The method of claim **16**, wherein the subject is a dog, horse, cow, pig, sheep, chicken, or human.

21. The method of claim **16**, wherein the subject is a dog.

* * * * *

```
3061 GTTGAAAGCA CATATACAAC TGTAGGAGCT GAAGGTCCGA GAACACCAGA AGGTGAAGAT
3121 CTGTATGCTA CTGTGGGAGC TGCAATTACT TCCGAGGCGC AAGCATCAGA TGCGGCGTCA
3181 TCTAAGGGAG AAAGGCCGGA ATCCATTTAT GCTGATCCAT TTGATATAGT GAAACCTAGG
3241 CAGGAAAGGC CTGAATCTAT CTATGCCTGAC CCATTTGCTG CGGAACGAAC ATCTTCTGGA
3301 GTAACGACAT TTGGCCCTAA GGAAGAGCCG ATTTATGCAA CAGTGAAAAA GGGTCCTAAG
3361 AAGAGTGATA CTTCTCAAAA AGAAGGAACA GCTTCTGAAA AAGTCTGCTC AACAATAACT
3421 GTGATTAAGA AGAAAGTGAA ACCTCAGGTT CCAGCTAGGA CAAGTAGTTT GCCTACTAAA
3481 GAAGGTATAG GTTCTGATAA AGACCTGAGT TCAGGAACTA GTAGCTCTTT TGCAGCTGAG
3541 CTGCAAGCAC AAAGGGGTAA ATTGCGTCCT GTGAAGGGAG GTGCTCCGGA TTCTACCAAA
3601 GACAAAACAG CTACTTCTAT ATTCTCCAGT AAAGAGTTCA AAAGGAACT AACAAAAGCT
3661 GCCGAAGGAT TACAGGGAGC AGTTGAAGAA GCTCAGAAGG GTGATGGAGG AGCTGCAAAG
3721 GCAAAGCAAG ATCTTGGCAT GGAATCTGGT GCCCCAGGAT CTCAACCAGA AGCTCCTCAA
3781 AGTGAAGGCC CTAAGTCTGT AAAAGGAGGT CGCGGTAGGT AGAATTATAC CGAAAAATCG
3841 CTGAGGTACT TTGATCAATA TAATTCGCGC TTCTGAGTAT TTAGGCGATG ATCTCGCCAC
3901 TTTAATAATA CCCCTTTTAG AGTACATAAC GCTCTAAAGG GGGCAGATTA TTTTAAGTAG
3961 TAGGGTTTTG ATTCTGAGAT CTTTTGAGTA CAACTATTCC TTAGTGTTTT TTTGGAATGC
4021 TATGTGCTTG ATAAAGAAAA AACTTGCTCT GGGGTGGGAT GCACTCTTGA GTACTTTCCG
4081 CGCTCTGTAT ATTCCTTTTT TTGCATCTGC ATAATCTGCT GCATATGTGA TTATGTGATA
4141 ATGACGGAAT TACCCAGAAA AGCCTTAGCG TGTGAGGCCT ATCATTCTCA GAAAGTCACA
4201 GTAGGAAACT TGCATTTTCA TCTTGTATTT TTGTAAGTTG GCTAAGAGCA CTAGCTATAA
4261 CAAATGCATC TATGGCATTT TTTGAGAGTT ATAATAATGA GAGCAACAAA GGGTGGTACT
4321 ATTGTTCAAA ATTTGTTTAT GTGCTTTGTC TCACAATGGA GTTTTAAAGTC ATCTCCGAGT
4381 AGTACTACGA CTTTAAGTAG AGAATACTTT GTATTTTCTT TATAGAGCTC AGAGATATAC
4441 TTCAGTATGT GTCGGAGGTT GTTCCCTTGG GAAAAGGGC ATTTTATCAA CTGTGAACTA
4501 TCGCTACTAT GGCTGAGGAA AAGTAGATAG CAACAAAGAT AGTATTCTGG TTTTATAATC
4561 AAACCGTAAT CTTTCAACAT GTTCGAAGAT CGCTTTCACT TTATAATCCT TTTTGACTGC
4621 CCTGCTGAAA GGGCTTTTTT GTTATGAAAC TATCCTCGCT CGATTTTCTT ATCTTTGGAT
4681 TCTATTACCA CGGATAATGT TTGTTGGAAT TATTTTAGAA GAAG (SEQ ID NO:3)
```

FIG. 6C

```
   1  MLRCNSMDVD ARSHDGRTII HYAAKDGNLE ILQQALGRKS SYSKFPVKDG VPTPGVYAIR
  61  EASGGKVSLQ ALDMLMRYEP HPQHVAVEAV RTGAVGVLEH LITTEVISVN EEITTPEGKK
 121  TTLTAEALTS GKYGVVKALI KNSADVNASP EPATTLGIQG RCFQGSKAIK HLKRVVEAGA
 181  HINTPTGSMS PLAAAVQAAN EASNLKEANK IVNFLLHRGA DLSSTEHTGT PALHLATAAG
 241  NHRTAMLLLD KGAPATQRDA RGRTALHIAA ANGDGKLYRM IAKKCPDSCQ PLCSDMGDTA
 301  LHEALYSDNV TEKCFLKMLK ESRKHLSNSS FFGDLLNTPQ EANGDTLLHL AASRGFGKAC
 361  KILLKAGASV SVVNVEGKTP VDVADPSLKT RPWFFGKSVV TMMAERVQVP EGGFPPYLPP
 421  ESPTPSLGSI SSFESVSALS SLGSGLDTAG AEESIYEEIK DTAKGTTEVE STYTTVGAEE
 481  SIYEEIKDTA KGTTEVESTY TTVGAEGPRT PEGEDLYATV GAAITSEAQA SDAASSKGER
 541  PESIYADPFD IVKPRQERPE SIYADPFAAE RTSSGVTTFG PKEEPIYATV KKGPKKSDTS
 601  QKEGTASEKV CSTTTVIKKK VKPQVPARTS SLPTKEGIGS DKDLSSGTSS SFAAEILQAQR
 661  GKLRPVKGGA PDSTKDKTAT SIFSSKEFKK ELTKAAEGLQ GAVEEAQKGD GGAAKAKQDL
 721  GMESGAPGSQ PEAPQSEGPK SVKGGRGR
```

(SEQ ID NO:4)

FIG. 7A

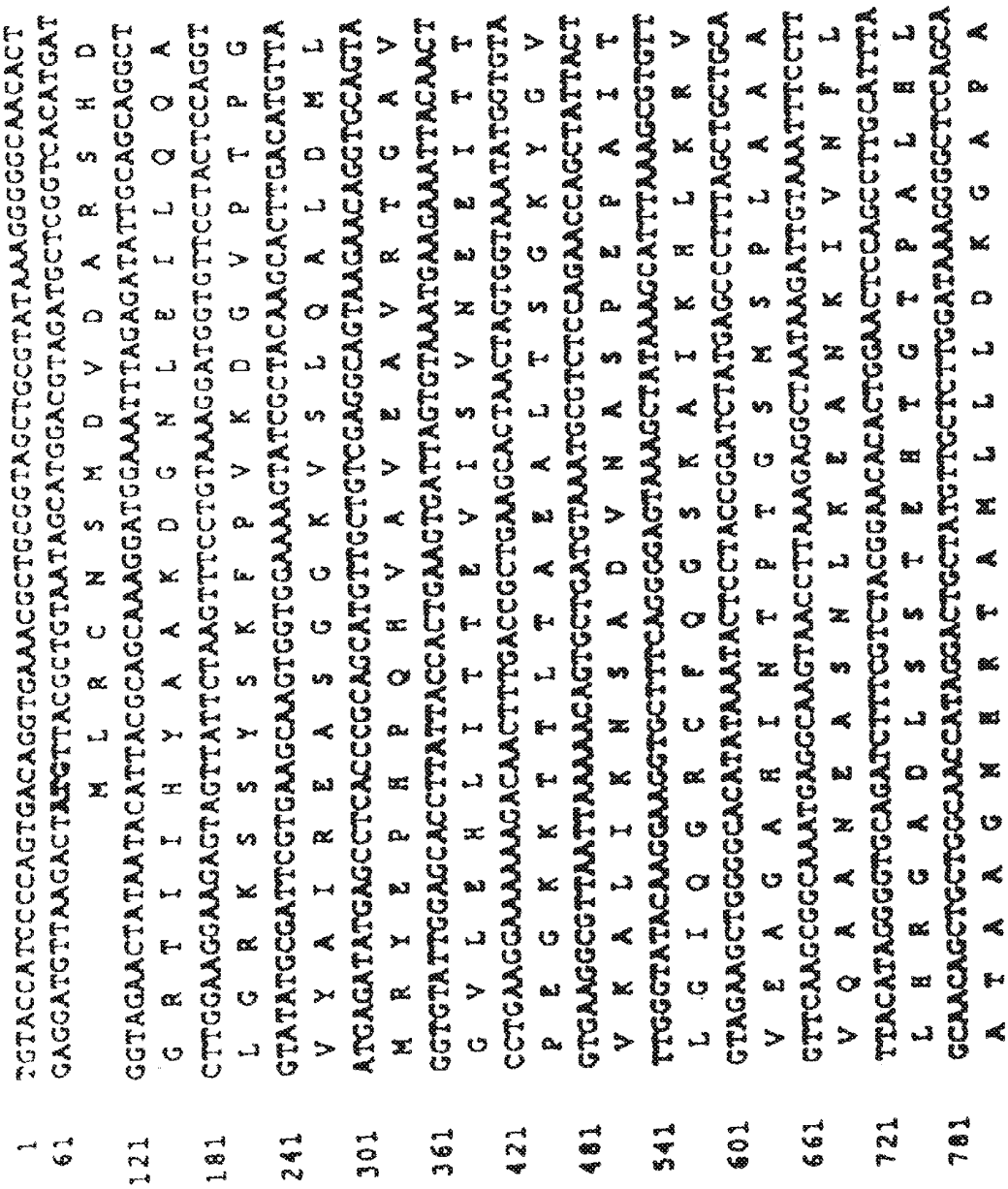
1 TGTACCATCCCCAGTGACAGGTGAAAGGCTGCCGGTAGCTGCGTATAAAGGGGGCAACACT
61 GAGGATGTTAAGACTAGGTTACGGCTGTAATAGCATGGACGTAGATGCTCGGTCACATGAT
M L R C N S M D V D A R S H D
121 GGTAGAACTATAATACATTACGGCAGCAAAGGATGGAAATTTAGAGATATTGCAGCAGGCT
G R T I I H Y A A K D G N L E I L Q Q A
181 CTTGGAAGGAAGAGTAGTTATTCTAAGTTTCCTGTAAAGGATGGTGTTCCTACTCCAGGT
L G R K S S Y S K F P V K D G V P T P G
241 GTATATGCGATTCGTGAAGCAAGTGGTGGAAAAGTATCGCTACAAGCACTTGACATGTTA
V Y A I R E A S G G K V S L Q A L D M L
301 ATGAGATATGAGCCTCACCGCCAGCATGTTGCTGTCGAGGCAGTAAGAACAGGTGCAGTA
M R Y E P H R Q H V A V E A V R T G A V
361 GGTGTATTGGAGCACCTTATTACCACTGAAGTGATTAGTGTAAATGAAGAAATTACAACT
G V L E H L I T T E V I S V N E E I T T
421 CCTGAAGAAAAAGACAACTTTGACCGCTGAAGCACTAACTAGTGGTAAATATGGTGTA
P E G K K T L T A E A L T S G K Y G V
481 GTGAAGGCGTTAATTAAAACAGTGCTGATGTAAATGCCTCTCCAGAACCAGCTATTACT
V K A L I K N S A D V N A S P E P A I T
541 TTGGGTATACAAGGAAGGTGCTTTCAGGGAGTAAAGCTATAAAGCATTTAAGCGTGTT
L G I Q G R C F Q G S K A I K H L K R V
601 GTAGAAGCTGGGGCACATATAAATACTCCTACCGATCTATGAGCCCTTTAGCTGCTGCA
V E A G A R I N T P T G S M S P L A A A
661 GTTCAAGCGGCAAATGAGCAAGTAACCTTAAAGAGGCTAATAAGATTGTAAATTTCCTT
V Q A A N E A S N L K E A N K I V N F L
721 TTACATAGGGGTGCAGATCTTTCGTCTACGGAACACACTGGAACTCCAGCCTTGCATTTA
L H R G A D L S S T E H T G T P A L H L
781 GCAACAGCTGCTGCCAACCATAGGACTGCTATGTTCCTCTTGGATAAAGGGCTCCAGCA
A T A A G M H R T A M L L L D K G A P A

FIG. 7B-1

```
841   ACGCAGAGATGCTAGCGTAGGACGGCTTTACATATGCAGCTGCTAATGGTGACGGT
        T  Q  R  D  A  R  G  R  T  A  L  H  I  A  A  A  N  G  D  G
901   AGCTATATAGGATGATTGCGAAAAATGCCCAGATAGCTGTCAACCACTCTGTTCTGAT
        K  L  Y  R  M  I  A  K  K  C  P  D  S  C  Q  P  L  C  S  D
961   ATGGAGATACAGCGTTACATGAGGCTTTATATTCTGATAATGTTACAGAAAATGCTTT
        M  G  D  T  A  L  H  E  A  L  Y  S  D  N  V  T  E  K  C  F
1021  TTAAGATGCTTAAGAGTCTCGAAGCATTGTCAACTCATCTTTTTCGGAGCTTG
        L  K  M  L  K  E  S  R  K  R  L  S  M  S  S  F  F  G  D  L
1081  CTTAATACTCCTCAAGAAGCAAATGGTGACACGTTACTGCATCTGGCTGCATCGCGTGGT
        L  N  T  P  Q  E  A  N  G  D  T  L  L  H  L  A  A  S  R  G
1141  TTCGGTAAAGCATGTAAAATACTACTATAAGGCTGGGGGCGTCAGTATCAGTCGTGAATGTA
        F  G  K  A  C  K  I  L  L  K  A  G  A  S  V  S  V  N  V
1201  GAGGGAAAACACCGGTAGATGTTGCGGATCCATCATTGAAACTCGTCCGTGGTTTTTT
        E  G  K  T  P  V  D  V  A  D  P  S  L  K  T  R  P  W  F  F
1261  GGAAGTCCGTTGTCACAATGATGGCTGAACGTTCCTGAAGGGGGATTCCCA
        G  K  S  V  V  T  M  M  A  E  R  V  Q  V  P  E  G  G  F  P
1321  CCATATCTGCCGCCTGAAGTCCAACTCCTTCTTTAGATCTATTTCAGTTTTGAGAGT
        P  Y  L  P  P  E  S  P  T  P  S  L  G  S  I  S  S  F  E  S
1381  GTCTTGGCTATCATCCTTGGGTAGTGGCCTAGATACTGCAGGAGCTGAGGAGTCTATC
        V  S  A  L  S  S  L  G  S  G  L  D  T  A  G  A  E  E  S  I
1441  TACGAAGAATTAAGGATACAGCAAAAGGTACAACGGAAGTTGAAAGCACATATACAACT
        Y  E  E  I  K  D  T  A  K  G  T  T  E  V  E  S  T  Y  T  T
1501  GTAGGAGCTGAGGAGTCTATCTACGAAGAAATTAAGGATACAGCAAAAGGTACAACGGAA
        V  G  A  E  E  S  I  Y  E  E  I  K  D  T  A  K  G  T  T  E
1561  GTTGAAGCACATATACAACTGTAGGAGCTGAAGGTCCGAGAACACCAGAAGGTGAAGAT
        V  E  S  T  Y  T  T  V  G  A  E  G  P  R  T  P  E  G  E  D
```

FIG. 7B-2

```
1621  CTGTATGCTACTGTGGAGCTGCAATTACTTCCGAGGCGCAAGCATCAGATGCGGCGTCA
        L  Y  A  T  V  G  A  A  I  T  S  E  A  Q  A  S  D  A  A  S
1681  TCTAAGGGAGAAAGGCCGGAATCCATTTATGCTGATCCATTTGATATAGTCAAACCTAGG
        S  K  G  E  R  P  E  S  I  Y  A  D  P  F  D  I  V  K  P  R
1741  CAGGAAAGGCCTGAATCTATCTATGCTGACCCATTTGCTGCGGAACGACATCTTCTGGA
        Q  E  R  P  E  S  I  Y  A  D  P  F  A  A  E  R  T  S  S  G
1801  GTAAGACATTTGCCCCTAAGGAAGCCGATTTATGCAACAGTGAAAAAGGGTCCTAAG
        V  T  I  F  G  P  K  E  E  P  I  Y  A  T  V  K  K  G  P  K
1861  AAGAGTGATACTTCTCAAAAGAAGGAACAGCTTCTGAAAAGTCTGCTCAACAATACT
        K  S  D  T  S  Q  K  E  G  T  A  S  E  K  V  C  S  T  I  T
1921  GTGATTAAGAGAAAGTGAAACCTCAGGTTCCAGCTAGGACAAGTAGTTTGCCTACTAAA
        V  I  K  K  K  V  K  P  Q  V  P  A  R  T  S  S  L  P  T  K
1981  GAAGGTATAGGTTCTGATAAAGACCTGAGTTCAGGAACTAGTAGCTCTTTTGCAGCTGAG
        E  G  I  G  S  D  K  D  L  S  S  G  T  S  S  F  A  A  E
2041  CTGCAAGCACAAAGGGTAAATTGCGTCCTGTGAAGGAGGTGCTCCGGATTCTACCAAA
        L  Q  A  Q  R  G  K  L  R  P  V  K  G  G  A  P  D  S  T  K
2101  GACAAAACAGCTACTTCTATATTCTCCAGTAAAGAGTTCAAAAAGGAACTAACAAAAGCT
        D  K  T  A  T  S  I  F  S  S  K  E  F  K  K  E  L  T  K  A
2161  GCCGAAGGATTACAGGAGCAGTTGAAAAGCTCAGAAGGGTGATGGAGGAGCTGCAAAG
        A  E  G  L  Q  G  A  V  E  E  A  Q  K  G  D  G  G  A  A  K
2221  GCAAAGCAAGATCTTGGCATGGAATCTGGTGCCCCAGGATCTCAACCAGAAGCTCCTCAA
        A  K  Q  D  L  G  M  E  S  G  A  P  G  S  Q  P  E  A  P  Q
2281  AGTGAAGGCCCTAAGTCTGTAAAAGGAGGTCGCGGTAGGCGAATTATACCGAAAAATCG
        S  E  G  P  K  S  V  K  G  G  R  G  R
2341  CTGAGGTACT
```

FIG. 7B-3

```
   1 GAATTCCTGA TAGTATTTTA GAGGATAGTA GGCAATATGG TTTAGGGGAT TTCTTCGCAT
  61 ACTTGTTATC ATCGTCCTTA TTTGTGTGCTTA GTTGGTCGGA TATTTGTGCA AGTTGTTGTA
 121 AAATATGCAT ATTGTATGTA TAGGTGTGCA AGATATCATC TCTTTTAGGTG TATCGTGTAG
 181 CACTTAAACA AATGCTGGTG AACGTAGAGG GATTAAAGGA GGATTTGCGT ATATGTATGG
 241 TATAGATATA GAGCTAAGTG ATTACAGAAT TGGTAGTGAA ACCATTTCCA GTGGAGATGA
 301 TGGCTACTAC GAAGGATGTG CTTGTGACAA AGATGCCAGC ACTAATGCGT ACTCGTATGA
 361 CAAGTGTAGG GTAGTACGGG GAACGTGGAG ACCGAGCGAA CTGGTTTTAT ATGTTGGTGA
 421 TGAGCATGTG GCATGTAGAG ATGTTGCTTC GGGTATGCAT CATGGTAATT TGCCAGGGAA
 481 GGTGTATTTT ATAGAGGCAG AAGCGGGCAG AGCTGCTACT GCTGAAGGTG GTGTTTATAC
 541 TACCGTTGTG GAGGCATTAT CGCTGGTGCA AGAGGAAGAG GGTACAGGTA TGTACTTGAT
 601 AAACGCACCA GAAAAAGCGG TCGTAAGGTT TTTCAAGATA GAAAAGAGTG CAGCAGAGGA
 661 ACCTCAAACA GTAGATCCTA GTGTAGTTGA GTCAGCAACA GGGTCGGGTG TAGATACGCA
 721 AGAAGAACAA GAAATAGATC AAGAAGCACC AGCAATTGAA GAAGTTGAGA CAGAAGAGCA
 781 AGAAGTTATT CTGGAAGAAG GTACTTTGAT AGATCTTGAG CAACCTGTAG CGCAAGTACC
 841 TGTAGTAGCT GAAGCAGAAT TACCTGGTGT TGAAGCTGCA GAAGCGATTG TACCATCACT
 901 AGAAGAAAAT AAGCTTCAAG AAGTGGTAGT TGCTCCAGAA GCGCAACAAC TAGAATCAGC
 961 TCCTGAAGTT TCTGCGCCAG CACAACCTGA GTCTACAGTT CTTGGTGTTG CTGAAGGTGA
1021 TCTAAAGTCT GAAGTATCTG TAGAAGCTAA TGCTGATGTA CCGCAAAAAG AAGTAATCTC
1081 TGGTCAACAA GAGCAAGAAA TTGCAGAAGC ACTAGAGGGA ACTGAAGCTC CTGTAGAAGT
1141 AAAAGAAGAA ACAGAAGTTC TTCTAAAGGA AGATACTTTG ATAGATCTTG AGCAACCTGT
1201 AGCACAAGTA CCTGTAGTAG CTGAAGCAGA ATTACCTGGT GTTGAAGCTG CAGAAGCGAT
1261 TGTACCATCA CTAGAAGAAA ATAAGCTTCA AGAAGTGGTA GTTGCTCCAG AAGCGCAACA
1321 ACTAGAATCA GCTCCTGAAG TTTCTGCGCC AGCACAACCT GAGTCTACAG TTCTTGGTGT
```

FIG. 8A

Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 35 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 37 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 38 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 50 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 51 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 52 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 53 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 54 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 55 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 56 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 57 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 58 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 59 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 60 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 61 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 62 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 63 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 64 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 65 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 66 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 67 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 68 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 69 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 70 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 71 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 72 in U.S. Patent No. 6,231,869, 2007.
Blast comparison of Seq ID Nos. 4, 6, 2, 8, 21, 22, 39, 27, 29 and 30 in U.S. Appl. No. 09/792,957 with Seq ID No. 73 in U.S. Patent No. 6,231,869, 2007.

* cited by examiner

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1381  TACTGAAGGT GATCTGAAGT CTGAAGTATC TGTAGAAGCT GATGCTGGTA TGCAGCAAGA
1441  AGCAGGAATC TCTGATCAAG AGACACAAGC AACTGAAGAA GTTGAAAAGG TTGAAGTATC
1501  TGTAGAAACA AAAACGGAAG AGCCAGAAGT TATTCTAGAA GAAGGTACTT TGATAGATCT
1561  TGAGCAACCT GTAGCGCAAG TACCTGTAGT AGCTGAAGCA GAATTACCTG GTGTTGAAGC
1621  TGCAGAAGCG ATTGTACCAT CACTAGAAGA AAATAAGCTT CAAGAAGTGG TAGTTGCTCC
1681  AGAAGCGCAA CAACTAGAAT CAGCTCCTGA AGTTTCTGCG CCAGTACAAC CTGAGTCTAC
1741  AGTTCTTGGT GTTACTGAAG GTGATCTGAA GTCTGAAGTA TCTGTAGAAG CTGATGCTGG
1801  TATGCAGCAA GAAGCAGGAA TCTCTGATCA AGAGACACAA GCAACTGAAG AAGTTGAGAA
1861  GGTTGAAGTA TCTGTAGAAG CTGATGCTGG TATGCAGCAA GAGTTAGTAG ATGTTCCGAC
1921  TGCTTTGCCG TTAAAGGATC CTGACGATGA AGATGTTCTA AGTTATTAGG ATATCTTTCT
1981  CGTGAAAAGT ATGGGGAAGG TTCGATGTGT TGAACCGTGC CCCATGCTTT TTCTTTAAGA
2041  TTTCTTCAAA AAGAGGTAAA ACTCTCCTAT GTTTTTTGTG AGCAGTAATT TCTTGCAGTT
2101  TTGCGACTGA GTTGTGTGTT ATTGCGAAGT TTTTCTTCTG ATTATTGGAC GAAGGTGGTG
2161  CTTGTCATGT CTGTGGTGCG TGCTTTCCAT GCTTGATAGA GCTCCTGATT ATTTTCTTTA
2221  TACGCAAGCC AGGTAAATCG TGTATGTGGC GACTTTTCGA ATCAGTGTTT AGATTACATA
2281  GAAGTAATTG TGGCTTATAC GCTGTTAATT GCGCTGCAAT CTGTCAAAAG TGATGCAGTA
2341  ACTTCCTCTA TATGTCCTAA TGCTGTTACA TGACATGGGT AATGCATAGC ATTATCAATG
2401  GTCATGGTGT CTTTAGTAGG CATACCAGCG GTTTTATATA CCAGTGATGC GCGAGCCTTG
2461  TTCTCCGCTT TCATAAAAGA TTTATTACTC AAGATATTGG TATACCTAGC GATTCACGTG
2521  TAATTTGAGT ACTTACCTGC GTATTTCGAA GGTAACGTAC TAATAGCGTA TGGTAAAACT
2581  ATCTATTATC CCAATCCCTA AGAATAACTA TGCTGTTTTG GAGCTGTTGC ATGCTGAAAG
2641  ATGTCTTATA GCATCGCGGT TATATATTTT CACATTTTAG AGATTTTAAG AGTATAACTT
2701  TCTAGCATCT TAGAGAACTA TACTCAAAGT TAAACACAAT AAAAACATGA AGCATTAAAA
2761  CTCAAGTATA CTAAACCAGC CTTAGACCTT AAAGGAAAGT AAGGAATGCT TATCTATGTT
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FIG. 8B

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2821  CAATTGTGCC ATTACTTAAA AAGCGAACCT AACACCGAAT TCCCCACCGA CATAAGCCAT
2881  GGAGAAATTA GCAATAGCAG TATCCTTAGT ACGACCCGCC GGACTAGTAT CATCTACAAG
2941  ACGTTGAGCC GGCAGATCAT CATAAACGCC ATCTCCAACA ACGCGATGAT AGAATCCACC
3001  CGCAAAAGCG GAGATTTCAG GTGAGAGCTG ATAACTCAAG CCAGCCTTTA ACCTCAAGCT
3061  TAGGAGTGAT GTTCTAGACA CCATCCGTAT TAGTCACAGA TTAGCTTCCT CTCGAAGTAC
3121  AGATAACCTC TGGAAAGTTT TAGAAAGGAC GGAATGTGTA ACGCCGCTCC GTGCCATCAA
3181  CCACGCCAAC GAAGTTACCG CCTAAACCAA CACAAGCATA AGGAACACAA CCTAAACCTT
3241  CACTAAGAAG ATCATAACAA GCATTAACCA TTACAGATGT AGAAGAAACA GCTCTGATCT
3301  CAACAACCTC TCCCCCTTCA ATAGTTTTAG CAAGTAATCC TGCTACTATG GTTTTTTTCAT
3361  CACGATTAAG ACCTAATAGG TCTTTAGCCA TAGCGTTTGC ATTACTATTA GGTTCTCCCT
3421  CGACGTTTTG ACTGCTGCCA TTACTCCCTC GTCCCCTAGG CCAGTTTTTA CCTTCACCGA
3481  CTTTCACAGT ATTAACAAAA CCACTCAACG TCTTTGGTCC TGTCGCCCCC GTCGTATTTC
3541  CCAAACCGCT ACACTGTGTT GTCTCCTCGT TGCCCGTGTGT CGTCGACAAC TCCGCAACAT
3601  ACTTCTTCCC CTTAGCCTTA GTTATAGCAG CATGATCCCC ACTACAAACC TTCCCATCAA
3661  TTTCAGGGCT GGAAATTTTC ACAGCATTGG CAAACTGAAC GATGTCTTTC CCAGAGGTTT
3721  TGGCAAGAGC AGCGGCAAGG TTATCAGTCT GCCCAGTAAC AACATCATAA GCTAACTCCT
3781  TAGCTAGTAG ATATACTGTA TCAGCTTCAT CTTCCTTACT ACCACTATCT CTAATACCCT
3841  TGGTCTTCCT TTTAATAATA AGAGTTATTG CATAGGATAT TGATATACCT ATCGATTTAT
3901  AGGCAGTTGA GCCGGAAGAT CATCTTAAAC ACCATCTCCC ACAACACGAT GGTAAAAGCC
3961  ACCCGCAGGA ATTCCGGAAT TCCGGAATTC CGGAATTC (SEQ ID NO:5)
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FIG. 8C

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   1  MYGIDIELSD YRIGSETISS GDDGYYEGCA CDKDASTNAY SYDKCRVVRG TWRPSELVLY
  61  VGDEHVACRD VASGMHHGNL PGKVYFIEAE AGRAATAEGG VYTTVVEALS LVQEEEGTGM
 121  YLINAPEKAV VRFFKIEKSA AEEPQTVDPS VVESATGSGV DTQEEQEIDQ EAPAIEEVET
 181  EEQEVILEEG TLIDLEQPVA QVPVVAEAEL PGVEAAEAIV PSLEENKLQE VVVAPEAQQL
 241  ESAPEVSAPA QPESTVLGVA EGDLKSEVSV EANADVPQKE VISGQQEQEI AEALEGTEAP
 301  VEVKEETEVL LKEDTLIDLE QPVAQVPVVA EAELPGVEAA EAIVPSLEEN KLQEVVVAPE
 361  AQQLESAPEV SAPAQPESTV LGVTEGDLKS EVSVEADAGM QQEAGISDQE TQATEEVEKV
 421  EVSVETKTEE PEVTLEEGTL IDLEQPVAQV PVVAEAELPG VEAAEAIVPS LEENKLQEVV
 481  VAPEAQQLES APEVSAPVQP ESTVLGVTEG DLKSEVSVEA DAGMQQEAGI SDQETQATEE
 541  VEKVEVSVEA DAGMQQELVD VPTALPLKDP DDEDVLSY   (SEQ ID NO:6)
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FIG. 9A

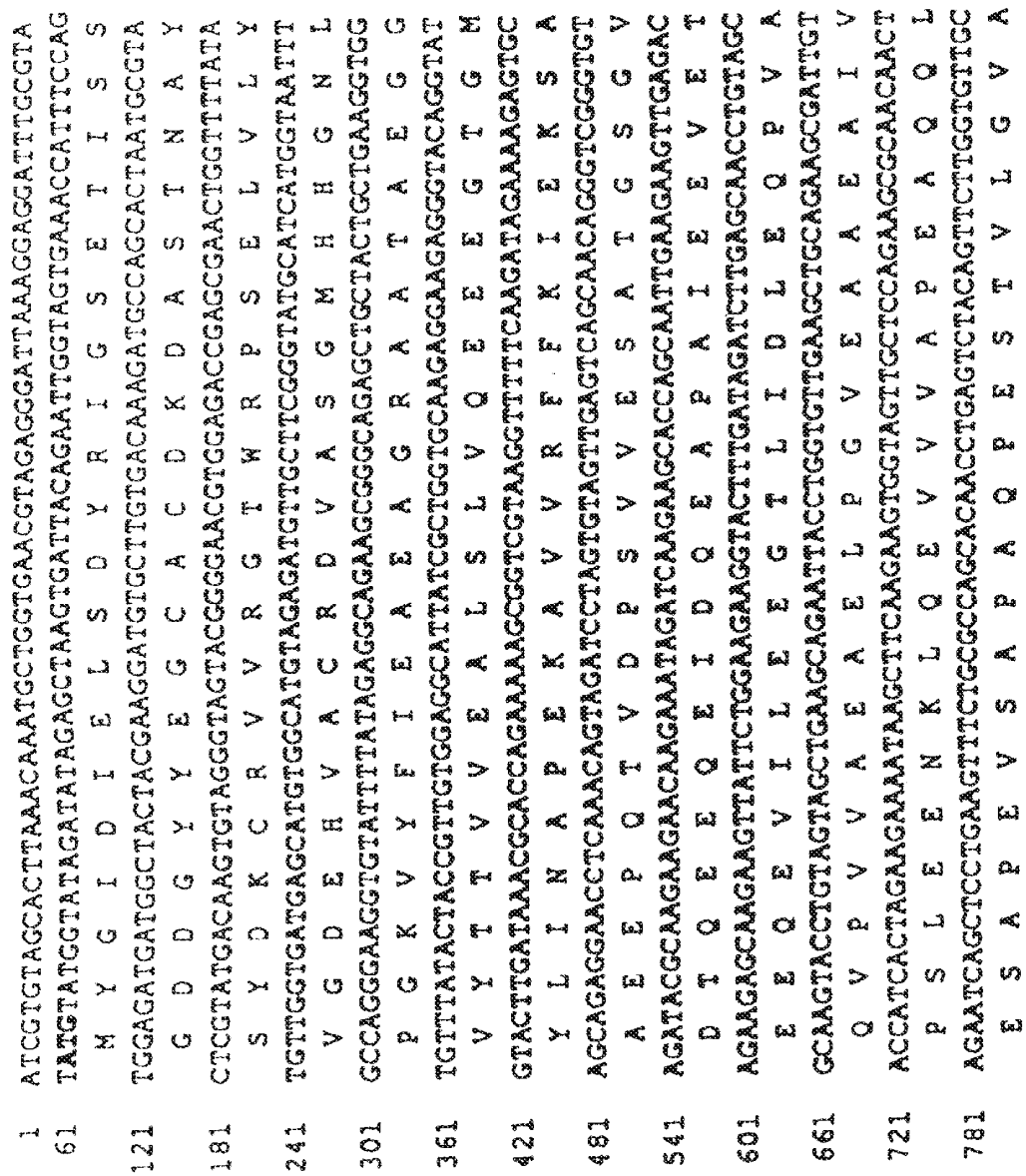
1 ATCGTGTAGCACTTAAACAAATGCTGGTGAACGTAGAGGGATTAAAGGAGGATTTGCGTA
61 TATGTATGGTATAGATATAGAGCTAAGTGATTACAGAATTGGTAGTGAAACCATTTCCAG
M Y G I D I E L S D Y R I G S E T I S S
121 TGGAGATGATGGCTACTACGAAGGATGTGCTTGTGACAAAGATGCCAGCACTAATGCGTA
G D D G Y Y E G C A C D K D A S T N A Y
181 CTCGTATGACAAGTGTAGGGTAGTACGGGGAACGTGGAGACCGAGCGAACTGGTTTTATA
S Y D K C R V V R G T W R P S E L V L Y
241 TGTTGGTGATGAGCATGTGGCATGTAGAGATGTTGCTTCGGGTATGCATCATGGTAATTT
V G D E H V A C R D V A S G M H H G N L
301 GCCAGGGAAGGTGTATTTTATAGAGGCAGAAGCGGGCAGAGCTGCTACTGCTGAAGGTGG
P G K V Y F I E A E A G R A A T A E G G
361 TGTTTATACTACCGTTGTGGAGGCATTATCGCTGGTGCAAGAGGAAGAGGGTACAGGTAT
V Y T T V V E A L S L V Q E E E G T G M
421 GTACTTGATAAACGCACCAGAAAAAGCGGTCGTAAGGTTTTTCAAGATAGAAAAGAGTGC
Y L I N A P E K A V V R F F K I E K S A
481 AGCAGAGGAACCTCAAACAGTAGATCCTAGTGTAGTTGAGTCAGCAACAGGGTCGGGTGT
A E E P Q T V D P S V V E S A T G S G V
541 AGATACGCAAGAAGAACAAGAAATAGATCAAGAAGCACCAGCAATTGAAGAGTTGAGAC
D T Q E E Q E E I D Q E A P A I E E V E T
601 AGAAGAGCAAGAAGTTATTCTGGAAGAAGGTACTTTGATAGATCTTGAGCAACCTGTAGC
E E Q E V I L E E G T L I D L E Q P V A
661 GCAAGTACCTGTAGTAGCTGAAGCAGAATTACCTGGTGTTGAAGCTGCAGAAGCGATTGT
Q V P V V A E A E L P G V E A A E A I V
721 ACCATCACTAGAAGAAATAAGCTTCAAGAAGTGGTAGTTGCTCCAGAAGCGCAACAACT
P S L E E N K L Q E V V V A P E A Q Q L
781 AGAATCAGCTCCTGAAGTTTCTGGCCAGCACAACCTGAGTCTACAGTTCTTGGTGTTGC
E S A P E V S A P A Q P E S T V L G V A

FIG. 9B-1

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841  TGAAGGTGATCTAAAGTCTGAAGTATCTGTAGAAGCTAATGCTGATGTACCGCAAAAAGA
       E  G  D  L  K  S  E  V  S  V  E  A  N  A  D  V  P  Q  K  E
901  AGTAATCTCTGGTCAACAAGAGCAAGAAATTGCAGAAGCACTAGAGGGAACTGAAGCTCC
       V  I  S  G  Q  Q  E  Q  E  I  A  E  A  L  E  G  T  E  A  P
961  TGTAGAAGTAAAAGAAGAAACAGAAGTTCTTCTAAAGGAAGATACTTTGATAGATCTTGA
       V  E  V  K  E  E  T  E  V  L  L  K  E  D  T  L  I  D  L  E
1021 GCAACCTGTAGCACAAGTACCTGTAGTAGCTGAAGCAGAATTACCTGGTGTTGAAGCTGC
       Q  P  V  A  Q  V  P  V  V  A  E  A  E  L  P  G  V  E  A  A
1081 AGAAGCGATTGTACCATCACTAGAAGAAAATAAGCTTCAAGAAGTGGTAGTTGCTCCAGA
       E  A  I  V  P  S  L  E  E  N  K  L  Q  E  V  V  V  A  P  E
1141 AGCGCAACAACTAGAATCAGCTCCTGAAGTTTCTGCGCCAGCACAACCTGAGTCTACAGT
       A  Q  Q  L  E  S  A  P  E  V  S  A  P  A  Q  P  E  S  T  V
1201 TCTTGGTGTTACTGAAGGTGATCTGAAGTCTGAAGTATCTGTAGAAGCTGATGCTGGTAT
       L  G  V  T  E  G  D  L  K  S  E  V  S  V  E  A  D  A  G  M
1261 GCAGCAAGAAGCAGGAATCTCTGATCAAGAGACACAAGCAACTGAAGAAGTTGAAAAGGT
       Q  Q  E  A  G  I  S  D  Q  E  T  Q  A  T  E  E  V  E  K  V
1321 TGAAGTATCTGTAGAACAAAAACGGAAGAGCCAGAAGTTATTCTAGAAGAAGGTACTTT
       E  V  S  V  E  T  K  T  E  E  P  E  V  I  L  E  E  G  T  L
1381 GATAGATCTTGAGCAACCTGTAGCGCAAGTACCTGTAGTAGCTGAAGCAGAATTACCTGG
       I  D  L  E  Q  P  V  A  Q  V  P  V  V  A  E  A  E  L  P  G
1441 TGTTGAAGCTGCAGAAGCGATTGTACCATCACTAGAAGAAATAAGCTTCAAGAAGTGGT
       V  E  A  A  E  A  I  V  P  S  L  E  E  N  K  L  Q  E  V  V
1501 AGTTGCTCCAGAAGCGCAACAACTAGAATCAGCTCCTGAAGTTTCTGCGCCAGTACAACC
       V  A  P  E  A  Q  Q  L  E  S  A  P  E  V  S  A  P  V  Q  P
1561 TGAGTCTACAGTTCTTGGTGTTACTGAAGGTGATCTGAAGTCTGAAGTATCTGTAGAAGC
       E  S  T  V  L  G  V  T  E  G  D  L  K  S  E  V  S  V  E  A
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FIG. 9B-2

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1621  TGATGCTGGTATGCAGCAAGAAGGCAGGAATCTCTGATCAAGAGACACAAGCAACTGAAGA
        D  A  G  M  Q  Q  E  E  A  G  G  I  S  D  D  Q  E  E  T  Q  A  T  E  E
1681  AGTTGAGAAGGTTGAAGTATCTGTAGAAGCTGATGCTGGTATGCAGCAAGAGTTAGTAGA
        V  E  K  V  E  V  S  V  E  E  A  D  D  A  G  G  M  Q  Q  Q  E  E  L  V  D
1741  TGTTCCGACTGCTTTGCCGTTAAAGGATCCTGACGATGAAGATGTTCTAAGTTATTAGGA
        V  P  T  A  L  P  L  K  D  D  P  D  D  D  E  D  V  L  S  Y
1801  TATCTTTCTCGTGAAAAGTATGGGGAAGGT
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FIG. 9B-3

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   1 GAATTCCCTG TGGTTATTAG GCGTGGTTTC GCCTGATAAT AAAGATACTT TAGAGGGTAT
  61 AAACTTGGAA AAAATAATGA AAAACCCTCC TTAGTGCCTC CCCGTTTTTG ACAACATACT
 121 CTTATGGAAA AGCGTTAGGG AGTTGCTTCG CTTGTCACGC GTGCGTTAGG TTTTACGTAT
 181 ACGTGTCTGG GACTTCACGA AAACTCGACG CAGGCGGATT TTGTACTATG TTTCACTTAA
 241 CAAGGTATTA TAAATGTTTG AACACAATAT TCCTGATACA TACACAGGAA CAACTGCAGA
 301 AGGTTCTCCT GGCTTAGCAG GCGGGGATTT TAGCTTAAGT TCTATTGACT TTACAAGGGA
 361 CTTTACAATT GAATCACATA GAGGAAGCTC AGCTGATGAC CCAGGTTACA TCAGCTTTAG
 421 GGATCAAGAC GGAAACGTCA TGTCACGTTT TCTTGATGTG TACGTAGCTA ATTTCAGCTT
 481 GCGATGCAAG CATTCTCCCT ATAACAACGA CAGAATGGAA ACAGCTGCGT TCTCTCTAAC
 541 TCCCGACATA ATAGAGCCTT CTGCTTTATT GCAAGAATCA CATAGTACAC AAACAATGT
 601 AGAAGAGGCA GTACAAGTTA CAGCTCTTGA GTGCCCTCCA TGTAATCCAG TCCCTGCCGA
 661 GGAAGTAGCT CCTCAACCGT CTTTTCTAAG CAGAATAATT CAGGCGTTCT TGTGGTTATT
 721 CACGCCTTCT TCTACTACCG ACACTGCTGA AGACAGCAAG TGTAATAGTA GCGATACTTC
 781 AAAATGTACC TCTGCTAGCA GTGAGTCATT AGAGCCAGCAA CAAGAATCAG TGGAAGTGCA
 841 ACCATCTGTA CTTATGTCTA CTGCCCCTAT AGCAACAGAG CCTCAAAATG CGGTTGTTAA
 901 CCAAGTAAAC ACTACTGCAG TACAAGTAGA ATCATCCATT ATTGTGCCAG AATCGCAACA
 961 CACTGACGTT ACCGTGCTCG AAGATACTAC TGAGACGATA ACTGTTGATG GGGAATATGG
1021 ACATTTTAGT GACATTGCTT CAGGTGAACA CAATAACGAT CTGCCTGCCA TGTTGTTAGA
1081 TGAAGCAGAC TTCACTATGT TATTAGCGAA CGAGGAGTCA AAGACCCTGG AGTCTATGCC
1141 TTCTGATAGC CTAGAAGACA ATGTTCAGGA ACTAGGTACA TTGCCTTTAC AAGAAGGTGA
1201 AACAGTTTCT GAGGGCAACA CACGAGAGTC ACTACCCACT GACGTTTCAC AAGACTCAGT
1261 TGGTGTAAGT ACAGATCTTG AAGCTCATTC TCAAGAAGTT GAAACAGTTT CTGAGGTCAG
1321 CACACAAGAT TCACTATCCA CTAACATTTC ACAAGACTCA GTTGGTGTAA GTACAGATCT
1381 TGAAGTTCAT TCTCAAGAAG TTGAAATAGT TTCTGAGGGC GGCACACAAG ATTCACTATC
1441 CACTAACATT TCACAAGACT CAGTTGGTGT AAGTACAGAT CTTGAAGCTC ATTCTAAAGG
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FIG. 10A

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1501    AGTTGAAATA GTTTCTGAGG GCGGCACACA AGATTCACTA TCCGCTGATT TTCCAATAAA
1561    CACAGTTGAA AGTGAAAGTA CAGATCTTGA AGCTCATTCC CCAGAAGGTG AAATAGTTTC
1621    TGAGGTCAGC ACACAAGATG CGCCATCCAC TGGAGTAGAG ATCAGATTTA TGGATCGTGA
1681    TTCTGATGAT GACGTGCTCG CGTTGTGAAG TGATCATGGT AGGGGAAACA GTTATGGCGT
1741    AAAGACATCT TTGATGACTT GTCTTGCGTG AATAAGTAGT GCAAGTTTTT TATGCATTGA
1801    TGTGCATGAT CATTGCCCCT AAGGAAAGCA GTACTAATGG TAGTCTAAGA TCTTATACAG
1861    GGTTTCGGAC TACCACTTTT GGTGTTTTAA AACGTCTTAT TCGCGTTGGG TGCTTGCTTA
1921    CAATGTACCT GTACGTGCCC AACACTAAAA ATGGTCAGTA TTACTTAGGG GAGTTCGTAG
1981    ACGAGGCATC TCGATTTACT CTAAGTAAGC TACAAATAAC TCAGTCATAT CAAGGTAGTT
2041    CAAGATGAAA GCAGTGCTAT GCTTATCATG GAGAATTCCT GCGGTTCTCT TCAAAATTCT
2101    CTTTTCCCGC AAGGGCAGAC TCTTATTTGT TAAAATAACA AAATTTCTCT ACAGGAAGCG
2161    ACATTTCATA TCAAAGCTGA TTGTGAAATA ATGGCATTGA GTATTTTTCT CGCCCTAGAA
2221    GATAATCATT TCGGCACTAT CAAAGCATTT ACGATATTCT CCATTATCTT GTAATCAGAT
2281    GGCTATCTTG AAAGCAACCA AGGATATCCG TACATGGTAG CTTACATACT GCTATCAATC
2341    TCCTATACGA CCTTCAATGA AACGGTAACT GTTGCTGACA GCTTGCACAT GCTGTGATTC
2401    AATTCCTGGT TCCTAGATGT TCTACTACGT TTATCCGGTA CTAATATTAT TCTTTGGCGC
2461    TCTATTATCT AGCAACTCAG AGTCCATTAT TGGATCTCTA ATACCAAGGG TATAAGGGAA
2521    AGTGGAAGAG TATTATTAGA GAGAAGAAGC AAATACAGTA TATCTACTAG CTAAGGAGTT
2581    AGCTTATGAT GTTGTTACTG GACAGACTGA TAAGCTTGCT GCTGCTCTTG CCAAGACCTC
2641    CGGGAAAGAT ATCGTTCAGT TTGCTAAGGC AGTTGAGATT TCGGCTCCTA AGATCGATAA
2701    GCAAGTTTGT GTGACTAATA AGAATGGGGA TAGCGGAACA AGATATGCTA AGTACCTCGA
2761    AGAAGCTGGA ACGTCTAGCA ATGCTGGCAC GTCGTTGTGT GGTGGTAAAA ACCTAAAGAC
2821    GACTGACTCC AACACAGGAG TAGAGAAAGG ACAGGTGTTA CATGACTTTG TTTCTGGAAC
2881    GTTGAGTGGG GGTACTAAGA ACTGGCCGAC ATCTAGTGAA AGTACTAAAG AAAATAACGA
2941    CAACGCAGGG AAGGTAGCTA AAGACCTGAC AAAACTAACC CCTGAAGAAA AAACCATAGT
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FIG. 10B

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3001  AGCAGGGTTA CTAGCTAAGA CTATTGAAGG GGGTGAAGTT GTTGAGATCA GGGCGGTTTC
3061  TTCTACTTCT GTGATGGTTA ATGCTTGTTA TGATCTTCTT AGTGAAGGTT TAGGTGTCGT
3121  TCCTTACGCT TGTGTTGGTC TTGGGGGTAA CTTCGTGGGG GGTTGTTGAT GGCACGGCGC
3181  AGCGTTACAC AATCCGTCCT TGACCTGAAT ACTCTAGTTA AGCACTAGGC AAAATTAGTG
3241  CTGGATCACT TACGCAACAT ACTACGGTCA GCGATTTTCC ATACTGAGCA GGTACGTACA
3301  GTGGCTTTAT ACTCTTACCC AGCATGAAAT TACTTGTTAT CTAAGAATCT CCACAGCTGA
3361  CCTTAGAAAG GTTATCTGTC C-TTCGAGAG AAAGCTAATC TGTGTCTTAT GCGGATGGCG
3421  TTGAACGTAT TACAGGTCCC AAGCTGTCTT GCAAGTTTCT AAGGATATTA TAAGGGCACA
3481  CCTATAAAAC TGCGCAATAT ATCACCTGCA ATACGGTCCC GATTCGAAAA CACTGGGAAG
3541  TGCGCTCATT ATCTATGAAT CGCTAGCTAG GCATAAATAA GAGTATACGC AATAACGCTT
3601  ATTATTAAAA ACAAGACCAA GGGTATTAGA GATAGTGGTA GTAAGGAAGA TGAAGCTGAT
3661  ACAGTATATC TACTAGCTAA GGAGTTAGCT TATGATGTTG TTACTGGGCA GACTGATAAC
3721  CTTGCTGCTG CTCTTGCCAA GACTTCTGGT AAAGATATTG TTCAGTTTGC TAAGACTCTT
3781  AATATTTCTC ACTCTAATAT CGATGGGAAG GTTTGTAGGA GGGAAAAGCA TGGGAGTCAA
3841  GGTTTGACTG GAACCAAAGC AGGTTCGTGT GATAGTCAGC CACAAACGGC GGGTTTCGAT
3901  TCCATGAAAC AAGGTTTGAT GGCAGCTTTA GGCGAACAAG GCGCTGAAAA GTGGCCCAAA
3961  ATTAACAATG GTGGCCACGC AACAATTTAT AGTAGTAGCG CAGGTCCAGG AAATGCGTAT
4021  GCTAGAGATG CATCTACTAC GGTAGCTACA GACCTAACAA AGCTCACTAC TGAAGAAAAA
4081  ACCATAGTAG CAGGGTTACT AGCTAGAACT ATTGAAGGGG GTGAAGTTGT TGAGATTAGG
4141  GCAGTTTCTT CTACTTCTGT GATGGTTAAT GCTTGTTATG ATCTTCTGAA GGGAAACCCA
4201  TCACATTATT TATCTGGTTG CTGTAATCTG ATCTTCCCGT TGCTATGATC GCATCTCCCC
4261  CTCACTTCTC TCGCAAACTC TGGATTAACC TCTGGATGCG AATAATGTTT ATCAGCTTTG
4321  AGAAAAACAT ATTAGAGTTT TATACAGCAC CAATGATAAG CGTGGGCACT TAAATAAAGG
4381  TTCATATCCC TAGAAATTTA TCCCACTAGC TAAAACTATT GAAGGGGGTG AGGTCGTTGA
4441  GATAAGGGCA GTTTCTTCTA CTTCTGTGAT GGTTAATGCT TGTTATGATC TTCTTAGTGA
4501  AGGTTTAGGC GTTGTTCCTT ATGCTTGCGT GGGTCTTGGT GGGAACTTCG TGGGCGTGGT
```

FIG. 10C

```
4561  TGATGGGCAT ATCACAAACC ACTCCATCTC TGACCCTGTA TGCACTAGCA AGTAACTAGG
4621  CAAAATTATT GCTGCATCAC TTTGAAACAA ACTACGATCA GCAATGTTCA ATACTTAGCA
4681  GGTCTGTACA GTGGCTTTAC ACTCTTACCC AGCATGAAAT ACTTGCTATC TAAGAATCTC
4741  CTCTAAAACT TTCCAGAGGT TATCTGTACT TTGAGGGAAG CTAATCTGTG GCTAATGAGG
4801  ATGGTGTCTA GAATATCACT CCTAAGCTTG CTTATAGGTT AAAGGCTGGG TTGAGTTATC
4861  AGCTTTCTCC TGAAATCTCT GCTTTTGTAG GTGGTTTCTA TCATTGGTTT GTTGGTTATG
4921  GTGTTTATGA TGATCTTCCG GCTCAACGTC TTGTAGATGA TACTAGTCCG GCGGGTCGTA
4981  CTAAGGATAC TGCTATTTGCT AACTTCTCCA TGGCCTATGT TGGTGCATCA TTTCAGCCAT
5041  ATTACGCCAGT TCTTCTAGTA GAACTGTATG AGTATCGATA AAAAGATATA CGACGCAGCA
5101  TGCTCTGAAA AAGTGACAGG GTGAACAGCA GTGCAGCATA CATTAGCCTG TCTGTATCAG
5161  TGTGCAATCT GTTACAAAAC TACTAGGATC ATTCTTTTAA CACCTATCGG GTTCATCAAG
5221  AAAATGAGTG CATACGTGAC GGCAAGCGCT AAAACGCTAA AGCTGATGCA TCACATTAAA
5281  ATGTGTTGAC GTTAGACCTT CCAAGCACTG TAAAGTGTGG CGATTCCCCC GCATACGTTC
5341  TTATGCGTGA TTTTGAAAAA CCCGGCGTCT GCAACTTCGG AAGAGAATTG TTTTTGTGTG
5401  GGAAAAGCTC TTATGCTTTC AACTAAGTAG GAATATGCAT CAATATCCCC GGCTACTATT
5461  TTGCCAATTT TCGGTATAAC AAAGAAAGAA TACAGGTCAT AAGCTGTTTT GAATGCACTG
5521  TCTTCGAGTA TAGGAGAAAA CTCCAAGCAT AAAAACCTAC CACATGGTTT CAGGAATTC
```

(SEQ ID NO:7)

FIG. 10D

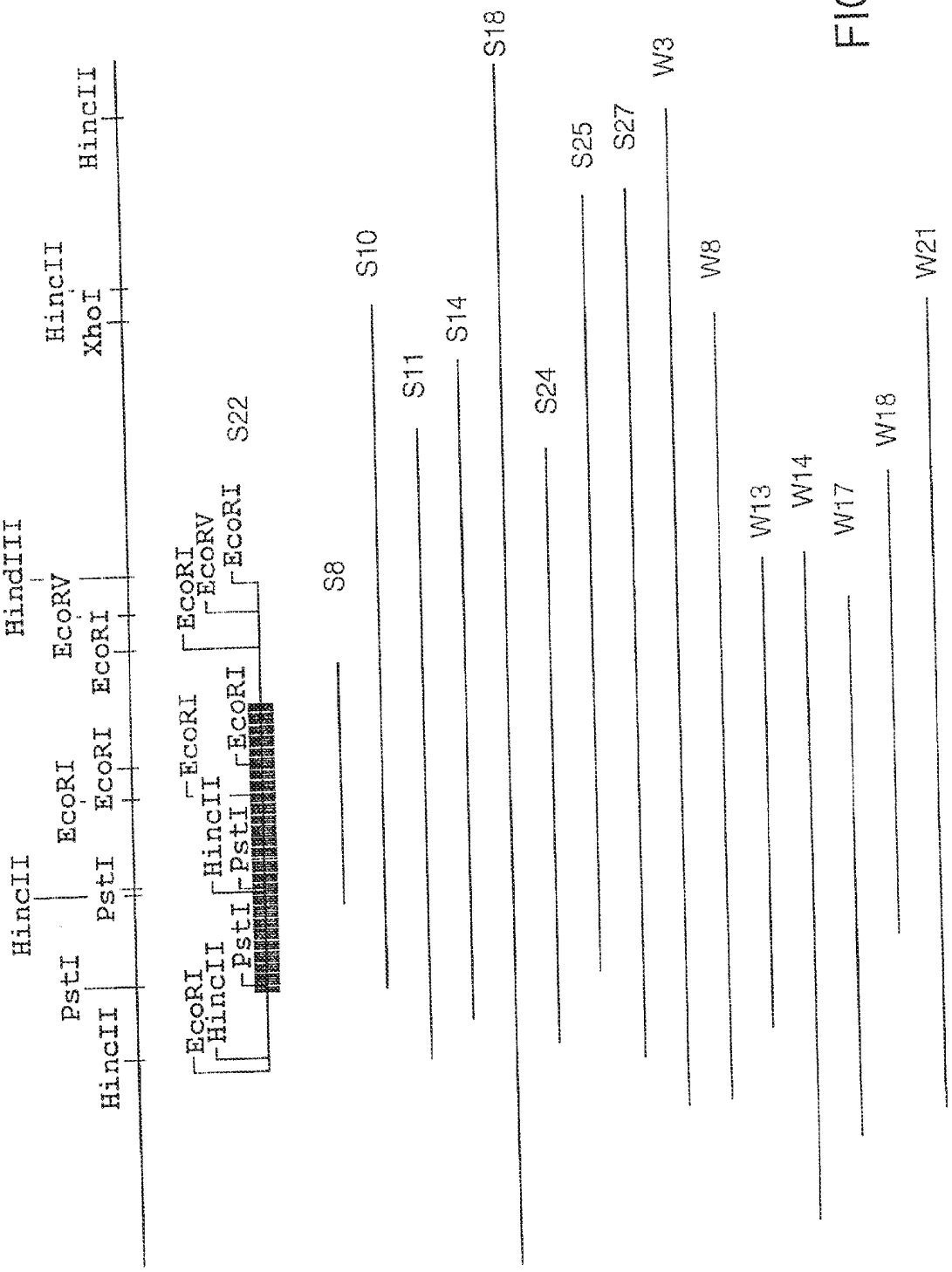

HincII
PstI
HincII
EcoRI
PstI
EcoRI
HindIII
EcoRV
EcoRI
HincII
XhoI
HincII
EcoRI
HincII
PstI
EcoRI
EcoRV
EcoRI
S22
S8
S10
S11
S14
S18
S24
S25
S27
W3
W8
W13
W14
W17
W18
W21

FIG. 1

```
   1  MFEHNIPDTY TGTTAEGSPG LAGGDFSLSS IDFTRDFTIE SHRGSSADDP GYISFRDQDG
  61  NVMSRFLDVY VANFSLRCKH SPYNNDRMET AAFSLTPDII EPSALLQESH STQNNVEEAV
 121  QVTALECPPC NPVPAEEVAP QPSFLSRJIQ AFLWLFTPSS TTDTAEDSKC NSSDTSKCTS
 181  ASSESLEQQQ ESVEVQPSVL MSTAPIATEP QNAVVNQVNT TAVQVESSII VPESQHTDVT
 241  VLEDTTETIT VDGEYGHFSD IASGEHNNDL PAMLLDEADF TMLLANEESK TLESMPSDSL
 301  EDNVQELGTL PLQEGETVSE GNTRESLPTD VSQDSVGVST DLEAHSQEVE TVSEVSTQDS
 361  LSTNISQDSV GVSTDLEVHS QEVEIVSEGG TQDSLSTNIS QDSVGVSTDL EAHSKGVEIV
 421  SEGGTQDSLS ADFPINTVES ESTDLEAHSP EGEIVSEVST QDAPSTGVEI RFMDRDSDDD
 481  VLAL  (SEQ ID NO:8)
```

FIG. 11

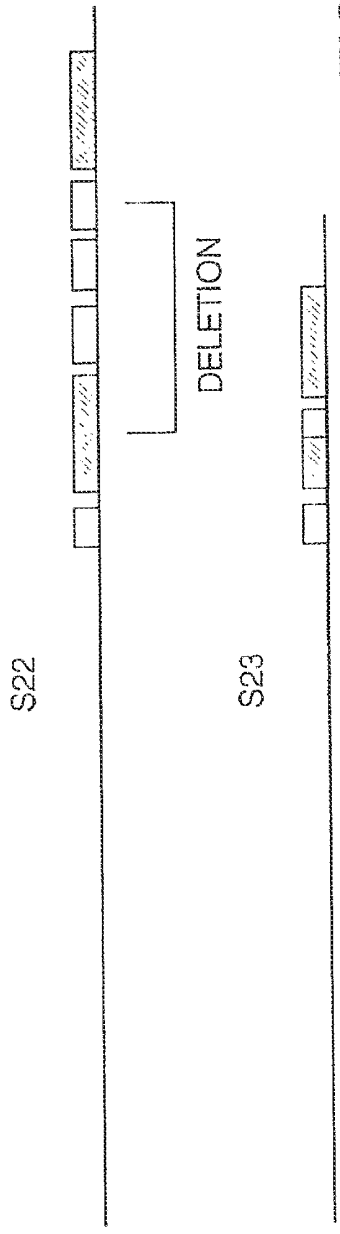


FIG. 12

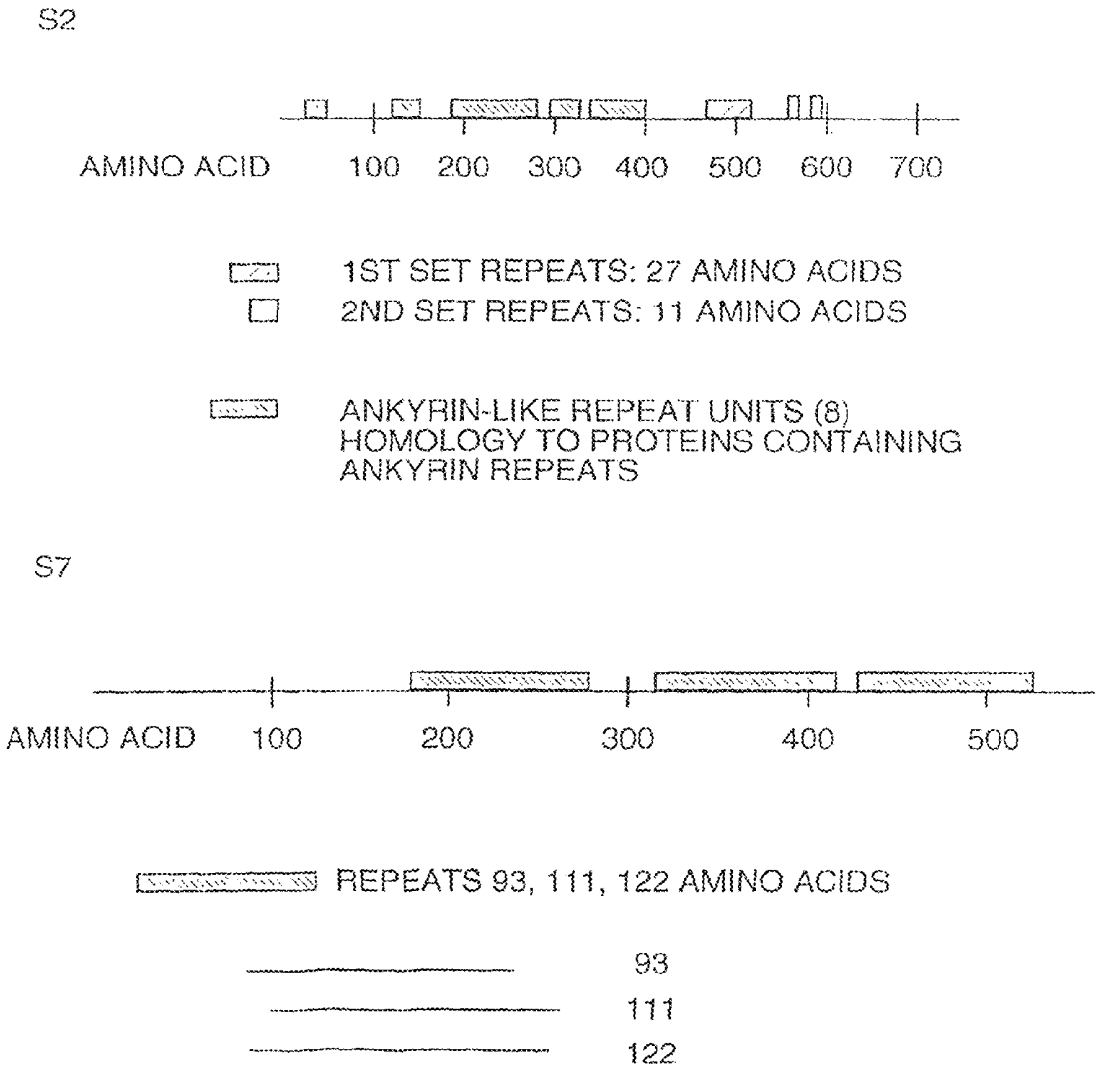
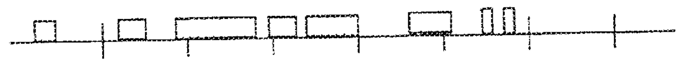

S2
AMINO ACID
100
200
300
400
500
600
700
1ST SET REPEATS: 27 AMINO ACIDS
2ND SET REPEATS: 11 AMINO ACIDS
ANKYRIN-LIKE REPEAT UNITS (8)
HOMOLOGY TO PROTEINS CONTAINING
ANKYRIN REPEATS
S7
AMINO ACID
100
200
300
400
500
REPEATS 93, 111, 122 AMINO ACIDS
93
111
122

FIG. 13

S2
160 kDa PROTEIN



ANKYRIN-LIKE REPEAT UNITS (1-8)
27 AMINO ACIDS REPEATS
11 AMINO ACIDS REPEATS

S2
160 kDa PROTEIN ANKYRIN REPEATS

CONSENSUS:		t S/TPLH Ah tt thht LLt t t (SEQ ID NO:17)
(SEQ ID NO:9) REPEAT	1	DGRTIIHYAAKDGNLEILQQALGRKSSYSKFPV
(SEQ ID NO:10)	2	KKTTLTAEALTSGKYGVVKALIKNSADVNASPE
(SEQ ID NO:11)	3	AVQAANEASNLKEANKIVNFLLHRGADLSSREH
(SEQ ID NO:12)	4	TGTPALHLATAAGNHRTAMLLLDKGAPATQRDA
(SEQ ID NO:13)	5	RGRTALHIAAANGDGKLYRMIAKKCPDSCQPLC
(SEQ ID NO:14)	6	MGDTALHEALYSDNBTEKCFLKMLKESRKRLSN
(SEQ ID NO:15)	7	NGDRLLHLAASRGFGKACKILLKAGASVSVVNV
(SEQ ID NO:16)	8	EGKTPVDVADPSLKTRPWFFGKSVVTMMAERVQ

FIG. 14

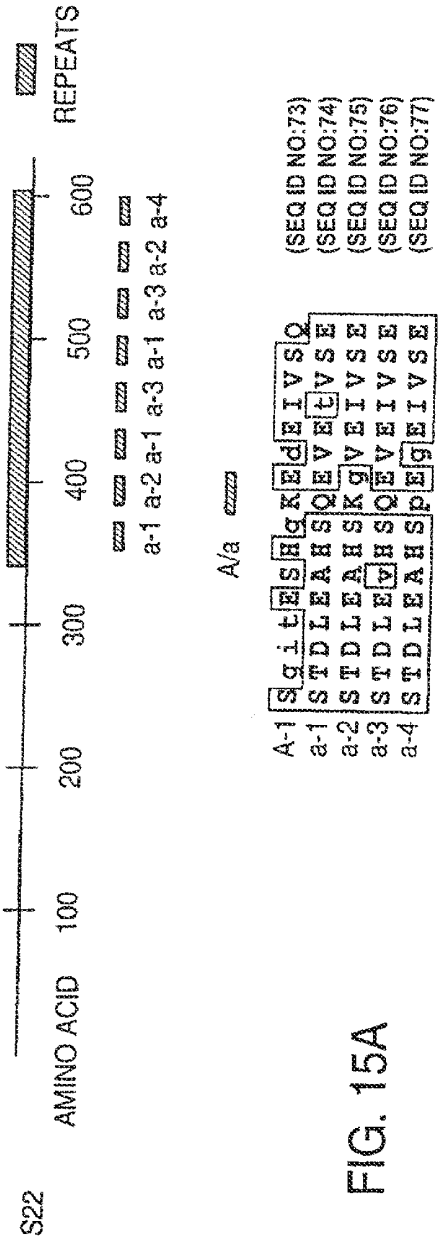

S22
AMINO ACID
100
200
300
400
500
600
REPEATS
a-1 a-2 a-1 a-3 a-1 a-3 a-2 a-4
A/a
A-1 SqitEsHgKEdEIVSQ (SEQ ID NO:73)
a-1 STDLEAHSQEVEtVSE (SEQ ID NO:74)
a-2 STDLEAHSKgVEIVSE (SEQ ID NO:75)
a-3 STDLEyHSQEVEIVSE (SEQ ID NO:76)
a-4 STDLEAHSpEgEIVSE (SEQ ID NO:77)

FIG. 15A

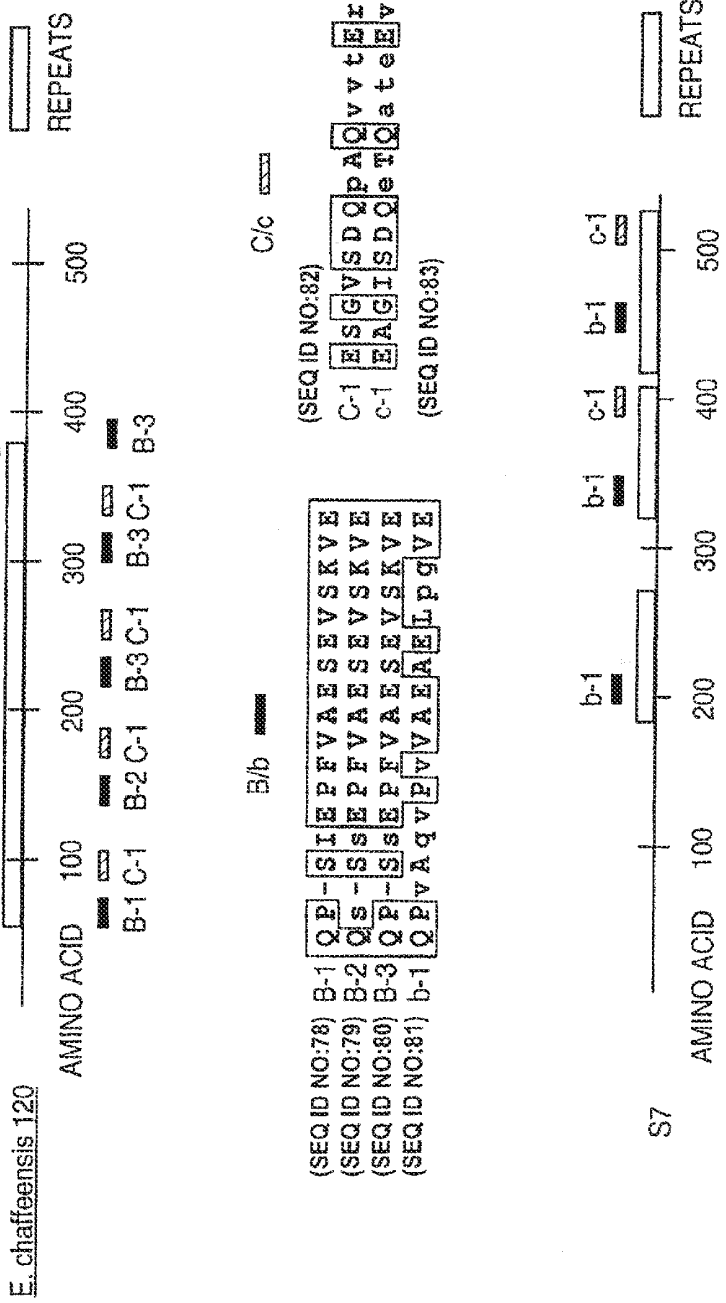

E. chaffeensis 120
AMINO ACID
100
200
300
400
500
REPEATS
A-1
A-1
A-1
A-1
B-1 C-1
B-2 C-1
B-3 C-1
B-3 C-1
B-3
B/b
C/c
(SEQ ID NO:78) B-1 QP-SIEPFVAESEVSKVE
(SEQ ID NO:79) B-2 Qs-SsEPFVAESEVSKVE
(SEQ ID NO:80) B-3 QP-SsEPFVAESEVSKVE
(SEQ ID NO:81) b-1 QPvAqvPvVAEAELpgVE
(SEQ ID NO:82)
C-1 ESGVSDQpAQvvtEIrE
c-1 EAGISDQeTQateEvE
(SEQ ID NO:83)
S7
AMINO ACID
100
200
300
400
500
REPEATS
b-1
b-1
c-1
b-1
c-1

FIG. 15B

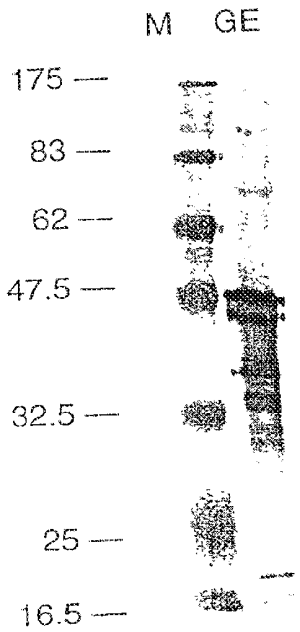


FIG. 16A

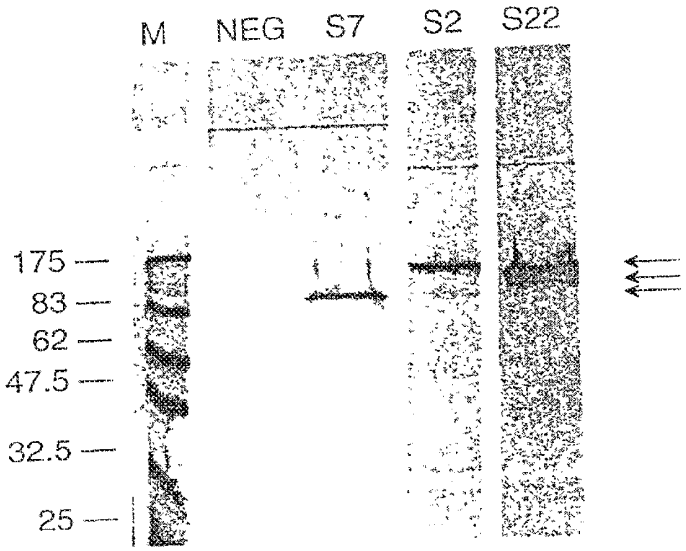


FIG. 16B

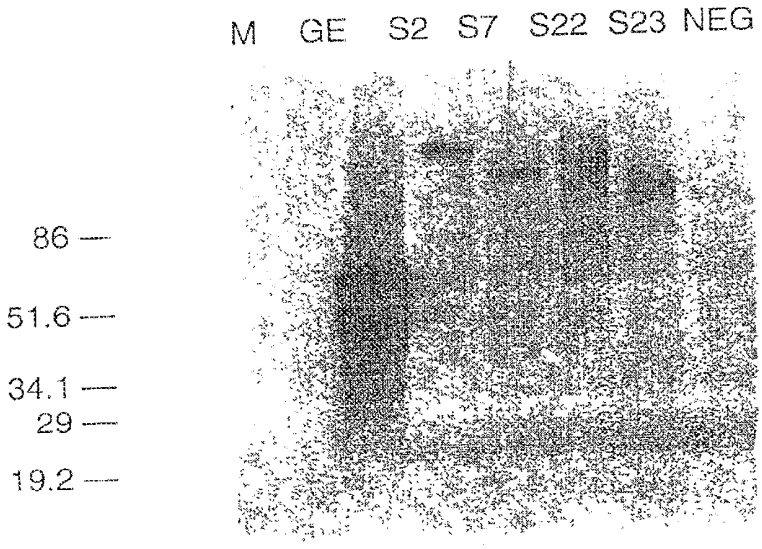


FIG. 17

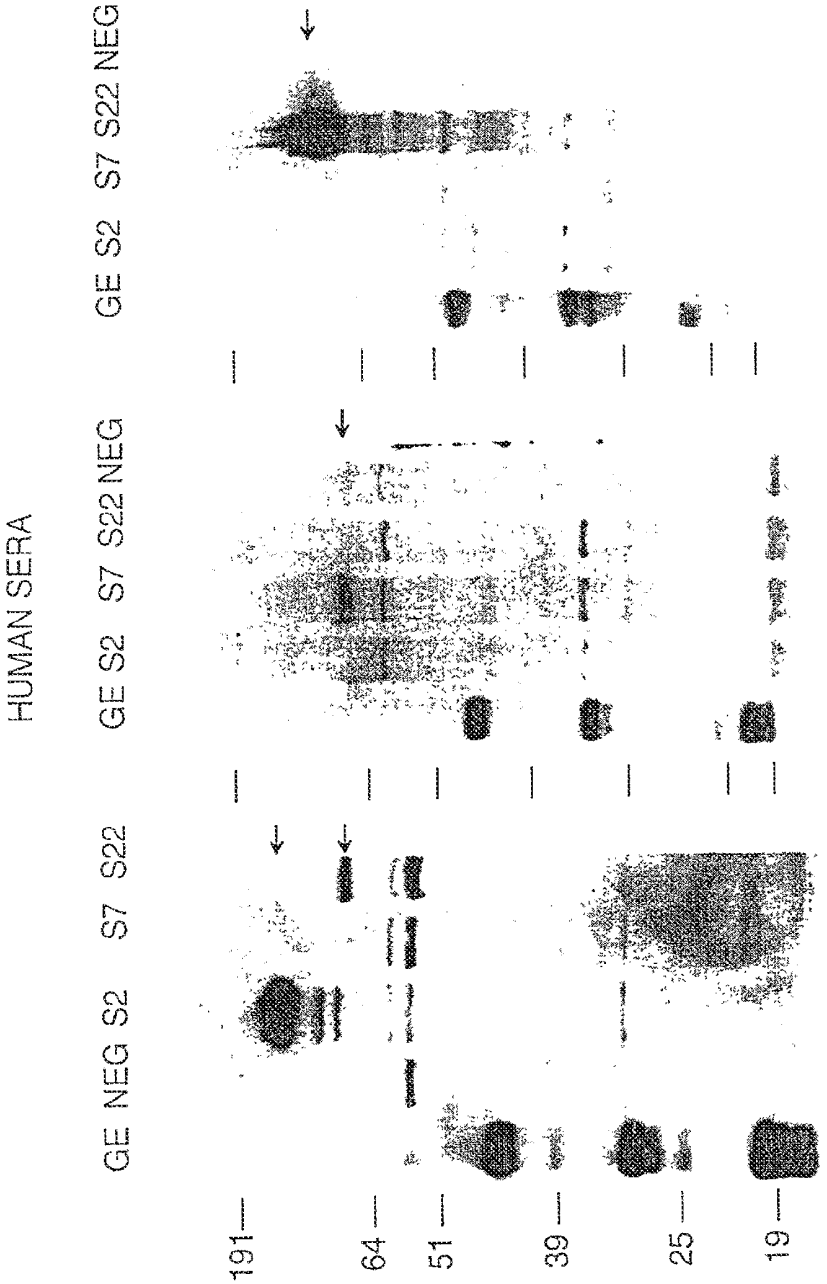


FIG. 18A

FIG. 18B

FIG. 18C

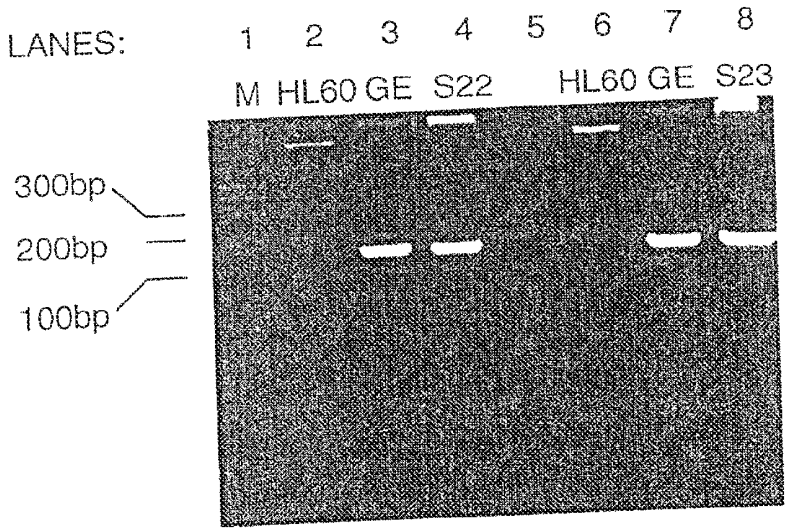


FIG. 19A

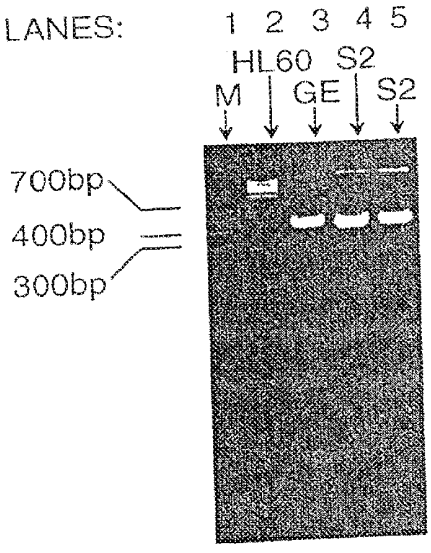


FIG. 19B

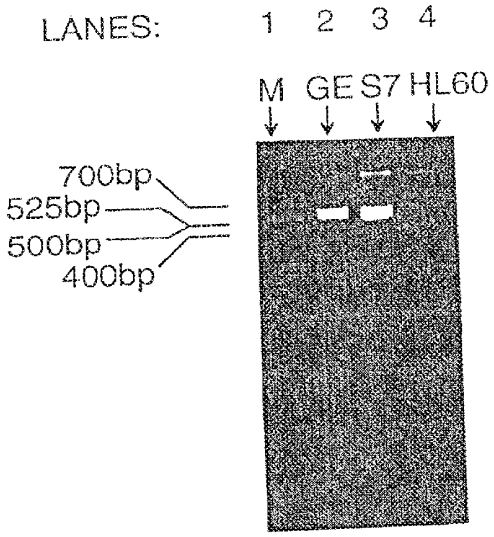


FIG. 19C

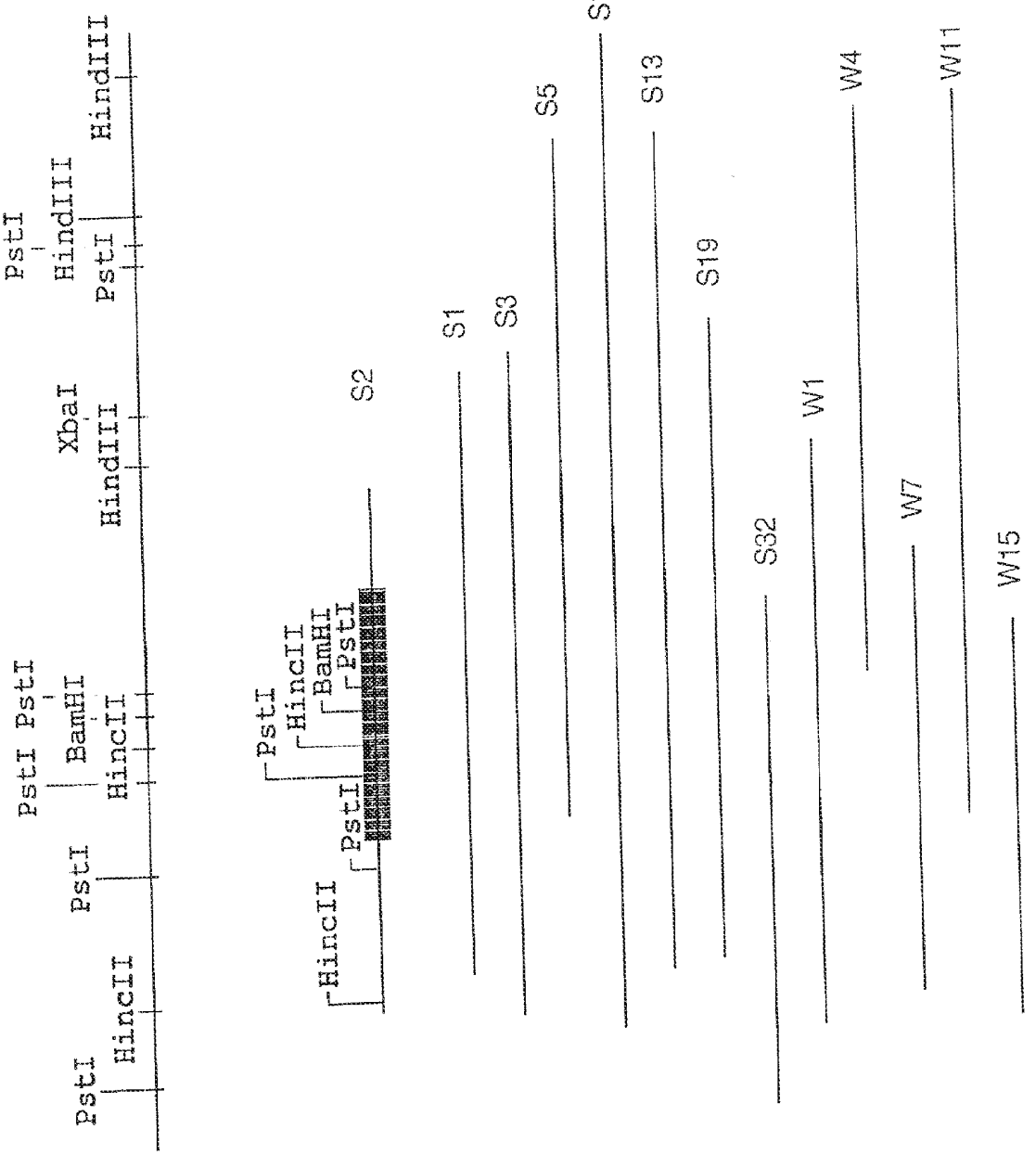

PstI
HindIII
PstI
HindIII
XbaI
HindIII
PstI PstI
BamHI
HincII
PstI
HincII
PstI
HincII
BamHI
PstI
PstI
HincII
PstI
S2
S1
S3
S5
S12
S13
S19
S32
W1
W4
W7
W11
W15

FIG. 2

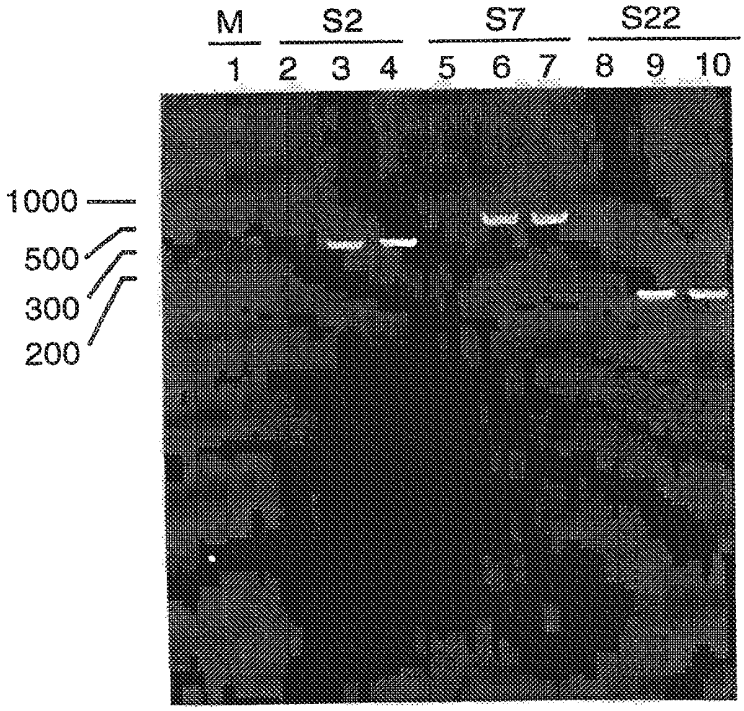


FIG. 20

```
  1  MGDAVEVRAE NLGGESILEA PIRVMKKVGD TVSAEDVLFI VETDKTSLEI SAPVAGVLTE
 61  LRVADEEVIT KGQVLAIIRP QGEATAEGVN KEPESKEEVP AQPVVAQAVS TQKPQEKTII
121  EGKGLVTPTV EDFVAGINTT PTSRALGMSA KSEQDKKIVA SQPSKDLMSC HGDVVGERRV
181  KMSKIRQVIA ARLKESQNTS ATLSTFNEVD MSKVMELRAK YKDAFVKRYD VKLGFMSFFI
241  RAVVLVLSEI PVLNAEISGD DIVYRDYCNI GVAVGTDKGL VVPVIRRAET MSLAEMEQAL
301  VDLSTKARSG KLSVSDMSGA TFTITNGGVY GSLLSTPIIN PPQSGILGMH AIQQRPVAVD
361  GKVEIRPMMY LALSYDHRIV DGQGAVTFLV RVKQYIEDPN RLALGI
```

(SEQ ID NO:21)

FIG. 21

```
  1  MGRGTITIHS KEDFACMRRA GMLAAKVLDF ITPHVVPGVT TNALNDLCHD FIISAGAIPA
 61  PLGYRGYPKS ICTSKNFVVC HGIPDDIALK NGDIVNIDVT VILDGWHGDT SRMYWVGDNV
121  SIKAKRICEA SYKALMAAIG VIQPGKKLNS IGLAIEEEIR GYGYSIVRDY CGHGIGREFH
181  AAPNIVHYYD EEDDVTIQEG MFFTVEPMIN AGKYHTVLDK KDGWTVTTRD FSLSAQFEHT
241  LGVTETGVEI FTMSPKNWHC PPYL
```

(SEQ ID NO:22)

FIG. 22

```
1     GAATTCCGGA ATTCCGGAAT TCCTATGGAT CGTGCAGTGA TGGAAGAGGG CAGCATGTTA
61    GCTGCAGGTT CACTGCTCAC TAGGGGTAAG ATTGTAAAAT CTGGAGAGTT ATGGGCAGGT
121   AGGCCTGCAA AATTTCTACG TATGATGACT GAAGAGGAGA TTTTATACCT ACAAAAATCT
181   GCTGAAAATT ACATAGCGTT ATCGCGTGGA TACTTATAAC AAGGTATTCA TCTATGGTTT
241   GACATTAGTG TCTTTTGGTG ATTACACTGC CTTTCAATCT GTGTTTTTTG TTTTAGTTCT
301   GGTTTGTATT TATGGGTGAT GCTGTAGAAG TTAGGGCTGA GAATCTTGGT GGCGAATCCA
361   TTCTAGAAGC TCCGATTCGG GTAATGAAAA AGGTGGGAGA TACTGTATCT GCAGAAGATG
421   TGCTCTTCAT TGTTGAAACA GACAAGACTT CTCTTGAAAT ATCAGCCCCT GTTGCTGGTG
481   TTCTCACAGA GTTGAGAGTT GCAGATGAAG AAGTGATTAC CAAGGGGCAG GTCTTGGCTA
541   TCATACGGCC ACAGGGTGAG GCTACTGCAG AGGGTGTTAA TAAGGAGCCA GAGAGCAAGG
601   AGGAGGTGCC TGCTCAACCC GTTGTTGCAC AGGCAGTGAG CACTCAAAAA CCGCAGGAAA
661   AGACAATTAT TGAAGGCAAA GGTCTAGTAA CTCCTACTGT AGAAGATTTT GTTGCAGGAA
721   TCAACACAAC TCCTACTTCT AGAGCTTTGG GTATGAGTGC TAAGAGTGAA CAAGACAAGA
781   AGATAGTTGC TAGCCAGCCG TCTAAGGATC TGATGAGTTG CCATGGCGAC GTGGTGGGTG
841   AAAGACGCGT GAAGATGAGC AAAATCCGCC AAGTTATAGC TGCTAGGCTT AAGGAGTCAC
901   AAAATACCTC TGCTACACTC AGCACCTTTA ATGAAGTTGA TATGAGCAAA GTGATGGAGC
961   TCAGAGCTAA GTACAAAGAT GCCTTTGTGA AGAGGTATGA TGTTAAGCTT GGGTTTATGT
1021  CCTTCTTTAT CAGAGCGGTT GTGCTAGTCC TTTCCGAAAT TCCTGTGCTG AATGCGGAGA
1081  TTTCAGGCGA TGATATAGTC TACAGGGACT ATTGTAACAT TGGAGTCGCG GTAGGTACCG
1141  ATAAGGGGTT AGTGGTGCCT GTTATCAGAA GAGCGGAAAC TATGTCACTT GCTGAAATGG
1201  AGCAAGCACT TGTTGACTTA AGTACAAAAG CAAGAAGTGG CAAGCTCTCT GTTTCTGATA
1261  TGTCTGGTGC AACCTTTACT ATTACCAATG GTGGTGTGTA TGGGTCGCTA TTGTCTACCC
1321  CTATAATCAA CCCTCCTCAA TCTGGAATCT TGGGTATGCA TGCTATACAG CAGCGTCCTG
1381  TGGCAGTAGA TGGTAAGGTA GAGATAAGGC CTATGATGTA TTTGGCGCTA TCATATGATC
1441  ATAGAATAGT TGACGGGCAA GGTGCTGTGA CGTTTTTGGT AAGAGTGAAG CAGTACATAG
1501  AAGATCCTAA CAGATTGGCT CTAGGAATTT AGGGGGTTTT TATGGGGCGG GGTACAATAA
```

FIG. 23A

```
1561 CCATCCACTC CAAAGAGGAT TTTGCCTGTA TGAGAAGGGC TGGGATGCTT GCAGCTAAGG
1621 TGCTTGATTT TATAACGCCG CATGTTGTTC CTGGTGTGAC TACTAATGCT CTGAATGATC
1681 TATGTCACGA TTTCATCATT TCTGCCGGGG CTATTCCAGC GCCTTTGGGC TATAGAGGGT
1741 ATCCTAAGTC TATTTTGTACT TCGAAGAATT TTGTGGTTTG CCATGGCATT CCAGATGATA
1801 TTGCATTAAA AAACGGCGAT ATAGTTAACA TAGACGTTAC TGTGATCCTC GATGGTTGGC
1861 ACGGGGATAC TAGTAGGATG TATTGGGTTG GTGATAACGT CTCTATTAAG GCTAAGCGCA
1921 TTTGTGAGGC AAGTTATAAG GCATTGATGG CGGCGATTGG TGTAATACAG CCAGGTAAGA
1981 AGCTCAATAG CATAGGGTTA GCTATAGAGG AAGAAATCAG AGGTTATGGA TACTCCATTG
2041 TTAGAGATTA CTGCGGACAT GGGATAGGTC GCGAATTTCA TGCTGCTCCT AACATAGTTC
2101 ACTACTATGA CGAAGAGGAT GATGTTACGA TTCAGGAGGG AATGTTTTTC ACTGTTGAGC
2161 CAATGATCAA TGCTGGAAAG TATCATACTG TGCTAGATAA GAAAGACGGA TGGACAGTTA
2221 CAACGAGAGA CTTTTCCCTT TCAGCGCAGT TTGAACATAC CTTGGGTGTA ACTGAAACTG
2281 GCGTTGAGAT TTTTACTATG TCGCCAAAAA ATTGGCATTG TCCGCCATAC CTTTAAGTAG
2341 GATATTTTTG TTATGTGTAA AGCGTGTGGC AGGGTAATGT TAGGTGCATG TTCTGTTGAC
2401 GATGTGTGCT GATAAGAAAT TGTACAATCA TACTGCGTTG GAAGTTAGGA ATATGTACTT
2461 ATGAGTGCTA ATAAGCTTGC TGTGTTATTA AGCGAAGCCG CTTCAGTTTT GAAAAGAGTA
2521 GGAATAGATA CACCGGGGTT AGACGCTCGA CTAATTGCGG GACATGTTTT GGGTTTAAGT
2581 GAGCATGAGG TGCTAATAAA TCCAGATTTA GTTGTTACTG CTGCTAAAAC AAAAGAATTT
2641 TTTGAAGTTA TTGCAAGACG TTTAGCCGGG GTACCAGTTT CGCATATTTT ACGCAGACGA
2701 GAATTC
```

(SEQ ID NO:23)

FIG. 23B

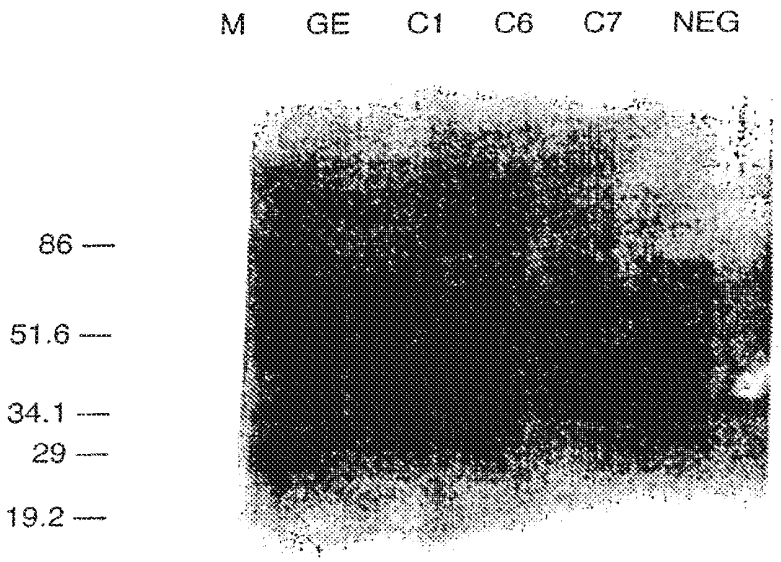


FIG. 24

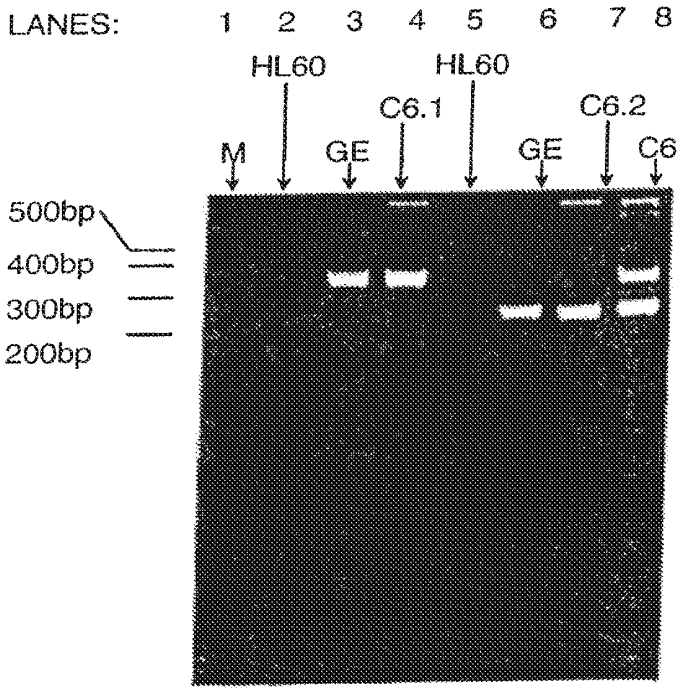

LANES:
1
2
3
4
5
6
7
8
HL60
HL60
C6.1
C6.2
M
GE
GE
C6
500bp
400bp
300bp
200bp

FIG. 25

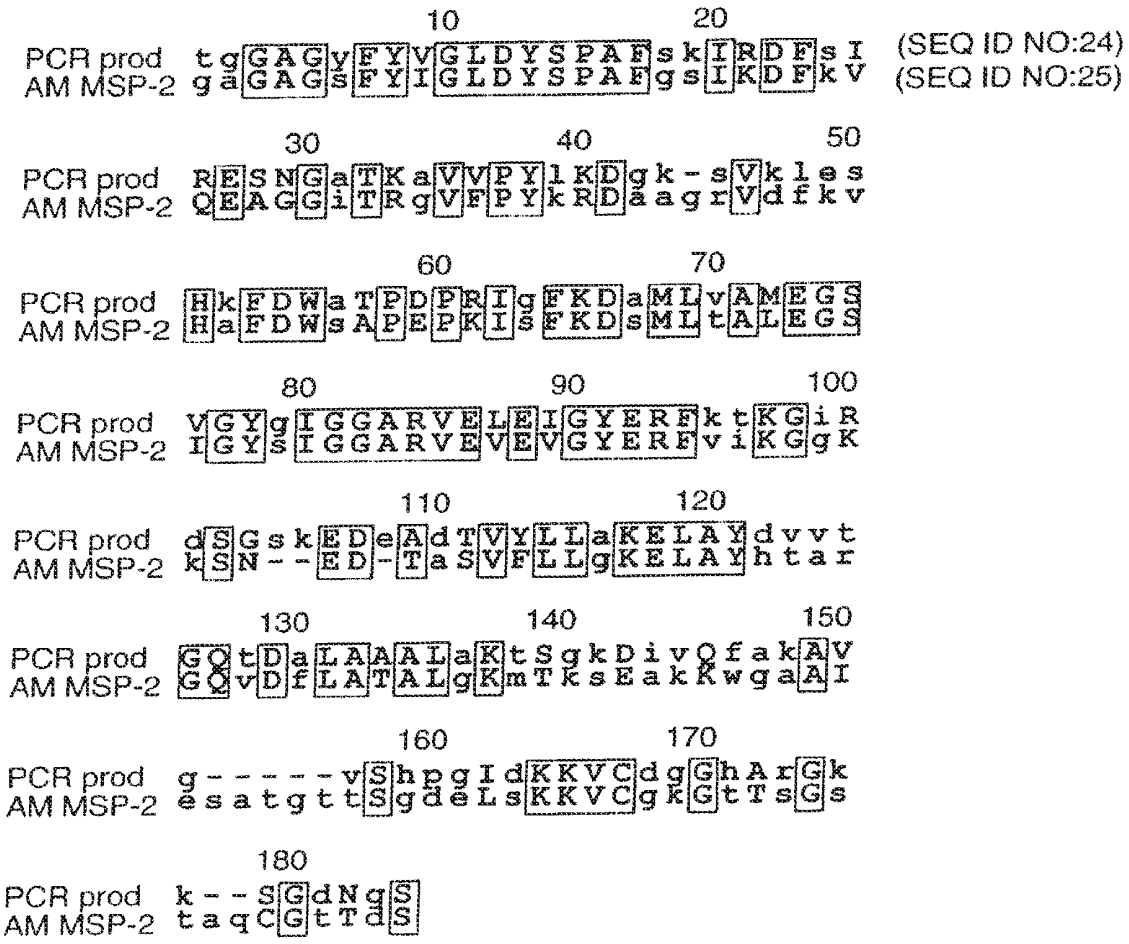

10 20
PCR prod tgGAGyFYVGLDYSPAFskIRDFsI (SEQ ID NO:24)
AM MSP-2 gaGAGsFYIGLDYSPAFgsIKDFkV (SEQ ID NO:25)
30 40 50
PCR prod RESNGaTKaVVPYlKDgk-sVkles
AM MSP-2 QEAGGiTRgVFPYkRDaagrVdfkv
60 70
PCR prod HkFDWaTPDPRIgFKDaMLvAMEGS
AM MSP-2 HaFDWsAPEPKIsFKDsMLtALEGS
80 90 100
PCR prod VGYgIGGARVELEIGYERFktKGiR
AM MSP-2 IGYsIGGARVEVEVGYERFviKGgK
110 120
PCR prod dSGskEDeAdTVYLLaKELAYdvvt
AM MSP-2 kSN--ED-TaSVFLLgKELAYhtar
130 140 150
PCR prod GQtDaLAAALaKtSgkDivQfakAV
AM MSP-2 GQvDfLATALgKmTksEakKwgaAI
160 170
PCR prod g-----vShpgIdKKVCdgGhArGk
AM MSP-2 esatgttSgdeLsKKVCgkGtTsGs
180
PCR prod k--SGdNgS
AM MSP-2 taqCGtTdS

FIG. 26

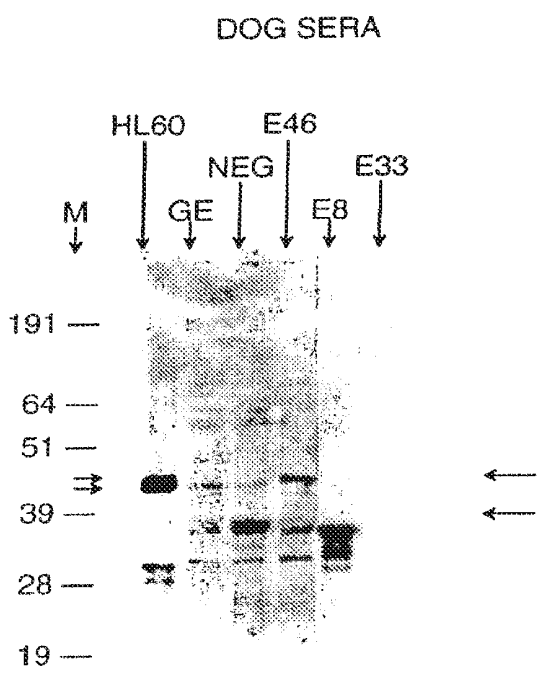


FIG. 27A

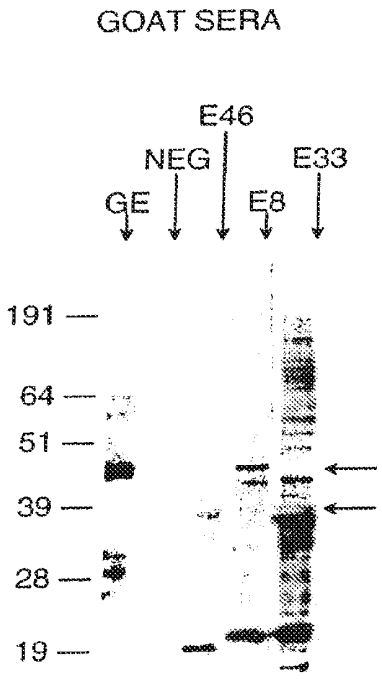


FIG. 27B

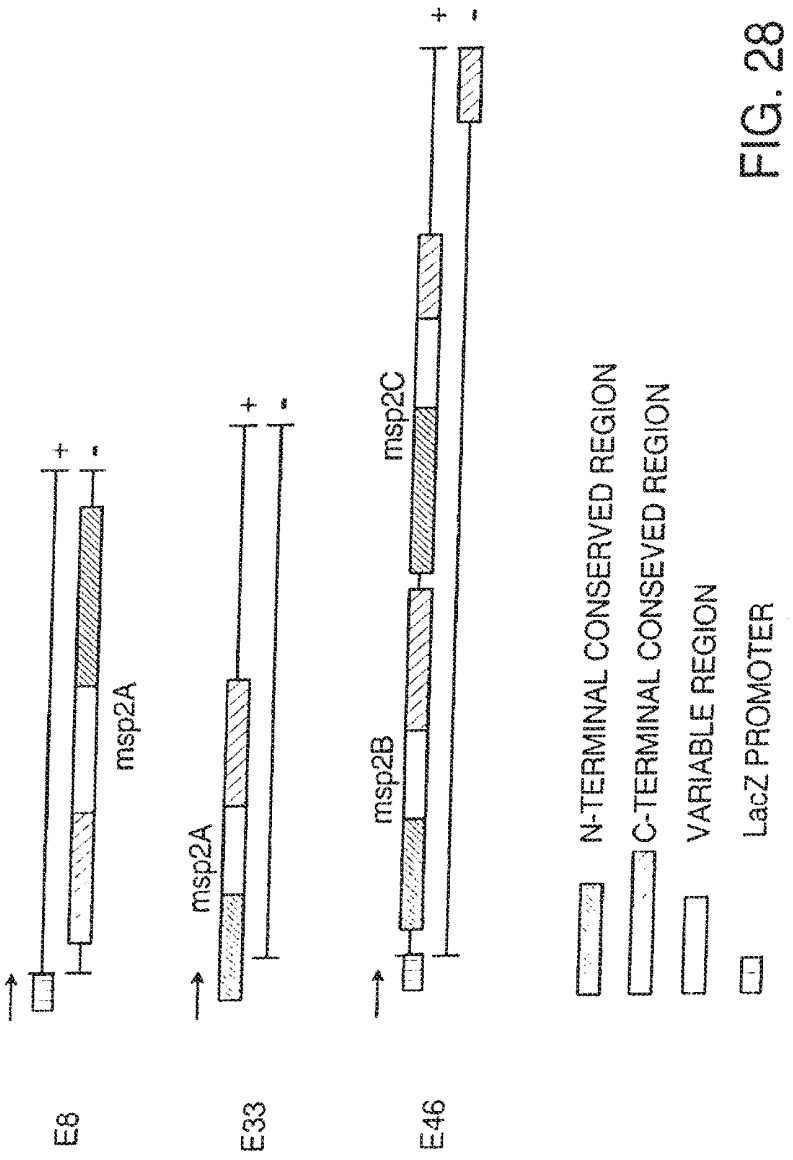

+
-
msp2A
msp2A
msp2B
msp2C
E8
E33
E46
N-TERMINAL CONSERVED REGION
C-TERMINAL CONSEVED REGION
VARIABLE REGION
LacZ PROMOTER

FIG. 28

FIG. 29

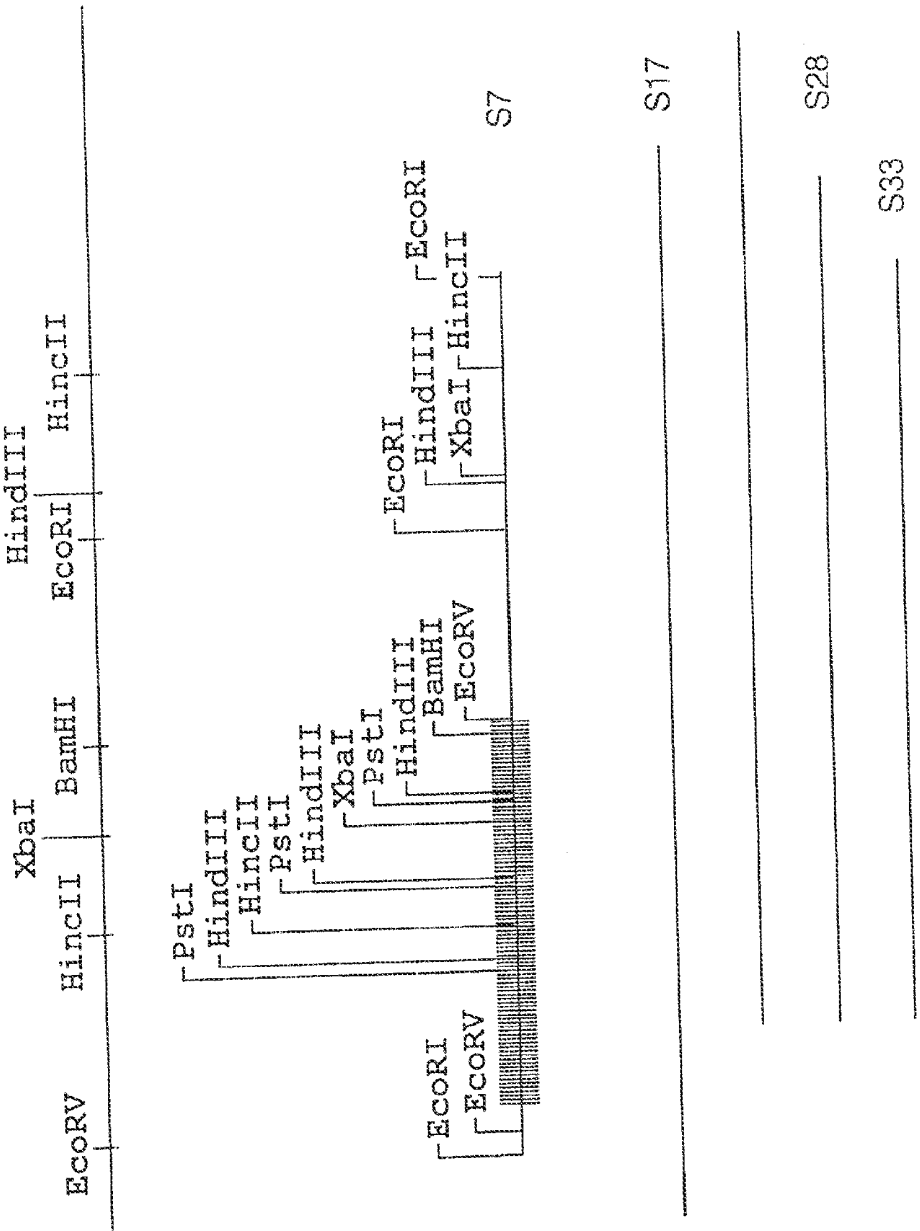

EcoRV
HincII
XbaI
BamHI
EcoRI
HindIII
HincII
PstI
HindIII
HincII
PstI
HindIII
XbaI
PstI
HindIII
BamHI
EcoRV
EcoRI
EcoRV
EcoRI
HindIII
XbaI
HincII
S7
S17
S21
S28
S33

FIG. 3

Sequence Range: 1 to 3435

```
        10        20        30        40        50        60
         *         *         *         *         *         *
TTTTTATATC TGGAGCTCTT GTACTGTGTT TACCACGGGA TTTATTATTG GGTAGGCTTG

        70        80        90       100       110       120
         *         *         *         *         *         *
ATATTCAGGC TCTATCAACG CAGCTATTCA TGGCATTATT ACAGATAAAT TTGGCATTTT

       130       140       150       160       170       180
         *         *         *         *         *         *
GGAGATAGGC GATCTAGGGT TCTATTATTA GGAATCTATT ATTTAGATAT ATAGGGATAT

       190       200       210       220       230       240
         *         *         *         *         *         *
AAGGGAGAGT AACGGAGAGA CTAAGGCAGT ATATCCATAC TTAAAGGATG GAAAGAGTGT

       250       260       270       280       290       300
         *         *         *         *         *         *
AAAGCTAGAG TCACACAAGT TTGACTGGAA CACTCCTGAT CCTCGGATTG GGTTTAAGGA
```

FIG. 30A

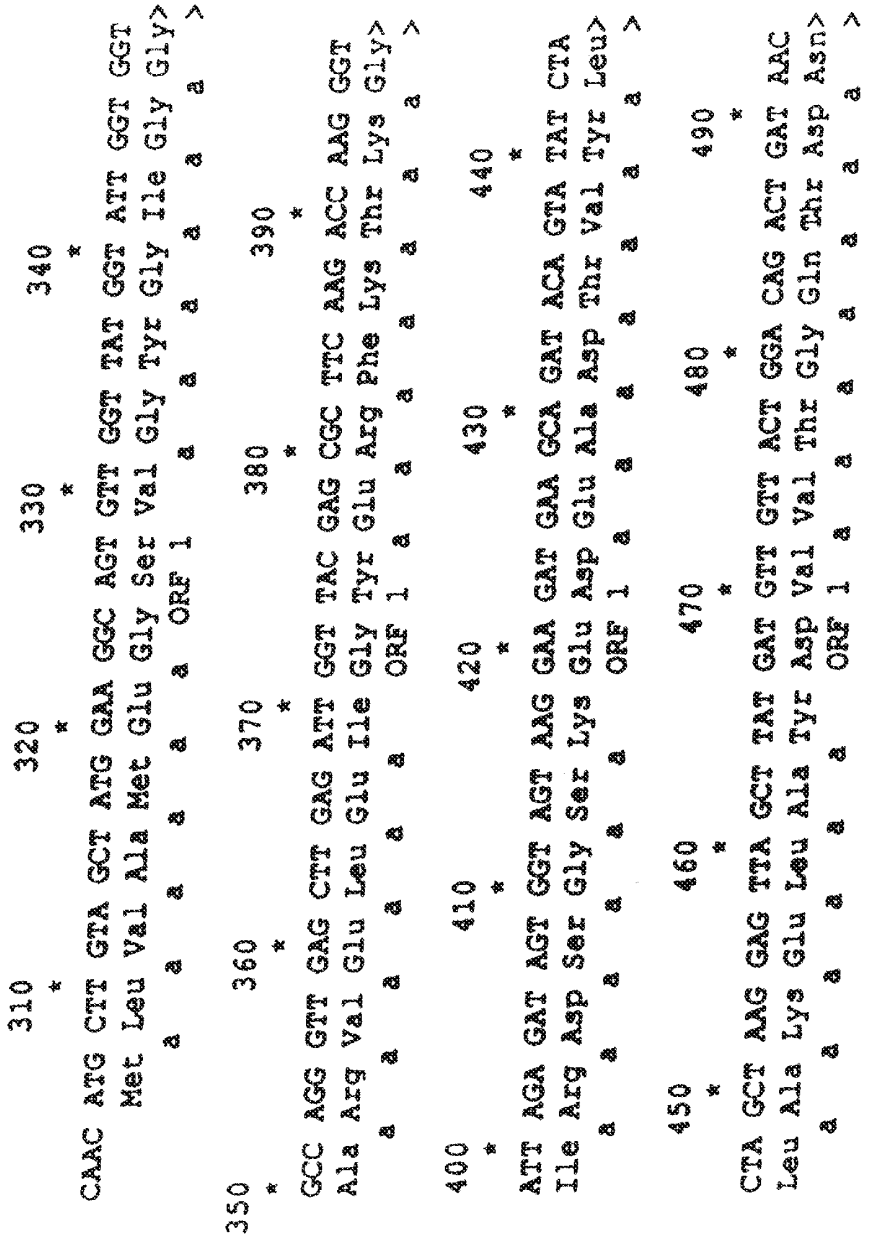

310 320 330 340
CAAC ATG CTT GTA GCT ATG GAA GGC AGT GTT GGT TAT GGT ATT GGT GGT
Met Leu Val Ala Met Glu Gly Ser Val Gly Tyr Gly Ile Gly Gly>
a a a a a a ORF 1 a a a a a a >
350 360 370 380 390
GCC AGG GTT GAG CTT GAG ATT GGT TAC GAG CGC TTC AAG ACC AAG GGT
Ala Arg Val Glu Leu Glu Ile Gly Tyr Glu Arg Phe Lys Thr Lys Gly>
a a a a a a ORF 1 a a a a a a >
400 410 420 430 440
ATT AGA GAT AGT GGT AGT AAG GAA GAT GAA GCA GAT ACA GTA TAT CTA
Ile Arg Asp Ser Gly Ser Lys Glu Asp Glu Ala Asp Thr Val Tyr Leu>
a a a a a a ORF 1 a a a a a a >
450 460 470 480 490
CTA GCT AAG GAG TTA GCT TAT GAT GTT GTT ACT GGA CAG ACT GAT AAC
Leu Ala Lys Glu Leu Ala Tyr Asp Val Val Thr Gly Gln Thr Asp Asn>
a a a a a a ORF 1 a a a a a a >

FIG. 30B

```
500        510        520        530        540
*          *          *          *          *
CTT GCC GCT GCT CTT GCC AAA ACC TCG GGG AAG GAC ATC GTT CAG TTT
Leu Ala Ala Ala Leu Ala Lys Thr Ser Gly Lys Asp Ile Val Gln Phe>
   a   a   a   a   a   a     ORF 1   a   a   a   a   a   a   >

      550        560        570        580
      *          *          *          *
GCC AAT GCT GTG AAA ATT TCT TAC CCT AAA ATT GAT GAG CAG GTT TGT
Ala Asn Ala Val Lys Ile Ser Tyr-Pro Lys Ile Asp Glu Gln Val Cys>
   a   a   a   a   a   a     ORF 1   a   a   a   a   a   a   >

590        600        610        620        630
*          *          *          *          *
AAT AAA AAT CAT ACA GTG TTG AAT ACG GGG AAA GGG ACA ACC TTT AAT
Asn Lys Asn His Thr Val Leu Asn Thr Gly Lys Gly Thr Thr Phe Asn>
```

FIG. 30C

```
    a   a   a   a   a   a    ORF 1  a   a   a   a   a   a   a   >

640                 650                 660                 670                 680
 *                   *                   *                   *                   *
CCA GAT CCC AAG ACA ACC GAA GAT AAT ACA GCG CAG TGC AGT GGG TTG
Pro Asp Pro Lys Thr Thr Glu Asp Asn Thr Ala Gln Cys Ser Gly Leu>
    a   a   a   a   a   a    ORF 1  a   a   a   a   a   a   a   >

    690                 700                 710                 720                 730
     *                   *                   *                   *                   *
AAC ACG AAG GGA ACG AAT AAG TTT AGC GAT TTT GCT GAA GGT GTA GGT
Asn Thr Lys Gly Thr Asn Lys Phe Ser Asp Phe Ala Glu Gly Val Gly>
    a   a   a   a   a   a    ORF 1  a   a   a   a   a   a   a   >

        740                 750                 760                 770                 780
         *                   *                   *                   *                   *
TTG AAA GAT AAT AAG AAT TGG CCT ACT GGT CAG GCT GGG AAG AGC AGT
Leu Lys Asp Asn Lys Asn Trp Pro Thr Gly Gln Ala Gly Lys Ser Ser>
    a   a   a   a   a   a    ORF 1  a   a   a   a   a   a   a   >

            790                 800                 810                 820
             *                   *                   *                   *
GGT GGT CCT GTG GTG GGT GCA TCT AAT AGT AAT GCC AAC GCT ATG GCT
Gly Gly Pro Val Val Gly Ala Ser Asn Ser Asn Ala Asn Ala Met Ala>
    a   a   a   a   a   a    ORF 1  a   a   a   a   a   a   a   >
```

FIG. 30D

```
830        840        850        860        870
*          *          *          *          *
AGA GAC CTA GTA GAT CTT AAT CGA GAC GAA AAA ACC ATA GTA GCA GGG
Arg Asp Leu Val Asp Leu Asn Arg Asp Glu Lys Thr Ile Val Ala Gly>
   a   a   a   a   a   a   a   ORF 1   a   a   a   a   a   a   >

880        890        900        910        920
*          *          *          *          *
TTA CTA GCT AAA ACT ATT GAA GGT GGT GAG GTT GTT GAG ATT AGG GCG
Leu Leu Ala Lys Thr Ile Glu Gly Gly Glu Val Val Glu Ile Arg Ala>
   a   a   a   a   a   a   a   ORF 1   a   a   a   a   a   a   >

930        940        950        960        970
*          *          *          *          *
GTT TCT TCT ACT TCT GTA ATG GTC AAT GCT TGT TAT GAT CTT CTT AGT
Val Ser Ser Thr Ser Val Met Val Asn Ala Cys Tyr Asp Leu Leu Ser>
   a   a   a   a   a   a   a   ORF 1   a   a   a   a   a   a   >

980        990        1000       1010       1020
*          *          *          *          *
GAA GGT CTA GGC GTT GTT CCT TAC GCT TGT GTC GGT CTT GGA GGT AAC
Glu Gly Leu Gly Val Val Pro Tyr Ala Cys Val Gly Leu Gly Gly Asn>
   a   a   a   a   a   a   a   ORF 1   a   a   a   a   a   a   >
```

FIG. 30E

```
                1030      1040      1050      1060
                   *         *         *         *
TTC GTG GGC GTT GTT GAT GGG CAT ATC ACT CCT AAG CTT GCT TAT AGA
Phe Val Gly Val Val Asp Gly His Ile Thr Pro Lys Leu Ala Tyr Arg>
    a   a   a   a   a   a     ORF 1   a   a   a   a   a   a   >

1070      1080      1090      1100      1110
   *         *         *         *         *
TTA AAG GCT GGG TTG AGT TAT CAG CTC TCT CCT GAA ATC TCC GCT TTT
Leu Lys Ala Gly Leu Ser Tyr Gln Leu Ser Pro Glu Ile Ser Ala Phe>
    a   a   a   a   a   a     ORF 1   a   a   a   a   a   a   >
```

FIG. 30F

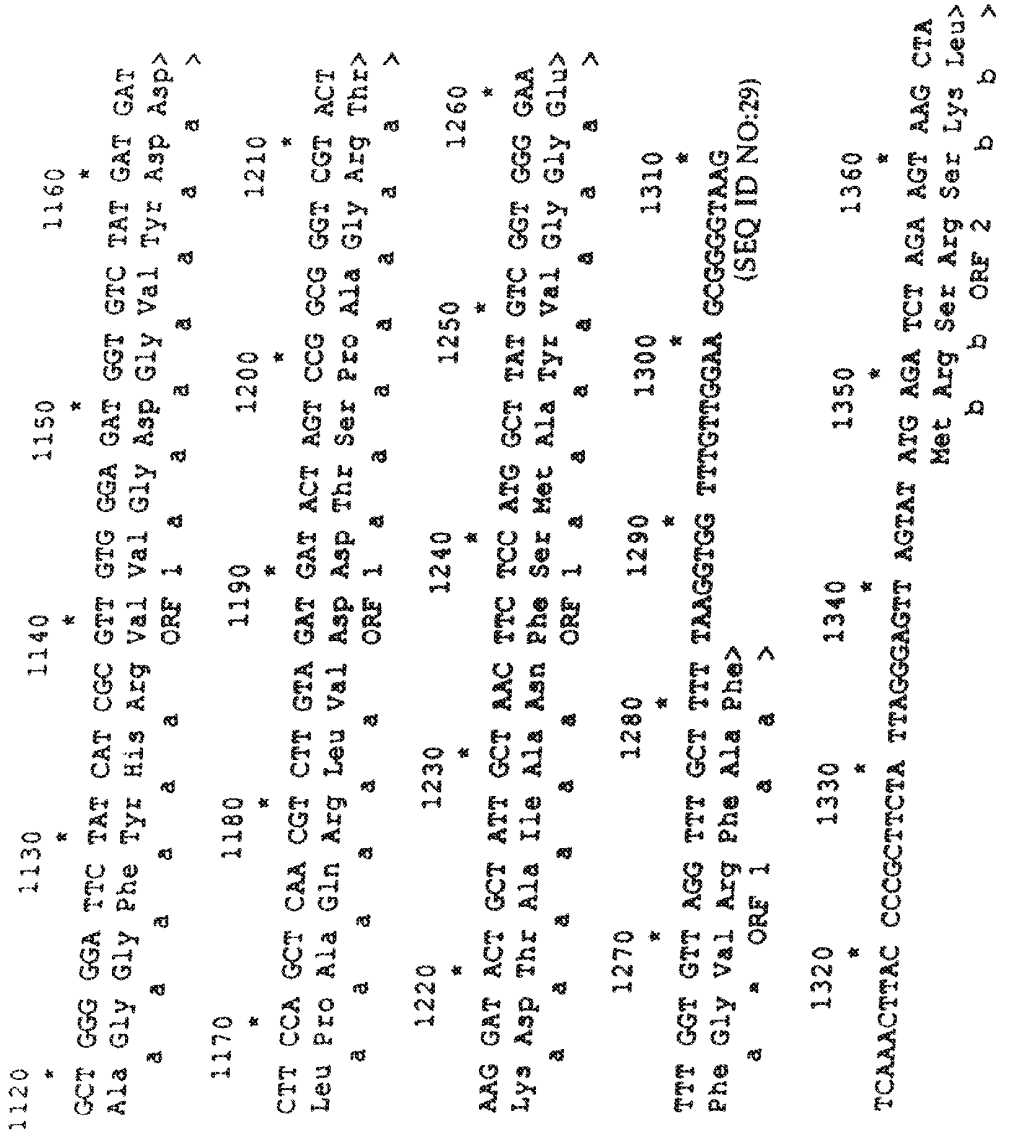

1120 1130 1140 1150 1160
GCT GGG GGA TTC TAT CAT CGC GTT GTG GGA GAT GGT GTC TAT GAT GAT
Ala Gly Gly Phe Tyr His Arg Val Val Gly Asp Gly Val Tyr Asp Asp>
a a a a a a ORF 1 a a a a a >
1170 1180 1190 1200 1210
CTT CCA GCT CAA CGT CTT GTA GAT GAT ACT AGT CCG GCG GGT CGT ACT
Leu Pro Ala Gln Arg Leu Val Asp Asp Thr Ser Pro Ala Gly Arg Thr>
a a a a a a ORF 1 a a a a a a >
1220 1230 1240 1250 1260
AAG GAT ACT GCT ATT GCT AAC TTC TCC ATG GCT TAT GTC GGT GGG GAA
Lys Asp Thr Ala Ile Ala Asn Phe Ser Met Ala Tyr Val Gly Gly Glu>
a a a a a a ORF 1 a a a a a a >
1270 1280 1290 1300 1310
TTT GGT GTT AGG TTT GCT TTT TAAGGTGG TTTGTTGGAA GCGGGGTAAG
Phe Gly Val Arg Phe Ala Phe> (SEQ ID NO:29)
a a ORF 1 a a >
1320 1330 1340 1350 1360
TCAAACTTAC CCCGCTTCTA TTAGGGAGTT AGTAT ATG AGA TCT AGA AGT AAG CTA
Met Arg Ser Arg Ser Lys Leu>
b b ORF 2 b b >

FIG. 30G

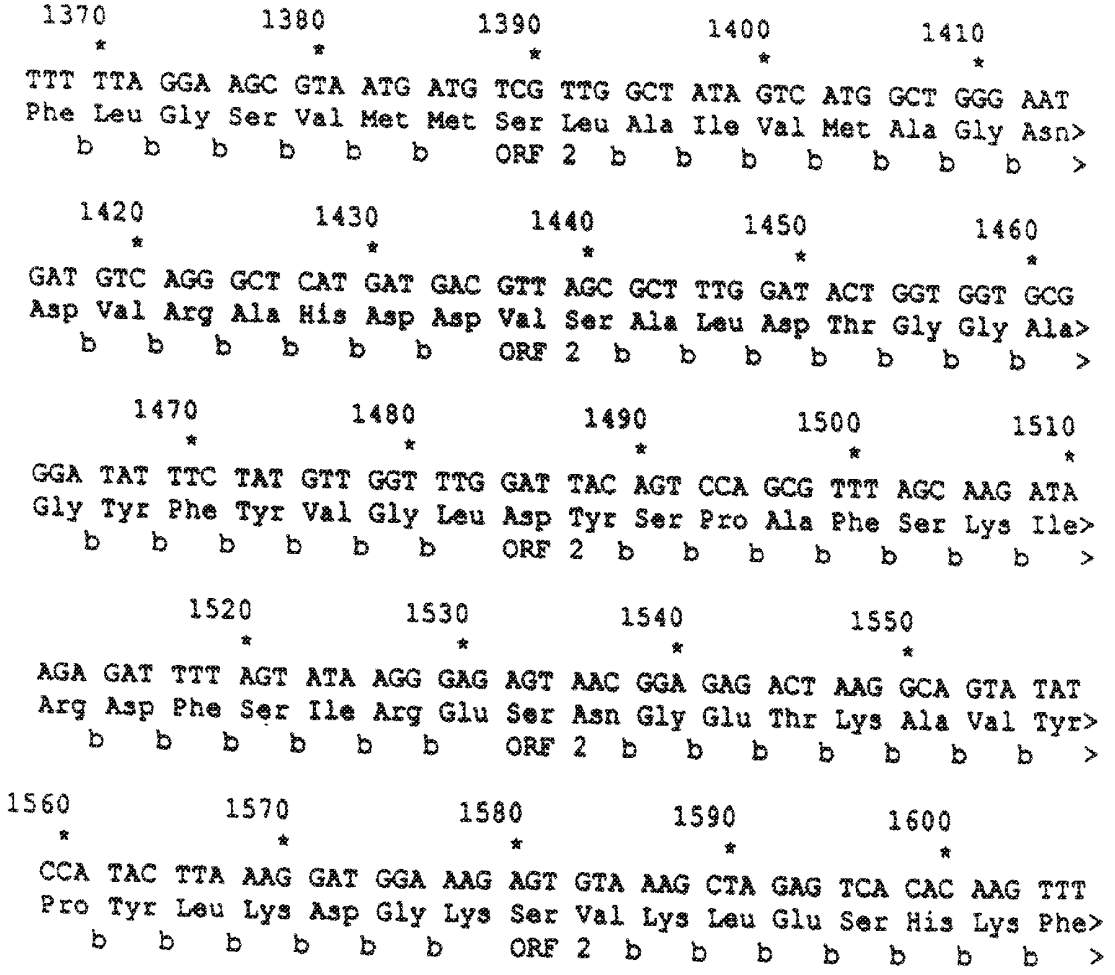

1370 1380 1390 1400 1410
TTT TTA GGA AGC GTA ATG ATG TCG TTG GCT ATA GTC ATG GCT GGG AAT
Phe Leu Gly Ser Val Met Met Ser Leu Ala Ile Val Met Ala Gly Asn>
b b b b b b ORF 2 b b b b b b b >
1420 1430 1440 1450 1460
GAT GTC AGG GCT CAT GAT GAC GTT AGC GCT TTG GAT ACT GGT GGT GCG
Asp Val Arg Ala His Asp Asp Val Ser Ala Leu Asp Thr Gly Gly Ala>
b b b b b b ORF 2 b b b b b b b >
1470 1480 1490 1500 1510
GGA TAT TTC TAT GTT GGT TTG GAT TAC AGT CCA GCG TTT AGC AAG ATA
Gly Tyr Phe Tyr Val Gly Leu Asp Tyr Ser Pro Ala Phe Ser Lys Ile>
b b b b b b ORF 2 b b b b b b b >
1520 1530 1540 1550
AGA GAT TTT AGT ATA AGG GAG AGT AAC GGA GAG ACT AAG GCA GTA TAT
Arg Asp Phe Ser Ile Arg Glu Ser Asn Gly Glu Thr Lys Ala Val Tyr>
b b b b b b ORF 2 b b b b b b b >
1560 1570 1580 1590 1600
CCA TAC TTA AAG GAT GGA AAG AGT GTA AAG CTA GAG TCA CAC AAG TTT
Pro Tyr Leu Lys Asp Gly Lys Ser Val Lys Leu Glu Ser His Lys Phe>
b b b b b b ORF 2 b b b b b b b >

FIG. 30H

```
1610        1620        1630        1640        1650
  *           *           *           *           *
GAC TGG AAC ACT CCT GAT CCT CGG ATT GGG TTT AAG GAC AAC ATG CTT
Asp Trp Asn Thr Pro Asp Pro Arg Ile Gly Phe Lys Asp Asn Met Leu>
   b   b   b   b   b   b   ORF 2  b   b   b   b   b   b   >

   1660        1670        1680        1690        1700
     *           *           *           *           *
GTA GCT ATG GAA GGT AGT GTT GGT TAT GGT ATT GGT GGT GCC AGG GTT
Val Ala Met Glu Gly Ser Val Gly Tyr Gly Ile Gly Gly Ala Arg Val>
   b   b   b   b   b   b   ORF 2  b   b   b   b   b   b   >

      1710        1720        1730        1740        1750
        *           *           *           *           *
GAG CTT GAG ATT GGT TAC GAG CGC TTC AAG ACC AAG GGT ATT AGA GAT
Glu Leu Glu Ile Gly Tyr Glu Arg Phe Lys Thr Lys Gly Ile Arg Asp>
   b   b   b   b   b   b   ORF 2  b   b   b   b   b   b   >

         1760        1770        1780        1790
           *           *           *           *
AGT GGT AGT AAG GAA GAT GAA GCT GAT ACA GTA TAT CTA CTA GCT AAG
Ser Gly Ser Lys Glu Asp Glu Ala Asp Thr Val Tyr Leu Leu Ala Lys>
   b   b   b   b   b   b   ORF 2  b   b   b   b   b   b   >

800        1810        1820        1830        1840
  *           *           *           *           *
GAG TTA GCT TAT GAT GTT GTT ACT GGG CAG ACT GAT AAC CTT GCC GCT
Glu Leu Ala Tyr Asp Val Val Thr Gly Gln Thr Asp Asn Leu Ala Ala>
   b   b   b   b   b   b   ORF 2  b   b   b   b   b   b   >
```

FIG. 30I

```
1850        1860        1870        1880        1890
   *           *           *           *           *
GCT CTG GCC AAA ACC TCC GGT AAA GAC TTT GTC CAG TTT GCT AAG GCG
Ala Leu Ala Lys Thr Ser Gly Lys Asp Phe Val Gln Phe Ala Lys Ala>
    b   b   b   b   b   b     ORF 2  b   b   b   b   b   b   >

   1900        1910        1920        1930        1940
      *           *           *           *           *
GTT GGG GTT TCT CAT CCT AGT ATT GAT GGG AAG GTT TGT AAG ACG AAG
Val Gly Val Ser His Pro Ser Ile Asp Gly Lys Val Cys Lys Thr Lys>
    b   b   b   b   b   b     ORF 2  b   b   b   b   b   b   >
                <Gly Leu Ile Ser Pro Phe Thr Gln Leu Val Phe
              < d   d   d   d   d ORF 4  d   d   d   d   d

   1950        1960        1970        1980        1990
      *           *           *           *           *
GCG GAT AGC TCG AAG AAA TTT CCG TTA TAT AGT GAC GAA ACG CAC ACG
Ala Asp Ser Ser Lys Lys Phe Pro Leu Tyr Ser Asp Glu Thr His Thr>
    b   b   b   b   b   b     ORF 2  b   b   b   b   b   b   >
<Ala Ser Leu Glu Phe Phe Asn Gly Asn Tyr Leu Ser Ser Val Cys Val
  <   d   d   d   d   d   d     ORF 4  d   d   d   d   d   d

      2000        2010        2020        2030
         *           *           *           *
AAG GGG GCA AGT GAG GGG AGA ACG TCT TTG TGC GGT GAC AAT GGT AGT
Lys Gly Ala Ser Glu Gly Arg Thr Ser Leu Cys Gly Asp Asn Gly Ser>
    b   b   b   b   b   b     ORF 2  b   b   b   b   b   b   >
<Phe Pro Ala Leu Ser Pro Leu Val Asp Lys His Pro Ser Leu Pro Leu
  <   d   d   d   d   d   d     ORF 4  d   d   d   d   d   d
```

FIG. 30J

```
   1 GAATTCCTTA CCTCCCTATA TTTCGTACAG GTTATTTCGC AGTCTAGCTA TGATGCTTTA
  61 CCAGGATACG TTAAACGTTG ACGTTCTACG CTGTCATAGC CTTTTATTCT GCAAAAATAG
 121 CTTAACTGTG TCACTTCCTG AGAAGTAAG ATACATATTT AGTTTTTGCA CAGCCAAAAA
 181 ACTTCTAGTG AACTGTGGTT TCTCTGGAAT CAATAACCTG TTTTATATTC GTGCGTTCTA
 241 TAACAATCTA CAGCTGTGGT TATTAGGCGT GGTTTCGCCT GATAATAAAG ATACTTTAGA
 301 GGGTATAAAC TTGGAAAAAA TAATGAAAAA CCCTCCTTAG TGCCTCCCCG TTTTTGACAA
 361 CATACTCTTA TGGAAAAGCG TTAGGGAGTT GCTTCGCTTG TCACGCGTGC GTTAGGTTTT
 421 ACGTATACGT GTCTGGGACT TCACGAAAAC TCGACGCAGG CGGATTTTGT ACTATGTTTC
 481 ACTTAACAAG GTATTATAAA TGTTTTGAACA CAATATTCCT GATACATACA CAGGAACAAC
 541 TGCAGAAGGT TCTCCTGGCT TAGCAGGCGG GGATTTTAGC TTAAGTTCTA TTGACTTTAC
 601 AAGGGACTTT ACAATTGAAT CACATAGAGG AAGCTCAGCT GATGACCCAG GTTACATCAG
 661 CTTTAGGGAT CAAGACGGAA ACGTCATGTC ACGTTTTCTT GATGTGTACG TAGCTAATTT
 721 CAGCTTGCGA TGCAAGCATT CTCCCTATAA CAACGACAGA ATGGAAACAG CTGCGTTCTC
 781 TCTAACTCCC GACATAATAG AGCCTTCTGC TTTATTGCAA GAATCACATA GTACACAAAA
 841 CAATGTAGAA GAGGCAGTAC AAGTTACAGC TCTTTGAGTGC CCTCCATGTA ATCCAGTCCC
 901 TGCCGAGGAA GTAGCTCCTC AACCGTCTTT TCTAAGCAGA ATAATTCAGG CGTTCTTGTG
 961 GTTATTCACG CCTTCTTCTA CTACCGACAC TGCTGAAGAC AGCAAGTGTA ATAGTAGCGA
1021 TACTTCAAAA TGTACCTCTG CTAGCAGTGA GTCATTAGAG CAGCAACAAG AATCAGTGGA
1081 AGTGCAACCA TCTGTACTTA TGTCTACTGC CCCTATAGCA ACAGAGCCTC AAAATGCGGT
1141 TGTTAACCAA GTAAACACTA CTGCAGTACA AGTAGAATCA TCCATTATTG TGCCAGAATC
1201 GCAACACACT GACGTTACCG TGCTCGAAGA TACTACTGAG ACGATAACTG TTGATGGGGA
1261 ATATGGACAT TTTTAGTGACA TTGCTTCAGG TGAACACAAT AACGATCTGC CTGCCATGTT
1321 GTTAGATGAA GCAGACTTCA CTATGTTATT AGCGAACGAG GAGTCAAAGA CCCTGGAGTC
1381 TATGCCTTCT GATAGCCTAG AAGACAATGT TCAGGAACTA GGTACATTGC CTTTACAAGA
1441 AGGTGAAACA GTTTCTGAGG GCAACACACG AGAGTCACTA CCCACTGACG TTTCACAAGA
1501 CTCAGTTGGT GTAAGTACAG ATCTTGAAGC TCATTCTCAA GAAGTTGAAA CAGTTTCTGA
1561 GGTCAGCACA CAAGATTCAC TATCCACTAA CATTTCACAA GACTCAGTTG GTGTAAGTAC
```

FIG. 4A

```
2040        2050        2060        2070        2080
  *           *           *           *           *
 TCT ACG ATA ACA AAC AGT GGT GCG AAT GTA AGT GAA ACT GGG CAG GTT
 Ser Thr Ile Thr Asn Ser Gly Ala Asn Val Ser Glu Thr Gly Gln Val>

    b   b   b   b   b   b   ORF 2  b   b   b   b   b   b   >
<Glu Val Ile Val Phe Leu Pro Ala Phe Thr Leu Ser Val Pro Cys Thr
 <  d   d   d   d   d   d   ORF 4  d   d   d   d   d   d

  2090        2100        2110        2120        2130
    *           *           *           *           *
 TTT AGG GAT TTT ATC AGG GCA ACG CTG AAA GAG GAT GGT AGT AAA AAC
 Phe Arg Asp Phe Ile Arg Ala Thr Leu Lys Glu Asp Gly Ser Lys Asn>
    b   b   b   b   b   b   ORF 2  b   b   b   b   b   b   >
<Lys Leu Ser Lys Ile Leu Ala Val Ser Phe Ser Ser Pro Leu Leu Phe
 <  d   d   d   d   d   d   ORF 4  d   d   d   d   d   d

  2140        2150        2160        2170        2180
    *           *           *           *           *
 TGG CCA ACT TCA AGC GGC ACG GGA ACT CCA AAA CCT GTC ACG AAC GAC
 Trp Pro Thr Ser Ser Gly Thr Gly Thr Pro Lys Pro Val Thr Asn Asp>
    b   b   b   b   b   b   ORF 2  b   b   b   b   b   b   >
<Gln Gly Val Glu Leu Pro Val Pro Val Gly Phe Gly Thr Val Phe Ser
 <  d   d   d   d   d   d   ORF 4  d   d   d   d   d   d

    2190        2200        2210        2220        2230
      *           *           *           *           *
 AAC GCC AAA GCC GTA GCT AAA GAC CTA GTA CAG GAG CTA ACC CCT GAA
 Asn Ala Lys Ala Val Ala Lys Asp Leu Val Gln Glu Leu Thr Pro Glu>
    b   b   b   b   b   b   ORF 2  b   b   b   b   b   b   >
<Leu Ala Leu Ala Thr Ala Leu Ser Arg Thr Cys Ser Ser Val Gly Ser
 <  d   d   d   d   d   d   ORF 4  d   d   d   d   d   d
```

FIG. 30K

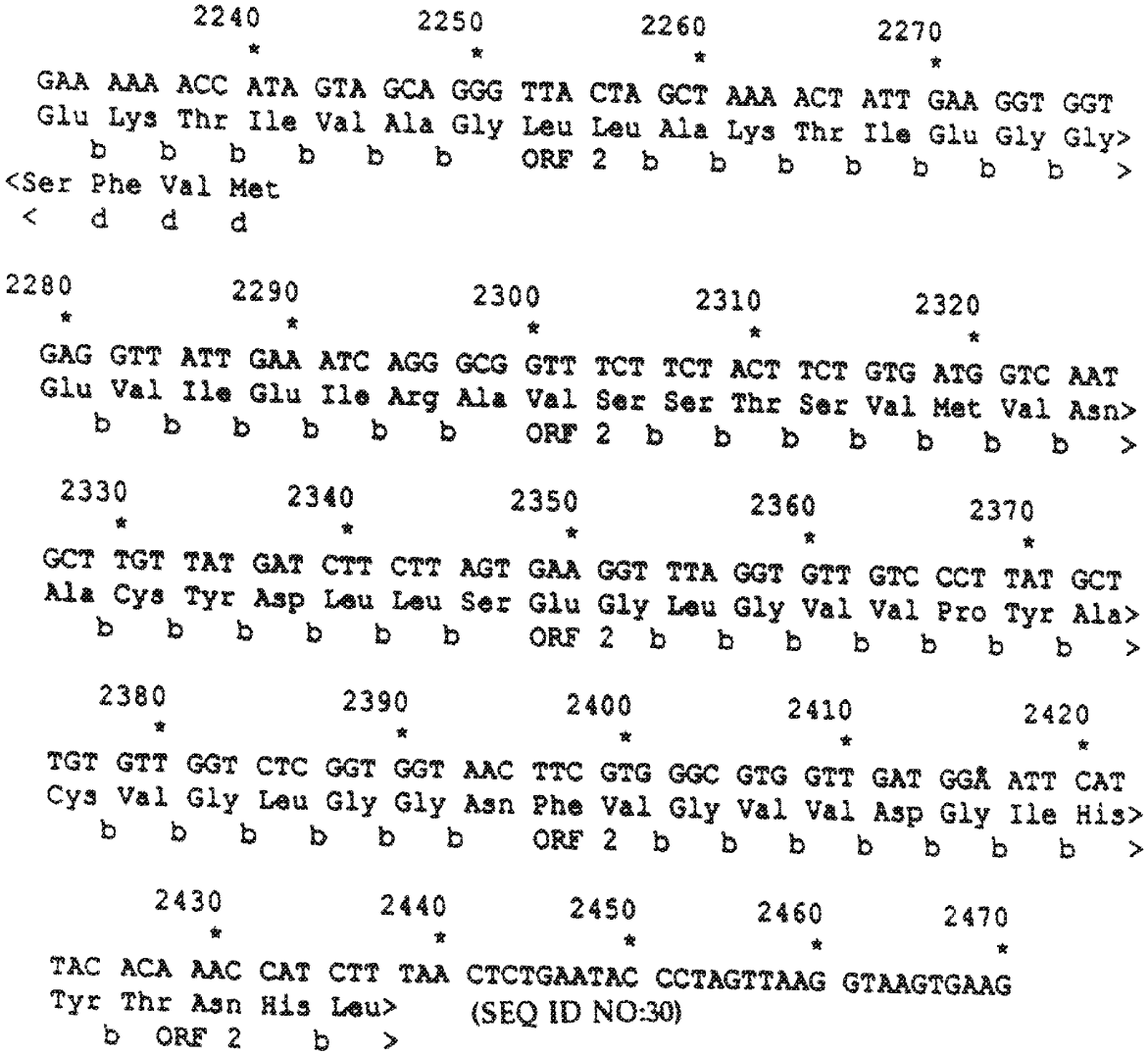

2240 2250 2260 2270
GAA AAA ACC ATA GTA GCA GGG TTA CTA GCT AAA ACT ATT GAA GGT GGT
Glu Lys Thr Ile Val Ala Gly Leu Leu Ala Lys Thr Ile Glu Gly Gly>
b b b b b b ORF 2 b b b b b b b >
<Ser Phe Val Met
< d d d
2280 2290 2300 2310 2320
GAG GTT ATT GAA ATC AGG GCG GTT TCT TCT ACT TCT GTG ATG GTC AAT
Glu Val Ile Glu Ile Arg Ala Val Ser Ser Thr Ser Val Met Val Asn>
b b b b b b ORF 2 b b b b b b b >
2330 2340 2350 2360 2370
GCT TGT TAT GAT CTT CTT AGT GAA GGT TTA GGT GTT GTC CCT TAT GCT
Ala Cys Tyr Asp Leu Leu Ser Glu Gly Leu Gly Val Val Pro Tyr Ala>
b b b b b b ORF 2 b b b b b b b >
2380 2390 2400 2410 2420
TGT GTT GGT CTC GGT GGT AAC TTC GTG GGC GTG GTT GAT GGA ATT CAT
Cys Val Gly Leu Gly Gly Asn Phe Val Gly Val Val Asp Gly Ile His>
b b b b b b ORF 2 b b b b b b b >
2430 2440 2450 2460 2470
TAC ACA AAC CAT CTT TAA CTCTGAATAC CCTAGTTAAG GTAAGTGAAG
Tyr Thr Asn His Leu>
b ORF 2 b >
(SEQ ID NO:30)

FIG. 30L

```
2480       2490       2500       2510       2520       2530
*          *          *          *          *          *
TAACTAGGCA AATTAGTGCT GCACCACTCG TGAAACAAAC TACGATCAGC GATTCACCAT

2540       2550       2560       2570       2580       2590
*          *          *          *          *          *
ACTTAGTAAG TCCGTACAGT GGCTTTACGC TCTTACCCAT CATGAAAAAT ACTTGCTATC

2600       2610       2620       2630       2640       2650
*          *          *          *          *          *
TAGGAATCTC CTCTAAAACT TTACAGAGGT TATCTGTACT TCGAGAGGAA GCTAATCTGT

2660       2670       2680       2690       2700       2710
*          *          *          *          *          *
GGCTCATGAG GATGGTATTT AGCGTATCAC AGGTTCCAGC TGTCTTACAG TCTCTGGAGA

2720       2730       2740       2750       2760       2770
*          *          *          *          *          *
TGTTATAAGG GTGCACATAT AAACTATGC AATATTTCGC TGCAATACGA TTCCGATTCG

2780       2790       2800       2810       2820       2830
*          *          *          *          *          *
AAAACACTGA AAAGTATTCC CATTATCTAT GAATCTCTGT GTAGATATAA ATAAGGGTAT

2840       2850       2860       2870       2880       2890
*          *          *          *          *          *
ACGCAGTAAC TCTTACTTGT TAAAAACAAG ACCAATGGTA TAAGGAAAAA GCCTCAGTGT

2900       2910       2920       2930       2940       2950
*          *          *          *          *          *
TGTTCCTCAT GCTTGCAGCT TACCCGATGC ACTCTTATTT AATAAGGTTG AATGTTAATC

2960       2970       2980       2990       3000       3010
*          *          *          *          *          *
AGTGTTTCTG GGAAGGGAAT ATCTTATTGC AAAAACCTCA GCAGCTGCTT AGATATTGAA
```

FIG. 30M

```
      3020        3030        3040        3050        3060        3070
       *           *           *           *           *           *
ACAAATGCGA TCATGCCGTC AGCACAATTA TGACATCTCT TAAGGCTCTG TAGTGCGCTT

      3080        3090        3100        3110        3120        3130
       *           *           *           *           *           *
ATTTAGTCTA ACATGTGGTA AAGCTTTGCC AGTRCTTTAC CACATGTTCA CCATCAGTTA

          3140        3150        3160        3170
           *           *           *           *
ATT GAA AGC AAA TCT TGC TCC TAT GTT GAA GCC GTA ACT AGC TAT ATT
<Asn Phe Ala Phe Arg Ala Gly Ile Asn Phe Gly Tyr Ser Ala Ile Asn
<   c   c   c   c   c   c   ORF 3   c   c   c   c   c   c   c

3180        3190        3200        3210        3220
 *           *           *           *           *
TGC CTT TAC CTT GGC TGC AGC ACC ACC TGC TAT GTT TAC ACG GTT ACT
<Ala Lys Val Lys Ala Ala Ala Gly Gly Ala Ile Asn Val Arg Asn Ser
<   c   c   c   c   c   c   ORF 3   c   c   c   c   c   c   c

   3230        3240        3250        3260        3270
    *           *           *           *           *
AGC GGG AAT ACC TGC ATA CTG TTC ATC GAA AAT TCC GTG GTA AAA ACC
<Ala Pro Ile Gly Ala Tyr Gln Glu Asp Phe Ile Gly His Tyr Phe Gly
<   c   c   c   c   c   c   ORF 3   c   c   c   c   c   c   c
```

FIG. 30N

```
   3280         3290         3300         3310         3320
     *            *            *            *            *
TCC AGC TAT TAA AGA TAT TTC AGG AGT AAG CTT GTA ACT TAC GCC TAC
<Gly Ala Ile Leu Ser Ile Glu Pro Thr Leu Lys Tyr Ser Val Gly Val
<   c   c   c   c   c   c   ORF 3   c   c   c   c   c   c   c

      3330         3340         3350         3360         3370
        *            *            *            *            *
CTT TCC TCT ATA AGC CAA CTT ACT TGT AAC GTG ATC GGC GAT ATT AAT
<Lys Gly Arg Tyr Ala Leu Lys Ser Thr Val His Asp Ala Ile Asn Ile
<   c   c   c   c   c   c   ORF 3   c   c   c   c   c   c   c

         3380         3390         3400         3410
           *            *            *            *
AAA GCT CGC CCC TAA CCC AGC ACA CAT GTA AGG AGG GAA TTC GAT ATC
<Phe Ser Ala Gly Leu Gly Ala Cys Met Tyr Pro Pro Glu Ile Asp
<   c   c   c   c   c   c   ORF 3   c   c   c   c   c   c   c

3420         3430
  *            *
AAG CTT ATC GAT ACC GT     (SEQ ID NO:28)
<Leu Lys Asp Ile Gly
<   c  ORF 3    c
```

FIG. 30O

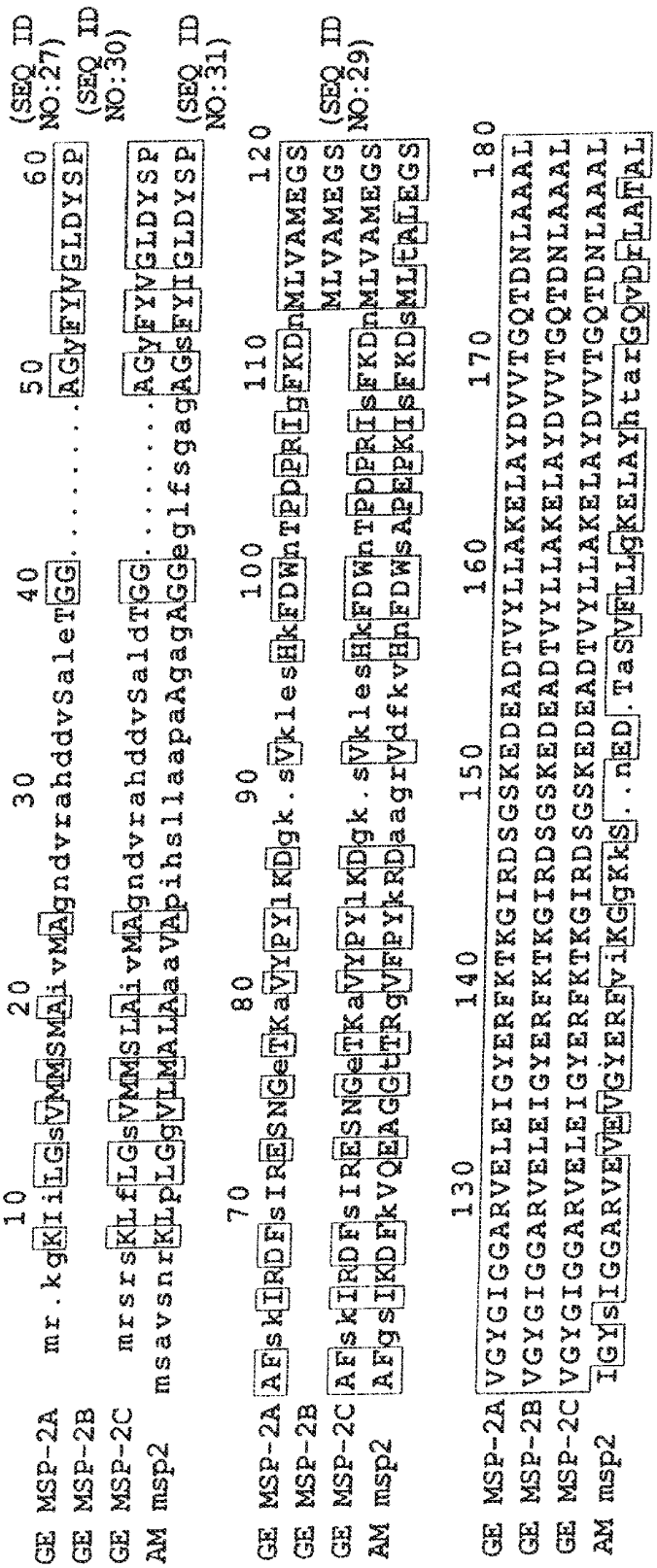

GE MSP-2A
GE MSP-2B
GE MSP-2C
AM msp2
(SEQ ID NO:27)
(SEQ ID NO:30)
(SEQ ID NO:31)
(SEQ ID NO:29)

FIG. 31A

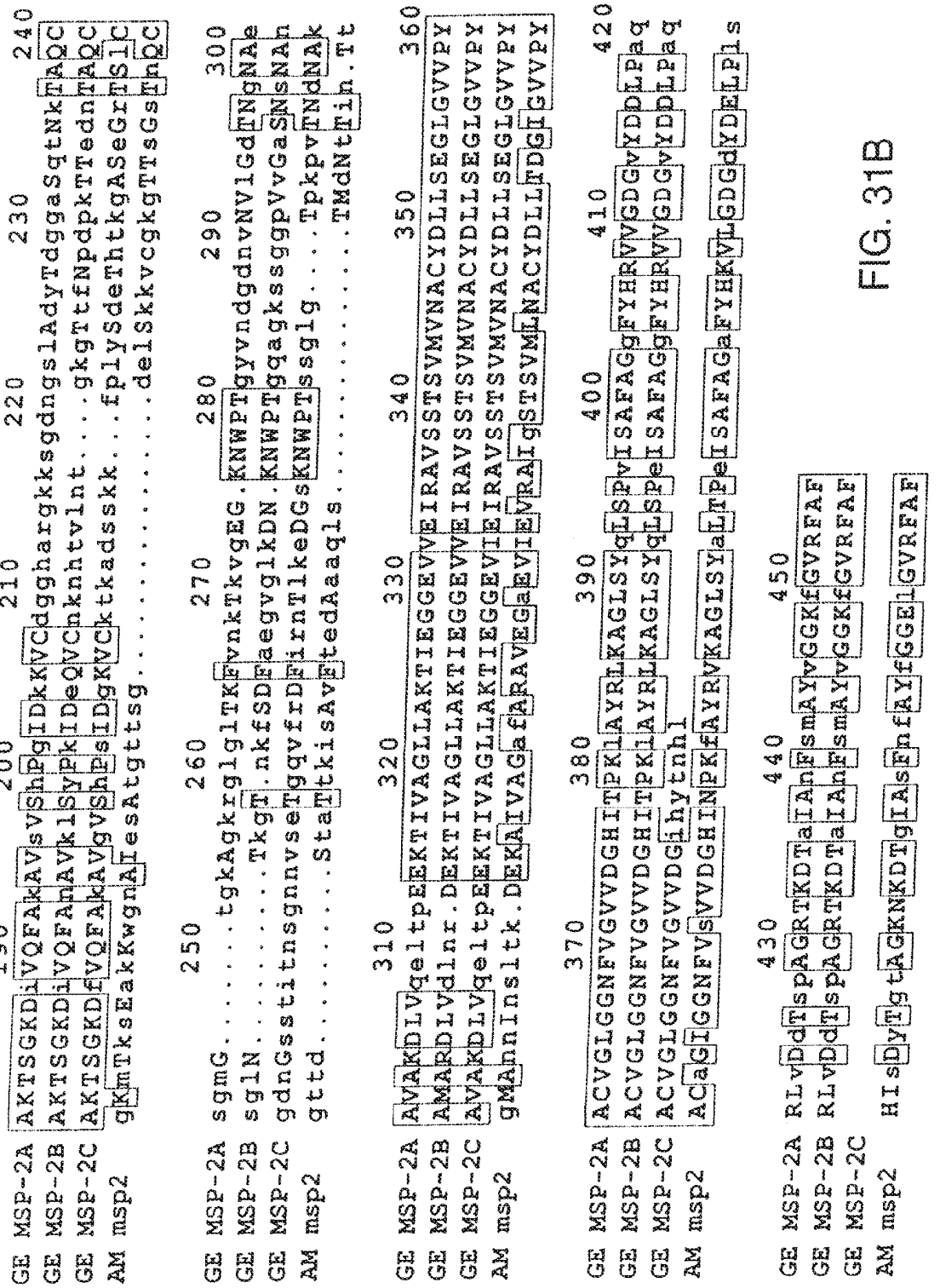


FIG. 31B

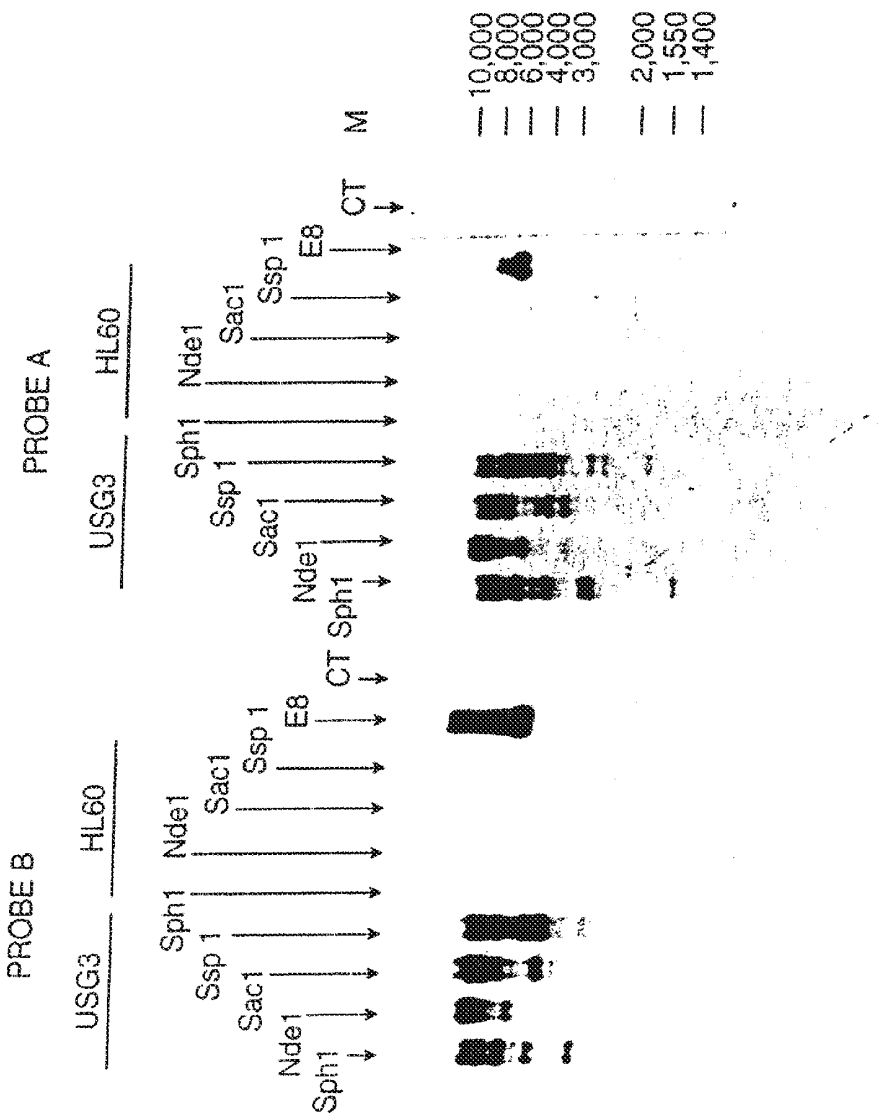


FIG. 32A

FIG. 32B

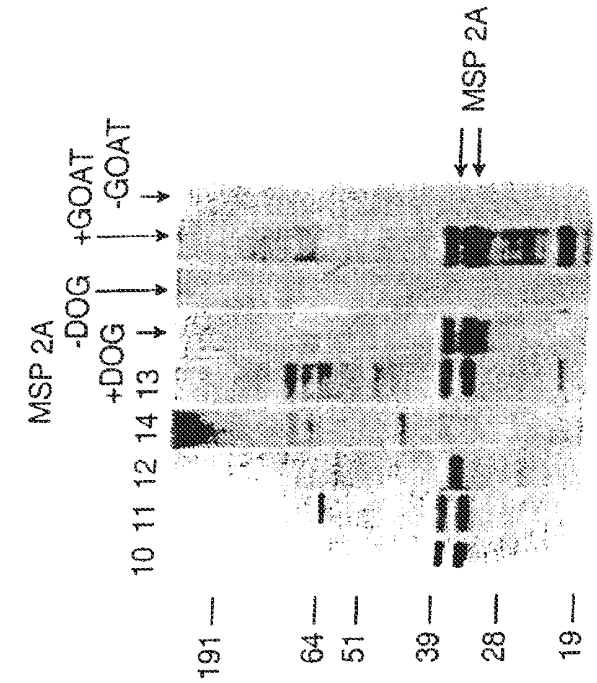


FIG. 33B

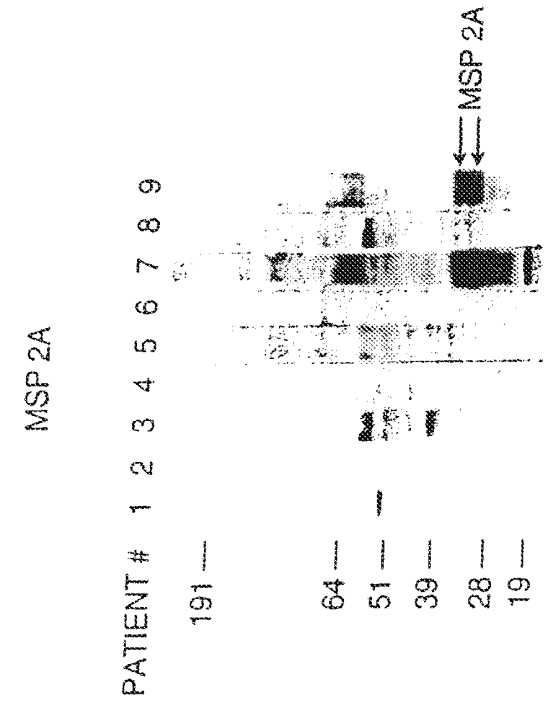


FIG. 33A

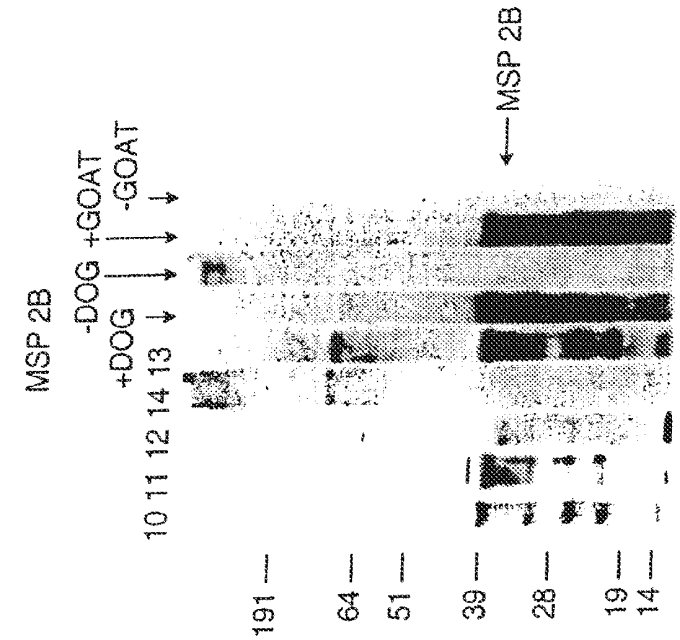


FIG. 33D

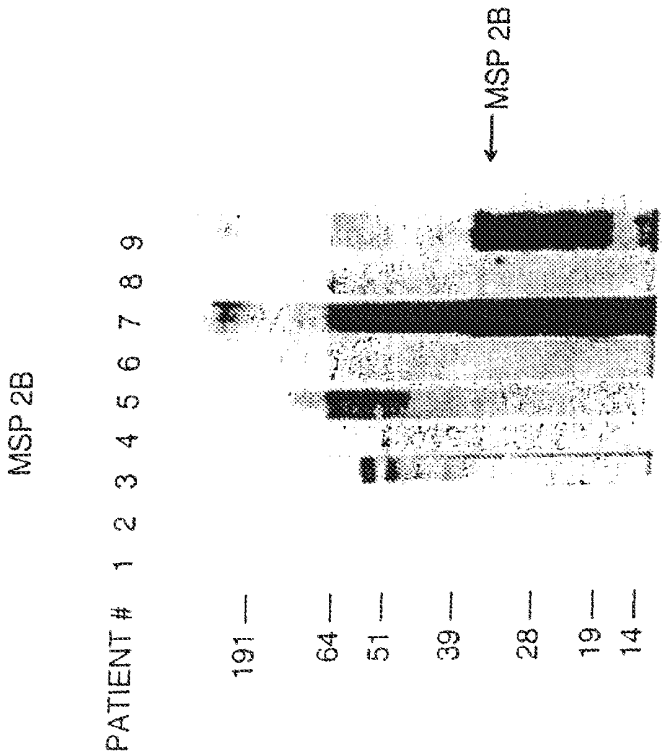


FIG. 33C

```
1621 AGATCTTGAA GCTCATTCTA AAGGAGTTGA AATAGTTTCT GAGGGCGGCA CACAAGATTC
1681 ACTATCCGCT GATTTTCCAA TAAACACAGT TGAAAGTGAA AGTACAGATC TTGAAGCTCA
1741 TTCTCAAGAA GTTGAAACTG TTTCTGAATT CACACAAGAT TCACTATCCA CTAACATTTC
1801 ACAAGACTCA GTTGGTGTAA GTACAGATCT TGAAGTTCAT TCTCAAGAAG TTGAAATAGT
1861 TTCTGAGGGC GGCACACAAG ATTCACTATC CACTAACATT TCACAAGACT CAGTTGGTGT
1921 AAGTACAGAT CTTGAAGCTC ATTCTCAAGA AGTTGAAACT GTTTCTGAAT TCACACAAGA
1981 TTCACTATCC ACTAACATTT CACAAGACTC AGTTGGTGTA AGTACAGATC TTGAAGTTCA
2041 TTCTCAAGAA GTTGAAATAG TTTCTGAGGG CGGCACACAA GATTCACTAT CCACTAACAT
2101 TTCACAAGAC TCAGTTGGTG TAAGTACAGA TCTTTGAAGCT CATTCTAAAG GAGTTGAAAT
2161 AGTTTCTGAG GGCGGCACAC AAGATTCACT ATCCGCTGAT TTTCCAATAA ACACAGTTGA
2221 AAGTGAAAGT ACAGATCTTG AAGCTCATTC CCCAGAAGGT GAAATAGTTT CTGAGGTCAG
2281 CACACAAGAT GCGCCATCCA CTGGAGTAGA GATCAGATTT ATGGATCGTG ATTCTGATGA
2341 TGACGTGCTC GCGTTGTGAA GTGATCATGG TAGGGGAAAC AGTTATGGCG TAAAGACATC
2401 TTTGATGACT TGTCTTGCGT GAATAAGTAG TGCAAGTTTT TTATGCATTG ATGTGCATGA
2461 TCATTGCCCC TAAGCAAAGC AGTACTAATG GTAGTCTAAG ATCTTATACA GGGTTTCGGA
2521 CTACCACTTT TGGTGTTTTA AAACGTCTTA TTCGCGTTGG GTGCTTGCTT ACAATGTACC
2581 TGTACGTGCC CAACACTAAA AATGGTCAGT ATTACTTAGG GGAGTTCGTA GACGAGGCAT
2641 CTCGATTTAC TCTAAGTAAG CTACAAATAA CTCAGTCATA TCAAGGTAGT TCAAGATGAA
2701 AGCAGTGCTA TGCTTATCAT GGAGAATTCC TGCGGTTCTC TTCAAAATTC TCTTTTCCCG
2761 CAAGGGCAGA CTCTTATTTG TTAAAATAAC AAAATTTCTC TACAGGAAGC GACATTTCAT
2821 ATCAAAGCTG ATTGTGAAAT AATGGCATTG AGTATTTTTC TCGCCCTAGA AGATAATCAT
2881 TTCGGCACTA TCAAAGCATT TACGATATTC TCCATTATCT TGTAATCAGA TGGCTATCTT
2941 GAAAGCAACC AAGGATATCC GTACATGGTA GCTTACATAC TGCTATCAAT CTCCTATACG
3001 ACCTTCAATG AAACGGTAAC TGTTGCTGAC AGCTTGCACA TGCTGTGATT CAATTCCTGG
3061 TTCCTAGATG TTCTACTACG TTTATCCGGT ACTAATATTA TTCTTTTGGCG CTCTATTATC
3121 TAGCAACTCA GAGTCCATTA GGAATTC (SEQ ID NO:1)
```

FIG. 4B

PEPTIDE #23

-E-L--*-V-V-*-G-E-N-T-L (SEQ ID NO:36)

PEPTIDE #24

--P-F-H-M-Y-P-G-L-Y-S-E-N-L-F-R-S-T-R-D-L-R-G-V-S-G-V (SEQ ID NO:34)

PRIMER 5F: CCNTTYCAYATGTAYCCNGG (SEQ ID NO:32)

PEPTIDE #25

F-R-L-S-L-A-G-E-Y-A-R-P-K (SEQ ID NO:35)

PEPTIDE #26

--E-D-T-V-R-D-G-I-A-G-F-D-S-L (SEQ ID NO:37)

PRIMER 6R: GGNCKNGCRTAYTCNCCNGC (SEQ ID NO:33)

FIG. 34

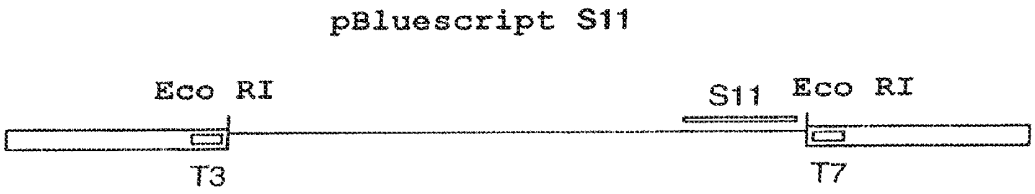


FIG. 35

```
   1 GAATTCCTAGCAACAAGGGTGGATATTTCACGCTTGCTAGGCTGAGTGATTTAGGACTGA
  61 GGGTGAGCTATGAGATGTATAGGGGGGGAGAGTATGCGCCTGCGTGCTTTTTACTCAGCTTC
 121 ATAAGATAGCGGCGAGCTACAGCTTTGCTACGGGGTTCGTAGAAAAGCGTTATTGTCGCT
 181 ATAACACTCGTGATGTATATCATCGTGATGTCGGTTATAAGGATCATGGATGTGCTATGG
 241 TTAAGCCTTTGAAGTATGACTTTGGCTTGATGGCTTTAGGTGTGAAGCTGGTCTTCTAAG
 301 AAGAGTGTGGGTGTTTGTGGATTTTTGAAGGTTTTGTATGAGAGGTTCTCTGGTAGTTGT
                                 M  R  G  S  L  V  V  V
 421 GAGTATGGCGATGCTTCTCCTGGGGTCCTCTGGTGTAGTTGCTGCATCTTCTGGAGG
       S  M  A  M  L  L  L  G  S  S  G  G  V  V  A  A  S  S  G  G
 481 GGGGTTTGAAGGAGAGCGTGCGTCGGTAACGGGTAAGGTGTTATCTTATGCCTGGTTGTT
         G  F  E  G  E  R  A  S  V  T  G  K  V  L  S  Y  A  W  L  L
 541 GAGTGATCGGGCTGTAAAAGGGCAAGGTAACAGTGAAGGTCAGAAGCTCGCGCTGGAAAT
       S  D  R  A  V  K  G  Q  G  N  S  E  G  Q  K  L  A  L  E  M
 601 GTATGGCGCAAAGTTGGGCTATAAGGGTTATGGTTATCCAGGAGTTGGAGATGTCTTTTC
         Y  G  A  K  L  G  Y  K  G  Y  P  G  V  G  D  V  F  S
 661 TTCGCCGTTGGAGCATGGTCTTGATTCTTGGGGAGCTAGCTATGATGCGATGTTATCTCT
       S  P  L  E  H  G  L  D  S  W  G  A  S  Y  D  A  M  L  S  L
 721 TGGATTGCGTACGGGTCGTGATGTGCTAGGTACCCAATATGGGGCAAATTTTTCCCTTAT
         G  L  R  T  G  R  D  V  L  G  T  Q  Y  G  A  N  F  S  L  M
 781 GGTTCCTGCGGGTTCTGGTGGATCTATGGTGTTTCATGGTGCGCCTGGTATAGAGAGCAG
         V  P  A  G  S  G  G  S  M  V  F  H  G  A  P  G  I  E  S  R
 841 GGTTTTTGCTGATACTTCCTTGGGAAATTTTTCTGTTGGTTACCAGGAAGGTGTCGAGTC
         V  F  A  D  T  S  L  G  N  F  S  V  G  Y  Q  E  G  V  E  S
 901 AAAAATGAAGGTGGATGTCTTCGGTGGCTTATCAGGTGAAAATGGAAGCGCTTGGGGTCG
       K  M  K  V  D  V  F  G  G  L  S  G  E  N  G  S  A  W  G  R
 961 GTACTTGCGTGGCTTTTTAAAGTATGCGAAGGGTGTACCTTTTTCACATGTATCCAGGGCT
         Y  L  R  G  F  L  K  Y  A  K  G  V  P  F  H  M  Y  P  G  L
1021 TTACAGTGAGAATTTATTCCGGTCTACAAGAGACTTACGGGGTGTTAGTGGTGTTTCTGC
       Y  S  E  N  L  F  R  S  T  R  D  L  R  G  V  S  G  V  S  A
```

FIG. 36A

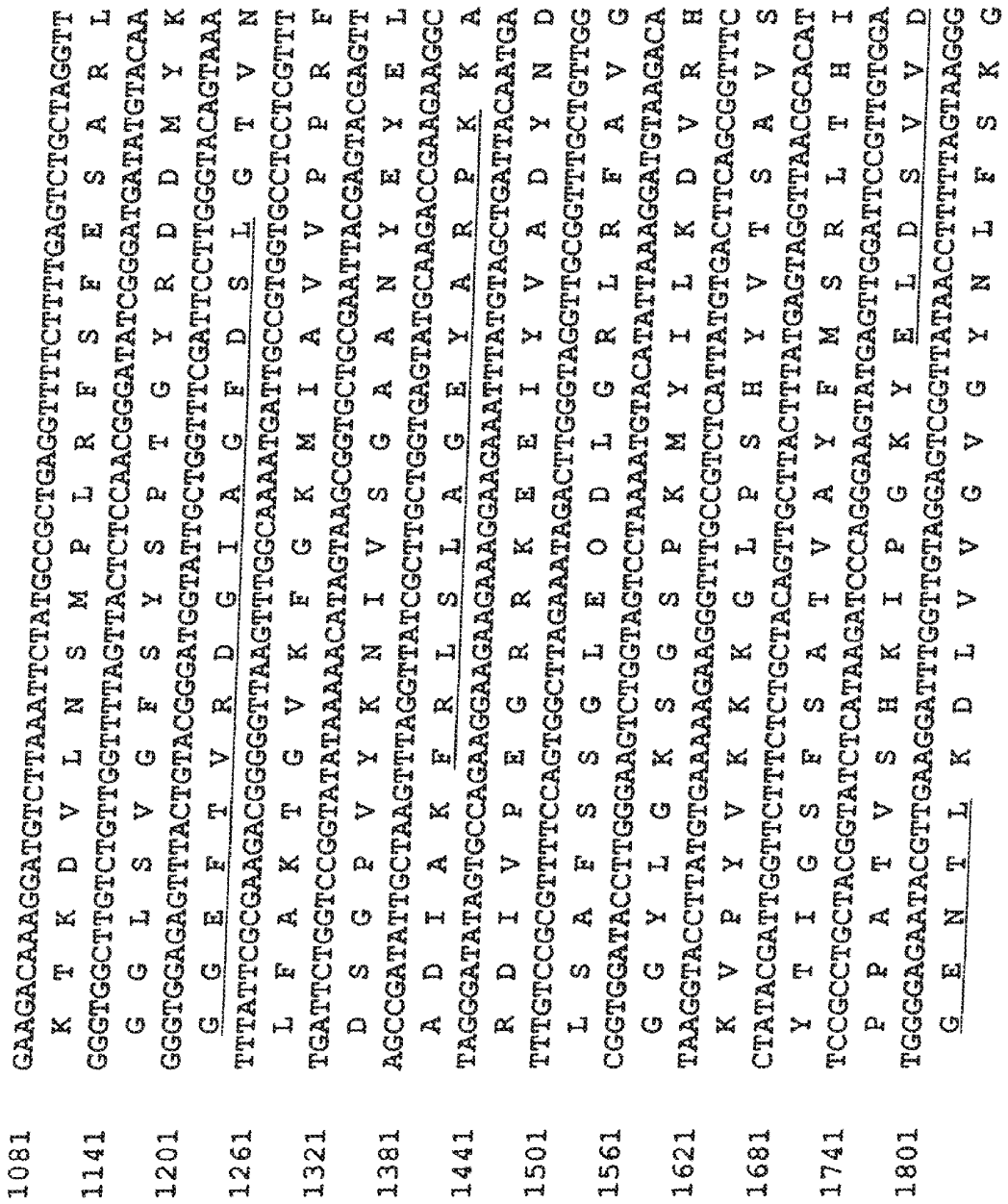

1081 GAAGACAAAGGATGTCTTAAATTCTATGCCGCTGAGGTTTTCTTTTGAGTCTGCTAGGTT
K T K D V L N S M P L R F S F E S A R L
1141 GGGTGGCTTGTCTGTTGGTTTTAGTTACTCTCCAACGGGATATCGGGATGATATGTACAA
G G L S V G F S Y S P T G Y R D D M Y K
1201 GGGTGGAGAGTTTACTGTACGGATGGTATTGCTGGTTTCGATTCCTTGGGTACAGTAAA
G G E F T V R D G I A G F D S L G T V N
1261 TTTATTCGCGAAGACGGGGGTTAAGTTTGGCAAAATGATTGCCGTGGTGCCTCCTCGTTT
L F A K T G V K F G K M I A V V P P R F
1321 TGATTCTGGTCCGGTATATAAAACATAGTAAGCGGTGCTGCGAATTACGAGTACGAGTT
D S G P V Y K N I V S G A A N Y E Y E L
1381 AGCCGATATTGCTAAGTTTAGGTTATCGCTTGCTGGTGAGTATGCAAGACCGAAGAAGGC
A D I A K F R L S L A G E Y A R P K K A
1441 TAGGGATATAGTGCCAGAAGGAAGAGAAAGGAAGAAATTTATGTAGCTGATTACAATGA
R D I V P E G R R K E E I Y V A D Y N D
1501 TTTGTCCGCGTTTTCCAGTGGCTTAGAAATAGACTTGGGTAGGTTGCGGTTTGCTGTTGG
L S A F S S G L E O D L G R L R F A V G
1561 CGGTGGATACCTTGGGAAGTCTGGTAGTCCTAAAATGTACATATTAAAGGATGTAGACA
G G Y L G K S G S P K M Y I L K D V R H
1621 TAAGGTACCTTATGTGAAAAAGAAGGGTTTGCCGTCTCATTATGTGACTTCAGCGGTTTC
K V P Y Y K K K G L P S H Y V T S A V S
1681 CTATACGATTGGTTCTTTCTCTGCTACAGTTGCTTACTTTATGAGTAGGTTAACGCACAT
Y T I G S F S A T V A Y F M S R L T H I
1741 TCCGCCTGCTACGGTATCTCATAAGATCCCAGGGAAGTATGAGTTGGATTCCGTTGTGGA
P P A T V S H K I P G K Y E L D S V V D
1801 TGGGGAGAATACGTTGAAGGATTTGGTTGTAGGAGTCGGTTATAACCTTTTTAGTAAGGG
G E N T L K D L V V G V G Y N L F S K G

FIG. 36B

```
1861  AAGTACGAGCTTAGAAGTATTTCTAAATTGTCACATGTTCTCTGTGCAACATAAATTCAA
        S  T  S  L  E  V  F  L  N  C  H  M  F  S  V  Q  H  K  F  N
1921  CATCCATGAGTACAAATCTACTGAGAGTAGTGGGTTTGTATTGAAAGAAGGTGGAGAGCG
        I  H  E  Y  K  S  T  E  S  S  G  F  V  L  K  E  G  G  E  R
1981  TGCAAATACTAAATAATGGCGCTGTGGCGTTATTAGGAATGAAGTTTTGCGTTTTTAATAACA
        A  N  T  N  N  G  A  V  A  L  L  G  M  K  F  A  F          (SEQ ID NO:39)
2041  AGGGGTTGTTGCAAGAATACTCTTGTGGTTTATTTAGCCAAGTCTTCTTATTGGGGCGTG
2101  TACTGAGGTACGGCGCCCCTTTTTTTTGTGGAGAGTCTAAGGTTTGTTATGTTGTAGA  (SEQ ID NO:38)
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FIG. 36C

CHARACTERIZATION OF GRANULOCYTIC EHRLICHIA AND METHODS OF USE

RELATED APPLICATIONS

This Application claims the benefit under 35 U.S.C. §120 of U.S. application Ser. No. 12/859,236, now U.S. Pat. No. 8,093,008, entitled "CHARACTERIZATION OF GRANULOCYTIC EHRLICHIA AND METHODS OF USE" filed on Aug. 18, 2010, which is herein incorporated by reference in its entirety. Application Ser. No. 12/859,236 claims the benefit under 35 U.S.C. §120 of U.S. application Ser. No. 09/792,957, entitled "CHARACTERIZATION OF GRANULOCYTIC EHRLICHIA AND METHODS OF USE" filed on Feb. 26, 2001, now U.S. Pat. No. 7,863,434, which is herein incorporated by reference in its entirety. Application Ser. No. 09/792,957 claims the benefit under 35 U.S.C. §120 of U.S. application Ser. No. 09/066,046, now U.S. Pat. No. 6,204,252, entitled "CHARACTERIZATION OF GRANULOCYTIC EHRLICHIA AND METHODS OF USE" filed on Apr. 24, 1998, which is herein incorporated by reference in its entirety. Application Ser. No. 09/066,046 claims priority under 35 U.S.C. §119(e) to U.S. Provisional Application Ser. No. 60/044,933, entitled "GRANULOCYTIC EHRLICHIA NUCLEIC ACIDS, PROTEINS, AND METHODS OF USE" filed on Apr. 25, 1997, which is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates, in general, to Granulocytic Ehrlichia (GE) proteins. In particular, the present invention relates to nucleic acid molecules coding for GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 proteins; purified GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 proteins and polypeptides; recombinant nucleic acid molecules; cells containing the recombinant nucleic acid molecules; antibodies having binding affinity specifically to GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 proteins and polypeptides; hybridomas containing the antibodies; nucleic acid probes for the detection of nucleic acids encoding GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 proteins; a method of detecting nucleic acids encoding GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 proteins or polypeptides in a sample; kits containing nucleic acid probes or antibodies; bioassays using the nucleic acid sequence, protein or antibodies of this invention to diagnose, assess, or prognose a mammal afflicted with ehrlichiosis; therapeutic uses, specifically vaccines comprising GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 proteins or polypeptides; and methods of preventing ehrlichiosis in an animal.

2. Related Art

Granulocytic ehrlichiosis is an acute, potentially fatal tick-borne infection. The causative agent, granulocytic Ehrlichia (GE), has been identified by the polymerase chain reaction (PCR) using universal primers for eubacterial 16S ribosomal RNA (rRNA) to amplify the DNA of infected patients' blood (Chen et al., *J. Clin. Micro.* 32:589-595 (1994)). Comparison of the 16S rRNA gene sequence of GE to other known 16S rDNA sequences revealed a nearly identical match to the 16S genes of *Ehrlichia phagocytophila* and *Ehrlichia equi* (Chen et al., 1994). Two other groups of Ehrlichia species have also been categorized according to their 16S rRNA gene sequences, the *Ehrlichia canis* and *Ehrlichia sennetsu* groups. The *E. canis* and *E. sennetsu* species predominantly infect mononuclear phagocytes (Dumler et al., *N. Eng. J. Med.* 325:1109-1110 (1991)), whereas members of the *E. phagocytophila* group including GE are tropic for granulocytes (Ristic et al., in *Bergey's Manual of Systemic Bacteriology*, Krieg et al., eds., (1984), pp. 704-709). The near identity of the 16S rRNA gene sequences and the sharing of significant antigenicity by IFA and immunoblot (Dumler et al., *J. Clin. Micro.* 33:1098-1103 (1995)) indicate that *E. phagocytophila, E. equi*, and GE are closely related.

Full classification of the *E. phagocytophila* species including antigenic relationships among the individual isolates has been impeded by the inability to cultivate these organisms in cell culture. It has been shown that GE can be successfully cultivated in HL60 cells, a human promyelocytic leukemia cell line (Coughlin et al., PCT Application No. PCT/US96/10117; Goodman et al., *N. Eng. J. Med.* 334:209-215 (1996)). Walker et al., PCT Application No. PCT/US97/09147 teaches an isolated gene encoding a 120 kDa immunodominant antigen of *E. chaffeensis* that stimulates production of specific antibodies in infected humans.

The present invention describes GE specific genes encoding ten proteins (S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2) which can be used as diagnostic reagents and vaccines.

SUMMARY OF INVENTION

The invention provides isolated nucleic acid molecules coding for polypeptides comprising amino acid sequences corresponding to GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 proteins.

The invention further provides purified polypeptides comprising amino acid sequences corresponding to GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 proteins.

The invention also provides nucleic acid probes for the specific detection of the presence of GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 proteins or polypeptides in a sample.

The invention further provides a method of detecting nucleic acid encoding GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein in a sample.

The invention also provides a kit for detecting the presence of nucleic acid encoding GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein in a sample.

The invention further provides a recombinant nucleic acid molecule comprising, 5' to 3', a promoter effective to initiate transcription in a host cell and the above-described isolated nucleic acid molecule.

The invention also provides a recombinant nucleic acid molecule comprising a vector and the above-described isolated nucleic acid molecule.

The invention further provides a recombinant nucleic acid molecule comprising a sequence complimentary to an RNA sequence encoding an amino acid sequence corresponding to the above-described polypeptide.

The invention also provides a cell that contains the above-described recombinant nucleic acid molecule.

The invention further provides a non-human organism that contains the above-described recombinant nucleic acid molecule.

The invention also provides an antibody having binding affinity specifically to a GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein or polypeptide.

The invention further provides a method of detecting GE S2, S7, S22, S23, C6.1, C6.2, 511, E8, E46#1, or E46#2 protein or polypeptide in a sample.

The invention also provides a method of measuring the amount of GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein or polypeptide in a sample.

The invention further provides a method of detecting antibodies having binding affinity specifically to a GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein or polypeptide.

The invention further provides a diagnostic kit comprising a first container means containing the above-described antibody, and a second container means containing a conjugate comprising a binding partner of the monoclonal antibody and a label.

The invention also provides a hybridoma which produces the above-described monoclonal antibody.

The invention further provides diagnostic methods for ehrlichiosis. More specifically, the invention further provides a method for identifying granulocytic *Ehrlichia* in an animal comprising analyzing tissue or body fluid from the animal for a S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 nucleic acid, protein, polysaccharide, or antibody.

The invention also provides methods for therapeutic uses involving all or part of the GE S2, S7, S22, S23, C6.1, C6.2, S11, E46#1, or E46#2 nucleic acid or protein. More specifically, the invention further provides a vaccine comprising a GE S2, S7, S22, S23, C6.1, C6.2, S11, E46#1, or E46#2 protein or nucleic acid together with a pharmaceutically acceptable diluent, carrier, or excipient, wherein the protein or nucleic acid is present in an amount effective to elicit a beneficial immune response in an animal to the protein.

The invention also provides a method of preventing or inhibiting ehrlichiosis in an animal comprising administering to the animal the above-described vaccine.

Further objects and advantages of the present invention will be clear from the description that follows.

DEFINITIONS

In the description that follows, a number of terms used in recombinant DNA (rDNA) technology are extensively utilized. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

Isolated Nucleic Acid Molecule. An "isolated nucleic acid molecule", as is generally understood and used herein, refers to a polymer of nucleotides, and includes but should not be limited to DNA and RNA.

Recombinant DNA. Any DNA molecule formed by joining DNA segments from different sources and produced using recombinant DNA technology (i.e., molecular genetic engineering).

DNA Segment. A DNA segment, as is generally understood and used herein, refers to a molecule comprising a linear stretch of nucleotides wherein the nucleotides are present in a sequence that can encode, through the genetic code, a molecule comprising a linear sequence of amino acid residues that is referred to as a protein, a protein fragment or a polypeptide.

Gene. A DNA sequence related to a single polypeptide chain or protein, and as used herein includes the 5' and 3' untranslated ends. The polypeptide can be encoded by a full-length sequence or any portion of the coding sequence, so long as the functional activity of the protein is retained.

Complementary DNA (cDNA). Recombinant nucleic acid molecules synthesized by to reverse transcription of messenger RNA ("mRNA").

Structural Gene. A DNA sequence that is transcribed into mRNA that is then translated into a sequence of amino acids characteristic of a specific polypeptide.

Open Reading Frame ("orf"). The property of some nucleic acid sequences to encode for more than one peptide within the same sequence, which is possible because these sequences contain a series of triplets coding for amino acids without any termination codons interrupting the relevant reading frames.

Restriction Endonuclease. A restriction endonuclease (also restriction enzyme) is an enzyme that has the capacity to recognize a specific base sequence (usually 4, 5, or 6 base pairs in length) in a DNA molecule, and to cleave the DNA molecule at every place where this sequence appears. For example, EcoRI recognizes the base sequence GAATTC/CTTAAG.

Restriction Fragment. The DNA molecules produced by digestion with a restriction endonuclease are referred to as restriction fragments. Any given genome can be digested by a particular restriction endonuclease into a discrete set of restriction fragments.

Agarose Gel Electrophoresis. To determine the length of restriction fragments, an analytical method for fractionating double-stranded DNA molecules on the basis of size is required. The most commonly used technique (though not the only one) for achieving such a fractionation is agarose gel electrophoresis. The principle of this method is that DNA molecules migrate through the gel as though it were a sieve that retards the movement of the largest molecules to the greatest extent and the movement of the smallest molecules to the least extent. Note that the smaller the DNA fragment, the greater the mobility under electrophoresis in the agarose gel.

The DNA fragments fractionated by agarose gel electrophoresis can be visualized directly by a staining procedure if the number of fragments included in the pattern is small. The DNA fragments of genomes can be visualized successfully. However, most genomes, including the human genome, contain far too many DNA sequences to produce a simple pattern of restriction fragments. For example, the human genome is digested into approximately 1,000,000 different DNA fragments by EcoRI. In order to visualize a small subset of these fragments, a methodology referred to as the Southern hybridization procedure can be applied.

Southern Transfer Procedure. The purpose of the Southern transfer procedure (also referred to as blotting) is to physically transfer DNA fractionated by agarose gel electrophoresis onto a nitrocellulose filter paper or another appropriate surface or method, while retaining the relative positions of DNA fragments resulting from the fractionation procedure. The methodology used to accomplish the transfer from agarose gel to nitrocellulose involves drawing the DNA from the gel into the nitrocellulose paper by capillary action or electrophoretic transfer.

Nucleic Acid Hybridization. Nucleic acid hybridization depends on the principle that two single-stranded nucleic acid molecules that have complementary base sequences will reform the thermodynamically favored double-stranded structure if they are mixed under the proper conditions. The double-stranded structure will be formed between two complementary single-stranded nucleic acids even if one is immobilized on a nitrocellulose filter as by the Southern hybridization transfer procedures. In the Southern hybridization procedure, the latter situation occurs. As noted previously, the DNA of the individual to be tested is digested with a restriction endonuclease, fractionated by agarose gel electrophoresis, converted to the single-stranded form, and transferred to nitrocellulose paper, making it available for reannealing to the hybridization probe. Examples of hybridization

conditions can be found in Ausubel, F. M. et al., *Current Protocols in Molecular Biology*, John Wily & Sons, Inc., New York, N.Y. (1989). For example, a nitrocellulose filter is incubated overnight at 68° C. with labeled probe in a solution containing 50% formamide, high salt (either 5×SSC [20×: 3M NaCl/0.3M trisodium citrate] or 5×SSPE [20×: 3.6M NaCl/0.2M NaH_2PO_4/0.02M EDTA, pH 7.7]), 5×Denhardt's solution, 1% SDS, and 100 μg/ml denatured salmon sperm DNA. This is followed by several washes in 0.2×SSC/0.1% SDS at a temperature selected based on the desired stringency: room temperature (low stringency), 42° C. (moderate stringency) or 68° C. (high stringency). The temperature selected is determined based on the melting temperature (Tm) of the DNA hybrid.

Hybridization Probe. To visualize a particular DNA sequence in the Southern hybridization procedure, a labeled DNA molecule or hybridization probe is reacted to the fractionated DNA bound to the nitrocellulose filter. The areas on the filter that carry DNA sequences complementary to the labeled DNA probe become labeled themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling are visualized. The hybridization probe is generally produced by molecular cloning of a specific DNA sequence.

Oligonucleotide or Oligomer. A molecule comprised of two or more deoxyribonucleotides or ribonucleotides, preferably more than three. Its exact size will depend to on many factors, which in turn depend on the ultimate function or use of the oligonucleotide. An oligonucleotide can be derived synthetically or by cloning.

Sequence Amplification. A method for generating large amounts of a target sequence. In general, one or more amplification primers are annealed to a nucleic acid sequence. Using appropriate enzymes, sequences found adjacent to, or in between the primers are amplified.

Amplification Primer. An oligonucleotide which is capable of annealing adjacent to a target sequence and serving as an initiation point for DNA synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is initiated.

Vector. A plasmid or phage DNA or other DNA sequence into which DNA can be inserted to be cloned. The vector can replicate autonomously in a host cell, and can be further characterized by one or a small number of endonuclease recognition sites at which such DNA sequences can be cut in a determinable fashion and into which DNA can be inserted. The vector can further contain a marker suitable for use in the identification of cells transformed with the vector. Markers, for example, are tetracycline resistance or ampicillin resistance. The words "cloning vehicle" are sometimes used for "vector."

Expression. Expression is the process by which a structural gene produces a polypeptide. It involves transcription of the gene into mRNA, and the translation of such mRNA into polypeptide(s).

Expression Vector. A vector or vehicle similar to a cloning vector but which is capable of expressing a gene which has been cloned into it, after transformation into a host. The cloned gene is usually placed under the control of (i.e., operably linked to) certain control sequences such as promoter sequences.

Expression control sequences will vary depending on whether the vector is designed to express the operably linked gene in a prokaryotic or eukaryotic host and can additionally contain transcriptional elements such as enhancer elements, termination sequences, tissue-specificity elements, and/or translational initiation and termination sites.

Functional Derivative. A "functional derivative" of a sequence, either protein or nucleic acid, is a molecule that possesses a biological activity (either functional or structural) that is substantially similar to a biological activity of the protein or nucleic acid sequence. A functional derivative of a protein can contain post-translational modifications such as covalently linked carbohydrate, depending on the necessity of such modifications for the performance of a specific function. The term "functional derivative" is intended to include the "fragments," "segments," "variants," "analogs," or "chemical derivatives" of a molecule.

As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties can improve the molecule's solubility, absorption, biological half life, and the like. The moieties can alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, and the like. Moieties capable of mediating such effects are disclosed in *Remington's Pharmaceutical Sciences* (1980). Procedures for coupling such moieties to a molecule are well known in the art.

Variant. A "variant" of a protein or nucleic acid is meant to refer to a molecule substantially similar in structure and biological activity to either the protein or nucleic acid. Thus, provided that two molecules possess a common activity and can substitute for each other, they are considered variants as that term is used herein even if the composition or secondary, tertiary, or quaternary structure of one of the molecules is not identical to that found in the other, or if the amino acid or nucleotide sequence is not identical.

Allele. An "allele" is an alternative form of a gene occupying a given locus on the chromosome.

Mutation. A "mutation" is any detectable change in the genetic material which can be transmitted to daughter cells and possibly even to succeeding generations giving rise to mutant cells or mutant individuals. If the descendants of a mutant cell give rise only to somatic cells in multicellular organisms, a mutant spot or area of cells arises. Mutations in the germ line of sexually reproducing organisms can be transmitted by the gametes to the next generation resulting in an individual with the new mutant condition in both its somatic and germ cells. A mutation can be any (or a combination of) detectable, unnatural change affecting the chemical or physical constitution, mutability, replication, phenotypic function, or recombination of one or more deoxyribonucleotides; nucleotides can be added, deleted, substituted for, inverted, or transposed to new positions with and without inversion. Mutations can occur spontaneously and can be induced experimentally by application of mutagens. A mutant variation of a nucleic acid molecule results from a mutation. A mutant polypeptide can result from a mutant nucleic acid molecule.

Species. A "species" is a group of actually or potentially interbreeding natural populations. A species variation within a nucleic acid molecule or protein is a change in the nucleic acid or amino acid sequence that occurs among species and can be determined by DNA sequencing of the molecule in question.

Purified. A "purified" protein or nucleic acid is a protein or nucleic acid that has been separated from a cellular component. "Purified" proteins or nucleic acids have been purified to a level of purity not found in nature.

BRIEF DESCRIPTION OF FIGURES

FIG. **1**. Restriction enzyme map of group I clones. The top line represents a composite map of all the group I clones and

contains the recognition sites for selected enzymes. Each group I clone is listed individually below this map and the relative length of the DNA insert is indicated by the line next to the clone name. A more detailed map of S22 is shown with the open reading frame indicated by the black box.

FIG. **2**. Restriction enzyme map of group II clones. Individual group II clones are depicted as described in the legend for FIG. **1**. S2 is the representative clone for this group and the open reading frame is indicated by the black box.

FIG. **3**. Restriction enzyme map of group III clones. Individual group III clones are depicted as described in the legend for FIG. **1**. S7 is the representative clone for this group and the open reading frame is indicated by the black box.

FIG. **4**. DNA sequence of S22 (SEQ ID NO:1). The complete DNA sequence of the S22 insert in Lambda Zap II is shown. The nucleotide number is indicated in the left margin.

FIG. **5**. FIG. **5**A shows the amino acid sequence of S22 (SEQ ID NO:2). This sequence constitutes the translated amino acid sequence for the open reading frame of S22 beginning at nucleotide 500 and ending with the stop codon at nucleotide 2359 of SEQ ID NO:1 (See, FIG. **4**). FIG. **5**B shows the nucleic acid sequence of the 130 kDa protein, corresponding to nucleotides 451-2379 of SEQ ID NO:1. Nucleotide numbers are indicated at the left. The ATG start codon and TAA stop codon are shown in bold type. The translated amino acid sequence for the open reading frame is displayed underneath the DNA sequence using the single-letter amino acid code (SEQ ID NO:2).

FIG. **6**. DNA sequence of S2 (SEQ ID NO:3). The complete DNA sequence of the S2 insert in Lambda Zap II is shown in FIG. **6**A and continued in FIGS. **6**B and **6**C. The nucleotide number is indicated in the left margin.

FIG. **7**. FIG. **7**A shows the amino acid sequence of S2 (SEQ ID NO:4) for the open reading frame beginning at nucleotide 1576 and ending with the stop codon at nucleotide 3801 (See, FIG. **6**). FIG. **7**B shows the nucleic acid sequence of the 160 kDa protein gene (nucleotides 1501-3850 of SEQ ID NO:3). Nucleotide numbers are indicated at the left. The ATG start codon and TAA stop codon are shown in bold type. The translated amino acid sequence for the open reading frame is displayed underneath the DNA sequence using the single-letter amino acid code (SEQ ID NO:4).

FIG. **8**. DNA sequence of S7 (SEQ ID NO:5). The complete DNA sequence of the S7 insert in Lambda Zap II is shown in FIG. **8**A and continued in FIGS. **8**B and **8**C. The nucleotide number is indicated in the left margin.

FIG. **9**. FIG. **9**A shows the amino acid sequence of S7 (SEQ ID NO:6) for the open reading frame beginning at nucleotide 233 and ending with the stop codon at nucleotide 1969 (See, FIG. **8**). FIG. **9**B also shows the nucleic acid sequence of the 100 kDa protein gene (nucleotide 172-2001 of SEQ ID NO:5). Nucleotide numbers are indicated at the left. The ATG start codon and TAA stop codon are shown in bold type. The translated amino acid sequence for the open reading frame is displayed underneath the DNA sequence using the single-letter amino acid code (SEQ ID NO:6).

FIG. **10**. DNA sequence of S23 (SEQ ID NO:7). The complete DNA sequence of the S23 insert in Lambda Zap II is shown in FIG. **10**A and continued in FIGS. **10**B, **10**C and **10**D. The nucleotide number is indicated in the left margin.

FIG. **11** Amino acid sequence of S23 for the open reading frame which begins at nucleotide 254 and ends at nucleotide 1708 of SEQ ID NO:7 (See, FIG. **10**) is shown (SEQ ID NO:8). Two smaller open reading frames are found at nucleotides 2656-2997 (complementary strand) and nucleotides 3904-4248 (See, FIG. **10**).

FIG. **12**. Schematic diagram of S22 and S23 proteins. The boxes represent amino acid repeat regions. Lighter boxes: 28 amino acid repeats; Darker boxes: 59 amino acid repeats. Note: the 28 amino acid repeats are also contained within the 59 amino acid repeat regions. The approximate size and location of the S22 deletion which results in S23 is indicated.

FIG. **13**. Schematic diagrams of S2 (top) and S7 (bottom) proteins. Repeat regions are indicated by the boxes.

FIG. **14**. Schematic diagram of GE 160 kDa protein. Repeat regions are indicated by the boxes. Sequences of proposed ankyrin repeats, numbered 1-8 (SEQ ID NOS:9-16), are aligned using the consensus sequence (SEQ ID NO:17) at the top: h, hydrophobic; t, turn-like or to polar; S/T, serine or threonine; capitals, conserved amino acids.

FIG. **15**. Amino acid sequence alignments of selected regions of GE 130 kDa and *E. chaffeensis* 120 kDa proteins (A) (SEQ ID NOS:73-77) and GE 100 kDa (SEQ ID NOS: 78-81) and *E. chaffeensis* 120 kDa proteins (SEQ ID NOS: 82-83) (B). Each protein is shown as a linear amino acid sequence and amino acids are numbered in hundreds. Boxed regions on the linear sequence represent repeated amino acids. FIG. **15**A shows the amino acid alignments of a sequence which occurs 4 times in the *E. chaffeensis* protein (top line of alignment, A-I) and 8 times in the GE 130 kDa protein (a-1 to a-4). Sequence a-1 is repeated 3 times, related sequences a-2 and a-3 are each repeated twice, and related sequence a-4 is found once. The position of these sequences in the proteins is indicated by the small bold lines. FIG. **15**B shows the amino acid alignments of two different sequence motifs which occur in the *E. chaffeensis* 120 kDa protein (B-1 to B-3 and C-1) and the GE 100 kDa protein (b-1 and c-1). Bold and cross-hatched boxes indicate the position of these sequences in the proteins. Identical amino acids are surrounded by boxes and conserved amino acids are in capital letters.

FIG. **16**. Western blot analysis of: A) Purified USG3 disrupted in SDS (lane GE). B) Individual recombinant clones of GE 100 kDa (S7), GE 160 kDa (S2), GE 130 kDa (S22), and a negative control (NEG, no insert), were grown and incubated with IPTG to induce protein expression according to Materials and Methods. Samples of each were electrophoresed on SDS-PAGE gels and transferred to nitrocellulose for Western blotting. Blots were probed with convalescent dog sera. Molecular weight markers (in kilodaltons) are shown to the left of each figure.

FIG. **17**. Western blot analysis of S2, S7, S22, and S23 proteins. Individual recombinant clones of S2, S7, S22, S23, and a negative control were grown and induced by IPTG to induce protein expression. Samples of each were electrophoresed on a SDS-PAGE gel and transferred to nitrocellulose for Western blotting. SDS-disrupted GE was used as a positive control. The blot probed with convalescent dog sera and samples are indicated at the top of the gel. Molecular weight markers (in kilodaltons) are shown to the left of each figure.

FIG. **18**. Western blot analysis of GE proteins. Three different human serum samples were used to probe Western blots containing SDS-disrupted USG3 (GE lanes), GE160, GE100, and GE130. A pBluescript library clone containing no insert was used as a negative control (NEG). Origin of sera is indicated at the bottom of each panel (WI, Wisconsin; NY, New York). Molecular weight markers (in kilodaltons) are shown to the left of each panel.

FIG. **19**. PCR analysis of groups I, II and III. PCR reactions were performed and the products analyzed using 4% Nusieve gels. Primer sequences are listed in Table 5. A) S22 primers were used to amplify a 159 bp region of S22 DNA using as

templates: S22 plasmid DNA (lane 4), S23 plasmid DNA (lane 8), HL60 DNA (lanes 2 and 6) and GE DNA (lanes 3 and 7). B) S2 primers were used to amplify a 395 by region of S2 DNA using as templates: S2 plasmid DNA (lanes 4 and 5), HL60 DNA (lane 2) and GE DNA (lane 3). C) S7 primers were used to amplify a 643 bp region of S7 DNA using as templates: S7 plasmid DNA (lane 3), HL60 DNA (lane 4) and GE DNA (lane 2). DNA molecular weight markers (50-1000 bp, FMC) are present in lane 1 of each figure.

FIG. **20**. PCR analysis of GE genes. PCR reactions were performed as described in Materials and Methods and the products analyzed using 4% Nusieve gels. S2 primers were used to amplify a 395 by region of S2 DNA using as templates: HL60 DNA (lane 2), S2 plasmid DNA (lane 3), and USG3 DNA (lane 4). S7 primers were used to amplify a 643 bp region of S7 DNA using as templates: HL60 DNA (lane 5), S7 plasmid DNA (lane 6), and USG3 DNA (lane 7). S22 primers were used to amplify a 159 bp region of S22 DNA using as templates: HL60 DNA (lane 8), S22 plasmid DNA (lane 9), and USG3 DNA (lane 10). DNA molecular weight markers (50-1000 bp, FMC, Rockland, Me.) are present in lane 1.

FIG. **21**. Amino acid sequence (SEQ ID NO:21) which is the translated amino acid sequence for the open reading frame of the C6.1 gene, which begins at nucleotide 312 and ends at nucleotide 1532 of SEQ ID NO:23 (See, FIG. **23**).

FIG. **22**. Amino acid sequence (SEQ ID NO:22) which is the translated amino acid sequence for the open reading frame of the C6.2 gene, which begins at nucleotide 1542 and ends at nucleotide 2336 of SEQ ID NO:23 (See, FIG. **23**).

FIG. **23**. DNA sequence of C6 (SEQ ID NO:23). The complete double strand DNA sequence of the C6 insert in Lambda Zap II is shown.

FIG. **24**. Western blot analysis of three C clones. Individual recombinant clones of C1, C6, and C7 were grown and induced by IPTG to induce protein expression according to Materials and Methods. Samples of each were electrophoresed on SDS-PAGE gels and transferred to nitrocellulose for Western blotting. SDS-disrupted GE was used as a positive control. The blot was probed with vaccinated mouse "C" sera. Samples are indicated at the top of the gel. Molecular weight markers (in kilodaltons) are shown to the left of the figure.

FIG. **25**. PCR analysis of C6. PCR reactions were performed and the products analyzed using 4% Nusieve gels. Primer sequences are listed in Table 5. C6.1 primers (from the first open reading frame, lanes 2, 3, 4) were used to amplify a 500 bp region of C6 DNA using as templates: C6 plasmid DNA (lane 4), HL60 DNA (lane 2) and GE DNA (lane 3). C6.2 primers (from the second open reading frame, lanes 5, 6, 7) were used to amplify a 300 bp region of C6 DNA using as templates: C6 plasmid DNA (lane 7), HL60 DNA (lane 5) and GE DNA (lane 6). Both primer sets were also used together in the same PCR reaction using C6 plasmid DNA as template (lane 8). DNA molecular weight markers (50-1000 bp, FMC) are present in lane 1.

FIG. **26**. ClustalW alignment of amino acids encoded by the 550 bp PCR product (SEQ ID NO:24) and the MSP-2 protein of *A. marginate* (GenBank accession number U07862) (SEQ ID NO:25). Identical amino acids are enclosed by boxes Amino acids which represent conservative codon changes are shown in capital letters.

FIG. **27**. Western blot of GE proteins. Samples containing purified USG3 antigen (GE lanes), uninfected HL60 cell proteins (HL60), a pBluescript library clone with no insert (NEG), E46, E8, or E33 were analyzed by SDS-PAGE and transferred to nitrocellulose blots. Blots were probed with either dog (FIG. **27**A) or goat (FIG. **27**B) sera. Molecular size markers are indicated on the left of each blot. Positions of expressed proteins are indicated by arrows at the right side of each blot. The double arrow on the left indicates the proteins that were excised for peptide sequencing.

FIG. **28**. Schematic diagram of E8, E33 and E46 pBluescript inserts. Each strand of the DNA insert is shown as a line; +) plus strand of DNA; −) minus strand of DNA. Boxed regions indicate related open reading frames. The position and orientation (arrows) of the lacZ promoter is indicated.

FIG. **29**. Sequence of the GE E8 msp2 gene (SEQ ID NO:26). Nucleotide numbers are indicated at the left. The ATG start codon and TAA stop codon are shown in bold type. The translated amino acid sequence for the open reading frame is displayed underneath the DNA sequence using the single letter amino acid code (SEQ ID NO:27). A possible ribosome binding site upstream of the ATG codon is also underlined.

FIG. **30**. Complete sequence of E46. The nucleotide number is indicated above the sequences. The complete DNA sequence of the E46 insert in Lambda Zap II is shown (SEQ ID NO:28). The translated amino acid sequences for the open reading frames are displayed underneath the DNA sequences. The amino acid sequence of E46#1 which begins at nucleotide 305 and ends at nucleotide 1282, is shown (SEQ ID NO:29). The amino acid sequence of E46#2 which begins at nucleotide 1346 and ends at nucleotide 2437, is shown (SEQ ID NO:30). The amino acid sequence of ORF3 corresponds to SEQ ID NO: 84. The amino acid sequence of ORF4 corresponds to SEQ ID NO: 85.

FIG. **31**. ClustalW alignment of GE MSP-2 and *A. marginale* MSP-2 (U07862) protein sequences (SEQ ID NOS:27, 29-31). Identical amino acids are enclosed by boxes. Amino acids which represent conservative codon changes are indicated by capital letters. The symbol-denotes a gap used to achieve optimal alignment between the sequences.

FIG. **32**. Southern blot analysis of USG3 genomic DNA. Genomic DNA from USG3 or HL60 cells was digested with the restriction enzymes indicated above the lanes and Southern blotted. Eco RI-digested E8 plasmid DNA was used as a positive control for probe hybridization and calf thymus DNA (CT) as a negative control. The blots were hybridized with digoxigenin-labeled probe A (5' end of E8 msp-2A) (FIG. **32**B) or probe B (3' end of E8 msp-2A) (FIG. **32**A).

FIG. **33**. Western blot analysis of E33 bacterial cultures expressing MSP-2A and MSP-2B probed with HGE patient sera. Bacterial cultures of E33 MSP-2A (top) and MSP-2B (bottom) were analyzed by SDS-PAGE and the proteins transferred to nitrocellulose blots. The blots were cut into strips and probed with patient sera #1-14 as indicated above the lanes. These numbers correspond to the patient numbers shown in Table 7 Immune(+) and preimmune(−) dog and goat sera were also used as positive and negative controls. Molecular size markers are indicated on the left side of each blot. The arrows show the positions of the MSP-2 proteins.

FIG. **34** Amino acid sequence of 64 kDa protein degenerate primer sequences derived therefrom (SEQ ID NOS:32-33) are listed for SEQ ID NOS:34 and 35 (peptides 24 and 25, respectively). Amino Acids from which the primer sequences were generated are underlined. Two other peptides are listed: peptide #23 (SEQ ID NO:36) and peptide #26 (SEQ ID NO:37). Undetermined positions of the peptide sequences are designated with an asterisk (*).

FIG. **35**. Linear map of pBluescript S11. Boxes on either end represent vector sequences and the solid center line denotes the insert. The T3 and T7 promoter sequences are positioned as indicated and the S11 gene is shown as a bold line.

```
   1  MFEHNIPDTY TGTTAEGSPG LAGGDFSLSS IDFTRDFTIE SHRGSSADDP GYISFRDQDG
  61  NVMSRFLDVY VANFSLRCKH SPYNNDRMET AAFSLTPDII EPSALLQESH STQNNVEEAV
 121  QVTALECPPC NPVPAEEVAP QPSFLSRIIQ AFLWLFTPSS TTDTAEDSKC NSSDTSKCTS
 181  ASSESLEQQQ ESVEVQPSVL MSTAPIATEP QNAVVNQVNT TAVQVESSII VPESQHTDVT
 241  VLEDTTETIT VDGEYGHFSD IASGEHNNDL PAMLLDEADF TMLLANEESK TLESMPSDSL
 301  EDNVQELGTL PLQEGETVSE GNTRESLPTD VSQDSVGVST DLEAHSQEVE TVSEVSTQDS
 361  LSTNISQDSV GVSTDLEAHS KGVETVSEGG TQDSLSADFP INTVESESTD LEAHSQEVET
 421  VSEFTQDSLS TNISQDSVGV STDLEVHSQE VETVSEGGTQ DSLSTNISQD SVGVSTDLEA
 481  HSQEVETVSE FTQDSLSTNI SQDSVGVSTD LEVHSQEVEI VSEGGTQDSL STNISQDSVG
 541  VSTDLEAHSK GVETVSEGGT QDSLSADFPI NTVESESTDL EAHSPEGEIV SEVSTQDAPS
 601  TGVEIRFMDR DSDDDVLAL  (SEQ ID NO:2)
```

FIG. 5A

FIG. **36**. Nucleic acid sequence (SEQ ID NO:38) and amino acid sequence (SEQ ID NO:39) of S11/GE 59 kDa. Start and stop codons are in bold type. Sequenced peptides are to underlined in FIG. **36**.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The sequencing and protein analysis of nine recombinant clones (S2, S7, S22, S23, C6, S11, E8, E46#1, and E46#2) identified by immunological screening of a GE genomic library is described. Two of these clones, S22 and S23, encode identical proteins which differ only by the loss of a repeated region in S23. One clone, C6, contains two open reading frames encoding polypeptides C6.1, C6.2. Clones E8, E46#1, and E46#2 contain conserved amino- and carboxy-terminus regions. These genomic DNA isolates were proven to be specific to GE based on PCR analysis of GE DNA and HL60 DNA.

Of the hundreds of phage plaques that came up positive using either convalescent dog sera or vaccinated mouse sera, the vast majority were identified as either group I (e.g., S22 or S23), group II (e.g., S2), group III (e.g., S7). The genes described herein most likely encode immunodominant GE antigens which may also be present in more than one copy in the GE genome. Other immunodominant rickettsial antigens have been shown to be important diagnostic reagents and vaccine targets including the outer membrane polypeptides of *Anaplasma marginale* (Tebele et al., *Infect. Immun.* 59:3199-3204 (1991)), immunogenic proteins of *Cowdria rumantiun* (Mahan et al., *Microbiology* 140:2135-2142 (1994); van Vliet et al., *Infect. Immun.* 62:1451-1456 (1994)), the 120 kDa immunodominant protein of *E. chaffeensis* (Yu et al., *J. Clin. Micro.* 34:2853-2855 (1996)), the immuno-dominant surface protein antigen of *Rickettsia prowazekii* (Dasch et al., in *Microbiology*, D. Schlessinger (ed.), American Society for Microbiology, Washington, D.C., (1984), pp. 251-256) and two *Rickettsia rickettsii* surface proteins (Anacker et al., *Infect. Immun.* 55:825-827 (1987); Sumner et al., *Vaccine* 13:29-35 (1995)). Many of these proteins contain highly repeated regions similar to those found for GE proteins. Repetitive protein domains have been shown to function in ligand binding—(Wren, *Mol. Microbiol.* 5:797-803 (1991)) and may function to facilitate rickettsial uptake by host cell membranes.

For purposes of clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the following subsections:

I. Isolated Nucleic Acid Molecules Coding for S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 Polypeptides;

II. Recombinantly Produced S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 Polypeptides;

III. A Nucleic Acid Probe for the Specific Detection of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2;

IV. A Method of Detecting The Presence of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 in a Sample;

V. A Kit for Detecting the Presence of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 in a Sample;

VI. DNA Constructs Comprising S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 Nucleic Acid Molecule and Cells Containing These Constructs;

VII. An Antibody Having Binding Affinity to S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 Polypeptide and a Hybridoma Containing the Antibody;

VIII. A Method of Detecting a S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 Polypeptide or Antibody in a Sample;

IX. A Diagnostic Kit Comprising S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 Protein or Antibody;

X. Diagnostic Screening; and

XI. Vaccines

I. Isolated Nucleic Acid Molecules Coding for S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 Polypeptides

In one embodiment, the present invention relates to isolated nucleic acid molecules comprising a polynucleotide sequence at least 90% identical (more preferably, 95%, 96%, 97%, 98%, 99%, or 100% identical) to a sequence selected from:

(a) a nucleotide sequence encoding the S2, S7, S22, S23, C6.1, C6.2, S11, E8, or E46#1, E46#2 polypeptide comprising the complete amino acid sequence in SEQ ID NOS:4, 6, 2, 8, 21, 22, 39, 27, 29, and 30, respectively;

(b) a nucleotide sequence encoding the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 polypeptide comprising the complete amino acid sequence encoded by the polynucleotide clone contained in ATCC Deposit Nos. 97844, 97840, 97842, 97843, 97841, 97841, 209740, 209736, 209743, and 209743 respectively (note, C6.1 and C6.2, are encoded by the polynucleotide clone contained in ATCC Deposit No. 97841 and that E46#1 and E46#2 are encoded by the polynucleotide clone contained in ATCC Deposit No. 209743); and

(c) a nucleotide sequence complementary to any of the nucleotide sequences in (a) or (b).

The S2, S7, S22, S23, and C6 (encoding C6.1 and C6.2) nucleic acids were deposited at the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md. 20852, USA on Dec. 31, 1996 as ATCC Deposit Nos. 97844, 97840, 97842, 97843, and 97841, respectively. The S11, E8, and E46 (encoding E46#1 and E46#2) nucleic acids were deposited at the ATCC on Mar. 31, 1998 as ATCC Deposit Nos. 209740, 209736 and 209743, respectively.

In one preferred embodiment, the isolated nucleic acid molecule comprises a GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 nucleotide sequence with greater than 90% identity or similarity to the nucleotide sequence present in SEQ ID NOS:3, 5, 1, 7, 23, 23, 38, 26, 28 or 28 (preferably greater than 95%, 96%, 97%, 98%, 99% or 100%), respectively. In another preferred embodiment, the isolated nucleic acid molecule comprises the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 nucleotide sequence present in SEQ ID NOS:3, 5, 1, 7, 23, 23, 38, 26, 28, or 28, respectively. In another embodiment, the isolated nucleic acid molecule encodes the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 amino acid sequence present in SEQ ID NOS:4, 6, 2, 8, 21, 22, 39, 27, 29, or 30, respectively.

Also included within the scope of this invention are the functional equivalents of the herein-described isolated nucleic acid molecules and derivatives thereof. For example, the nucleic acid sequences depicted in SEQ ID NOS:3, 5, 1, 7, 23, 23, 38, 26, 28, or 28 can be altered by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as depicted in SEQ ID NOS:4, 6, 2, 8, 21, 22, 39, 27, 29, or 30 can be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or portions of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 nucleic acid depicted in SEQ ID NOS:3, 5, 1, 7, 23, 23, 38, 26, or 28,

respectively which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence.

In addition, the nucleic acid sequence can comprise a nucleotide sequence which results from the addition, deletion or substitution of at least one nucleotide to the 5'-end and/or the 3'-end of the nucleic acid formula shown in SEQ ID NOS:3, 5, 1, 7, 23, 23, 38, 26, 28, or 28 or a derivative thereof. Any nucleotide or polynucleotide can be used in this regard, provided that its addition, deletion or substitution does not substantially alter the amino acid sequence of SEQ ID NOS: 4, 6, 2, 8, 21, 22, 39, 27, 29, or 30 which is encoded by the nucleotide sequence. Moreover, the nucleic acid molecule of the present invention can, as necessary, have restriction endonuclease recognition sites added to its 5'-end and/or 3'-end. All variations of the nucleotide sequence of the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 gene and fragments thereof permitted by the genetic code are, therefore, included in this invention.

Further, it is possible to delete codons or to substitute one or more codons by codons other than degenerate codons to produce a structurally modified polypeptide, but one which has substantially the same utility or activity of the polypeptide produced by the unmodified nucleic acid molecule. As recognized in the art, the two polypeptides are functionally equivalent, as are the two nucleic acid molecules which give rise to their production, even though the differences between the nucleic acid molecules are not related to degeneracy of the genetic code.

A. Isolation of Nucleic Acid

In one aspect of the present invention, isolated nucleic acid molecules coding for polypeptides having amino acid sequences corresponding to S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 are provided. In particular, the nucleic acid molecule can be isolated from a biological sample (preferably of mammalian or tick origin) containing GE RNA or DNA.

The nucleic acid molecule can be isolated from a biological sample containing GE RNA using the techniques of cDNA cloning and subtractive hybridization. The nucleic acid molecule can also be isolated from a cDNA library using a homologous probe.

The nucleic acid molecule can be isolated from a biological sample containing genomic DNA or from a genomic library. Suitable biological samples include, but are not limited to, whole organisms, organs, tissues, blood and cells. The method of obtaining the biological sample will vary depending upon the nature of the sample.

One skilled in the art will realize that genomes can be subject to slight allelic variations between individuals. Therefore, the isolated nucleic acid molecule is also intended to include allelic variations, so long as the sequence is a functional derivative of the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 coding sequence. When an S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2, allele does not encode the identical sequence to that found in SEQ ID NOS: 3, 5, 1, 7, 23, 23, 38, 26, 28 or 28 it can be isolated and identified as S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 using the same techniques used herein, and especially PCR techniques to amplify the appropriate gene with primers based on the sequences disclosed herein.

One skilled in the art will realize that organisms other than GE will also contain S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 genes. The invention is intended to include, but not be limited to, S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 nucleic acid molecules isolated from the above-described organisms. Also, infected eukaryotes (for example, mammals, birds, fish and humans) may contain the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 genes.

B. Synthesis of Nucleic Acid

Isolated nucleic acid molecules of the present invention are also meant to include those chemically synthesized. For example, a nucleic acid molecule with the nucleotide sequence which codes for the expression product of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 gene can be designed and, if necessary, divided into appropriate smaller fragments. Then an oligomer which corresponds to the nucleic acid molecule, or to each of the divided fragments, can be synthesized. Such synthetic oligonucleotides can be prepared, for example, by the triester method of Matteucci et al., *J. Am. Chem. Soc.* 103:3185-3191 (1981) or by using an automated DNA synthesizer.

An oligonucleotide can be derived synthetically or by cloning. If necessary, the 5'-ends of the oligomers can be phosphorylated using T4 polynucleotide kinase. Kinasing of single strands prior to annealing or for labeling can be achieved using an excess of the enzyme. If kinasing is for the labeling of probe, the ATP can contain high specific activity radioisotopes. Then, the DNA oligomer can be subjected to annealing and ligation with T4 ligase or the like.

II. Recombinantly Produced S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 Polypeptides

In another embodiment, the present invention relates to a purified polypeptide (preferably, substantially pure) having an amino acid sequence corresponding to S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 or a functional derivative thereof. In a preferred embodiment, the polypeptide has the amino acid sequence set forth in SEQ ID NOS:4, 6, 2, 8, 21, 22, 39, 27, 29, or 30, respectively, or mutant or species variation thereof, or at least 60% identity or at least 70% similarity thereof (preferably, at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity or at least 95%, 96%, 97%, 98%, or 99% similarity thereof), or at least 6 contiguous amino acids thereof (preferably, at least 10, 15, 20, 25, or 50 contiguous amino acids thereof).

In a preferred embodiment, the invention relates to S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 epitopes. The epitope of these polypeptides is an immunogenic or antigenic epitope. An immunogenic epitope is that part of the protein which elicits an antibody response when the whole protein is the immunogen. An antigenic epitope is a fragment of the protein which can elicit an antibody response. Methods of selecting antigenic epitope fragments are well known in the art. (Sutcliffe et al., *Science* 219:660-666 (1983)). Antigenic epitope-bearing peptides and polypeptides of the invention are useful to raise an immune response that specifically recognizes the polypeptides. Antigenic epitope-bearing peptides and polypeptides of the invention comprise at least 7 amino acids (preferably, 9, 10, 12, 15, or 20 amino acids) of the proteins of the invention. Non-limiting examples of antigenic polypeptides or peptides include those listed in Table 1, below.

TABLE 1

Antigenic Epitopes		
	Size[1]	Amino Acids[2]
S2	10	181-190
	22	411-432
	15	636-650
S7	16	13-28
	10	73-82
	11	496-506

TABLE 1-continued

Antigenic Epitopes		
	Size[1]	Amino Acids[2]
S22	13	41-53
	17	168-184
	19	317-335
S23	15	6-20
	11	78-88
	18	387-404
C6.1	9	110-118
	9	338-346
	11	353-363
C6.2	12	65-76
	9	104-112
	9	170-178
S11	12	90-101
	17	144-160
	9	334-342
E8	10	40-49
	12	132-143
	15	261-275
E46.#1	9	32-41
	12	125-136
	20	222-241
E46.#2	12	55-66
	14	177-190
	10	291-300

[1]Number of amino acids.

[2]See FIGS. 7, (S2), 9 (S7), 5 (S22), 11 (S23), 17 (C6.1), 18 (C6.2) and 23 (S11) for amino acid numbering.

Amino acid sequence variants of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 can be prepared by mutations in the DNA. Such variants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence shown in SEQ ID NOS:4, 6, 2, 8, 21, 22, 39, 27, 29, or 30. Any combination of deletion, insertion, and substitution can also be made to arrive at the final construct, provided that the final construct possesses the desired activity.

While the site for introducing an amino acid sequence variation is predetermined, the mutation per se need not be predetermined. For example, to optimize the performance of a mutation at a given site, random mutagenesis can be conducted at the target codon or region and the expressed S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 variants screened for the optimal combination of desired activity. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example, site-specific mutagenesis.

Preparation of an S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 variant in accordance herewith is preferably achieved by site-specific mutagenesis of DNA that encodes an earlier prepared variant or a nonvariant version of the protein. Site-specific mutagenesis allows the production of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 variants through the use of specific oligonucleotide sequences that encode the DNA sequence of the desired mutation. In general, the technique of site-specific mutagenesis is well known in the art, as exemplified by publications such as Adelman et al., *DNA* 2:183 (1983) and Ausubel et al., "Current Protocols in Molecular Biology", J. Wiley & Sons, New York, N.Y., 1996.

As will be appreciated, the site-specific mutagenesis technique can employ a phage vector that exists in both a single-stranded and double-stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage, for example, as disclosed by Messing et al., *Third Cleveland Symposium on Macromolecules and Recombinant DNA*, A. Walton (ed.), Elsevier, Amsterdam (1981). These phage are readily commercially available and their use is generally well known to those skilled in the art. Alternatively, plasmid vectors that contain a single-stranded phage origin of replication (Vieira et al., *Meth. Enzymol.* 153:3 (1987)) can be employed to obtain single-stranded DNA.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector that includes within its sequence a DNA sequence that encodes the relevant protein. An oligonucleotide primer bearing the desired mutated sequence is prepared, generally synthetically, for example, by the method of Crea et al., *Proc. Natl. Acad. Sci.* (*USA*) 75:5765 (1978). This primer is then annealed with the single-stranded protein-sequence-containing vector, and subjected to DNA-polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, to complete the synthesis of the mutation-bearing strand. Thus, a mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells and clones are selected that include recombinant vectors bearing the mutated sequence arrangement.

After such a clone is selected, the mutated protein region can be removed and placed in an appropriate vector for protein production, generally an expression vector of the type that can be employed for transformation of an appropriate host.

Amino acid sequence deletions generally range from about 1 to 30 residues, more preferably 1 to 10 residues, and typically are contiguous.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions of from one residue to polypeptides of essentially unrestricted length, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions (i.e., insertions within the complete S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 sequence) can range generally from about 1 to 10 residues, more preferably 1 to 5.

The third group of variants are those in which at least one amino acid residue in the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 molecule, and preferably, only one, has been removed and a different residue inserted in its place. Such substitutions preferably are made in accordance with the following Table 2 when it is desired to modulate finely the characteristics of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2.

TABLE 2

Original Residue	Exemplary Substitutions
Ala	gly; ser
Arg	lys
Asn	gln; his
Asp	glu
Cys	ser
Gln	asn
Glu	asp
Gly	ala; pro
His	asn; gln
Ile	leu; val
Leu	ile; val
Lys	arg; gln; glu
Met	leu; tyr; ile
Phe	met; leu; tyr
Ser	thr
Thr	ser
Trp	tyr
Tyr	trp; phe
Val	ile; leu

Substantial changes in functional or immunological identity are made by selecting substitutions that are less conser-

vative than those in Table 2, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions that in general are expected are those in which (a) glycine and/or proline is substituted by another amino acid or is deleted or inserted; (b) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl, or alanyl; (c) a cysteine residue is substituted for (or by) any other residue; (d) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) a residue having an electronegative charge, e.g., glutamyl or aspartyl; or (e) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having such a side chain, e.g., glycine.

Some deletions and insertions, and substitutions are not expected to produce radical changes in the characteristics of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays. For example, a variant typically is made by site-specific mutagenesis of the native S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2, encoding-nucleic acid, expression of the variant nucleic acid in recombinant cell culture, and, optionally, purification from the cell culture, for example, by immunoaffinity adsorption on a column (to absorb the variant by binding it to at least one remaining immune epitope). The activity of the cell lysate or purified S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 molecule variant is then screened in a suitable screening assay for the desired characteristic. For example, a change in the immunological character of the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 molecule, such as affinity for a given antibody, is measured by a competitive type immunoassay. Changes in immunomodulation activity are measured by the appropriate assay. Modifications of such protein properties as redox or thermal stability, hydrophobicity, susceptibility to proteolytic degradation or the tendency to aggregate with carriers or into multimers are assayed by methods well known to the ordinarily skilled artisan.

A variety of methodologies known in the art can be utilized to obtain the peptide of the present invention. In one embodiment, the peptide is purified from tissues or cells which naturally produce the peptide. Alternatively, the above-described isolated nucleic acid fragments can be used to express the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein in any organism. The samples of the present invention include cells, protein extracts or membrane extracts of cells, or biological fluids. The sample will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts used as the sample.

Any prokaryotic (preferably, a granulocytic ehrlichia) organism can be used as a source for the peptide of the invention, as long as the source organism naturally contains such a peptide. A eukaryotic organism infected with granulocytic ehrlichia can also be used as the source organism. As used herein, "source organism" refers to the original organism from which the amino acid sequence of the subunit is derived, regardless of the organism the subunit is expressed in and ultimately isolated from.

One skilled in the art can readily follow known methods for isolating proteins in order to obtain the peptide free of natural contaminants. These include, but are not limited to: immunochromotography, size-exclusion chromatography, HPLC, ion-exchange chromatography, and immuno-affinity chromatography.

M. A Nucleic Acid Probe for the Specific Detection of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1 and E46#2

In another embodiment, the present invention relates to a nucleic acid probe for the specific detection of the presence of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 nucleic acid in a sample comprising the above-described nucleic acid molecules or at least a fragment thereof which binds under stringent conditions to S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 nucleic acid.

In one preferred embodiment, the present invention relates to an isolated nucleic acid probe consisting of 10 to 1000 nucleotides (preferably, 10 to 500, 10 to 100, 10 to 50, 10 to 35, 20 to 1000, 20 to 500, 20 to 100, 20 to 50, or 20 to 35) which hybridizes preferentially to RNA or DNA of granulocytic ehrlichia but not to RNA or DNA of non-granulocytic ehrlichia organisms (example, humans), wherein said nucleic acid probe is or is complementary to a nucleotide sequence consisting of at least 10 consecutive nucleotides (preferably, 15, 20, 25, or 30) from the nucleic acid molecule comprising a polynucleotide sequence at least 90% identical to a sequence selected from:

(a) a nucleotide sequence encoding the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 polypeptide comprising the complete amino acid sequence in SEQ ID NOS:4, 6, 2, 8, 21, 22, 39, 27, 29, or 30, respectively;

(b) a nucleotide sequence encoding the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, E46#2 polypeptide comprising the complete amino acid sequence encoded by the polynucleotide clone contained in ATCC Deposit Nos. 97844, 97840, 97842, 97843, 97841, 97841, 209740, 209736, 209743 or 209743 respectively (note, C6.1 and C6.2 are encoded by the polynucleotide clone contained in ATCC Deposit No. 97841 and E46#1 and E46#2 are encoded by the polynucleotide clone contained in ATCC Deposit No. 209743);

(c) a nucleotide sequence complementary to any of the nucleotide sequences in (a) or (b); and

(d) a nucleotide sequence as previously described above.

Examples of specific nucleic acid probes which can be used in the present invention are set forth in Table 3, below.

TABLE 3

Nucleic Acid Probes		
	Size[3]	Nucleotides[4]
S2	20	2660-2679
	37	2643-2679
	75	1820-1894
	450	2150-2599
S7	20	489-508
	35	321-355
	75	420-494
	450	300-749
S22	23	1220-1242
	36	1187-1222
	75	1220-1294
	450	570-1019
S23	23	974-996
	35	962-996
	75	720-794
	450	600-1049
C6	19	530-548
	35	1097-1131
	75	1710-1784
	450	1850-2299

TABLE 3-continued

Nucleic Acid Probes		
	Size[3]	Nucleotides[4]
S11	20	570-589
	35	1045-1079
	75	1600-1674
	450	500-949
E8	20	520-539
	35	650-684
	75	900-974
	450	700-1149
E46	20	1450-1469
	35	1800-1834
	75	1030-1104
	450	400-849

[3]Number of bases.
[4]See FIGS. 6 (S2), 8 (S7), 4 (S22), 10 (S23), 16 (C6) and 23 (S11) for nucleotide numbering.

The nucleic acid probe can be used to probe an appropriate chromosomal or cDNA library by usual hybridization methods to obtain another nucleic acid molecule of the present invention. A chromosomal DNA or cDNA library can be prepared from appropriate cells according to recognized methods in the art (cf. *Molecular Cloning: A Laboratory Manual, 2nd edition*, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989).

In the alternative, chemical synthesis is carried out in order to obtain nucleic acid probes having nucleotide sequences which correspond to amino-terminal and carboxy-terminal portions of the S2, S7, S22, S23, C6.1, C6.2, S11 amino acid sequence (See, Table 3) or E8, E46#1, or E46#2 amino acid sequence. Thus, the synthesized nucleic acid probes can be used as primers to in a polymerase chain reaction (PCR) carried out in accordance with recognized PCR techniques, essentially according to *PCR Protocols, A Guide to Methods and Applications*, edited by Michael et al., Academic Press, 1990, utilizing the appropriate chromosomal, cDNA or cell line library to obtain the fragment of the present invention.

One skilled in the art can readily design such probes based on the sequence disclosed herein using methods of computer alignment and sequence analysis known in the art (cf. *Molecular Cloning: A Laboratory Manual, 2nd edition*, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989).

The hybridization probes of the present invention can be labeled by standard labeling techniques such as with a radiolabel, enzyme label, fluorescent label, biotin-avidin label, chemiluminescence, and the like. After hybridization, the probes can be visualized using known methods.

The nucleic acid probes of the present invention include RNA, as well as DNA probes, such probes being generated using techniques known in the art.

In one embodiment of the above described method, a nucleic acid probe is immobilized on a solid support. Examples of such solid supports include, but are not limited to, plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, and acrylic resins, such as polyacrylamide and latex beads. Techniques for coupling nucleic acid probes to such solid supports are well known in the art.

The test samples suitable for nucleic acid probing methods of the present invention include, for example, cells or nucleic acid extracts of cells, or biological fluids. The sample used in the above-described methods will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts to be assayed. Methods for preparing nucleic acid extracts of cells are well known in the art and can be readily adapted in order to obtain a sample which is compatible with the method utilized.

IV. A Method of Detecting the Presence of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 in a Sample

In another embodiment, the present invention relates to a method of detecting the presence of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 nucleic acid in a sample comprising a) contacting the sample with the above-described nucleic acid probe, under specific hybridization conditions such that hybridization occurs, and b) detecting the presence of the to probe bound to the nucleic acid molecule. Alternatively, in another preferred embodiment, the method of detecting the presence of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 nucleic acid in a sample may comprise a) amplifying the nucleic acid in the sample with the nucleic acid probe wherein the amplification uses PCR techniques and b) detecting the presence of the amplified nucleic acid molecules. One skilled in the art would select the nucleic acid probe according to techniques known in the art as described above. Samples to be tested include but should not be limited to RNA samples from human tissue.

V. A Kit for Detecting the Presence of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 in a Sample

In another embodiment, the present invention relates to a kit for detecting the presence of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 nucleic acid in a sample comprising at least one container means having disposed therein the above-described nucleic acid probe. In a preferred embodiment, the kit further comprises other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound nucleic acid probe. Examples of detection reagents include, but are not limited to radiolabelled probes, enzymatic labeled probes (horse radish peroxidase, alkaline phosphatase), and affinity labeled probes (biotin, avidin, or streptavidin).

In detail, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allow the efficient transfer of reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the probe or primers used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, and the like), and containers which contain the reagents used to detect the hybridized probe, bound antibody, amplified product, or the like.

One skilled in the art will readily recognize that the nucleic acid probes described in the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

VI. DNA Constructs Comprising an S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 Nucleic Acid Molecule and Cells Containing These Constructs

In another embodiment, the present invention relates to a recombinant DNA molecule comprising, 5' to 3', a promoter effective to initiate transcription in a host cell and the above-described nucleic acid molecules. In another embodiment, the present invention relates to a recombinant DNA molecule comprising a vector and an above-described nucleic acid molecule.

In another embodiment, the present invention relates to a nucleic acid molecule comprising a transcriptional control

region functional in a cell, a sequence complimentary to an RNA sequence encoding an amino acid sequence corresponding to the above-described polypeptide, and a transcriptional termination region functional in the cell.

Preferably, the above-described molecules are isolated and/or purified DNA molecules.

In another embodiment, the present invention relates to a cell or non-human organism that contains an above-described nucleic acid molecule.

In another embodiment, the peptide is purified from cells which have been altered to express the peptide.

As used herein, a cell is said to be "altered to express a desired peptide" when the cell, through genetic manipulation, is made to produce a protein which it normally does not produce or which the cell normally produces at low levels. One skilled in the art can readily adapt procedures for introducing and expressing either genomic, cDNA, or synthetic sequences into either eukaryotic or prokaryotic cells.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene sequence expression. The precise nature of the regulatory regions needed for gene sequence expression can vary from organism to organism, but shall in general include a promoter region which, in prokaryotes, contains both the promoter (which directs the initiation of RNA transcription) as well as the DNA sequences which, when transcribed into RNA, will signal synthesis initiation. Such regions will normally include those 5'-non-coding sequences involved with initiation of transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like.

If desired, the non-coding region 3' to the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 coding sequence can be obtained by the above-described methods. This region can be retained for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3'-region naturally contiguous to the DNA sequence encoding a S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 gene, the transcriptional termination signals can be provided. Where the transcriptional termination signals are not satisfactorily functional in the expression host cell, then a 3' region functional in the host cell can be substituted. Two DNA sequences (such as a promoter region sequence and a S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 coding sequence) are said to be operably linked if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region sequence to direct the transcription of a S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 coding sequence, or (3) interfere with the ability of the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 coding sequence to be transcribed by the promoter region sequence. Thus, a promoter region would be operably linked to a DNA sequence if the promoter were capable of effecting transcription of that DNA sequence.

The present invention encompasses the expression of the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 coding sequence (or a functional derivative thereof) in either prokaryotic or eukaryotic cells. Prokaryotic hosts are, generally, the most efficient and convenient for the production of recombinant proteins and, therefore, are preferred for the expression of the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 coding sequence.

Prokaryotes most frequently are represented by various strains of *E. coli*. However, other microbial strains can also be used, including other bacterial strains. In prokaryotic systems, plasmid vectors that contain replication sites and control sequences derived from a species compatible with the host can be used. Examples of suitable plasmid vectors include pBR322, pUC18, pUC19, pUC118, pUC119 and the like; suitable phage or bacteriophage vectors include λgt10, λgt11 and the like; and suitable virus vectors include pMAM-neo, pKRC and the like. Preferably, the selected vector of the present invention has the capacity to replicate in the selected host cell.

Recognized prokaryotic hosts include bacteria such as *E. coli, Bacillus, Streptomyces, Pseudomonas, Salmonella, Serratia*, and the like. However, under such conditions, the peptide will not be glycosylated. The prokaryotic host must be compatible with the replicon and control sequences in the expression plasmid.

To express S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 in a prokaryotic cell, it is necessary to operably link the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 coding sequence to a functional prokaryotic promoter. Such promoters can be either constitutive or, more preferably, regulatable (i.e., inducible or derepressible). Examples of constitutive promoters include the int promoter of bacteriophage λ, the bla promoter of the β-lactamase gene sequence of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene sequence of pBR325, and the like. Examples of inducible prokaryotic promoters include the major right and left promoters of bacteriophage λ (P_L and P_R), the trp, recA, lacZ, lacI, and gal promoters of *E. coli*, the α-amylase (Ulmanen et al., *J. Bacteriol.* 162:176-182 (1985)) and the ς -28-specific promoters of *B. subtilis* (Gilman et al., *Gene sequence* 32:11-20 (1984)), the promoters of the bacteriophages of *Bacillus* (Gryczan, In: *The Molecular Biology of the Bacilli*, Academic Press, Inc., NY (1982)), and *Streptomyces* promoters (Ward et al., *Mol. Gen. Genet.* 203:468-478 (1986)). Prokaryotic promoters are reviewed by Click (*J. Ind. Microbiol.* 1:277-282 (1987)); Cenatiempo (*Biochimie* 68:505-516 (1986)); and Gottesman (*Ann. Rev. Genet.* 18:415-442 (1984)).

Proper expression in a prokaryotic cell also requires the presence of a ribosome binding site upstream of the gene sequence-encoding sequence. Such ribosome binding sites are disclosed, for example, by Gold et al., (*Ann. Rev. Microbiol.* 35:365-404 (1981)).

The selection of control sequences, expression vectors, transformation methods, and the like, are dependent on the type of host cell used to express the gene. As used herein, "cell", "cell line", and "cell culture" can be used interchangeably and all such designations include progeny. Thus, the words "transformants" or "transformed cells" include the primary subject cell and cultures derived therefrom, without regard to the number of transfers. It is also understood that all progeny cannot be precisely identical in DNA content, due to deliberate or inadvertent mutations. However, as defined, mutant progeny have the same functionality as that of the originally transformed cell. Host cells which can be used in the expression systems of the present invention are not strictly limited, provided that they are suitable for use in the expression of the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 peptide of interest. Suitable hosts include eukaryotic cells.

Preferred eukaryotic hosts include, for example, yeast, fungi, insect cells, mammalian cells either in vivo, or in tissue

culture. Preferred mammalian cells include HeLa cells, cells of fibroblast origin such as VERO or CHO-K1, or cells of lymphoid origin and their derivatives.

In addition, plant cells are also available as hosts, and control sequences compatible with plant cells are available, such as the cauliflower mosaic virus 35S and 19S, and nopaline synthase promoter and polyadenylation signal sequences.

Another preferred host is an insect cell, for example *Drosophila* larvae. Using insect cells as hosts, the *Drosophila* alcohol dehydrogenase promoter can be used, (Rubin, *Science* 240:1453-1459 (1988)). Alternatively, baculovirus vectors can be engineered to express large amounts of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 in insect cells (Jasny, *Science* 238:1653 (1987); Miller et al., In: *Genetic Engineering* (1986), Setlow, J. K., et al., eds., *Plenum*, Vol. 8, pp. 277-297).

Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (e.g., glycosylation, cleavage) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed.

Any of a series of yeast gene sequence expression systems can be utilized which incorporate promoter and termination elements from the actively expressed gene sequences coding for glycolytic enzymes. These enzymes are produced in large quantities when yeast are grown in mediums rich in glucose. Known glycolytic gene sequences can also provide very efficient transcriptional control signals.

Yeast provides substantial advantages in that it can also carry out post-translational peptide modifications. A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number of plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on cloned mammalian gene sequence products and secretes peptides bearing leader sequences (i.e., pre-peptides). For a mammalian host, several possible vector systems are available for the expression of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2.

A wide variety of transcriptional and translational regulatory sequences can be employed, depending upon the nature of the host. The transcriptional and translational regulatory signals can be derived from viral sources, such as adenovirus, bovine papilloma virus, simian virus, or the like, where the regulatory signals are associated with a particular gene sequence which has a high level of expression. Alternatively, promoters from mammalian expression products, such as actin, collagen, myosin, and the like, can be employed. Transcriptional initiation regulatory signals can be selected which allow for repression or activation, so that expression of the gene sequences can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by varying the temperature, expression can be repressed or initiated, or are subject to chemical (such as metabolite) regulation.

As discussed above, expression of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 in eukaryotic hosts requires the use of eukaryotic regulatory regions. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis. Preferred eukaryotic promoters include, for example, the promoter of the mouse metallothionein I gene sequence (Hamer et al., *J. Mol. Appl. Gen.* 1:273-288 (1982)); the TK promoter of Herpes virus (McKnight, *Cell* 31:355-365 (1982)); the SV40 early promoter (Benoist et al., *Nature* (*London*) 290:304-310 (1981)); the yeast gal4 gene sequence promoter (Johnston et al., *Proc. Natl. Acad. Sci.* (*USA*) 79:6971-6975 (1982)); Silver et al., *Proc. Natl. Acad. Sci.* (*USA*) 81:5951-5955 (1984)) and the CMV immediate-early gene promoter (Thomsen et al., *Proc. Natl. Acad. Sci.* (*USA*) 81:659-663 (1984)).

As is widely known, translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine. For this reason, it is preferable to ensure that the linkage between a eukaryotic promoter and a S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 coding sequence does not contain any intervening codons which are capable of encoding a methionine (i.e., AUG). The presence of such codons results either in a formation of a fusion protein (if the AUG codon is in the same reading frame as the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 coding sequence) or a frame-shift mutation (if the AUG codon is not in the same reading frame as the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 coding sequence).

A S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 nucleic acid molecule and an operably linked promoter can be introduced into a recipient prokaryotic or eukaryotic cell either as a non-replicating DNA (or RNA) molecule, which can either be a linear molecule or, more preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the gene can occur through the transient expression of the introduced sequence. Alternatively, permanent expression can occur through the integration of the introduced DNA sequence into the host chromosome.

In one embodiment, a vector is employed which is capable of integrating the desired gene sequences into the host cell chromosome. Cells which have stably integrated the introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker can provide for prototrophy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals, such as copper, or the like. The selectable marker gene sequence can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements can also be needed for optimal synthesis of single chain binding protein mRNA. These elements can include splice signals, as well as transcription promoters, enhancer signal sequences, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama, *Molec. Cell. Biol.* 3:280 (1983).

In a preferred embodiment, the introduced nucleic acid molecule will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors can be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector can be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species. Preferred prokaryotic vectors include plasmids such as those capable of replication in *E. coli* (such as, for example, pBR322, ColE1, pSC101, pACYC 184, πVX). Such plasmids are, for example, disclosed by Sambrook (cf. *Molecular Cloning: A Laboratory Manual*, second edition, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989). *Bacillus* plasmids include pC194, pC221, pT127, and the like. Such plasmids are disclosed by Gryczan (In: *The Molecular Biology of the Bacilli*, Academic Press, NY (1982), pp. 307-329). Suitable *Streptomyces* plasmids include pIJ101 (Kendall et al., *J. Bacteriol.* 169:4177-4183 (1987)), and *streptomyces* bacteriophages such as ΦC31 (Chater et al., In: *Sixth Inter-*

national Symposium on Actinomycetales Biology, Akademiai Kaido, Budapest, Hungary (1986), pp. 45-54). *Pseudomonas* plasmids are reviewed by John et al., (*Rev. Infect. Dis.* 8:693-704 (1986)), and Izaki (*Jpn. J. Bacteriol.* 33:729-742 (1978)).

Preferred eukaryotic plasmids include, for example, BPV, vaccinia, SV40, 2-micron circle, and the like, or their derivatives. Such plasmids are well known in the art (Botstein et al., *Miami Wntr. Symp.* 19:265-274 (1982); Broach, In: *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., p. 445-470 (1981); Broach, *Cell* 28:203-204 (1982); Bollon et al., *J. Clin. Hematol. Oncol.* 10:39-48 (1980); Maniatis, In: *Cell Biology: A Comprehensive Treatise*, Vol. 3, Gene Sequence Expression, Academic Press, NY, pp. 563-608 (1980)).

Once the vector or nucleic acid molecule containing the construct(s) has been prepared for expression, the DNA construct(s) can be introduced into an appropriate host cell by any of a variety of suitable means, i.e., transformation, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate-precipitation, direct microinjection, and the like. After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene molecule(s) results in the production of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2. This can take place in the transformed cells as such, or following the induction of these cells to differentiate (for example, by administration of bromodeoxyuracil to neuroblastoma cells or the like).

VII. An Antibody having Binding Affinity to an S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 Polypeptide and a Hybridoma Containing the Antibody

In another embodiment, the present invention relates to an antibody having binding affinity specifically to an S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 polypeptide as described above or specifically to an S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 polypeptide binding fragment thereof. An antibody binds specifically to a S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 polypeptide or to consensus sequences described herein corresponding to the amino- and/or carboxy-terminus regions shared by E8, E46#1, and E46#2, or binding fragment thereof if it does not bind to non-S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 polypeptides. Those which bind selectively to S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 or to consensus sequences described herein corresponding to the amino- and/or carboxy-terminus regions shared by E8, E46#1, and E46#2, would be chosen for use in methods which could include, but should not be limited to, the analysis of altered S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 expression in tissue containing S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2.

The S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 proteins, or proteins including the consensus sequences corresponding to the amino- and/or carboxy-terminus regions shared by E8, E46#1, and E46#2 of the present invention can be used in a variety of procedures and methods, such as for the generation of antibodies, for use in identifying pharmaceutical compositions, and for studying DNA/protein interaction.

The S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 proteins, or proteins including the consensus sequences corresponding to the amino and/or carboxy terminus regions shared by E8, E46#1, and E46#2 of the present invention can be used to produce antibodies or hybridomas. One skilled in the art will recognize that if an antibody is desired, such a peptide would be generated as described herein and used as an immunogen.

The antibodies of the present invention include monoclonal and polyclonal antibodies, as well as fragments of these antibodies. The invention further includes single chain antibodies. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment; the Fab' fragments, Fab fragments, and Fv fragments.

Of special interest to the present invention are antibodies to S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, E46#2 or to proteins, or proteins including the consensus sequences corresponding to the amino- and/or carboxy-terminus regions shared by E8, E46#1, and E46#2 which are produced in humans, or are "humanized" (i.e.; non-immunogenic in a human) by recombinant or other technology. Humanized antibodies can be produced, for example by replacing an immunogenic portion of an antibody with a corresponding, but non-immunogenic portion (i.e., chimeric antibodies) (Robinson et al., PCT Application No. PCT/US86/02269; Akira et al., European Patent No. 184,187; Taniguchi, European Patent No. 171,496; Morrison et al., European Patent No. 173,494; Neuberger et al., PCT Application WO 86/01533; Cabilly et al., European Patent No. 125,023; Better, et al., *Science* 240:1041-1043 (1988); Liu et al., *Proc. Natl. Acad. Sci. USA* 84:3439-3443 (1987); Liu et al., *J. Immunol.* 139:3521-3526 (1987); Sun, et al., *Proc. Natl. Acad. Sci. USA* 84:214-218 (1987); Nishimura et al., *Canc. Res.* 47:999-1005 (1987); Wood et al., *Nature* 314:446-449 (1985)); Shaw et al., *J. Natl. Cancer Inst.* 80:1553-1559 (1988). General reviews of "humanized" chimeric antibodies are provided by Morrison (*Science,* 229:1202-1207 (1985)) and by Oi et al., *BioTechniques* 4:214 (1986)). Suitable "humanized" antibodies can be alternatively produced by CDR or CEA substitution (Jones et al., *Nature* 321:552-525 (1986); Verhoeyan et al., *Science* 239:1534 (1988); Beidler et al., *J. Immunol.* 141:4053-4060 (1988)).

In another embodiment, the present invention relates to a hybridoma which produces the above-described monoclonal antibody. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

In general, techniques for preparing monoclonal antibodies and hybridomas are well known in the art (Campbell, "*Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*," Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth et al., *J. Immunol. Methods* 35:1-21 (1980)).

Any animal (mouse, rabbit, and the like) which is known to produce antibodies can be immunized with the selected polypeptide. Methods for immunization are well known in the art. Such methods include subcutaneous or interperitoneal injection of the polypeptide. One skilled in the art will recognize that the amount of polypeptide used for immunization will vary based on the animal which is immunized, the antigenicity of the polypeptide and the site of injection.

The polypeptide can be modified or administered in an adjuvant in order to increase the peptide antigenicity. Methods of increasing the antigenicity of a polypeptide are well known in the art. Such procedures include coupling the antigen with a heterologous protein (such as globulin or β-galactosidase) or through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells.

Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, western blot analysis, or radioimmunoassay (Lutz et al., *Exp. Cell Res.* 175:109-124 (1988)).

Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell, *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*, supra (1984)).

For polyclonal antibodies, antibody containing antisera is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures.

In another embodiment of the present invention, the above-described antibodies are detectably labeled. Antibodies can be detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, and the like), enzymatic labels (such as horseradish peroxidase, alkaline phosphatase, and the like), fluorescent labels (such as FITC or rhodamine, and the like), paramagnetic atoms, and the like. Procedures for accomplishing such labeling are well-known in the art, for example, see (Sternberger et al., *J. Histochem. Cytochem.* 18:315 to (1970); Bayer et al., *Meth. Enzym.* 62:308 (1979); Engval et al., *Immunol.* 109:129 (1972); Goding, *J. Immunol. Meth.* 13:215 (1976)). The labeled antibodies of the present invention can be used for in vitro, in vivo, and in situ assays to identify cells or tissues which express a specific peptide.

In another embodiment of the present invention, the above-described antibodies are immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, and acrylic resins such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir et al., "*Handbook of Experimental Immunology*" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10 (1986); Jacoby et al., *Meth. Enzym.* 34 Academic Press, N.Y. (1974)). The immobilized antibodies of the present invention can be used for in vitro, in vivo, and in situ assays as well as in immunochromatography.

Furthermore, one skilled in the art can readily adapt currently available procedures, as well as the techniques, methods and kits disclosed above with regard to antibodies, to generate peptides capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby et al., "Application of Synthetic Peptides: Antisense Peptides", In *Synthetic Peptides, A User's Guide*, W. H. Freeman, NY, pp. 289-307 (1992), and Kaspczak et al., *Biochemistry* 28:9230-8 (1989).

Anti-peptide peptides can be generated in one of two fashions. First, the anti-peptide peptides can be generated by replacing the basic amino acid residues found in the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, and E46#2 peptide sequence or consensus sequences described herein with acidic residues, while maintaining hydrophobic and uncharged polar groups. For example, lysine, arginine, and/or histidine residues are replaced with aspartic acid or glutamic acid and glutamic acid residues are replaced by lysine, arginine or histidine.

VIII. A Method of Detecting an S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 Polypeptide or Antibody in a Sample

In another embodiment, the present invention relates to a method of detecting a S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 polypeptide including the consensus sequence corresponding to the amino- and/or carboxy-terminus regions shared by E8, E46#1, and E46#2 polypeptide in a sample, comprising: a) contacting the sample with an above-described antibody (or protein), under conditions such that immunocomplexes form, and b) detecting the presence of the antibody bound to the polypeptide. In detail, the methods comprise incubating a test sample with one or more of the antibodies of the present invention and assaying whether the antibody binds to the test sample. Altered levels of peptides S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2, or in a sample as compared to normal levels can indicate a specific disease.

In a further embodiment, the present invention relates to a method of detecting a S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 antibody in a sample, comprising: a) contacting the sample with an above-described S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 polypeptide, including the consensus sequence corresponding to the amino- and/or carboxy-terminus regions shared by E8, E46#1, and E46#2 polypeptide under conditions such that immunocomplexes form, and b) detecting the presence of the protein bound to the antibody or antibody bound to the protein. In detail, the methods comprise incubating a test sample with one or more of the proteins of the present invention and assaying whether the antibody binds to the test sample. The presence of antibodies to S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 may indicate exposure to GE, the potential need for therapy of the affected individual, or GE contamination of a biological sample.

Conditions for incubating an antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the antibody used in the assay. One skilled in the art will recognize that any one of the commonly available immunological assay formats (such as radioimmunoassays, enzyme-linked immunosorbent assays, diffusion based Ouchterlony, or rocket immunofluorescent assays) can readily be adapted to employ the antibodies of the present invention. Examples of such assays can be found in Chard, *An Introduction to Radioimmunoassay and Related Techniques*, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock et al., *Techniques in Immunocytochemistry*, Academic Press, Orlando, Fla. Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, *Practice and Theory of Enzyme Immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1985).

The immunological assay test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is capable with the system utilized.

IX. A Diagnostic Kit Comprising S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 Protein or Antibody

In another embodiment of the present invention, a kit is provided which contains all the necessary reagents to carry out the previously described methods of detection.

The kit can comprise: i) a first container means containing an above-described antibody, and ii) second container means containing a conjugate comprising a binding partner of the antibody and a label.

The kit can comprise: i) a first container means containing an above-described protein, and preferably, ii) second container means containing a conjugate comprising a binding partner of the protein and a label. More specifically, a diagnostic kit comprises S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, E46#2, or a peptide having consensus sequences corresponding to the amino and/or carboxy terminus regions shared by E8, E46#1, and E46#2 protein as described above, to detect antibodies in the serum of potentially infected animals or humans.

In another preferred embodiment, the kit further comprises one or more other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound antibodies. Examples of detection reagents include, but are not limited to, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the chromophoric, enzymatic, or antibody binding reagents which are capable of reacting with the labeled antibody. The compartmentalized kit can be as described above for nucleic acid probe kits.

One skilled in the art will readily recognize that the antibodies described in the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

X. Diagnostic Screening

It is to be understood that although the following discussion is specifically directed to human patients, the teachings are also applicable to any animal which can be infected with GE.

The diagnostic and screening methods of the invention are especially useful for a patient suspected of being at risk for developing ehrlichiosis.

According to the invention, a pre- and post-symptomatic screening of an individual in need of such screening is now possible using DNA encoding the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein or fragment thereof, or a protein having consensus sequences corresponding to the amino and/or carboxy terminus regions shared by E8, E46#1, and E46#2 of the invention. The screening method of the invention allows a presymptomatic diagnosis of the presence of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein or DNA in individuals, and thus an opinion concerning the likelihood that such individual would develop or has developed ehrlichiosis. Early diagnosis is desired to maximize appropriate timely intervention.

In one preferred embodiment of the method of screening, a tissue sample would be taken from an individual, and screened for (1) the presence of the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 DNA coding sequence; (2) the presence of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 mRNA; (3) the presence of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein; and/or (4) the presence of antibody to S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein.

A preferred method of detecting the presence of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein and/or the presence of antibody to S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein comprises: a) contacting the sample with a polypeptide or antibody to a polypeptide having the amino acid sequence of S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2, or a fragment thereof under conditions such that immunocomplexes form; and b) detecting the presence of the immunocomplexed antibody and polypeptide.

Individuals not infected with GE do not have GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 DNA, mRNA, or protein.

The screening and diagnostic methods of the invention do not require that the entire S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 coding sequence be used for the probe. Rather, it is only necessary to use a fragment or length of nucleic acid that is sufficient to detect the presence of the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 nucleic acid in a DNA preparation from an individual.

Analysis of nucleic acid specific to GE can be by PCR techniques or hybridization techniques (cf. *Molecular Cloning: A Laboratory Manual, 2nd edition*, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989; Eremeeva et al., *J. Clin. Microbiol.* 32:803-810 (1994) which describes differentiation among spotted fever group *Rickettsiae* species by analysis of restriction fragment length polymorphism of PCR-amplified DNA). Nucleic acid probes used to analyze GE genomic DNA via PCR analysis have been described in Chen et al., *J. Clin. Microbiol.* 32:589-595 (1994).

XI. Vaccines

In another embodiment, the present invention relates to a vaccine comprising a GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein or a fragment thereof, or a protein having consensus sequences corresponding to the amino and/or carboxy terminus regions shared by E8, E46#1, and E46#2 (preferably, an immunologically active fragment) together with a pharmaceutically acceptable diluent, carrier, or excipient, wherein the protein is present in an amount effective to elicit a beneficial immune response in an animal to GE. S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein, or a protein having consensus sequences corresponding to the amino- and/or carboxy-terminus regions shared by E8, E46#1, and E46#2 may be obtained as described above and using methods well known in the art. An immunologically active fragment comprises an epitope-bearing portion of the protein.

In a further preferred embodiment, the present invention relates to a composition comprising a GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 protein or fragment thereof, or a protein having consensus sequences corresponding to the amino- and/or carboxy-terminus regions shared by E8, E46#1, and E46#2 (preferably, an immunologically reactive fragment-antigenic epitope, examples are listed in Table 1) and a carrier.

In another embodiment, the present invention relates to a vaccine comprising a GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 nucleic acid (preferably, DNA) or a fragment thereof (preferably, a fragment encoding an immunologically active protein or peptide), or nucleic acid coding for a polypeptide, or a protein having consensus sequences corresponding to the amino- and/or carboxy-terminus regions shared by E8, E46#1, and E46#2 together with a pharmaceutically acceptable diluent, carrier, or excipient, wherein the nucleic acid is present in an amount effective to elicit a beneficial immune response in an animal to GE. S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 nucleic acid may be obtained as described above and using methods well known in the art. An immunologically active fragment to comprises an epitope-bearing portion of the nucleic acid.

In a further preferred embodiment, the present invention relates to a composition comprising a GE S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, or E46#2 nucleic acid (preferably, DNA) or fragment thereof (preferably, encoding an immunologically reactive protein or fragment-antigenic epitope) and a carrier.

```
1    TCGACGCAGGCGGATTTTGTACTATGTTTCACTTAACAAGGTATTATAAATGTTTGAACA
                                                    M  F  E  H

61   CAATATTCCTGATACATACACAGGAACAACTGCAGAAGGTTCTCCTGGCTTAGCAGGCGG
       N  I  P  D  T  Y  T  G  T  T  A  E  G  S  P  G  L  A  G  G

121  GGATTTTAGCTTAAGTTCTATTGACTTTACAAGGGACTTTACAATTGAATCACATAGAGG
       D  F  S  L  S  S  I  D  F  T  R  D  F  T  I  E  S  H  R  G

181  AAGCTCAGCTGATGACCCAGGTTACATCAGCTTTAGGGATCAAGACGGAAACGTCATGTC
       S  S  A  D  D  P  G  Y  I  S  F  R  D  Q  D  G  N  V  M  S

241  ACGTTTTCTTGATGTGTACGTAGCTAATTTCAGCTTGCGATGCAAGCATTCTCCCTATAA
       R  F  L  D  V  Y  V  A  N  F  S  L  R  C  K  H  S  P  Y  N

301  CAACGACAGAATGGAAACAGCTGCGTTCTCTCTAACTCCCGACATAATAGAGCCTTCTGC
       N  D  R  M  E  T  A  A  F  S  L  T  P  D  I  I  E  P  S  A

361  TTTATTGCAAGAATCACATAGTACACAAAACAATGTAGAAGAGGCAGTACAAGTTACAGC
       L  L  Q  E  S  H  S  T  Q  N  N  V  E  E  A  V  Q  V  T  A

421  TCTTGAGTGCCCTCCATGTAATCCAGTCCCTGCCGAGGAAGTAGCTCCTCAACCGTCTTT
       L  E  C  P  P  C  N  P  V  P  A  E  E  V  A  P  Q  P  S  F

481  TCTAAGCAGAATAATTCAGGCGTTCTTGTGGTTATTCACGCCTTCTTCTACTACCGACAC
       L  S  R  I  I  Q  A  F  L  W  L  F  T  P  S  S  T  I  D  T

541  TGCTGAAGACAGCAAGTGTAATAGTAGCGATACTTCAAAATGTACCTCTGCTAGCAGTGA
       A  E  D  S  K  C  N  S  S  D  T  S  K  C  T  S  A  S  S  E

601  GTCATTAGAGCAGCAACAAGAATCAGTGGAAGTGCAACCATCTGTACTTATGTCTACTGC
       S  L  E  Q  Q  Q  E  S  V  E  V  Q  P  S  V  L  M  S  T  A

661  CCCTATAGCAACAGAGCCTCAAAATGCGGTTGTTAACCAAGTAAACACTACTGCAGTACA
       P  I  A  T  E  P  Q  N  A  V  V  N  Q  V  N  T  T  A  V  Q

721  AGTAGAATCATCCATTATTGTGCCAGAATCGCAACACACTGACGTTACCGTGCTCGAAGA
       V  E  S  S  I  I  V  P  E  S  Q  H  T  D  V  T  V  L  E  D

781  TACTACTGAGACGATAACTGTTGATGGGGAATATGGACATTTTAGTGACATTGCTTCAGG
       T  T  E  T  I  T  V  D  G  E  Y  G  H  F  S  D  I  A  S  G
```

FIG. 5B-1

In a further preferred embodiment, the present invention relates to a method of producing an immune response which recognizes GE in a host comprising administering to the host the above-described composition.

In a preferred embodiment, the animal to be protected is selected from humans, horses, deer, cattle, pigs, sheep, dogs, and chickens. In a more preferred embodiment, the animal is a human or a dog.

In a further embodiment, the present invention relates to a method of preventing ehrlichiosis in an animal comprising administering to the animal the above-described vaccine, wherein the vaccine is administered in an amount effective to prevent or inhibit Ehrlichiosis. The vaccine of the invention is used in an amount effective depending on the route of administration. Although intranasal, subcutaneous or intramuscular routes of administration are preferred, the vaccine of the present invention can also be administered by an oral, intraperitoneal or intravenous route. One skilled in the art will appreciate that the amounts to be administered for any particular treatment protocol can be readily determined without undue experimentation. Suitable amounts are within the range of 2 μg of the S2, S7, S22, S23, C6.1, C6.2, S11, E8, E46#1, E46#2 protein, or a protein having consensus sequences corresponding to the amino and/or carboxy terminus regions shared by E8, E46#1, and E46#2 per kg body weight to 100 μg per kg body weight (preferably, 2 μg to 50 μg, 2 μg to 25 μg, 5 μg to 50 μg, or 5 μg to 10 μg).

Examples of vaccine formulations including antigen amounts, route of administration and addition of adjuvants can be found in Kensil, *Therapeutic Drug Carrier Systems* 13:1-55 (1996), Livingston et al., *Vaccine* 12:1275 (1994), and Powell et al., *AIDS RES, Human Retroviruses* 10:5105 (1994).

The vaccine of the present invention may be employed in such forms as capsules, liquid solutions, suspensions or elixirs for oral administration, or sterile liquid forms such as solutions or suspensions. Any inert carrier is preferably used, such as saline, phosphate-buffered saline, or any such carrier in which the vaccine has suitable solubility properties. The vaccines may be in the form of single dose preparations or in multi-dose flasks which can be used for mass vaccination programs. Reference is made to Remington's *Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa., Osol (ed.) (1980); and *New Trends and Developments in Vaccines, Voller* et al. (eds.), University Park Press, Baltimore, Md. (1978), for methods of preparing and using vaccines.

The vaccines of the present invention may further comprise adjuvants which enhance production of antibodies and immune cells. Such adjuvants include, but are not limited to, various oil formulations such as Freund's complete adjuvant (CFA), the dipeptide known as MDP, saponins (e.g., QS-21, U.S. Pat. No. 5,047,540), aluminum hydroxide, or lymphatic cytokines. Freund's adjuvant is an emulsion of mineral oil and water which is mixed with the immunogenic substance. Although Freund's adjuvant is powerful, it is usually not administered to humans. Instead, the adjuvant alum (aluminum hydroxide) may be used for administration to a human. Vaccine may be absorbed onto the aluminum hydroxide from which it is slowly released after injection. The vaccine may also be encapsulated within liposomes according to Fullerton, U.S. Pat. No. 4,235,877.

The present invention is described in further detail in the following non-limiting examples.

EXAMPLES

The following Protocols A-G and experimental details are referenced in the non-limiting examples, Examples 1-16.

Protocol A: Cultivation of GE in HL60 Cells

The GE-infected HL60 cell line, USG3, was obtained by co-culturing HL60 cells (ATCC CCL 240) with blood cells from dogs challenged with field collected *Ixodes* scapularis ticks. After degenerative cell morphology became noticeable, the infected cells were passed over fresh uninfected HL60 cells to maintain the culture. USG3 was grown in RPMI 1640 containing 10-20% heat-inactivated fetal bovine serum, 2 mM 1-glutamine, 1 mM sodium pyruvate, 0.1 mM MEM non-essential amino acids and was split into fresh HL60 cells two to three times per week. This procedure is also outlined in Coughlin et al., PCT Application No. PCT/US96/10117 and has also been demonstrated by Goodman et al., *N. Eng. J. Med.* 334:209-215 (1996).

Protocol B: DNA Isolation

USG3 cultures at approximately 80% cell lysis (monitored microscopically) were centrifuged at 840×g for 15 min at 4° C. to remove host HL60 cell debris. The supernatant was filtered through a Poretics (Livermore, Calif.) 5 μm polycarbonate membrane, 47 mm in diameter, followed by a Poretics 3 μm filter under negative pressure. The USG3 filtrate was centrifuged at 9460×g in a Sorvall centrifuge for 30 min at 4° C. Following centrifugation, the GE pellet was resuspended in 5 ml 25 mM Tris, pH 8.0, 10 mM MgCl, and 0.9% NaCl. DNase I (Life Technologies, Gaithersburg, Md.) was added to a final concentration of 9 μg per ml and the solution was incubated for 15 min at 37° C. Following incubation, the DNase was inactivated by the addition of 0.5 ml of 0.5M EDTA and the GE was pelleted at 14,000×g in a Sorvall centrifuge for 30 min at 4° C.

Protocol C: Construction of the GE Genomic Library

Genomic DNA was isolated from purified GE using the QIAamp Genomic DNA kit (Qiagen, Chatsworth, Calif.) for library preparation (Stratagene, La Jolla, Calif.). The DNA was mechanically sheared to a 4-10 kb size range and ligated to EcoRI linkers. Linkered fragments were ligated into the EcoRI site of Lambda Zap II and the library was amplified in *E. coli* strain XL1-Blue MFR' to a titer of 10^{10} Pfu/ml.

Protocol D: Preparation of the Screening Sera

Dog sera: Adult *Ixodes* scapularis ticks collected from regions of the eastern United States having a high incidence of human Lyme disease were applied to dogs as described (Coughlin et al., *J. Infect. Dis.* 171:1049-1052 (1995)). Sera from the dogs was tested for immunoreactivity to *E. equi* by an immunofluorescence assay. Positive sera from infected dogs was pooled and used for immuno screening of the GE genomic library.

Mouse sera: Proteins contained in SDS-disrupted whole GE were separated by SDS-PAGE and forty-six individual bands were excised from each of two gels, 10% and 15% acrylamide. Each gel fragment was mashed, added to buffer and Ribi adjuvant and used to immunize two mice. Sera with similar immuno reactivity patterns against GE antigen as determined by Western blot were pooled into 4 groups: A, B, C, and D.

Goat sera: Mixtures of 100 μg of purified heat-inactivated USG3 antigen were used to immunize goats. Goats received three subcutaneous doses of antigen at bi-weekly intervals. Serum was collected two weeks following the third immunization and used for immunoscreening of the GE genomic DNA library.

Protocol E: Screening of the GE Genomic DNA Library

Bacteriophage were diluted and plated with XL1-Blue MRF' cells on NZY agar plates. Plates were prepared giving approximately 50,000 plaques per plate. Phages were induced to express cloned protein with 10 mM IPTG (Sigma, St. Louis, Mo.) and transferred to nitrocellulose filters. For immuno screening, filters were blocked in TBS (25 mM Tris HCl, pH 7.5, 0.5 M NaCl) containing 0.1% polyoxyethylene 20 cetyl ether (Brij 58) and incubated with pooled dog sera, pooled mouse sera, or pooled goat sera. The filters were washed and then reacted with anti-dog HRP conjugated antibody, anti-mouse HRP conjugated antibody, or anti-goat HRP conjugated antibody. The filters were washed again and developed with 4-chloronapthol (Bio-Rad).

Positive plaques were isolated, replated and rescreened twice to achieve purity. Plasmid DNA containing the putative recombinant clones was obtained by plasmid rescue (Stratagene, La Jolla, Calif.).

Protocol F: DNA Analysis

Restriction enzyme analysis: Standard techniques were followed according to the protocols of Sambrook et al., *Molecular Cloning* (2nd ed.), Cold Spring Harbor Laboratory Press, New York (1989)).

DNA sequencing and sequencing analysis: DNA sequencing of recombinant clones was performed using the primer walking method and an ABI 373A DNA sequencer (ACGT, Northbrook, Ill.; Lark Technologies, Houston, Tex.; and Sequegen, Shrewsbury, Mass.). Sequences were analyzed by using the MacVector (Oxford Molecular Group) sequence analysis program, version 6.0. The BLAST algorithm, D version 1.4, was used to search for homologous nucleic acid and protein sequences available on the National Center for Biotechnology Information (NCBI) server.

PCR amplification of target sequences: DNA oligonucleotide primer sets were designed based on sequencing information from each individual clone. PCR primers were synthesized by Life Technologies (Gaithersburg, Md.). Templates for PCR were either purified plasmid DNA, purified GE or HL60 genomic DNA, or phage lysates. All reactions were performed using a Gene Amp 9600 thermal cycler (Perkin-Elmer, CT), GenAmp reagents from Perkin-Elmer, and TaqStart antibody (Clontech, CA). The cycling program consisted of 30 cycles, each of 30 s at 94° C., 30s at 48° C. to 55° C., and 1 min at 72° C., and an additional cycle of 10 min at 72° C. PCR products were analyzed on 4% Nusieve 3:1 agarose gels (FMC Bioproducts, Rockland, Me.).

Protocol G: Protein Isolation and Analysis

Overnight cultures of individual clones were diluted 1:25 into TP broth (per liter: 20 g bactotryptone, 2 g Na_2HPO_4, 1 g KH_2PO_4, 8 g NaCl, 15 g yeast extract) and grown at 37° C. until an OD_{600} of 0.5 to 1 was reached. A 1.5 ml aliquot of culture was harvested. IPTG was added to a concentration of 5 mM and growth was continued for 3 hours at 37° C. The OD_{600} was read and each culture was pelleted. Pellets were resuspended in 5× Laemmli buffer (12% glycerol, 0.2M Tris-HCl, pH 6.8, 5% SDS, 5% β-mercaptoethanol) at 200 μl per 1 OD unit. In the alternative, harvested GE protein preparations were pelleted and resuspended in 0.4% SDS, 12.5 mM Tris, pH 6.8 and heated at 90-100° C. for 20 min. For cell lysis, 50 μl of a cocktail consisting of RNase (33 μg/ml) and aprotinin (0.2 mg/ml) and 9 μl of DNase (0.17 mg/ml) was added per 5 mg of GE. 20 μl of 25× Boehringer/Mannheim protease inhibitor cocktail (Cat. #1697498) was added per 0.5 ml cell suspension and 2 μl of a PMSF solution (1M in DMSO) was added just prior to cell disruption. Cells were disrupted in 30 second intervals for a total of 3 min in a mini-beadbeater cell disrupter, Type BX-4(BioSpec), agitated at room temperature for 30 min and centrifuged at 15,000×g for 10 min. The pellet was suspended in Laemmli sample buffer and adjusted to 1.4 mg SDS/mg protein. Samples were boiled and 10 μl of each were electrophoresed on SDS-PAGE gels.

For Western blot analysis, gels were transferred to nitrocellulose filters, the filters were blocked in TBS/Brij 58 and the blots were probed with antisera. Blots were then washed and incubated with HRP conjugated secondary antibody. After a final washing step, blots were developed with 4-chloronapthol (Bio-Rad, Hercules, Calif.) or detected using enhanced chemiluminescence (Pierce, Rockford, Ill.).

Example 1

PCR Amplification and Cloning of GE 16S rDNA

GE was cultivated in HL60 cells as described in Protocol A (supra). Cell extracts were prepared by lysis protocols as described supra, PCR primers (specific for the 16S ribosomal DNA of the genogroup comprising *E. equi, E. phagocytophila*, and the HGE agent used to amplify DNA from the cell extracts) were modified to include restriction enzyme recognition sites as follows:

```
forward primer,                          (SEQ ID NO: 40)
5'-CTGCAGGTTTGATCCTGG-3' (PstI site);

reverse primer,                          (SEQ ID NO: 41)
5'-GGATCCTACCTTGTTACGACTT-3' (BamHI site),
```

These primers (0.5 μM) were added to a 100 μl reaction mixture containing 1×PCR buffer II (Perkin-Elmer Corp), 1.5 mM $MgCl_2$ (Perkin-Elmer Corp.), 200 μM each dATP, dGTP, dCTP and dTTP, 2.5 U of Amplitaq DNA polymerase and 20 μl of USG3 DNA. Amplification was performed as described in Protocol F. The amplified 1500 bp fragment was digested with Pst I and BamHI and ligated to pUC19 linearized with the same enzymes. The resulting clone, pUCHGE16S, was sequenced.

Example 2

Isolation of Clones Using Canine Sera

Western blot analysis of the individual recombinant plasmid was performed as described in Protocol G using canine sera prepared as described in Protocol D or a 1:1000 dilution of human sera prepared from two convalescent-phase sera from patients (No. 2 and 3, New York, kindly provided by Dr. Aguero-Rosenfeld) and from an individual in Wisconsin who was part of a seroprevalence study (No. 1, kindly provided by Dr. Bakken). Blots were washed and incubated with biotin-labeled goat anti-dog IgG (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) followed by peroxidase labeled streptavidin (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) or HRP conjugated anti-human IgG (Bio-Rad, Hercules, Calif.). After several additional washes, the dog sera blots were developed with 4-chloronapthol to (Bio-Rad, Hercules, Calif.). Over 1000 positive clones were identified. Three hundred of these clones (both strong (S) and weak (W) immunoreactivity) were further purified by a secondary screen of the library. From this group, 48 clones were purified as single plaques by a third immunoscreening. Plasmids were rescued according to the Stratagene protocol and DNA was purified using Qiagen plasmid purification kits. Of the original forty-eight clones, seven were not able to be analyzed due

to lack of sufficient DNA. A number of restriction digests were performed on each clone to assess their relatedness. Single enzyme digests were performed with EcoRI, HindIII, BamHI, HincII, XbaI, PstI and Alw26I and in some cases a number of double digests were done. Based on these digests restriction maps were generated and most of the clones could be placed into one of three groups—designated groups I, II and III. FIGS. **1-3** show the structures of the three groups based on the restriction enzyme analysis. Another five clones had lost the insert during the plasmid rescue and were not grouped.

Example 3

Characterization of Representative Clones S2, S7, S22, and S23

A representative clone was chosen for further characterization from each of the three groups (see Example 2, supra). These clones, S2, S7, and S22, were sequenced according to Protocol F. S23 was also sequenced since it did not appear to fall into one of these groups. The complete nucleic acid sequence of each of these clones is shown as follows: FIG. **4**, group I (S22); FIG. **6**, group II (S2); FIG. **8**, group III (S7); FIG. **10**, (S23). Sequence analysis (Mac Vector, Oxford Molecular Group) showed that each clone contained a single large open reading frame encoded by the plus strand of the insert and each one appeared to be a complete gene. The amino acid sequences encoded by each clone are shown in FIG. **5** (S22), FIG. **7**, (S2), and FIG. **9** (S7), and FIG. **11** (S23). There are also two additional small open reading frames in the S23 DNA insert, one on the negative strand and the other on the positive strand. A comparison of the DNA sequences of the 4 clones revealed that S23 is a group I clone which is missing a stretch of nucleotides in S22 containing two EcoRI sites. The nucleotide sequences of the genes described here have been assigned the following GenBank accession numbers: GE ank (GE 160), AF020521; GE rea (GE 130), AF020522; GE gra (GE 100), AF020523. Further sequence analysis of the four clones showed that all of them contain regions of repeated amino acids.

FIG. **12** represents a schematic diagram of the S22 and S23 proteins and the repeat regions within those proteins. Similarly, FIG. **13** shows the repeat regions of the S2 and S7 proteins in a schematic diagram Amino acid sequence analysis of the proteins encoded by the three gene clones S22, S2, and S7, showed that all contain regions of repeated amino acids. A schematic version of these repeat structures is shown in FIGS. **14** and **15**. The S2 encoded protein (160 kDa) has three groups of repeats. The first set consists of a number of ankyrin-like repeat units of 33 amino acids, the second consists of repeat units of 27 amino acids, and the third consists of repeat units of 11 amino acids. The ankyrin repeats were revealed by a BLAST database search for protein homologies. Ankyrin repeats occur in at least four consecutive copies and are present in yeast, plants, bacteria, and mammals. FIG. **14** shows a multiple alignment of the S2 encoded protein (160 kDa) ankyrin repeats under a consensus sequence derived from the analysis of several hundred similar ankyrin-like motifs. The eighth repeat sequence holds to the consensus only through the first half of the repeat unit and may not represent a full ankyrin-like repeat.

The S22 encoded protein (130 kDa) has a repeat unit of 26 to 34 amino acids which occurs eight times in the carboxy-terminal half of the protein (See FIG. **15**). The sequence varies somewhat from repeat to repeat. A database homology search with the NCBI BLAST algorithm revealed that the S22 encoded protein has limited homology to the *E. chaffeensis* 120 kDa protein. An amino acid sequence alignment of a motif common to both proteins is shown in FIG. **15**A. This motif is represented by a bold line and occurs four times in an identical fashion in the *E. chaffeensis* protein (designated A-1) and eight times with four variations in the 130 kDa protein (a-1, a-2, a-3, and a-4).

The S7 encoded protein (100 kDa) has three large repeat units, which differ somewhat in length (See FIG. **15**). A database search revealed that it is similar to the 120 kDa *E. chaffeensis* protein, which contains four repeats of 80 amino acids each. Both proteins contain large amounts of glutamic acid: 18% for the 100 kDa protein and 17% for the 120 kDa protein. When the two protein sequences are aligned, most of the homology occurs in the repeat regions. FIG. **15**B shows alignments for two homologous groups of amino acid motifs from the two proteins (designated B/b and C/c) found with the BLAST algorithm. These are not the only possible alignments of the two proteins but do provide an example of their similarities. The locations of the homologous sequences are indicated by bold or hatched lines above (S7 encoded 100 kDa protein) or below (*E. chaffeensis* 120 kDa protein) the respective proteins. The B sequence represented by the bold line varies slightly in the *E. chaffeensis* protein (shown as B-1, B-2, and B-3) and occurs a total of five times. The S7 encoded protein equivalent, b-1, is invariant and occurs three times. The sequence represented by the hatched line occurs four times in *E. chaffeensis* 120 kDa (C-1) and two times in S7 (C-1).

Samples of recombinant clones were induced to express the encoded protein and bacterial extracts were prepared for SDS-PAGE as outlined in Protocol G. FIG. **16** shows a Western blot containing samples of S2, S7, S22, and FIG. **17** shows a western blot also containing a sample of S23. SDS-disrupted whole GE was used as a positive control and a non-protein expressing clone was run as a negative control. Immunoreactive proteins for all 4 clones were detected by the dog sera. The same proteins were also detected when the blots were probed with sera obtained from a human patient with GE, as evident in FIG. **18**. The blots were probed with human antisera. Based on the amino acid sequences of these proteins, the calculated molecular weights are significantly lower than the apparent molecular weights by SDS-PAGE. The calculated (based on the amino acid sequence) and apparent (based on mobility in SDS-PAGE) molecular weights of each protein encoded by the open reading frames of the listed clones are compared in Table 4. This phenomenon has been observed in other proteins (see Barbet et al., *Infect. Immun.* 59:971-976 (1991); Hollingshead et al., *J. Biol. Chem.* 267:1677-1686 (1986); Yu et al., *Gene* 184:149-154 (1997)).

TABLE 4

Clone	Calculated Molecular Weight	Apparent Molecular Weight
S2	78 kDa	160 kDa
S7	61 kDa	100 kDa
S22	66 kDa	130 kDa
S23	52 kDa	90 kDa

Example 4

Verification that Clones S2, S7, S22, and S23 are GE Derived by PCR Analysis

PCR primer sets were designed based on the sequences of each of the three GE clones and are as described in Table 5.

The sequences of each primer set indicated in Table 5 were used to amplify regions of the listed clones (SEQ ID NOS: 47-52). Each oligonucleotide sequence is shown in the 5' to 3' orientation. Each 50 μl reaction contained 0.5 μM of each primer, 1×PCR Supermix (Life Technologies, Gaithersburg, Md.) and either 100 ng USG3 DNA, 100 ng HL60 DNA or 200 ng plasmid DNA. PCR amplification was performed as to described in Protocol F.

TABLE 5

Clone	Forward Primer	Reverse Primer
S22	CACGCCTTCTTCTAC (SEQ ID NO: 42)	CTCTGTTGCTATAGGGGC (SEQ ID NO: 43)
S7	GATGTTGCTTCGGGTATGC (SEQ ID NO: 44)	CAGAGATTACTTCTTTTTGCGG (SEQ ID NO: 45)
S2	GCGTCTCCAGAACCAG (SEQ ID NO: 46)	CCTATATAGCTTACCG (SEQ ID NO: 47)

These experiments established that the sequenced genes were derived from GE DNA and not HL60 DNA, and allowed the elimination of duplicate clones prior to plasmid rescue and DNA isolation by using them in PCR of phage lysates. Primer pairs specific for S22/S23, S2 and S7 were used in separate PCR reactions to amplify three different templates: GE DNA, HL60 DNA, or the purified plasmid DNA of each clone. FIGS. **19** and **20** show the results obtained for primers of S22, S23, S2, and S7 using the PCR conditions outlined above. All three clones were specific to GE and were not present in HL60 DNA. In each case the size of the PCR product using genomic DNA as a template was the same as that generated by purified plasmid DNA.

Example 5

Further Characterization of Isolated GE Clones

The same primer pairs (supra) were also used to confirm or establish the identity of each purified phage stock from all 48 clones derived from the library screening with the dog sera. Every isolate, with one exception (W20), was either a group I, II, or III clone, as evident in Table 6 below. Clones were isolated by immunoscreening with convalescent dog sera. Each clone is classified as a group I, II or III clone based on PCR with primers specific for the group I, II or III DNA sequences. Clone W20 was the only clone different from the other 3 groups.

TABLE 6

Clone Name	Group
S1	II
S2	II
S3	II
S5	II
S6	III
S7	III
S8	I
S9	I
S10	I
S11	I
S12	II
S13	II
S14	I
S19	II
S22	I
S23	I

TABLE 6-continued

Clone Name	Group
S24	I
S25	I
S27	I
S32	II
W1	II
W2	I
W3	I
W4	I
S16	III
S17	III
S18	I
S20	III
S21	III
S28	III
S30	II
S33	III
W5	II
W7	II
W8	I
W9	III
W10	III
W11	I
W13	I
W14	I
W15	II
W16	III
W17	I
W18	I
W19	III
W20	—
W21	I
W22	III

Example 6

Isolation of Clones Using Murine Sera

Four different pools of sera (designated A, B, C, and D) obtained from mice immunized with gel band samples of GE protein (Protocol D) were used to screen the GE genomic DNA library. Twenty-six clones were plaque purified and used for further analysis. These were designated A1, A2, A8, A11, A14, A16; B1, B3, B6, B8, B9, B12; C1, C3, C5, C6, C7, C10, C11; D1, D2, D7, D8, D9, D11, and D14. Plasmid DNA was rescued from each clone and restriction analyses were performed. Several of the clones (A14, B12, C3, C5, D1, D2, D9 and D11) had no insert. Of the remaining clones, nine could be placed into one of two groups due to similarities in their restriction enzyme patterns. The first group included all of the C clones and the second group consisted of all of the D clones plus B3. Some of the other clones were not grouped at this stage due to lack of sufficient DNA.

Example 7

Characterization of Representative Clone C6

One representative clone from the C group (C6) was selected for DNA sequencing. The insert of 2.7 kb contained two open reading frames (designated C6.1, C6.2, and whose amino acid sequences are given in FIGS. **21** and **22**, respectively) on the plus strand which were separated by 9 nucleotides (FIG. **23**). A search of the protein/nucleotide databases revealed that the first amino acid sequence (C6.1) has significant homology to dihydrolipoamide succinyltransferase, an enzyme involved in the oxidative decarboxylation of pyruvate and 2-oxoglutarate (Spencer et al., *Eur. J. Biochem.* 141:361-374 (1984)). The second amino acid sequence

(C6.2) is homologous to a methionine aminopeptidase found in several types of bacterial species.

Clones, C1, C6, and C7, were induced to express the encoded protein and bacterial extracts were prepared for SDS-PAGE. FIG. **24** shows a Western blot of these samples electrophoresed next to SDS-disrupted whole GE. The immune mouse serum designated "C" was used to probe the blot. All three recombinant clones expressed a protein of the same molecular weight, about 50 kDa. The calculated molecular weights of C6.1 and C6.2 are 44 kDa and 29 kDa, respectively. Thus, based on size, C6.1 is more likely to be the expressed recombinant protein detected by immunoscreening.

DNA sequencing also revealed that the group of clones consisting of all of the D clones and the B3 clone contained an open reading frame for a protein with homology to the heat shock protein hsp70.

Based on the DNA sequences of each clone, PCR primers were designed to amplify specific regions of each open reading frame contained in C6. The primers used were as follows:

```
forward primer for C6.1:          (SEQ ID NOS: 48)
5'-CAGGCAGTGAGCACTCAAAAACC-3';

reverse primer for C6.1:          (SEQ ID NOS: 49)
5'-GCGACTCCAATGTTACAATAGTCCC-3';

forward primer for C6.2:          (SEQ ID NOS: 50)
5'-TGTGATCCTCGATGGTTGGC-3';

reverse primer for C6.2:          (SEQ ID NOS: 51)
5'-CCCTCCTGAATCGTAACATCATCC-3'.
```

FIG. **25** shows the results obtained with each primer pair using GE DNA, HL60 DNA or the C6 plasmid DNA as templates in a PCR reaction. Both primer sets amplified a region of the expected size using GE or plasmid templates but not the HL60 template. Thus both C6 genes are GE specific.

The C6 primers were also used to amplify phage lysates from each of the other twenty-five clones isolated using the immune mouse sera. In addition to all of the C clones, the C6.1 and C6.2 genes were also found in A1, A11, A14 and A16.

The following examples (Examples 8-15) all relate to the characterization of the GE immunoreactive protein in the 42-45 kDa molecular mass range.

Example 8

SDS-PAGE and Peptide Sequencing of Immunoreactive Proteins

To characterize the GE proteins in the 42 to 45 kDa range, a 50 μl of a cocktail consisting of RNase (33 μg/ml) and aprotinin (0.2 mg/ml) and 9 μl of DNase (0.17 mg/ml) was added per 5 mg of USG3 pellet in 2 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.5 buffer. Twenty μl of 25× Boehringer/Mannheim protease inhibitor cocktail was added per 0.5 ml cell suspension and 2 μl of a PMSF solution (1M in DMSO) was added just prior to USG3 disruption. Cells were disrupted in 30 second intervals for a total of 3 min. in a mini-beadbeater cell disrupter, Type BX-4 (BioSpec), agitated at room temperature for 30 min and centrifuged at 15,000×g for 10 min. The pellet was suspended in Laemmli sample buffer and adjusted to 1.4 mg SDS per mg protein, and heated at 90-100° C. for 5 min. The protein concentration was determined by BCA to assay (Pierce Chemical Co., Rockford, Ill.). Electrophoresis was performed on a 15% SDS-PAGE gel and proteins were transferred onto a 0.2 μm PVDF membrane. Half of the blot was probed with anti-GE dog sera (6) and the other half was stained with Ponceau S. Two protein bands which matched the molecular mass of the two most immunoreactive bands on the Western blot (43 and 45 kDa) were excised. A portion of each band was used for direct N-terminal sequencing. The remaining material was digested with trypsin in situ and individual peptides were separated by RP-HPLC on a ZORBAX C18 (1 mm×150 mm) column. The peptides were analyzed and screened by MALDI-TOF mass spectrometry. Sequencing of peptides was performed by Edman degradation (Harvard Microchemistry, Cambridge, Mass.). An N-terminal peptide and two internal peptides were obtained for each protein (Table 7).

TABLE 7

Peptide Sequences from Transblotted GE Proteins

	N-terminal (N) or Internal (I)	Homology to *A. marginale* MSP-2	Location
45 kDa			
HDDVSALETGGAGYF[a] (SEQ ID NO: 66)	N	no	MSP2-A, MSP-2C (1)[b]
SGDNGSLADYTDGGASQTNK (SEQ ID NO: 67)	I	no	MSP-2A
AVGVSHPGIDK (SEQ ID NO: 68)	I	no	MSP-2A, MSP-2C(2)
43 kDa			
HDDVSALETGGAGYF (SEQ ID NO: 66)	N	no	MSP-2A, MSP-2C(1)
FDWNTPDPR (SEQ ID NO: 69)	I	yes	MSP-2A, MSP-2C
LSYQLSPVISAFAGGFYH (SEQ ID NO: 70)	I	yes	MSP-2A, MSP-2B(1)

[a]Amino acids are shown using the single letter code.
[b]Numbers in parentheses indicate the number of amino acid changes from the sequence shown.

The results show that the amino-terminal peptides from the two proteins are identical. A BLAST homology search showed that two of the internal peptides from the 43 kDa protein were homologous to the MSP-2 proteins of *Anaplasma marginale*, a rickettsial hemoparasite of livestock (Palmer et al., *Infect. Immun.* 62:3808-3816 (1994)) which is phylogenetically closely related to the GE (Dumler et al., *J. Clin. Microbiol.* 33:1098-1103 (1995)).

Example 9

PCR Amplification of USG3 Genomic DNA

To obtain additional sequence information for these proteins, degenerate pools of oligonucleotides were synthesized based on the reverse translation of the peptide sequences and used to amplify DNA from USG3. The reverse complement of each oligonucleotide was also synthesized with the exception of the one corresponding to the amino-terminal peptide. PCR amplifications were performed using one forward and one reverse primer set using USG3 genomic DNA as template and an annealing temperature of 55° C. Primer pairs either gave no PCR product or a single band. The primer pair that resulted in generating the longest product, 550 bp, consisted of the forward primer 5'-ACNGGNGGNGCWGGNTAYTTY-3' (SEQ ID NO:71) (amino-terminal peptide HDDVSALETGGAGYF (SEQ ID NO:66)) and the reverse primer 5'-CCNCCRTCNGTRTARTCNGC-3' (SEQ ID NO:72) (peptide SGDNGSLADYTDGGASQTNK (SEQ ID NO:67)). This DNA was sequenced and found to contain an open reading frame with homology to the MSP-2 protein of *A. marginale* (FIG. **26**). Two other peptides, one from the 45 kDa protein and one from the 43 kDa protein, were also contained within this sequence. The similarity in protein sequence between the two immunoreactive 43 and 45 kDa proteins may indicate that they are differentially modified or processed versions of the same protein or they may represent proteins expressed from two different members of a gene family.

Example 10

Isolation of Clones Using Goat Sera

A goat serum reactive against proteins of the HGE agent was obtained by immunizing animals 3 times with purified USG3 antigen. Western blot analysis showed that many proteins of various molecular mass were recognized by this serum including the 43 and 45 kDa proteins (FIG. **27**, GE lanes). The USG3 genomic expression library (prepared as described in Protocol C) was screened with immune goat serum and several immunoreactive plaques were identified for further analysis. To eliminate clones previously isolated using immune dog sera, phage supernatants from the plaques were screened by PCR using primers based on the sequences of those previously identified clones. Bacteriophage were plated with XL1-Blue MRF and induced to express protein with 10 mM IPTG (Sigma, St. Louis, Mo.). Proteins were transferred to nitrocellulose filters and the filters were washed with TBS (25 mM Tris HCl, pH 7.5, 0.5 M NaCl). Washed filters were blocked in TBS containing 0.1% polyoxyethylene 20 cetyl ether (Brij 58) and incubated with a 1:1000 dilution of goat serum depleted of anti-*E. coli* antibodies. The filters were washed and incubated with rabbit anti-goat Ig HRP conjugated antibody (1:2000 dilution), rewashed and developed with 4-chloronapthol. Positive plaques were isolated, replated and screened again. Plasmid DNA containing the putative recombinant clones was obtained by plasmid rescue (Stratagene, La Jolla, Calif.). pBluescript plasmids were rescued from the remaining clones and they were assessed for relatedness by restriction enzyme analysis. Two clones, E8 and E33, appeared to contain the same insert in opposite orientation from the lacZ promoter. Two other clones, E46 and E80, shared restriction enzyme fragments in common but E46 contained a larger insert than E80.

Example 11

DNA Sequencing and Sequence Analysis

Three clones, E8, E33, and E46, were sequenced by the primer walking method. Both strands of each insert were sequenced as described in Protocol F. The sequences of the three clones shared considerable homology. The E8 clone contained a larger version of the E33 insert but in opposite orientation with respect to the lacZ promoter (FIG. **28**). Both clones contained the same open reading frame but E33 was missing 420 nucleotides from the 5' end of the gene. The deduced amino acid sequence of the E33 open reading frame was in frame with the partial β-galactosidase amino acid sequence encoded by the vector (data not shown). The nucleotide and deduced amino acid sequences of the pBluescript E8 insert (which did contain the entire gene) are shown in FIG. **29**. The predicted molecular mass of the protein encoded by this gene was 45.9 kDa. The nucleotide and deduced amino acid sequences for E46 clone is shown in FIG. **30**. The E46 insert contained one partial and two complete open reading frames which all shared considerable homology with the protein encoded by the E8 gene. FIG. **28** shows how the DNA sequences (+ and −strands) and deduced amino acid sequences from E46 compare with those from E8 and E33. The boxed regions represent the open reading frames and shaded areas indicate homologous sequences. As shown in FIG. **31**, all three of the complete genes showed a similar pattern for the encoded proteins: a variable domain flanked by conserved regions having a consensus amino-terminal sequence as set forth in SEQ ID NOS:41-43, and/or a carboxy terminus having a consensus sequence as set forth in SEQ ID NOS:41-43. (See FIG. **31**). The length of the conserved regions varied among the encoded proteins, with the longest amino and carboxy-terminal conserved regions present in the E8 protein. The sequences present in the E8, E33 and E46 pBluescript plasmids were confirmed to be derived from USG3 genomic DNA and not HL60 DNA by PCR analysis using the primers described herein. When the sequences of the three full length genes isolated by expression library cloning were compared with the sequence of the PCR product derived from the peptide analysis, it was found that the PCR fragment was contained within the E8 sequence, by 232 to 760 (FIG. **29**). In fact, the amino-terminal peptide and all four internal peptides sequenced from the 43 kDa and 45 kDa proteins could be found within the amino acid sequence of the E8 protein. The sequenced peptides are underlined in FIG. **29**. The amino-terminal peptide (HDDVSALE . . .) was found beginning at amino acid 27 and this may indicate that the first 26 amino acids are part of a signal peptide which is cleaved to produce the mature protein. Since the PCR product had both nucleotide and amino acid homology to the *A. marginale* msp2 gene family, a BLAST homology search was performed to assess the relatedness of the E8 and E46 gene products to this family as well. Strong matches were observed for all of the GE proteins described here to the *A. marginals* MSP-2 proteins. A ClustalW amino acid alignment of the GE proteins (designated GE MSP-2A (E8), MSP-2B (E46#1), and MSP-2C (E46#2)) with one of the *A. marginale* MSP-2 proteins (GenBank accession number U07862) is shown in FIG. **31**. The homology of the GE MSP-2 proteins with *A. marginale* MSP-2 occurred primarily in the conserved regions shown in FIG. **28**. Amino acid identity ranged from 40 to 50% between the proteins of the two species and amino acid similarity was close to 60%. The *A. marginale* MSP-2 proteins contain signal peptides (data not shown) and the data indicating that GE MSP-2A has a signal peptide is consistent with

the homology observed between the MSP-2 proteins of the two species. The nucleotide sequences of the genes described here have been assigned the following GenBank accession numbers: GE msp2A (E8):AF029322; GE msp2B (E46#1) and GE msp2C (E46#2):AF029323.

The three GE clones E8, E33, and E46 thus appear to be part of a multigene family encoding proteins containing highly homologous amino- and carboxy-terminal regions related to the MSP-2 proteins of *A. marginale*. In addition to the three full length and one truncated msp2-like genes reported here, there are likely to be others present in the GE genome. Hybridization studies (infra) using probes from either the 5' or 3' end of the E8 msp2 gene identified multiple copies of homologous msp2 genes in the genome of USG3. Sequencing of several other GE library clones has revealed short (100 to 300 nucleotides) stretches of DNA homologous to msp2. Several different MSP-2 proteins ranging in size from 33 to 41 kDa have been reported for *A. marginale* and >1% of its genome may consist of msp2. The function of the GE MSP-2 to proteins is unknown. Zhi et al., supra, demonstrated that the antigens are present in outer membrane fractions of purified granulocytic ehrlichiae. Thus, they may play a role in the interaction between the pathogen and the host cell. In *A. marginale*, expression of antigenically unique MSP-2 variants by individual organisms during acute rickettsemia in cattle suggests that the multiple msp-2 gene copies may provide a mechanism for evasion of the beneficial immune response directed against these antigens. This may explain the observation that the GE MSP-2A protein is present in purified USG3 but the MSP-2B and MSP-2C are not.

Example 12

Southern Blot Analysis

To determine whether additional copies of msp-2 were present in the genome, genomic DNA was isolated from USG3 and digested with restriction enzymes. Digoxigenin-labeled probes were prepared by PCR using the PCR Dig Probe Synthesis kit (Boehringer Mannheim). Two sets of primers were used to generate a 240 bp product (probe A) from the 5' end of the E8 gene: (forward primer: 5'-CATGCTTGTAGCTATG-3' (SEQ ID NO:52); reverse primer: 5'-GCAAACTGAACAATATC-3' (SEQ ID NO:53)) and a 238 bp product (probe B) from the 3'-end of the E8 gene; (forward primer: 5'-GACCTAGTACAGGAGC-3' (SEQ ID NO:54); reverse primer: 5'-CTATAAGCAAGCTTAG-3' (SEQ ID NO:55) including the consensus sequence corresponding to the amino- and/or carboxy-terminus regions shared by E8, E46#1, and E46#2 polypeptide). Genomic DNA was prepared from USG3 or HL6O cells as described above and aliquots of 1 μg of DNA were digested with SphI, NdeI, SacI, or SspI (New England Biolabs, Beverly, Mass.). These restriction endonucleases do not cut within the sequence of E8 msp2A. Calf thymus DNA was digested identically as a control. Recombinant pBluescript E8 plasmid DNA was digested with EcoRI and used as a positive control for probe hybridization. Digested fragments were separated by gel electrophoresis in a 1% agarose gel. Southern blotting was performed under prehybridization and hybridization conditions of 65° C. in Dig Easy Hyb (Boehringer Mannheim) and hybridization was performed overnight. Two membrane washes in 2×SSC/0.1% SDS were performed at room temp for 5 min each followed by two washes in 0.5× SSC/0.1% SDS at 65° C. for 15 min each. Bound probe was detected by chemiluminescence using anti-digoxigenin alkaline phospate conjugated antibody (Boehringer Mannheim). FIG. **32** shows that multiple bands were present on the Southern blots using both probes, indicating the presence of multiple msp-2 copies. The exact number of genes cannot be determined since sequence differences may generate additional restriction enzyme sites in some of the msp-2 copies, resulting in more than one band from a single copy. Also, more than one msp-2 gene could be present on a single restriction fragment, an event which does occur with the msp-2B and msp-2C genes.

Example 13

Western Blot Analysis of Proteins Encoded by GE Clones

Bacterial lysates from the genomic library clones, E8, E33, and E46, were analyzed by SDS-PAGE and Western blotting. Individual recombinant plasmid containing cultures were induced to express protein with 5 mM IPTG. Bacterial cells were pelleted by centrifugation and resuspended in 5× Laemmli buffer (12% glycerol, 0.2M Tris-HCl, pH 6.8, 5% SDS, 5% p-mercaptoethanol) at 200 μl per 1 OD unit of culture. Samples were boiled and 10 μl of each were analyzed on NuPage gels (Novex, San Diego, Calif.). Proteins were transferred to nitrocellulose filters, the filters were blocked in TBS/Brij 58 and the blots were probed with either a 1:500 dilution of pooled sera from dogs that were infected with GE by tick exposure, a 1:500 dilution of the goat serum described above, or a 1:1000 dilution of human serum. Preimmune dog and goat sera were also used at a 1:500 dilution. Blots were washed and incubated with HRP conjugated secondary antibody (Bio-Rad, Hercules, Calif.). After several additional washes, the blots were developed using the Pierce (Rockford, Ill.) Super Signal Chemiluminescence kit and viewed by autoradiography. FIG. **27** shows that a protein of approximately 37 kDa from the E46 clone and a 45 kDa protein from the E8 clone were specifically detected by dog and goat sera (indicated by arrows on the right side of each blot). The reactivity of the sera differed somewhat in that the dog sera reacted much better than the goat sera with the E46 protein and the goat sera had better reactivity to the E8 protein. Whether the 37 kDa/E46 protein is encoded by the first or second E46 gene is unknown and the reason for the expression of two closely sized immunoreactive E33 proteins is also unclear. Preimmune sera did not detect these proteins and expression was observed in the absence of IPTG induction. The molecular mass of the proteins is consistent with the coding capacity of the msp-2 genes found in the library clones. The negative control (NEG lane) was a pBluescript library clone without an insert. FIG. **27** also shows a couple of proteins of smaller molecular mass from E46 and E8 that react specifically with the goat serum. It is not known whether they are breakdown products of the full length MSP-2 proteins or whether they are produced by internal initiation within the msp-2 genes.

Example 14

PCR Amplification of Isolated Clones

PCR primer sets were designed based on the sequences of each GE clone and are as follows:

```
E8 (forward 5'-GCGTCACAGACGAATAAGACGG-3'
(SEQ ID NO: 56);

reverse 5'-AGCGGAGATTACAGGAGAGAGCTG-3'
(SEQ ID NO: 57));
```

-continued

E46.1 (forward 5'-TGTTGAATACGGGGAAAGGGAC-3' (SEQ ID NO: 58);

reverse 5' AGCGGAGATTTCAGGAGAGAGCTG 3' (SEQ ID NO: 59);

E46.2 (forward 5'-TGGTTTGGATTACAGTCCAGCG 3' (SEQ ID NO: 60);

reverse 5' ACCTGCCCAGTTTCACTTACATTC 3' (SEQ ID NO: 61)).

Each 50 μl reaction contained 0.5 μM of each primer, 1×PCR Supermix (Life Technologies, Gaithersburg, Md.) and either 100 ng USG3 DNA, 100 ng HL60 DNA or 250 ng plasmid DNA. PCR amplification was performed using the following conditions: 94° C. for 30 s, 61° C. for 30 s, and 72° C. for 1 min. After 30 cycles, a single 10 min extension at 72° C. was done. PCR products were analyzed on 4% Nusieve 3:1 agarose gels (FMC Bioproducts, Rockland, Me.).

Example 15

Recognition of MSP-2A and MSP-2B by GE-Positive Human Sera

PCR amplification of the first gene in pBluescript clone E46 was performed to generate an insert for subcloning in *E. coli*. Primer sets were designed to contain restriction sites for cloning, a translation termination codon and a six residue histidine sequence for expressed protein purification

(forward 5-CCGGCATATGCTTGTAGCTATGGAAGGC-3' (SEQ ID NO: 62);

reverse. 5'-CCGGCTCGAGCTAGTGGTGGTGGTGGTGGTGAAAAGCA AACCTAACACCAAATTCCCC-3' (SEQ ID NO: 63)).

The 100 μl reaction contained 500 ng of each primer, 500 ng of E46 template, and 1×PCR Supermix (Life Technologies, Gaithersburg, Md.). Amplification was performed using the following conditions: 94° C. for 30 s, 58° C. for 30 s, 72° C. for 1 min. After 37 cycles a single 10 min extension at 72° C. was performed. Following analysis on a 1% TBE agarose gel, amplified product was purified using a QIAEX II gel extraction kit (QIAGEN Inc, Chatsworth, Calif.) and digested with restriction enzymes NdeI and XhoI (New England Biolabs, Beverly, Mass.) using the manufacturer's recommended conditions. The 1004 bp fragment was ligated into NdeI and XhoI digested pXA and transformed into *E. coli* strain MZ-1 (19). Expression vector pXA is a pBR322-based vector containing the bacteriophage lambda pL promoter, a ribosome binding site, ATG initiation codon and transcription and translation termination signals. Recombinant MSP-2B was induced by growing the Mz-1 transformed clone to an A_{550}=1.0 at 30° C. and then shifting the temperature to 38° C. for an additional 2 hr. Aliquots (1.5 ml) of pre-induced and induced cells were pelleted by centrifugation and resuspended in 5× Laemmli buffer.

The coding regions for MSP-2A and MSP-2B were recloned using a heat inducible *E. coli* expression system as outlined above. The expression of the MSP2-A protein using this system remained low. However, the recombinant MSP-2B protein was expressed and could be detected with both dog and goat GE-positive sera (FIG. **32**). The recombinant MSP-2B protein and the E33 MSP-2A protein were then tested for reactivity with human serum samples which had previously been shown to be positive for granulocytic Ehrlichia by immunofluorescence assay (IFA). Table 8 shows the patient profiles and diagnostic laboratory results from fourteen individuals. Ten of these individuals were clinically diagnosed with HGE (#1-9, 13), three of them participated in a seroprevalence study (#10-12), and one was a negative control (#14). Immune and preimmune dog and goat sera were also used as positive and negative controls in the Western blots. FIG. **33** shows the reactivity of each human serum sample with MSP-2A (top) and MSP-2B (bottom). All of the human samples with IFA titers of 512 or more (#7,9,10,11,13) reacted with the MSP-2 proteins as did the positive dog and goat sera. Human serum #8 also reacted faintly with both proteins. In addition, these same sera all reacted with purified GE on Western blots (data not shown). Human serum #12 reacted with an *E. coli* protein which migrates in between the two E33 MSP-2 proteins. This reactivity was seen with all of the library clones we have tested, including those which do not express any GE related proteins (data not shown). From these data it appears that the IFA assay is more sensitive than the Western blot for diagnosis of HGE.

TABLE 8

HGE Patient Profiles and Diagnostic Laboratory Test Results

Patient	Gender	Age	Loc'n (state)	Conval. Stage (months)	Morulae	PCR[1]	IFA[2]	Peak IFA[3]
1	F	57	MN	0.5	+	ND	320	>2560
2	M	56	WI	12	+	+	160	640
3	M	59	MN	6	+	ND	320	320
4	M	74	WI	12	+	+	160	>2560
5	M	40	WI	12	+	+	320	5120
6	M	71	WI	24	+	+	320	1280
7	M	80	WI	36	+	–	>2560	>2560
8	M	60	MN	6	–	ND	320	>2560
9	F	44	MN	42	–	–	>2560	5120
10	M	50	WI	random	ND	ND	>2560	ND
11	F	50	WI	random	ND	ND	>2560	ND
12	M	64	WI	random	ND	ND	60	ND
13[3]	F	65	RI	1	–	+	512	1024
14	F	29	MA	NA	–	ND	<32	<32

[1]PCR with GE9F and GE1 OR primers (6).

[2]Polyclonal IFA assay with *E. equi* antigen.

[3]Data taken from reference 27.

+ Positive, – negative, ND not done, NA not applicable.

Example 16

Characterization of Representative Clone S11

Purified GE protein preparations were obtained as described in Protocol G. Aliquots were run on four lanes to allow the staining of three lanes with Ponceau S (0.1% in 1 N acetic acid) and one lane with Coomassie blue staining. Molecular weight markers were also run in two lanes. Electrophoresis was performed on a 10% SDS-PAGE preparative gel and proteins were transferred onto a 0.2 μm PVDF membrane. The Ponceau S bands with the same molecular weight as the bands stained with Coomassie blue (five total) were cut out for sequencing. N-terminal sequence was obtained for one of the five bands. The proteins in the other four bands were digested with trypsin in situ for internal peptide sequencing. Peptides were separated by RP-HPLC on a ZORBAX C18 (1 mm×150 mm) column Potential candidates for sequencing were screened for molecular mass by MALDI-TOF Mass Spectrometry on a Finnigan Lasermet 2000 (Hemel, UK). Protein sequencing was performed by Edman degradation.

Four of the five gel bands contained either serum proteins (probably from the fetal bovine serum used to culture the cells) or heat shock proteins. The other band appeared to contain a unique protein. Four internal peptide sequences were obtained from this gel band, representing a protein of approximately 64 kDa, that did not match any protein sequences in the database. The sequences of these peptides are shown in FIG. **34**. (SEQ ID NOS:34-37). Based on these sequences, degenerate DNA oligonucleotides were designed for each peptide (both forward and reverse/complement orientation) and used in all possible combinations for PCR using GE DNA as template. One combination, primers 5F (SEQ ID NO:32) and 6R (SEQ ID NO:33) (shown in FIG. **34**), produced a PCR fragment of 450 base pairs. The DNA was cloned into pCR Script SK(+) and the insert was sequenced. When the insert DNA was translated, both peptides (#24 and 25) (SEQ ID NOS:34-35) were found in the sequence, one at each end as expected.

To obtain a clone containing the entire gene represented by the PCR fragment, two primers were designed based on the DNA sequence of the PCR fragment. These primers were used in PCR reactions to screen sublibraries of the GE genomic library.

Forward primer (250F2): 5' CCCCGGGCTTTACAGT 3' (SEQ ID NO: 64)

Reverse primer (250R2): 5' CCAGCAAGCGATAACC 3' (SEQ ID NO: 65)

The sublibraries were generated by the initial screening of the genomic library with convalescent dog sera.

When a positive phage stock was found by PCR screening, the lysate was serially diluted twice and replated with bacterial stock XL1-Blue MRF to obtain isolated plaques. Forty-eight of these plaques were picked and lysates screened by PCR with primers 250F2 and 250R2. A positive clone was obtained which was designated S11. The plasmid DNA was rescued and restriction enzyme analysis performed to determine the size of the insert DNA and the approximate location of the gene within the insert. Results indicated that the insert size was about 8 kb and that the gene of interest was located at the T7 end of the insert relative to the pBluescript vector (FIG. **35**). A 2 kb portion of the S11 insert was sequenced and found to contain an open reading frame of 545 amino acids. The complete sequence is shown in FIG. **36** (SEQ ID NO:39).

When the amino acid sequence of S11 (SEQ ID NO:39) was compared to the peptide sequences obtained from the excised gel band representing a protein of 64 kDa, all four peptide sequences were found. These are shown underlined in FIG. **36**. The only difference between the nucleic acid sequence and the peptide sequences was the presence of phenylalanine (F) instead of aspartic acid (D) in position 4 of peptide #26 (SEQ ID NO:37). The reason for this difference is unknown. The calculated molecular weight of the protein encoded by the S11 gene was 58.5 kDa. A search of the nucleic acid and protein databases did not reveal any significant homology between it and other proteins in the database. There were, however, some minor similarities to outer surface proteins of some bacterial species.

All publications mentioned hereinabove are hereby incorporated in their entirety by reference. While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention and appended claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 85

<210> SEQ ID NO 1
<211> LENGTH: 3147
<212> TYPE: DNA
<213> ORGANISM: Granulocytic Ehrlichia
<220> FEATURE:
<223> OTHER INFORMATION: S22 of Granulocytic Ehrlichia

<400> SEQUENCE: 1

gaattcctta cctccctata tttcgtacag gttatttcgc agtctagcta tgatgcttta 60

ccaggatacg ttaaacgttg acgttctacg ctgtcatagc cttttattct gcaaaaatag 120

cttaactgtg tcacttcctg agaaagtaag atacatattt agtttttgca cagccaaaaa 180

acttctagtg aactgtggtt tctctggaat caataacctg ttttatattc gtgcgttcta 240

taacaatcta cagctgtggt tattaggcgt ggtttcgcct gataataaag atactttaga 300

-continued

gggtataaac ttggaaaaaa taatgaaaaa ccctccttag tgcctccccg tttttgacaa 360

catactctta tggaaaagcg ttagggagtt gcttcgcttg tcacgcgtgc gttaggtttt 420

acgtatacgt gtctgggact tcacgaaaac tcgacgcagg cggattttgt actatgtttc 480

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ctcgatttac tctaagtaag ctacaaataa ctcagtcata tcaaggtagt tcaagatgaa 2700

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当前申请(专利权)人(译)	ANTIGENICS INC.		
[标]发明人	MURPHY CHERYL STOREY JAMES BELTZ GERALD A COUGHLIN RICHARD T		
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摘要(译)

本发明一般涉及具有来自粒细胞埃希氏（GE）蛋白的抗原表位的多肽及其使用方法。