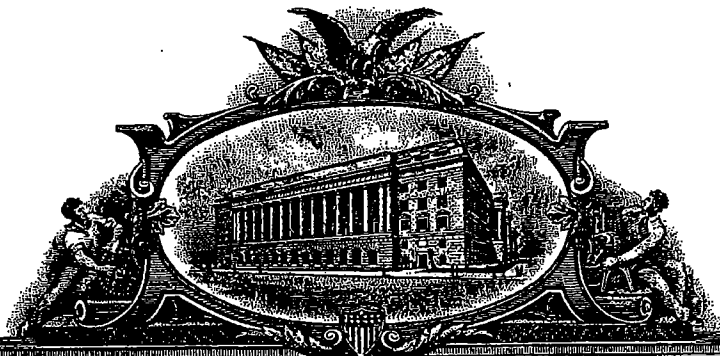


Exhibit 19
(part 1 of 2)

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ISSUE DATE: September 21, 1999**

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Certifying Officer**





US005955422A

United States Patent [19]

[11] Patent Number: **5,955,422**

Lin

[45] Date of Patent: ***Sep. 21, 1999**

[54] PRODUCTION OF ERTHROPOIETIN

FOREIGN PATENT DOCUMENTS

[75] Inventor: Fu-Kuen Lin, Thousand Oaks, Calif.
 [73] Assignee: Kirin-Amgen, Inc., Thousand Oaks, Calif.
 [*] Notice: This patent is subject to a terminal disclaimer.

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 [22] Filed: Aug. 2, 1993

Related U.S. Application Data

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[51] Int. Cl.⁶ A61K 38/16
 [52] U.S. Cl. 514/8; 514/12; 530/351; 530/363; 530/395; 530/350
 [58] Field of Search 424/439; 435/69.1, 435/69.2, 69.3, 69.6, 71.1, 71.2, 172.1, 172.3; 436/8; 514/8, 778, 970, 12; 530/351, 361, 395, 350

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[57] ABSTRACT

Disclosed are novel polypeptides possessing part or all of the primary structural conformation and one or more of the biological properties of mammalian erythropoietin ("EPO") which are characterized in preferred forms by being the product of procaryotic or eucaryotic host expression of an exogenous DNA sequence. Illustratively, genomic DNA, cDNA and manufactured DNA sequences coding for part or all of the sequence of amino acid residues of EPO or for analogs thereof are incorporated into autonomously replicating plasmid or viral vectors employed to transform or transfect suitable procaryotic or eucaryotic host cells such as bacteria, yeast or vertebrate cells in culture. Upon isolation from culture media or cellular lysates or fragments, products of expression of the DNA sequences display, e.g., the immunological properties and in vitro and in vivo biological activities of EPO of human or monkey species origins. Disclosed also are chemically synthesized polypeptides sharing the biochemical and immunological properties of EPO. Also disclosed are improved methods for the detection of specific single stranded polynucleotides in a heterologous cellular or viral sample prepared from, e.g., DNA present in a plasmid or viral-borne cDNA or genomic DNA "library".

2 Claims, 27 Drawing Sheets

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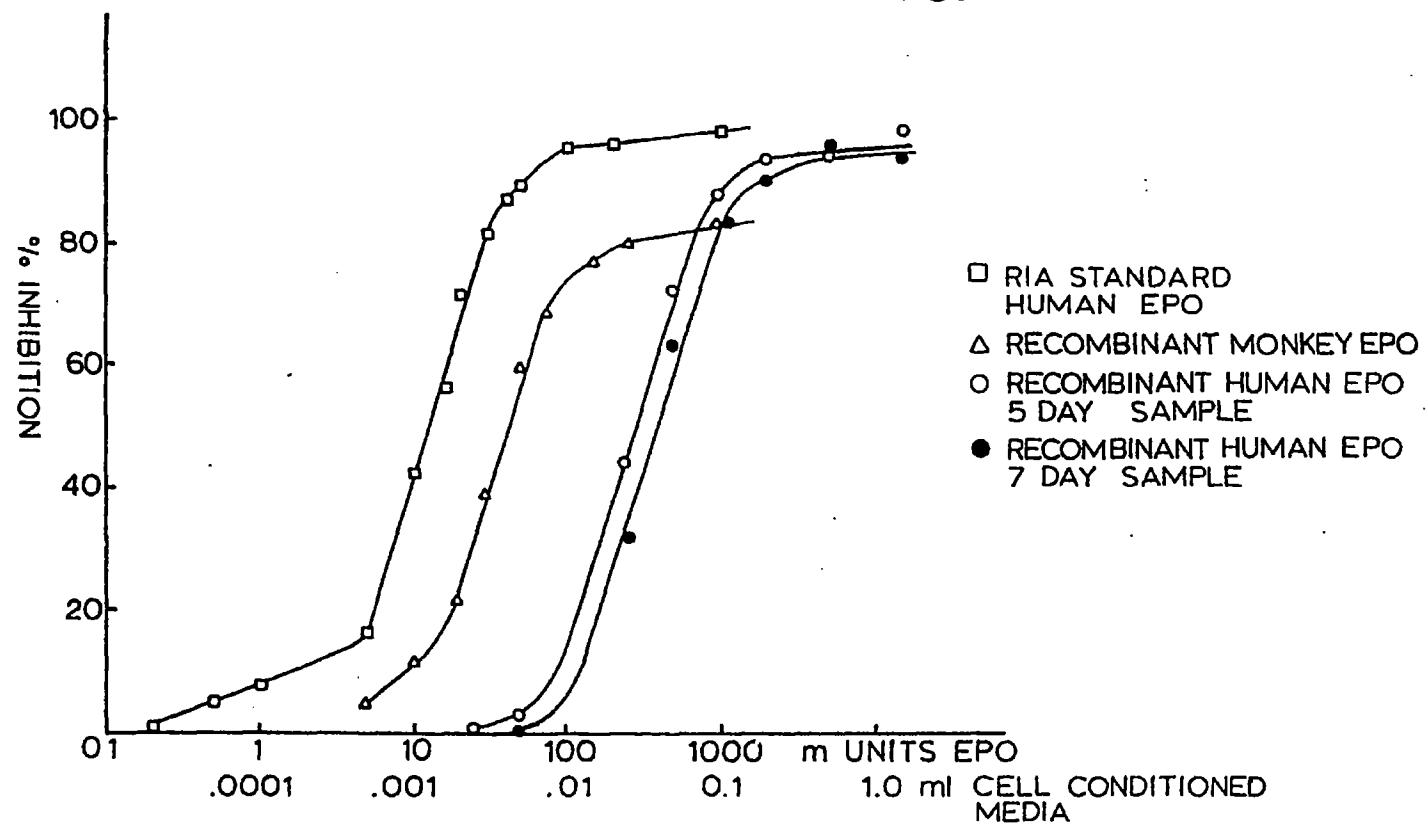
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FIG. 1



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FIG. 2

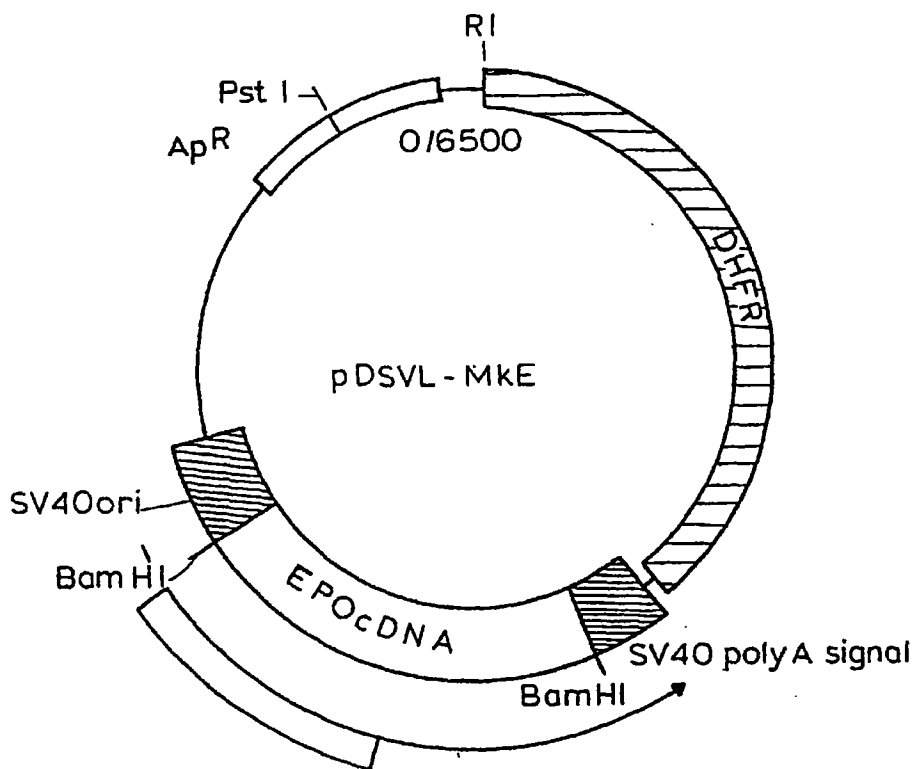
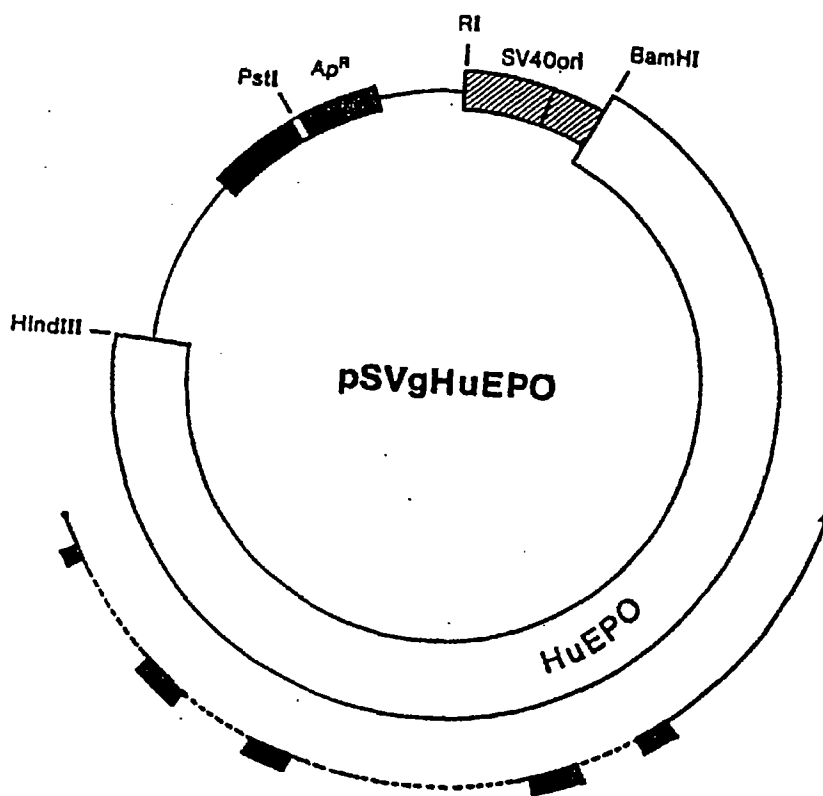


FIG. 3



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FIG. 4

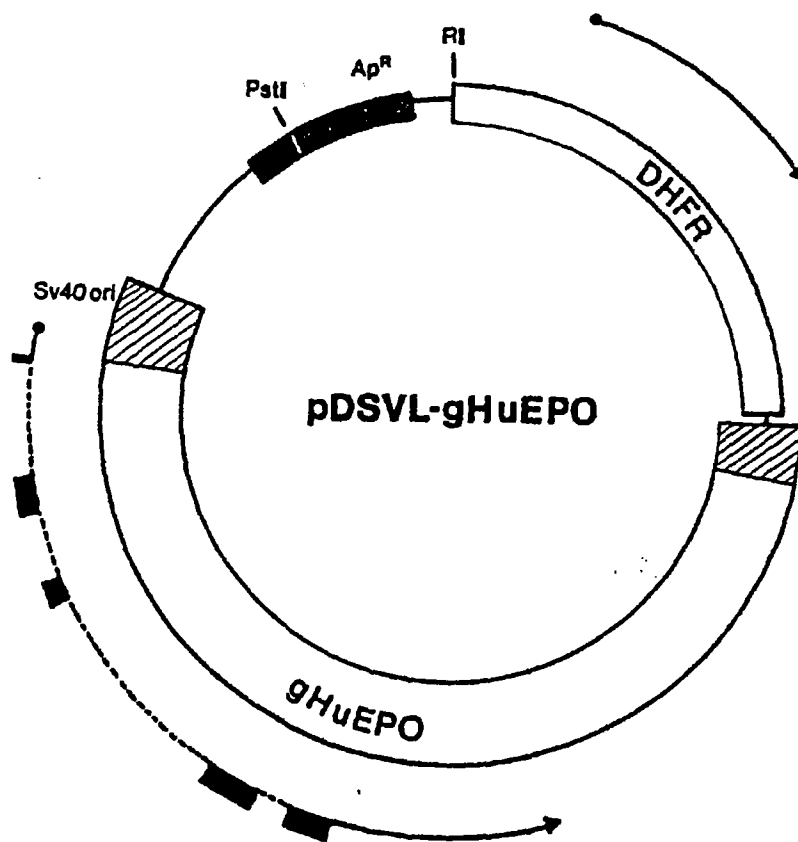


FIG. 5A

Sau3A

GATCCCGCGCCCCCTGGACAGCCGCCCTCTCCTCCAGGCCCGTGGGGCTGGCCCTGCC

CGCTGAACTTCCCGGGATGAGGACTCCCGGTGTGGTCACCGCGCGCCTAGGTCGCTGAG

-27

-20

Met Gly Val His Glu Cys Pro Ala Trp
 GGACCCCGGCCAGGCGCGGAGATG GGG GTG CAC GAA TGT CCT GCC TGG

-10

Leu Trp Leu Leu Leu Ser Leu Val Ser Leu Pro Leu Gly Leu Pro
 CTG TGG CTT CTC CTG TCT CTC GTG TCG CTC CCT CTG GGC CTC CCA

-1 +1

10

Val Pro Gly Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu
 GTC CCG GGC GCC CCA CCA CGC CTC ATC TGT GAC AGC CGA GTC CTG

20

*

Glu Arg Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn Val Thr Met
 GAG AGG TAC CTC TTG GAG GCC AAG GAG GCC GAG AAT GTC ACG ATG

30

*

40

Gly Cys Ser Glu Ser Cys Ser Leu Asn Glu Asn Ile Thr Val Pro
 GGC TGT TCC GAA AGC TGC AGC TTG AAT GAG AAT ATC ACC GTC CCA

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FIG.5B

```

                    50
Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg Met Glu Val Gly
GAC ACC AAA GTT AAC TTC TAT GCC TGG AAG AGG ATG GAG GTC GGG

                    60
Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu Leu Ser Glu
CAG CAG GCT GTA GAA GTC TGG CAG GGC CTG GCC CTG CTC TCA GAA

                    70
Ala Val Leu Arg Gly Gln Ala Val Leu Ala Asn Ser Ser Gln Pro
GCT GTC CTG CGG GGC CAG GCC GTG TTG GCC AAC TCT TCC CAG CCT

                    80
                    *
Phe Glu Pro Leu Gln Leu His Met Asp Lys Ala Ile Ser Gly Leu
TTC GAG CCC CTG CAG CTG CAC ATG GAT AAA GCC ATC AGT GGC CTT

                    90
                    100
Arg Ser Ile Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Glu Ala
CGC AGC ATC ACC ACT CTG CTT CGG GCG CTG GGA GCC CAG GAA GCC

                    110
Ile Ser Leu Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile
ATC TCC CTC CCA GAT GCG GCC TCG GCT GCT CCA CTC CGA ACC ATC

                    120
                    130
Thr Ala Asp Thr Phe Cys Lys Leu Phe Arg Val Tyr Ser Asn Phe
ACT GCT GAC ACT TTC TGC AAA CTC TTC CGA GTC TAC TCC AAT TTC
    
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FIG.6A

AAGCTTCTGGGCTTCCAGACCCAGCTACTTTGCGGAACTCAGCAACCCAGGCATCTCTGAGTCTCCGCCCA
AGACCGGGATGCCCCCAGGGGAGGTGTCCGGGAGCCCAGCCTTTCCAGATAGCACGCTCCGCCAGTCCC
AAGGGTGCGCAACCGGCTGCACTCCCCTCCCGGACCCAGGGCCCGGGAGCAGCCCCATGACCCACACGC
ACGTCTGCAGCAGCCCCGCTCACGCCCCGGCGAGCCTCAACCCAGGCGTCCTGCCCTGCTCTGACCCCGG
GTGGCCCCCTACCCCTGGCGACCCCTCACGCACACAGCCTCTCCCCACCCCAACCCGCGCACGCACACATG
CAGATAACAGCCCCGACCCCGGCCAGAGCCGXAGAGTCCCTGGGCCACCCCGGCCGCTCGCCTGCCGCTG
CGCCGCACCGCGCTGTCTCTCCCGGAGCCGGACCGGGGCCACCGCGCCXGCTCTGCTCCGACACCGCGCCC
CTTGGACAGCCGCCCTCTCTCTAGGCCCGTGGGGCTGGCCCTGCACCGCCGAGCTTCCCGGGATGAGGXX

-27 -24
Met Gly Val His
CCCGGTGACCGGCGCGCCCCAAGTCGCTGAGGGACCCCGGCCAAGCGCGGAG ATG GGG GTG CAC G
GTGAGTACTCGCGGGCTGGGCGCTCCCGGCGGCCGGGTTCTGTTTGTGAGCGGGATTTAGCGCCCCGGCT

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FIG.6B

ATTGGCCAAGAGGTGGCTGGGTTC AAGGACCGGCGACTTGTCAAGGACCCGGAAGGGGGAGGGGGGTGGG
 GCAGCCTCCACGTGCCGCGGGGACTTGGGGGAGTTCTTGGGGATGGCAAAAACCTGGCCTGTTGAGGGGCA
 CAGTTTGGGGTTGGGGAGGAGGTTTGGGGTTCTGCTGTGCAGTTGTGTCGTTGTCAAGTGTCTCG [I · S ·]
 TTGCACACGCACAGATCAATAAGCCAGAGGCAGCACCTGAGTGCTTGCATGGTTGGGACAGGAAGGACGAG
 CTGGGGCAGAGACGTGGGGATGAAGGAAGCTGTCCTTCCACAGCCACCCTTCTCCCCCCCCGCCTGACTCT

| | | | | |
|-----------------------|-----|-----|-----|-----|
| | -23 | | -20 | |
| CAGCCTGGCTATCTGTTCTAG | AA | TGT | CCT | GCC |
| | TGG | CTG | TGG | CTT |
| | CTC | CTG | TCC | CTG |

| | | | | | |
|-----|-----|-----|-----|-----|-----|
| | -10 | | -1 | +1 | |
| CTG | TCG | CTC | CCT | CTG | GGC |
| Leu | Ser | Leu | Pro | Leu | Gly |
| Leu | Ser | Leu | Pro | Val | Leu |
| Gly | Leu | Pro | Val | Leu | Gly |
| Ala | Pro | Pro | Arg | Leu | Ile |
| Cys | ATC | TGT | TGT | TGT | TGT |

| | | | | |
|-----|-----|-----|-----|-----|
| | 10 | | 20 | |
| GAC | AGC | CGA | GTC | CTG |
| Asp | Ser | Arg | Val | Leu |
| Glu | Arg | Tyr | Leu | Leu |
| Glu | Ala | Lys | Glu | Ala |
| Ala | Glu | Ala | Glu | Asn |
| Ile | AAT | ATC | ATC | ATC |

| | | |
|--------|--|---------------|
| | 26 | |
| ACG | GTGAGACCCCTTCCCCAGCACATTCCACAGAACTCACGCTCAGGGCTTCAGGGA | ACTCCTCCCAGAT |
| CCAGGA | AAGCTGACACTGCCCCCTACATAAGAATAAGTC | AAGTC |

FIG.6C

TGGTGGCCCCAAACCATACCTGAAACTAGGCAAGGAGCAAAGCCAGCAGATCCTACGCCTGTGGGCCAGGG

CCAGAGCCTTCAGGGACCCTTGACTCCCCGGGCTGTGTGCATTTACAG
27 30
Thr Gly Cys Ala Glu
ACG GGC TGT GCT GAA

* 40
His Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe Tyr
CAC TGC AGC TTG AAT GAG AAT ATC ACT GTC CCA GAC ACC AAA GTT AAT TTC TAT

50 55
Ala Trp Lys Arg Met Glu
GCC TGG AAG AGG ATG GAG GTGAGTTCCTTTTTTTTTTTTTTTTTCCTTTCTTTTGGAGAATCTCATT

TGCGAGCCTGATTTTGGATGAAAGGGAGAATGATCGGGGGAAAGGTAAAATGGAGCAGCAGAGATGAGGCT

GCCTGGGCGCAGAGGCTCACGTCTATAATCCCAGGCTGAGATGGCCGAGATGGGAGAATTGCTTGAGCCCT

GGAGTTTCAGACCAACCTAGGCAGCATAGTGAGATCCCCATCTCTACAAACATTTAAAAAATTAGTCAG

GTGAAGTGGTGCATGGTGGTAGTCCCAGATATTTGGAAGGCTGAGGCGGGAGGATCGCTTGAGCCCAGGAA

TTTGAGGCTGCAGTGAGCTGTGATCACACCACTGCACTCCAGCCTCAGTGACAGAGTGAGGCCCTGTCTCA

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FIG. 6E

130 140
 Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val Tyr Ser
 CCA CTC CGA ACA ATC ACT GCT GAC ACT TTC CGC AAA CTC TTC CGA GTC TAC TCC

150 160
 Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Thr Gly
 AAT TTC CTC CGG GGA AAG CTG AAG CTG TAC ACA GGG GAG GCC TGC AGG ACA GGG

166
 Asp Arg OP
 GAC AGA TGA CCAGGTGTGTCCACCTGGGCATATCCACCACCTCCCTCACCAACATTGCTTGTGCCACA
 CCCTCCCCGCCACTCCTGAACCCCGTCGAGGGGCTCTCAGCTCAGCGCCAGCCTGTCCCATGGACTCC
 AGTGCCAGCAATGACATCTCAGGGGCCAGAGGAACTGTCCAGAGAGCAACTCTGAGATCTAAGGATGTCAC
 AGGGCCAACTTGAAGGGCCCAGAGCAGGAAGCATTTCAGAGAGCAGCTTTAAACTCAGGGACAGAGCCATGC
 TGGGAAGACGCCTGAGCTCACTCGGCACCCTGCAAATTTGATGCCAGGACACGCTTTGGAGGCGATTTAC
 CTGTTTTTCGCACCTACCATCAGGGACAGGATGACCTGGAGAACTTAGGTGGCAAGCTGTGACTTCTCCAGG
 TCTCACGGGCATGGGCACCTCCCTTGGTGGCAAGAGCCCCCTTGACACCGGGGTGGTGGGAACCATGAAGAC
 AXGATXGGGGCTGGCCTCTGGCTCTCATGGGGTCCAAGTTTTGTGTATTCTCAACCTATTGACAGACTGAA
 ACACAATATGAC

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FIG. 7

| | | | | |
|------------|-------------|-------------|-------------|------------|
| | | | -1 | 1 |
| | <u>XbaI</u> | | MetA1a | |
| CTAG | AAACCATGAG | GGTAATAAAA | TAATGGCTCC | GCCGCGTCTG |
| | TTTGGTACTC | CCATTATTTT | ATTACCGAGG | CGGCGCAGAC |
| ATCTGCGACT | CGAGAGTTCT | GGAACGTTAC | CTGCTGGAAG | CTAAAGAAGC |
| TAGACGCTGA | GCTCTCAAGA | CCTTGCAATG | GACGACCTTC | GATTTCTTCG |
| TGAAAACATC | ACCACTGGTT | GTGCTGAACA | CTGTTCTTTG | AACGAAAACA |
| ACTTTTGTAG | TGGTGACCAA | CACGACTTGT | GACAAGAAAC | TTGCTTTTGT |
| TTACGGTACC | AGACACCAAG | GTTAACTTCT | ACGCTTGGAA | ACGTATGGAA |
| AATGCCATGG | TCTGTGGTTC | CAATTGAAGA | TGCGAACCTT | TGCATACCTT |
| GTGCGTCAAC | AAGCAGTTGA | AGTTTGGCAG | GGTCTGGCAC | TGCTGAGCGA |
| CAACCAGTTG | TTCGTCAACT | TCAAACCGTC | CCAGACCGTG | ACGACTCGCT |
| GGCTGTACTG | CGTGGCCAGG | CACCTGCTGGT | AAACTCCTCT | CAGCCGTGGG |
| CCGACATGAC | GCACCGGTCC | GTGACGACCA | TTTGAGGAGA | GTCGGCACCC |
| AACCGCTGCA | GCTGCATGTT | GACAAAGCAG | TATCTGGCCT | GAGATCTCTG |
| TTGGCGACGT | CGACGTACAA | CTGTTTCGTC | ATAGACCGGA | CTCTAGAGAC |
| ACTACTCTGC | TGCGTGCTCT | GGGTGCACAG | AAAGAGGCTA | TCTCTCCGCC |
| TGATGAGACG | ACGCACGAGA | CCCACGTGTC | TTTCTCCGAT | AGAGAGGCCG |
| GGATGCTGCA | TCTGCTGCAC | CGCTGCGTAC | CATCACTGCT | GATACCTTCC |
| CCTACGACGT | AGACGACGTG | GCGACGCATG | GTAGTGACGA | CTATGGAAGG |
| GCAAACGTGT | TCGTGTATAC | TCTAACCTCC | TGCGTGGTAA | ACTGAAACTG |
| CGTTTGACAA | AGCACATATG | AGATTGAAGG | ACGCACCATT | TGACTTTGAC |
| | | | <u>SalI</u> | |
| TATACTGGCG | AAGCATGCCG | TACTGGTGAC | CGCTAATAG | |
| ATATGACCGC | TTCGTACGGC | ATGACCACTG | GCGATTATCA | GCT |

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FIG. 8

| <u>HindIII</u> | -1 +1 | <u>ArgAla</u> | | | |
|----------------|------------|---------------|------------|------------|--|
| AGCTTGGATA | AAAGAGCTCC | ACCAAGATTG | ATCTGTGACT | CGAGAGTTTT | |
| ACCTAT | TTTCTCGAGG | TGGTTCTAAC | TAGACACTGA | GCTCTCAAAA | |
| GGAAAGATAC | TTGTTGGAAG | CTAAAGAAGC | TGAAAACATC | ACCACTGGTT | |
| CCTTTCTATG | AACAACCTTC | GATTTCTTCG | ACTTTTGTAG | TGGTGACCAA | |
| GTGCTGAACA | CTGTTCTTTG | AACGAAAACA | TTACGGTACC | AGACACCAAG | |
| CACGACTTGT | GACAAGAAAC | TTGCTTTTGT | AATGCCATGG | TCTGTGGTTC | |
| GTAACTTCT | ACGCTTGGA | ACGTATGGAA | GTTGGTCAAC | AAGCTGTTGA | |
| CAATTGAAGA | TGCGAACCTT | TGCATACCTT | CAACCAGTTG | TTGCACAAC | |
| AGTTTGGCAA | GGTTTGGCCT | TGTTATCTGA | AGCTGTTTTG | AGAGGTCAAG | |
| TCAAACCGTT | CCAAACCGGA | ACAATAGACT | TCGACAAAAC | TCTCCAGTTC | |
| CCTTGTTGGT | TAACCTTCT | CAACCATGGG | AACCATTGCA | ATTGCACGTC | |
| GGAACAACCA | ATTGAGAAGA | GTTGGTACCC | TTGGTAACGT | TAACGTGCAG | |
| GATAAAGCCG | TCTCTGGTTT | GAGATCTTTG | ACTACTTTGT | TGAGAGCTTT | |
| CTATTTCCGC | AGAGACCAA | CTCTAGAAAC | TGATGAAACA | ACTCTCGAAA | |
| GGGTGCTCAA | AAGGAAGCCA | TTTCCCCACC | AGACGCTGCT | TCTGCCGCTC | |
| CCCACGAGTT | TTCCTTCGGT | AAAGGGGTGG | TCTGCGACGA | AGACGGCGAG | |
| CATTGAGAAC | CATCACTGCT | GATACCTTCA | GAAAGTTATT | CAGAGTTTAC | |
| GTAACCTTTG | GTAGTGACGA | CTATGGAAGT | CTTCAATAA | GTCTCAAATG | |
| TCCAACCTTCT | TGAGAGGTAA | ATTGAAGTTG | TACACCGGTG | AAGCCTGTAG | |
| AGGTTGAAGA | ACTCTCCATT | TAACTTCAAC | ATGTGGCCAC | TTCCGACATC | |
| AAGTGGTGAC | AGATAAGCCC | GACTGATAAC | AACAGTGTAG | | |
| TTGACCACTG | TCTATTCGGG | CTGACTATTG | TTGTCACATC | | |

SalI

ATGTAACAAA G
TACATTGTTT CAGCT

FIG. 9

| | | | | | | | |
|--------|--|-----|-----|-----|-----|-----|-----|
| | -20 | -10 | +1 | 10 | 20 | 30 | 40 |
| Human | MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPDTK | | | | | | |
| | ***** | | | | | | |
| Monkey | MGVHECPAWLWLLLSLVSLPLGLPVPGAPPRLICDSRVLERYLLEAKEAENVTMGCSESCSLNENITVPDTK | | | | | | |
| | | | | | | | |
| | 50 | 60 | 70 | 80 | 90 | 100 | 110 |
| Human | VNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTLRALGAQKE | | | | | | |
| | ***** | | | | | | |
| Monkey | VNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQAVLANSSQPFEPLQLHMDKAISGLRSITTLRALGAQ-E | | | | | | |
| | | | | | | | |
| | 120 | 130 | 140 | 150 | 160 | | |
| Human | AISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKCLKLYTGEACRTGDR | | | | | | |
| | *** | | | | | | |
| Monkey | AISLPDAASAAPLRTITADTFCKLFRVYSNFLRGKCLKLYTGEACRRGDR | | | | | | |

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FIG. 10

1. AATTCTAGAAACCATGAGGGTAATAAAATA
2. CCATTATTTTATTACCCTCATGGTTTCTAG
3. ATGGCTCCGCCGCGTCTGATCTGCGAC
4. CTCGAGTCGCAGATCAGACGCGGCGGAG
5. TCGAGAGTTCTGGAACGTTACCTGCTG
6. CTTCCAGCAGGTAACGTTCCAGAACT
7. GAAGCTAAAGAAGCTGAAAACATC
8. GTGGTGATGTTTTTCAGCTTCTTTAG
9. ACCACTGGTTGTGCTGAACACTGTTC
10. CAAAGAACAGTGTTTCAGCACAACCA
11. TTTGAACGAAAACATTACGGTACCG
12. GATCCGGTACCGTAATGTTTTTCGTT

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FIG. 11

XbaI

EcoRI 1 3

AATTCTAG AAACCATGAG GGTAATAAAA TAATGGCTCC GCCGCGTCTG
 GATC TTTGGTACTC CCATTATTTT ATTACCGAGG CGGCGCAGAC

2 4

5

ATCTGCGACT CGAGAGTCT GGAACGTTAC CTGCTGGAAG CTAAAGAAGC
 TAGACGCTGA GCTCTCAAGA CCTTGCAATG GACGACCTTC GATTTCCTCG

6

7 9 11

TGAAAACATC ACCACTGGTT GTGCTGAACA CTGTTCTTIG AACGAAAAACA
 ACTTTTGTAG TGGTGACCAA CACGACTTGT GACAAGAAAC TTGCTTTTGT

8 10

0

KpnI BamHI

TTACGGTACC G
 AATGCCATGG CCTAG

12

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FIG. 12

1. AATTCGGTACCAGACACCAAGGT
2. GTTAACCTTGGTGTCTGGTACCG
3. TAACTTCTACGCTTGGAAACGTAT
4. TTCCATACGTTTCCAAGCGTAGAA
5. GGAAGTTGGTCAACAAGCAGTTGAAGT
6. CCAAACCTTCAACTGCTTGTGACCAAC
7. TTGGCAGGGTCTGGCACTGCTGAGCG
8. GCCTCGCTCAGCAGTGCCAGACCCTG
9. AGGCTGTACTGCGTGGCCAGGCA
10. GCAGTGCCTGGCCACGCAGTACA
11. CTGCTGGTAAACTCCTCTCAGCCGT
12. TTCCCACGGCTGAGAGGAGTTTACCA
13. GGAACCGCTGCAGCTGCATGTTGAC
14. GCTTTGTCAACATGCAGCTGCAGCGG
15. AAAGCAGTATCTGGCCTGAGATCTG
16. GATCCAGATCTCAGGCCAGATACT

FIG. 13

EcoRI KpnI
 A ¹ATTTCGGTACC AGACACCAAG ³GTTAACTTCT ACGCTTGGAA ACGTATGGAA
 GCCATGG TCTGTGGTTC CAATTGAAGA TCGGAACCTT TGCATACCTT
₂ ₄

⁵GTTGGTCAAC AAGCAGTTGA AGTTTGGCAG ⁷GGTCTGGCAC TGCTGAGCGA
 CAACCAGTTG TTCGTCAACT TCAAACCGTC CCAGACCGTG ACGACTCGCT
₆ ₈

⁹GGCTGTACTG CGTGGCCAGG CACTGCTGGT ¹¹AAACTCCTCT CAGCCGTGGG
 CCGACATGAC GCACCGGTCC GTGACGACCA TTTGAGGAGA GTCGGCACCC
₁₀ ₁₂

¹³AACCGCTGCA GCTGCATGTT GACAAAGCAG ¹⁵TATCTGGCCT GAGATCTG BglIII BamHI
 TTGGCGACGT CGACGTACAA CTGTTTCGTC ATAGACCGGA CTCTAGACCTAC
₁₄ ₁₆

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FIG. 14

1. GATCCAGATCTCTGACTACTCTGC
2. ACGCAGCAGAGTAGTCAGAGATCTG
3. TCGGTGCTCTGGGTGCACAGAAAGAGG
4. GATAGCCTCTTTCTGTGCACCCAGAGC
5. CTATCTCTCCGCCGGATGCTGCATCT
6. CAGCAGATGCAGCATCCGGCGGAGA
7. GCTGCACCGCTGCGTACCATCACTG
8. ATCAGCAGTGATGGTACGCAGCGGTG
9. CTGATACCTTCCGCAAACCTGTTTCG
10. ATACACGAAACAGTTTGCGGAAGGT
11. TGTATACTCTAACTTCCTGCGTGGTA
12. CAGTTTACCACGCAGGAAGTTAGAGT
13. AACTGAAACTGTATACTGGCGAAGC
14. GGCATGCTTCGCCAGTATACAGTTT
15. ATGCCGTACTGGTGACCGCTAATAG
16. TCGACTATTAGCGGTCACCAGTAC

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FIG. 15

BamHI BglII
GA TCCAGATCTCTG
GTCTAGAGAC

1 ACTACTCTGC TGCGTGCTCT 3 GGGTGCACAG AAAGAGGCTA 5 TCTCTCCGCC
TGATGAGACG 2 ACGCACGAGA CCCACGTGTC 4 TTTCTCCGAT AGAGAGGCCG

GGATGCTGCA 7 TCTGCTGCAC CGCTGCGTAC CATCACTGCT 9 GATACCTTCC
CCTACGACGT 6 AGACGACGTG 8 GCGACGCATG GTAGTGACGA CTATGGAAGG

GCAAACGTGT 11 TCGTGTATAC TCTAACTTCC TCGTGTTAA 13 ACTGAAACTG
CGTTTGACAA 10 AGCACATATG 12 AGATTGAAGG ACGCACCATT TGACTTTGAC

TATACTGGCG 15 AAGCATGCCG TACTGGTGAC CGCTAATAG SalI
ATATGACCGC 14 TTCGTACGGC ATGACCACTG GCGATTATC 16 AGCT

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FIG. 16

1. AATCAAGCTTGGATAAAAGAGCT
2. GTGGAGCTCTTTTATCCAAGCTTG
3. CCACCAAGATTGATCTGTGACTC
4. TCTCGAGTCACAGATCAATCTTG
5. GAGAGTTTTGGAAAGATACTTGTTG
6. CTTCCAACAAGTATCTTTCCAAAC
7. GAAGCTAAAGAAGCTGAAAACATC
8. GTGGTGATGTTTTTCAGCTTCTTTAG
9. ACCACTGGTTGTGCTGAACACTGTTC
10. CAAAGAACAGTGTTTCAGCACAACCA
11. TTTGAACGAAAACATTACGGTACCG
12. GATCCGGTACCGTAATGTTTTTCGTT

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FIG. 17

EcoRI HindIII 1
AATTCA AGCTTGGATA
GT TCGAACCTAT
2

3
AAAGAGCTCC ACCAAGATTG ATCTGTGACT CGAGAGTTTT
TTTCTCGAGG TGGTTCTAAC TAGACACTGA GCTCTCAAAA
4

5 7
GGAAAGATAC TTGTTGGAAG CTAAAGAAGC TGAAAACATC ACCACTGGTT
CCTTTCTATG AACAACTTC GATTTCTTCG ACTTTTGTAG TGGTGACCAA
6 8

9 11 KpnI BamHI
GTGCTGAACA CTGTTCTTTG AACGAAAACA TTACGGTACC G
CACGACTTGT GACAAGAAAC TTGCTTTTGT AATGCCATGG CCTAG
12

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FIG. 18

1. AATTCGGTACCAGACACCAAGGT
2. GTTAACCTTGGTGTCTGGTACCG
3. TAACTTCTACGCTTGGAAACGTAT
4. TTCCATACGTTTCCAAGCGTAGAA
5. GGAAGTTGGTCAACAAGCAGTTGAAGT
6. CCAAACCTTCAACTGCTTGTTGACCAAC
7. TTGGCAAGGTTTGGCCTTGTTATCTG
8. GCTTCAGATAACAAGGCCAAACCTTG
9. AAGCTGTTTTGAGAGGTGAAGCCT
10. AACAAAGGCTTGACCTCTCAAACA
11. TGTTGGTTAACTCTTCTCAACCATGGG
12. TGGTTCCCATGGTTGAGAAGAGTTAACC
13. AACCATTGCAATTGCACGTCGAT
14. CTTTATCGACGTGCAATTGCAA
15. AAAGCCGTCTCTGGTTTGAGATCTG
16. GATCCAGATCTCAAACCAGAGACGG

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FIG. 19

KpnI

EcoRI 1
A ATTCGGTACC AGACACCAAG
GCCATGG TCTGTGGTTC
2

3 5
GTTAACTTCT ACGCTTGGAA ACGTATGGAA GTTGGTCAAC AAGCTGTTGA
CAATTGAAGA TCGGAACCTT TGCATACCTT CAACCAGTTG TTCGACAACT
4 6

7 9
AGTTGGCAA GGGTTGGCCT TGTATCTGA AGCTGTTTGG AGAGGTCAAG
TCAAACCGTT CCAAACCGGA ACAATAGACT TCGACAAAAC TCTCCAGTTC
8 10

11 13
CCTTGTGGT TAACTCTTCT CAACCATGGG AACCATTGCA ATTGCACGTC
GGAACAACCA ATTGAGAAGA GTTGGTACCC TTGGTAACGT TAACGTGCAG
12 14

15 BglII BamHI
GATAAAGCCG TCTCTGGTTT GAGATCTG
CTATTTCCGC AGAGACCAA CTCTAGACCTA G
16

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FIG. 20

1. GATCCAGATCTTTGACTACTTTGTT
2. TCTCAACAAAGTAGTCAAAGATCTG
3. GAGAGCTTTGGGTGCTCAAAAGGAAG
4. ATGGCTTCCTTTTGAGCACCCAAAGC
5. CCATTTCCCCACCAGACGCTGCTT
6. GCAGAAGCAGCGTCTGGTGGGGAA
7. CTGCCGCTCCATTGAGAACCATC
8. CAGTGATGGTTCTCAATGGAGCG
9. ACTGCTGATACCTTCAGAAAGTT
10. GAATAACTTTCTGAAGGTATCAG
11. ATTCAGAGTTTACTCCAATTCT
12. CTCAGAAGTTGGAGTAAACTCT
13. TGAGAGGTAAATTGAAGTTGTACAC
14. ACCGGTGTACAACTTCAATTTACCT
15. CGGTGAAGCCTGTAGAACTGGT
16. CTGTCACCAGTTCTACAGGCTTC
17. GACAGATAAGCCCGACTGATAA
18. GTTGTATCAGTCGGGCTTAT
19. CAACAGTGTAGATGTAACAAAG
20. TCGACTTTGTTACATCTACACT

FIG. 21

BamHI BglII 1
 GATC CAGATCTTTG ACTACTTTGT TGAGAGCTTT
 GTCTAGAAAC TGATGAAACA ACTCTCGAAA
2

3 GGGTGCTCAA AAGGAAGCCA 5 TTTCCCACC AGACGCTGCT TCTGCCGCTC
 CCCACGAGTT TTCCTTCGGT AAAGGGGTGG TCTGCGACGA AGACGGCGAG
4 6

7 CATTGAGAAC CATCACTGCT 9 GATACCTTCA GAAAGTTATT 11 CAGAGTTTAC
 GTAACTCTTG GTAGTGACGA CTATGGAAGT CTTTCAATAA GTCTCAAATG
8 10 12

TCCAACCTTCT 13 TGAGAGGTAA ATTGAAGTTG TACACCGGTG 15 AAGCCTGTAG
 AGGTTGAAGA ACTCTCCATT TAACTTCAAC ATGTGGCCAC TTCGGACATC
14 16

17 AACTGGTGAC AGATAAGCCC GACTGATAAC 19 AACAGTGTAG
 TTGACCACTG TCTATTCGGG CTGACTATTG TTGTCACATC

SalI
 ATGTAACAAA G
 TACATTGTTT CAGCT
20