

United States Patent [19]

[11] **Patent Number:** **5,618,698**

Lin

[45] **Date of Patent:** ***Apr. 8, 1997**

[54] **PRODUCTION OF ERYTHROPOIETIN**
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 [*] Notice: The portion of the term of this patent subsequent to Aug. 15, 2012, has been disclaimed.
 [21] Appl. No.: **468,381**
 [22] Filed: **Jun. 6, 1995**

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Related U.S. Application Data

[63] Continuation of Ser. No. 113,179, Oct. 23, 1987, Pat. No. 5,441,868, which is a continuation of Ser. No. 675,298, Nov. 30, 1984, Pat. No. 4,703,008, which is a continuation-in-part of Ser. No. 655,841, Sep. 28, 1984, which is a continuation-in-part of Ser. No. 582,185, Feb. 21, 1984, abandoned, which is a continuation-in-part of Ser. No. 561,024, Dec. 13, 1983.
 [51] **Int. Cl.⁶** **C12P 71/02; C12N 15/27**
 [52] **U.S. Cl.** **435/69.4; 435/69.6; 435/325; 536/23.51**
 [58] **Field of Search** **435/240.2, 177.3, 435/320.1, 69.4, 240.1, 69.1, 69.6; 536/23.5, 23.51**

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Primary Examiner—James Martinell
Attorney, Agent, or Firm—Marshall, O'Toole, Gerstein, Murray & Borun

[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 end 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".

9 Claims, 27 Drawing Sheets

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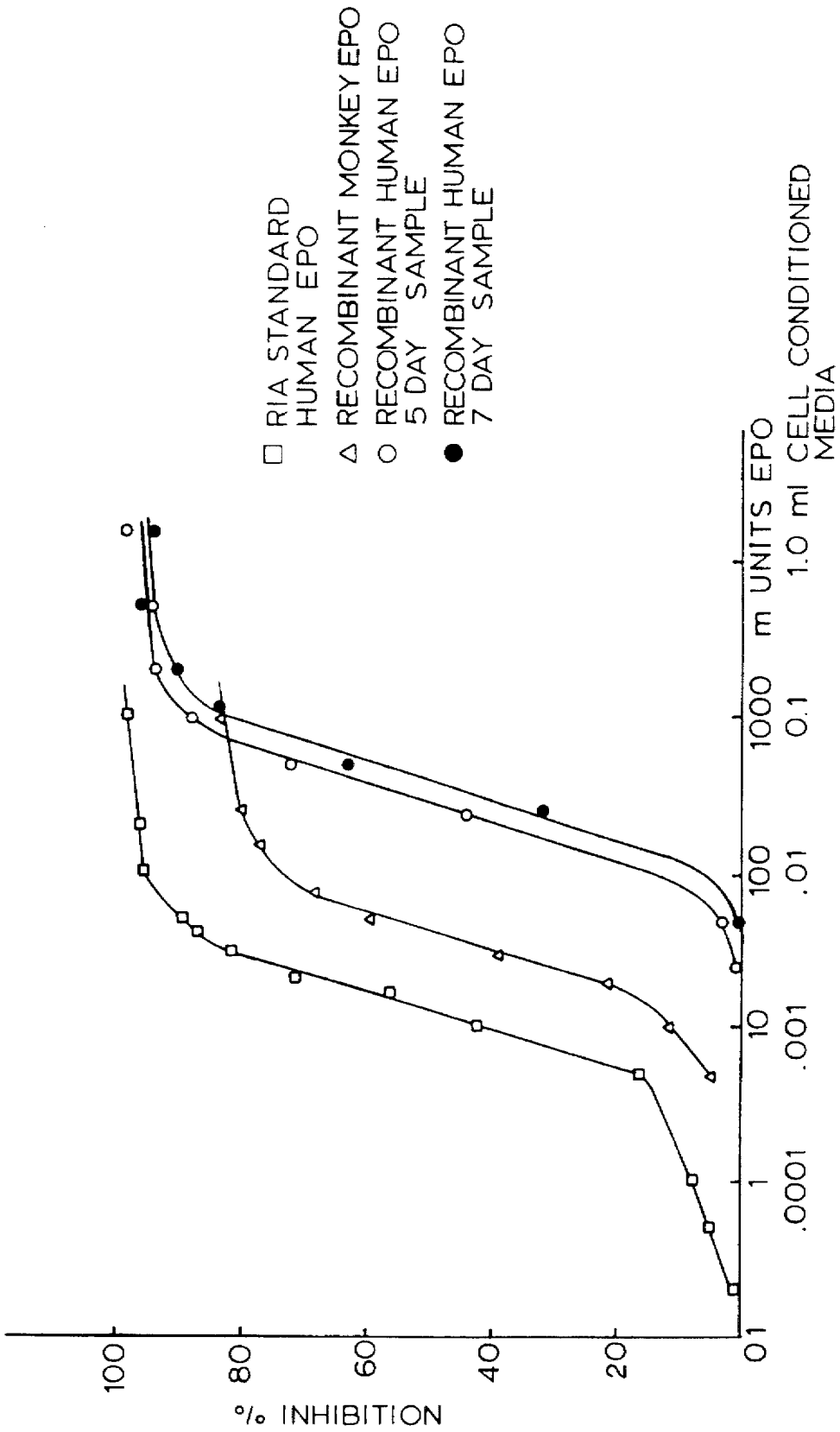
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FIG. 1



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FIG. 2

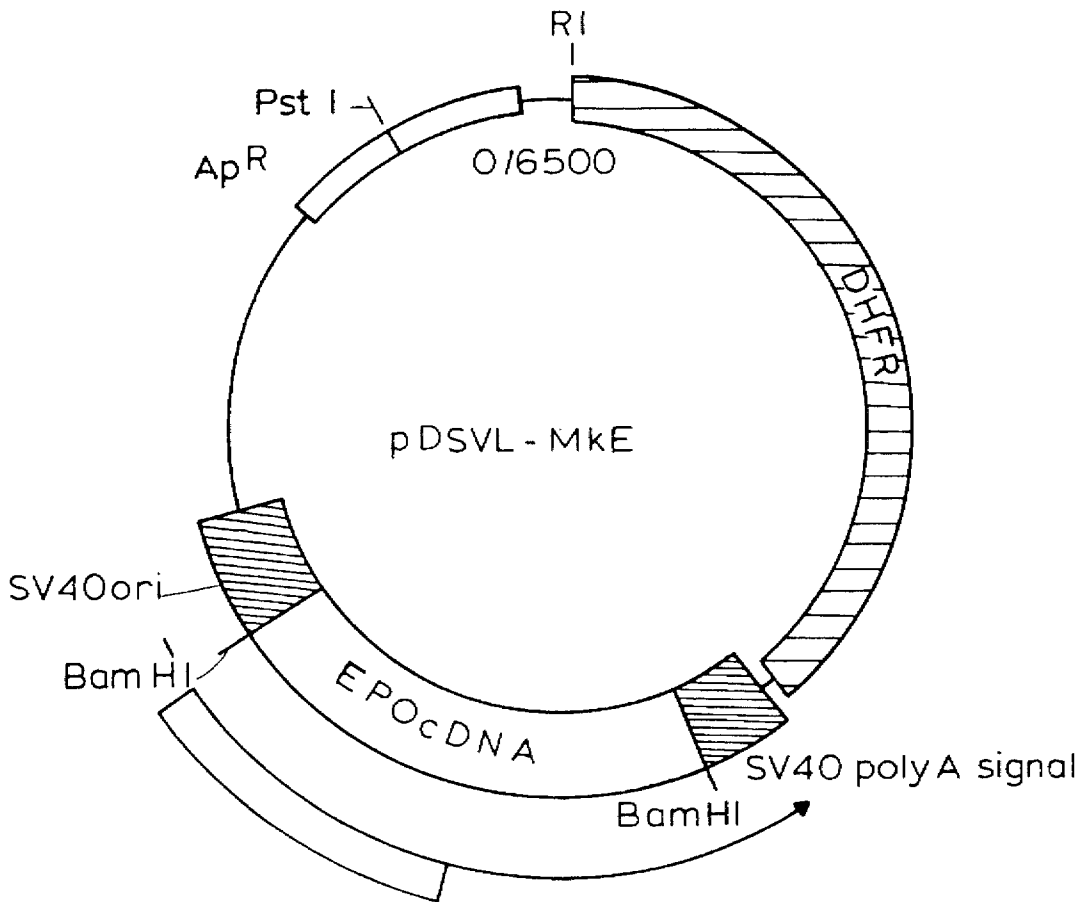


FIG. 3

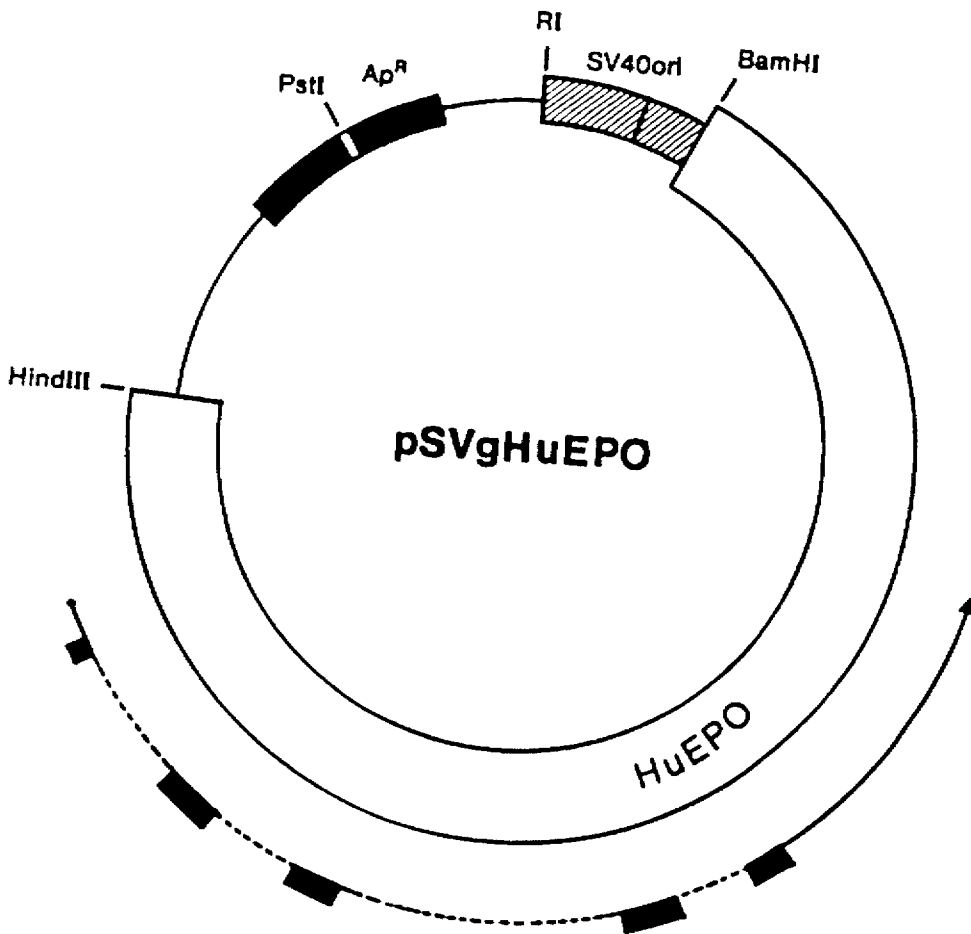


FIG. 4

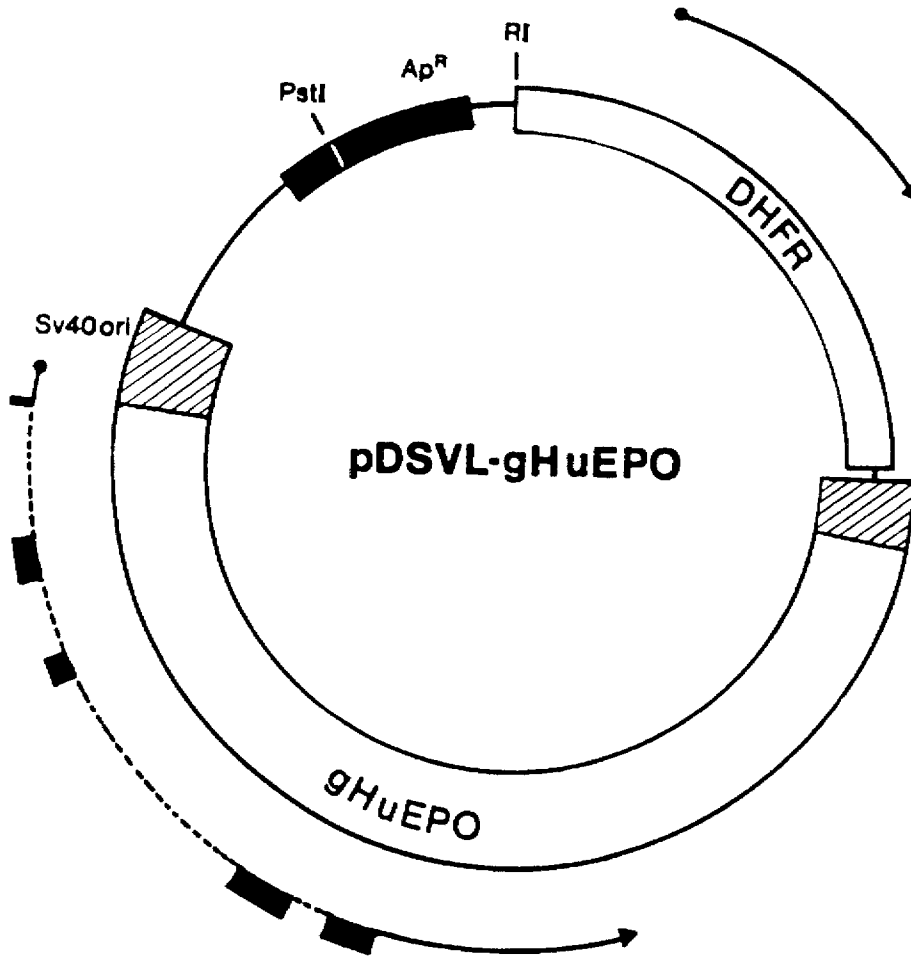


FIG. 5A

Sau3A
 GATCCGGGCCCTGTGACAGCCGCCCTCTCCTCCAGGCCCGTGGGGCTGGCCCTGCC
 CGCTGAACITCCCGGATGAGGACTCCCGGTGTGGTCAACCGCGCCTAGGTCGCTGAG

-27

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

-20

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

-10

Val Pro Gly Ala 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

+1

10

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

20

*

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

30

*

40

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FIG.5B

50 Asp Thr Lys Val 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 GAG GTC GGG
 60 Gln Gln Ala Val Glu Val Trp Gln Gln Gly GGC GGC CTG GCC CTG TCA GAA
 CAG CAG GCT GTA GAA GTC TGG CAG CAG GGC CTG GCC CTG TCC TCA GAA
 70 Ala Val Leu Arg Gly Gln Ala Val Leu Ala Asn Ser Ser Gln Pro
 GCT GTC CTG CCG 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 Arg Ser Ile Thr Thr Leu Leu Thr Leu Leu Arg Ala Leu Gly Ala Gln Glu Ala
 CGC AGC ATC ACC ACT ACT CTG CTT CGG GCG 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 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
 130
 140

FIG.6A

AAGCTTCTGGGCTTCCAGACCCAGCTACTTTGGGAACTCAGCAACCAGGCATCTCTGAGTCTCGGCCCA
AGACCGGATGCCCCAGGGAGGTGTCCGGGAGCCAGCCTTTCCAGATAGCACGCTCCGGCCAGTCCC
AAGGTGGCAACCGGCTGCACTCCCTCCCGACCCAGGGCCGGAGCAGCCCCCATGACCCACACGC
ACGTCTGCAGACCCCGCTCACGCCCGGAGCCTCAACCAGGGTCTGCCCCCTGCTCTGACCCCGG
GTGGCCCTACCCCTGGCGACCCCTCACGCACACAGCCTCTCCCCACCCCGCCGACGACACATG
CAGATAACAGCCCGACCCCGGCAGAGCCGXAGAGTCCCTGGGCCACCCCGGCCGCTCGCCGCTG
CGCCGACCGGCTGTCTCCGGAGCCGGACCGGGCCACCGGCCXGCTGTCTCGACACCGGCC
CTTGGACAGCCCTCTCTCTAGGCCCGTGGGGCTGGCCCTGCACCCCGAGCTTCCCAGGATGAGGXX
-27 -24
Met Gly Val His
ATG GGG GTG CAC G
GTGAGTACTCGCGGGCTGGCGCTCCCGGGCCGGTTCCGTGTTGAGCGGGATTAGGCCCCCGGCT

FIG.6B

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ATTGGCCAAGAGGTGGCTGGGTTCAAGGACCGGGACTTGTCAAGGACCCCGGAAGGGGAGGGGGTGGG
GCAGCCTCCACGTGCCCGGGGACTTGGGGAGTCTTGGGGATGGCAAAACCTGGCCCTGTTGAGGGGCA
CAGTTGGGGTTGGGAGGAGGTTTGGGGTCTGTGTCAGTTGTGTCGTTGTGTCAGTGTCTCG [ I · S · ]
TTGCCACACGACAGATCAATAAGCCAGAGGCACCTGAGTGTGTCATGGTTGGGACAGGAAGGACGAG
CTGGGCAGAGACGTGGGGATGAAGGAGCTGTCTTCCACAGCCACCCTTCTCCCCCGCCCTGACTCT
-23          -20
Glu Cys Pro Ala Trp Leu Trp Leu Trp Leu Leu Ser Leu
CAGCCTGGCTATCTGTTCTAG  AA TGT CCT GCC TGG CTG TGG CTT CTC CTG TCC CTG
-10          -1          +1
Leu Ser Arg Val Leu Gly Leu Pro Val Leu Gly Ala Pro Pro Arg Leu Ile Cys
CTG TCG CTC CCT CTG GGC CTC CCA GTC CTC CCA CCA CCA CCA CGC CTC ATC TGT
10          20          *
Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn Ile
GAC AGC CGA GTC CTG GAG AGG TAC CTC TTG GAG GCC AAG GAG GCC GAG AAT ATC
26
Thr
ACG GTGAGACCCCTTCCCAGCACATTCCACAGAACTCACGCTCAGGGCTTCAGGGAACCTCCTCCAGAT
CCAGGAACCTGGCAGCTTGGTTTGGGGTGGAGTTGGGAAGCTAGACACTGCCCCCCCTACATAAGAAATAGTC
    
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FIG.6C

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TGGTGGCCCAACCATACCTGAACACTAGGCAAGGAGCAAAAGCCAGCAGATCCTACGCCCTGTGGGCCAGGG
                27          30
                Thr Gly Cys Ala Glu
CCAGAGCCTTCAGGGACCCCTTGACTCCCGGGCTGTGTGCATTTTCAG   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 ACT GTC CCA GAC ACC AAA GTT AAT TTC TAT
50
Ala Trp Lys Arg Met Glu
GCC TGG AAG AGG ATG GAG GTGAGTCCCTTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT TTTT
TCCGAGCCTGATTTTGGATGAAAGGGAGAAATGATCGGGGAAAGGTAAATGGAGCAGCAGAGATGAGGCT
GCC TGG CCG CAG AGG C TCA TATAATCC CAGGCTGAGATGGCCGAGATGGGAGAAATGCTT GAGCCCT
GGAGTTTCAGACCAACCTAGGCAGCATAGTGAGATCCCCCATCTCTACAACAATTTAAAAAATTAGTCAG
GTGAAGTGGTGCATGGTGGTAGTCCCAGATATTTGGAAGGCTGAGCGGGAGGATCGCTTGAGCCCGAGAA
TTTGAGGCTGCAGTGAGCTGTGATCACACCACCTGCACCTCCAGCCCTCAGTGACAGAGTGAGGCCCTGTCTCA

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FIG. 6D

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AAAAAGAAAAGAAAAGAAAATAATGAGGGCTGTATGGAATACATTCAATTATTCACTCACTCACT
CACTCACTCACTCACTCACTCACTCACTCACTCACTCACTCACTCACTCACTCACTCACTCACTCACT
GGCTGCTGAGGGCAGGAGGGAGAGGGTGACATGGGTCAGCTCGACTCCAGAGTCCACTCCCTGTAG
56          60          70
Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu Leu Ser Glu Ala
GTC GGG CAG CAG GCC GTA GAA GTC TGG CAG GGC CAG CTG GCC CTG TCG GAA GCT
Val Leu Arg Gly Gln Ala Leu Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu
GTC CTG CGG GGC CAG GCC CTG TGG GTC AAC TCT TCC CAG CCG TGG GAG CCC CTG
80          *          90
Gln Leu His Val Asp Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu
CAG CTG CAT GTG GAT AAA GCC GTC AGT GGC CTT CGC AGC CTC ACC ACT CTG CTT
110          115
Arg Ala Leu Gly Ala Gln
CGG GCT CTG GGA GCC CAG GTGAGTAGGAGCGGACACTTCTGCTTGCCCTTCTGTAAGAAGGGGA
GAAGGGTCTTGCTAAGGAGTACAGGAACGTCCGTATTCCTTCCCTTCTGTGGCACTGCAGGACCCTCCT
116          120
Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala
GTTTTCTCCTTGGCAG AAG GAA GCC ATC TCC CCT CCA GAT GCG GCC TCA GCT GCT

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FIG. 6E

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130 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
140
150 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
160
166 ASP ARG OP
GAC AGA TGA CCAGGTGTGCCACCTGGGCATATCCACCACCTCCCTCACCACAATTGCTTGCCACA
CCCTCCCCCGCCACTCCTGAACCCCGTCGAGGGGCTCTCAGCTCAGCGCCAGCCCTGTCCCATGGACACTCC
AGTGCCAGCAATGACATCTCAGGGGCCAGAGGAACTGTCCAGAGAGCAACTCTGAGATCTAAGGATGTCAC
AGGGCCAACTTGAAGGCCCAGAGCAGGAAGCAATTCAGAGAGCAGCTTTAAACTCAGGGACAGAGCCATGC
TGGGAAGACGCCCTGAGCTCACTCGGCACCCCTGC AAAAATTGATGCCAGGACACGCTTTGGAGCGGATTTAC
CTGTTTTCGCACCTACCATCAGGGACAGGATGACCTGGAGAACTTAGGTGGCAAGCTGTGACTTCTCCAGG
TCTCACGGGCATGGGCAC TCCCCTGGTGGCAAGAGCCCCCTTGACACCCGGGTGGTGGAAACCATGAAGAC
AXGATXGGGGCTGGCCCTCTGGCTCTCATGGGGTCCAAAGTTTGTGTATTCTCAACCTATTGACAGACTGAA
ACACAATATGAC
    
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FIG. 7

			-1	1
	<u>XbaI</u>		MetAla	
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	GTAACTTCT	ACGCTTGGAA	ACGTATGGAA
AATGCCATGG	TCTGTGGTTC	CAATTGAAGA	TGCGAACCTT	TGCATACCTT
GTTGGTCAAC	AAGCAGTTGA	AGTTTGGCAG	GGTCTGGCAC	TGCTGAGCGA
CAACCAGTTG	TTCGTCAACT	TCAAACCGTC	CCAGACCGTG	ACGACTCGCT
GGCTGTACTG	CGTGGCCAGG	CACTGCTGGT	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	AGAGAGGCGG
GGATGCTGCA	TCTGCTGCAC	CGCTGCGTAC	CATCACTGCT	GATACCTTCC
CCTACGACGT	AGACGACGTG	GCGACGCATG	GTAGTGACGA	CTATGGAAGG
GCAAACCTGTT	TCGTGTATAC	TCTAACTTCC	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

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                -1 +1
HindIII      ArgAla
AGCTTGGATA AAAGAGCTCC ACCAAGATTG ATCTGTGACT CGAGAGTTTT
      ACCTAT TTTCTCGAGG TGGTTCCTAAC TAGACTGA GCTCTCAAAA

GGAAAGATAC TTGTTGGAAG CTAAAGAAGC TGAAAACATC ACCACTGGTT
CCTTTCTATG AACACCTTC GATTTCTTCG ACTTTTGTAG TGGTGACCAA

GTGCTGAACA CTGTTCTTTG AACGAAAACA TTACGGTACC AGACACCAAG
CACGACTTGT GACAAGAAAC TTGCTTTTGT AATGCCATGG TCTGTGGTTC

GTAACTTCT ACGCTTGGAA ACGTATGGAA GTTGGTCAAC AAGCTGTTGA
CAATTGAAGA TGCGAACCTT TGCATACCTT CAACCAGTTG TTCGACAACT

AGTTTGGCAA GTTTGGCCT TGTTATCTGA AGCTGTTTTG AGAGGTCAAG
TCAAACCGTT CCAAACCGGA ACAATAGACT TCGACAAAAC TCTCCAGTTC

CCTTGTTGGT TAACTCTTCT CAACCATGGG AACCATTGCA ATTGCACGTC
GGAACAACCA ATTGAGAAGA GTTGGTACCC TTGGTAACGT TAACGTGCAG

GATAAAGCCG TCTCTGGTTT GAGATCTTTG ACTACTTTGT TGAGAGCTTT
CTATTTTCGGC AGAGACCAA CTCTAGAAAC TGATGAAACA ACTCTCGAAA

GGGTGCTCAA AAGGAAGCCA TTTCCCACC AGACGCTGCT TCTGCCGCTC
CCCACGAGTT TTCCTTCGGT AAAGGGGTGG TCTGCGACGA AGACGGCGAG

CATTGAGAAC CATCACTGCT GATACCTTCA GAAAGTTATT CAGAGTTTAC
GTAACCTTTG GTAGTGACGA CTATGGAAGT CTTTCAATAA GTCTCAAATG

TCCAACCTTCT TGAGAGGTAA ATTGAAGTTG TACACCGGTG AAGCCTGTAG
AGGTTGAAGA ACTCTCCATT TAACTTCAAC ATGTGGCCAC TTCGGACATC

AACTGGTGAC AGATAAGCCC GACTGATAAC AACAGTGTAG
TTGACCACTG TCTATTCGGG CTGACTATTG TTGTCACATC

                SalI
ATGTAACAAA G
TACATTGTTT CAGCT

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FIG. 9

	-20	-10	+1	10	20	30	40
Human	MGVHECPAWLWLLSLSPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPTDK						
	*****	*****	*****	*****	*****	*****	*****
Monkey	MGVHECPAWLWLLSLSPLGLPVPGAPPRLICDSRVLERYLLEAKEAENVTMGCSSECSLNENITVPTDK						

	50	60	70	80	90	100	110
Human	VNIFYAWKRMEVGQQAQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSITLLRALGAQKE						
	*****	*****	*****	*****	*****	*****	*****
Monkey	VNIFYAWKRMEVGQQAQAVEVWQGLALLSEAVLRGQAVLANSSQPFEPQLHMDKAVISGLRSITLLRALGAQ-E						

	120	130	140	150	160
Human	AISLPPDAASAAPLRTITADTFRKLFRVYSNFLRGKLLKLYTGEACRTGDR				
	***	*****	*****	*****	*****
Monkey	AISLPPDAASAAPLRTITADTFCKLFRVYSNFLRGKLLKLYTGEACRRGDR				

FIG. 10

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

FIG. 12

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

FIG. 13

ECORI KpnI
A ATTGGTACC AGACACCAAG GTTAACTTCT ACGCTTGGAA ACGTATGGAA
GCCATGG TCTGTGGTTC CAATTGAAGA TGCGAACCTT TGCATACCTT

GTTGGTCAAC AAGCAGTTGA AGTTTGGCAG GGTCTGGCAC TGCTGAGCGA
CAACCAC TTG TTCGTCAACT TCAAACCGTC CCAGACCGTG ACGACTCGCT

GGCTGTACTG CGTGGCCAGG CAC TGTGGT AAACTCCTCT CAGCCGTGGG
CCGACATGAC GCACCGGTCC GTGACGACCA TTTGAGGAGA GTCGGCACCC

AACCGCTGCA GCTGCATGTT GACAAAGCAG TATCTGGCCT GAGATCTG
TTGGCGACGT CGACGTACAA CTGTTTCGTC ATAGACCGGA CTCTAGACCTAC

1 2 3 4
5 6 7 8
9 10 11 12
13 14 15 16
BglIII BamHI