

United States Patent [19]

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[54] **PRODUCTION OF ERYTHROPOIETIN**
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Related U.S. Application Data

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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".

7 Claims, 27 Drawing Sheets

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FIG. 1

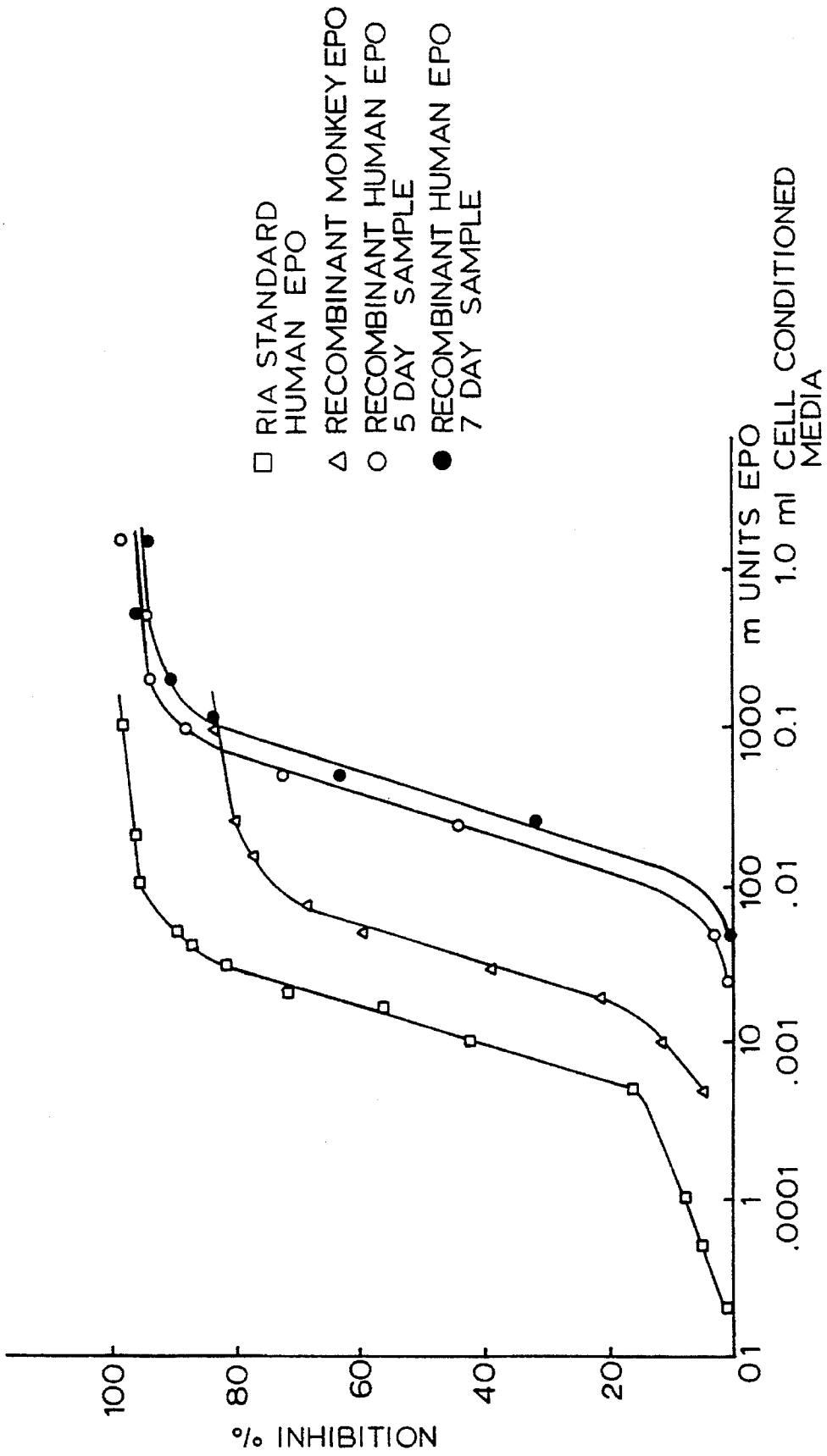


FIG. 2

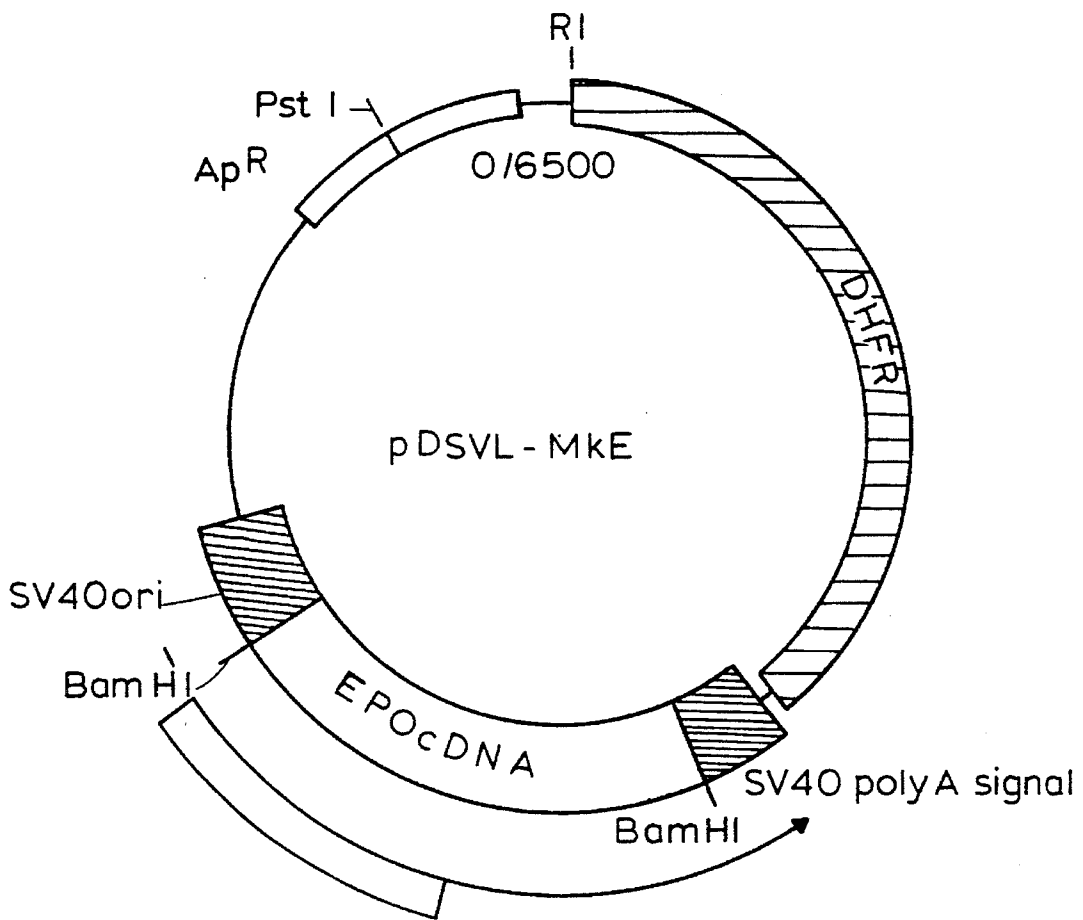


FIG. 3

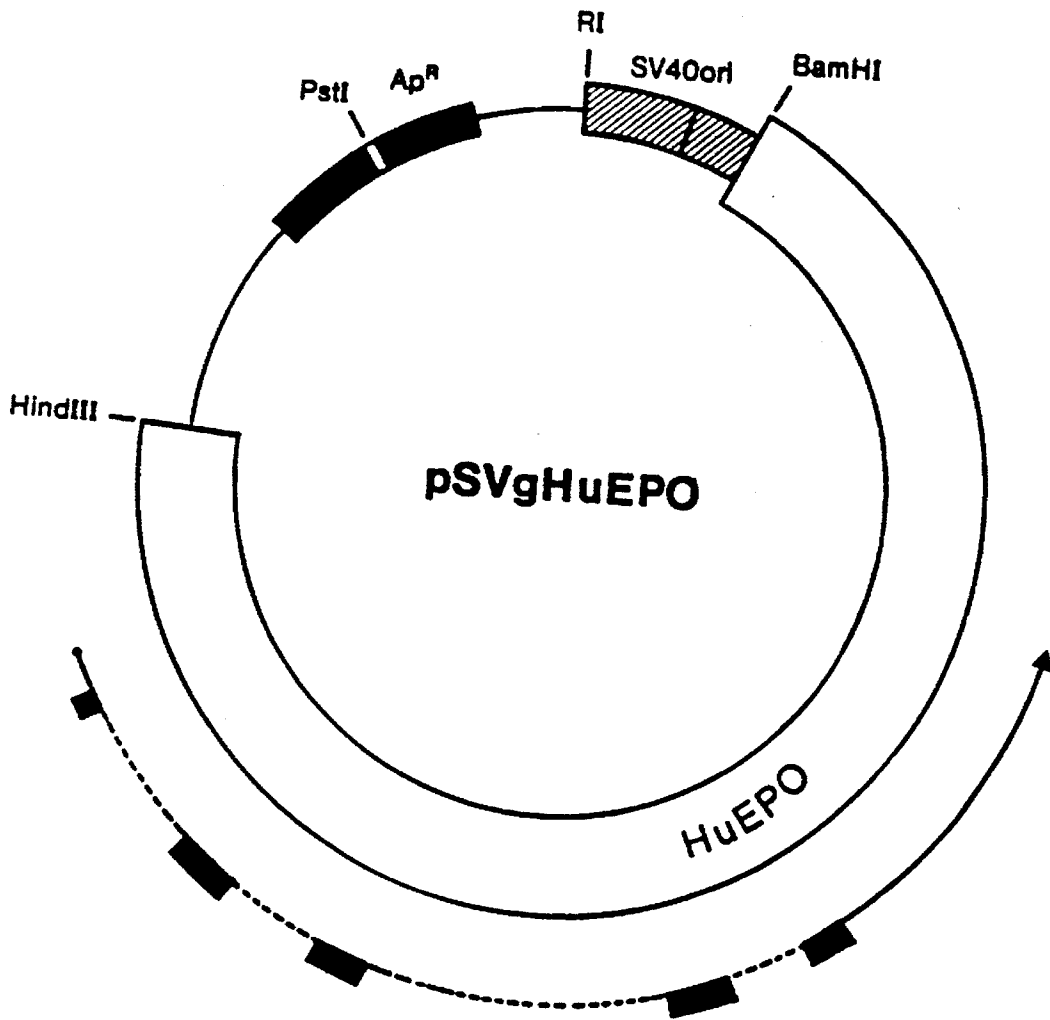


FIG. 4

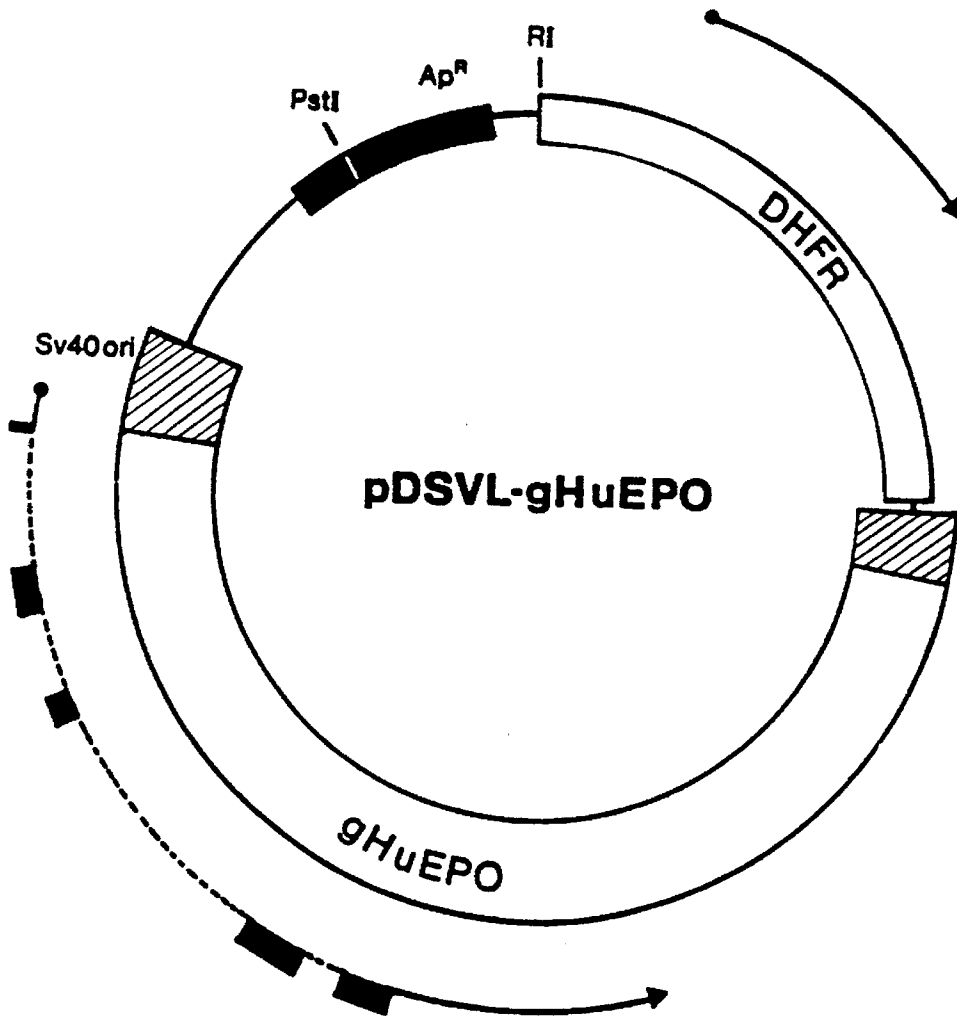


FIG. 5A

Sau3A
 GATCCCGGCCCTGGACAGCCCTCTCCTCCAGGCCCGTGGGGCTGGCCCTGCCC
 CGTGAACTTCCCGGATGAGGACTCCCGGTGTGGTACCCGCCCTAGGTCGCTGAG

-27
 Met Gly Val His Glu Cys Pro Ala Trp
 GGACCCCGCCAGCGCGGAGATG GGG GTG CAC GAA TGT CCT GCC TGG

-20

-10

-1 +1 10

Leu Trp Leu Leu Leu Ser Leu Val Ser Leu Pro Leu Gly Leu Pro
 CTG TGG CTT CTC CTG TCT CTC CTC GTG TCG CTC CCT CTG GGC CTC CCA
 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 Ala Trp Lys Arg Met Glu Val Gly
 GAC ACC AAA GTT AAC TTC TAT GCC TGG TGG AAG AGG ATG GAG GAG GTC GGG
 60 Gln Gln Ala Ala Val Glu Val Trp Gln Gln Gly GGC GGC CTG Ala Leu Leu Ser Glu
 CAG CAG GCT GTA GAA GTC GTC TGG CAG CAG GGC CTG GCC CTG CTG TCA GAA
 70 Ala Val Leu Arg Gly Gln Ala Val Leu Ala Asn Ser Ser Gln Pro
 GCT GTC CTG CTG CGG GGC GGC CAG CAG GCC GTG GTG TTG GCC AAC TCT TCC CAG CCT
 80 Phe Glu Pro Leu Leu Gln Leu His Met Asp Ala Lys Ala Ile Ser Gly Leu
 TTC GAG CCC CTG CTG CAG CAG CTG CAC ATG ATG GAT AAA GCC ATC AGT GGC CTT
 90 Arg Ser Ile Thr Thr Thr Leu Leu Leu Arg Ala Leu Gly Ala Gln Glu Ala
 CGC AGC ATC ACC ACT ACT ACT CTG CTT CGG CGG GCG GCG GGA GCC CAG GAA GCC
 100 Ile Ser Leu Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile
 ATC TCC CTC CCA GAT GAT GCG GCC TCG TCG GCT GCT CCA CTC CGA ACC ATC
 110 Thr Ala Asp Thr Phe Cys Lys Leu Phe Arg Val Tyr Ser Asn Phe
 ACT GCT GAC ACT TTC TTC AAA AAA CTC TTC TTC CGA GTC TAC TCC AAT TTC
 120
 130
 140

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FIG. 5C

150 Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Arg
CTC CGG GGA AAG CTG AAG CTG AAG CTG TAC ACG GGG GAG GCC TGC AGG AGA

160
Gly Asp Arg OP
GGG GAC AGA TGA CCAGGTGCCGTCCAGCTGGGCACATCCACCACCTCCCTCACCACA
CTGCCCTGTGCCACACCCCTCCCTCACCACCTCCCGAACCCCATCGAGGGGGCTCTCAGCTAAG
CGCCAGCCCTGTCCCATGGACACTCCAGTGCCAGCAATGACATCTCAGGGGCCAGAGGAAC
TGTCCAGAGCACAACCTGTGAGATCTAAGGATGTCCAGGGCCCACTTGAGGGCCAGAGC
AGGAAGCATTCAGAGAGCAGCTTTAAACTCAGGAGCAGAGACAATGCCAGGGAAACACCTT
GAGCTCACTGGCCACCTGC AAAATTGATGCAGGACACGCTTTGGAGGCAATTTACCTG
TTTTTGCACCTACCATCAGGGACAGGATGACTGGAGAACTTAGGTGGCAAGCTGTGACTT
CTCAAGGCTCAGGGCACTCCCTTGGTGGCAAGAGCCCTTGACACTGAGAGAAATATT
TTGCAATCTGCAGCAGGAAAAATTACGGACAGGTTTTGGAGGTTGGAGGTTACTTGACAG
GTGfGTGGGAAGCAGGGCGGJAGGGGTGGAGCTGGGATGCCAGfGAGAACCCTGAAGAC
AGGATGGGGGCTGGCCCTCTGGTTCTCTGTGGGGTCCAAGCTT

HindIII

FIG. 6A

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AAGCTTCTGGGCTTCCAGACCCAGCTACTTTGCGGAACCTCAGCAACCAGGCATCTCTGAGTCTCCGCCCA
AGACCGGGATGCCCCAGGGAGGTGTCCCGGAGCCAGCCTTTCAGATAGCACGCTCCGCCAGTCCC
AAGGTGCGCAACC GGCTGCACTCCCTCCCGGACCCAGGCCCGGAGCAGCCCCATGACCCACACGC
ACGTCTGCAGCAGCCCGCTCAGCCCGGAGCCTCAACCAGGCGTCTGCCCCCTGCTCTGACCCCGG
GTGGCCCTTACCCCTGGGACCCCTCACGCACACAGCCTCTCCCCACCCCGGCACGCACACATG
CAGATAACAGCCCCCGACCCCGGCCAGAGCCGXAGAGTCCCTGGGCCACCCCGGCCGCTGCCCCTG
CGCCGACCCGGCTGTCTCCCGGAGCCGGACCGGGCCACCGCCXGCTCTGCTCCGACACCGGCC
CTTGGACAGCCGCCCTCTCCTCTAGGCCCGTGGGCTGGCCCTGCACCGCCGAGCTTCCCGGATGAGGXX
          -27          -24
          Met Gly Val His
          ATG GGG GTG CAC G
GTGAGTACTCGGGCTGGGCGCTCCCGCGGCCGGTTCCTGTGTGAGCGGGATTTAGCGCCCGGGCT

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FIG. 6B

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ATTGCCAAGAGGTGGCTGGTTCAAGGACCGGACTTGTCAAGGACCCCGGAAGGGGAGGGGGTGGG
GCAGCCTCCACGTCGCCCGGACTTGGGGAGTCTTGGGGATGGCAAAACCTGGCCTGTGTGAGGGGCA
CAGTTTGGGTTGGGAGGAGGTTGGGTTCTGCTGTGCAGTTGTGTCGTTGTCAAGTGTCTCG [I · S · ]
TTGCACACGACAGATCAATAAGCCAGAGGCACCTGAGTGCTTGCAATGGTTGGACAGGAAGGACGAG
CTGGGGCAGAGACGTGGGGATGAAGGAAGCTGTCTTCCACAGCCACCTTCTCCCCCGCCTGACTCT
-23 -20
Glu Cys Pro Ala Trp Leu Trp Leu Trp Leu Leu Ser Leu
AA TGT CCT GCC TGG CTG TGG TGG CTT CTC CTG TCC CTG
CAGCCTGGCTATCTGTCTAG -1 +1
Leu Ser Leu Pro Leu Gly Leu Val Leu Gly Ala Pro Pro Arg Leu Ile Cys
CTG TCG CTC CCT CTG GGC CTC CCA GTC CTG GGC GGC CCA CCA CGC CTC ATC TGT
10
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 GTGAGACCCCTTCCCCAGCACATTCCACAGAACTCACGGCTCAGGGCTTCAGGGAACCTCTCCAGAT
CCAGGAACCTGGCACTTGGTTTGGGGTGGAGTTGGGAAGCTAGACACTGCCCCCTACATAAGAATAAGTC

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FIG.6C

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TGGTGGCCCCAAACCATACTGAAACTAGGCAAGGAGCAAAAGCCAGCAGATCCTACGCCCTGTGGGCCAGGG
                27          30
                Thr Gly Cys Ala Glu
                ACG GGC TGT GCT GAA
CCAGAGCCCTTCAGGGACCCTTGACTCCCCGGGCTGTGTGCATTTTCAG
                *          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 ATC ACT GTC CCA GAC ACC AAA GTT AAT TTC TAT
50
Ala Trp Lys Arg Met Glu
GCC TGG AAG AGG ATG GAG GTGAGTTCCTTTTTTTTTTTTTTCCCTTCTTTTGGAGAAATCTCATTT
                55
TGGAGCCCTGATTTGGATGAAAGGGAGAAATGATCGGGGAAAGGTAATAATGGAGCAGCAGAGATGAGGCT
GCC TGGCGCAGAGGCTCACGTCTATAATCCCAGGCTGAGATGGCCGAGATGGGAGAAATTGCTTGAGCCCT
GGAGTTTCAGACCAACCTAGGCAGCATAAGTGAGATCCCCCATCTACAAACAATTAATAAAAATTAGTCAG
GTGAAGTGGTGCA TGGTGTAGTCCCAGATAATTTGGAAGGCTGAGGGGGGAGGATCGCTTGAGCCCAGGAA
TTTGAGGCTGCAGTGAGCTGTGATCACACCACCTGCACCTCCAGCCTCAGTGACAGAGTGAGGCCCTGTCTCA
    
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FIG. 6D

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AAAAAGAAAAGAAAATAATGAGGGCTGTATGGAATACATTATTATTACTCACTCACTCACT
CACTCATTCAATTCATTCAACAAGTCTTATTGCATACCTTCTGTGTTGCTCAGCTTGGTGTGG
GGCTGCTGAGGGGCAGGAGGAGGGGTGACATGGGTGAGTCCAGACTCCAGAGTCCACTCCCTGTAG
56          60          70
Val Gly Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu Ser Glu Ala
GTC GGG CAG CAG GCC GTA GAA GTC TGG CAG GGC CTG GCC CTG TCG GAA GCT

Val Leu Arg Gly Gln Ala Leu Val Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu
GTC CTG CCG GGC CAG GCC CTG TTG 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
100
110 Arg Ala Leu Gly Ala Gln
CGG GCT CTG GGA GCC CAG GTGAGTAGGAGCGGACACTTCTGCTTGCCCTTCTGTAGAAGGGGA
GAAGGGTCTTGCTAAGGAGTACAGGAACGTCCGTATTCCCTTCTGTTGGCACTGCAGCGACCTCCT
115
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

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 CCAGGTGTGCCACCTGGGCATATCCACCACCTCCCTCACCACCATGCTTGTGCCACA
CCCCCCCCCACTCCTGAACCCCGTCCGAGGGGCTCTCAGCTCAGCCGCGCCCTGTCCCATGGACACTCC
AGTGCCAGCAATGACATCTCAGGGGCCAGAGGAACCTGTCCAGAGAGCAACTCTGAGATCTAAGGATGTCAC
AGGGCCAACTTGAAGGCCAGAGCAGGAAGCAATTCAGAGAGCAGCTTTAAACTCAGGGACAGAGCCATGC
TGGGAAGACGCCCTGAGCTCACTCGGCACCCCTGCAAAATTGATGCCAGGACACGCTTTGGAGCGGATTAC
CTGTTTTCGCACCTACCATCAGGGACAGGATGACCTGGAGAACTTAGGTGGCAAGCTGTGACTTCTCCAGG
TCTCACGGGCATGGGCACCTCCCTTGGTGGCAAGAGCCCCCTTGACACCGGGGTGGTGGAAACCATGAAGAC
AXGATXGGGGCTGGCCCTCTGGCTCTCATGGGGTCCAAGTTTTGTGTATTTCTCAACCCTATTGACAGACTGAA
ACACAATATGAC

FIG. 7

			-1	1
<u>XbaI</u>			<u>Met</u>	<u>Ala</u>
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	ACGCTTGGA	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

<u>HindIII</u>	-1	+1			
	Arg	Ala			
AGCTTGGATA	AAAGAGCTCC	ACCAAGATTG	ATCTGTGACT	CGAGAGTTTT	
ACCTAT	TTTCTCGAGG	TGGTTCTAAC	TAGACACTGA	GCTCTCAAAA	
GGAAAGATAC	TTGTTGGAAG	CTAAAGAAGC	TGAAAACATC	ACCACTGGTT	
CCTTCTATG	AACAACCTTC	GATTTCTTCG	ACTTTTGTAG	TGGTGACCAA	
GTGCTGAACA	CTGTTCTTTG	AACGAAAACA	TTACGGTACC	AGACACCAAG	
CACGACTTGT	GACAAGAAAC	TTGCTTTTGT	AATGCCATGG	TCTGTGGTTC	
GTTAACTTCT	ACGCTTGGAA	ACGTATGGAA	GTTGGTCAAC	AAGCTGTTGA	
CAATTGAAGA	TGCGAACCTT	TGCATACCTT	CAACCAGTTG	TTCGACAAC	
AGTTTGCCAA	GGTTTGGCCT	TGTTATCTGA	AGCTGTTTTG	AGAGGTCAAG	
TCAAACCGTT	CCAAACCGGA	ACAATAGACT	TCGACAAAAC	TCTCCAGTTC	
CCTTGTTGGT	TAACTCTTCT	CAACCATGGG	AACCATTGCA	ATTGCACGTC	
GGAACAACCA	ATTGAGAAGA	GTTGGTACCC	TTGGTAACGT	TAACGTGCAG	
GATAAAGCCG	TCTCTGGTTT	GAGATCTTTG	ACTACTTTGT	TGAGAGCTTT	
CTATTTCCGGC	AGAGACCAAA	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		
	<u>SalI</u>				
ATGTAACAAA	G				
TACATTGTTT	CAGCT				

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FIG. 9

	-20	-10	+1	10	20	30	40
Human	MGVHECPAWLWLLSLLSPLGLPVLGAPPRLLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVDPDK						
Monkey	MGVHECPAWLWLLSLLSPLGLPVLGAPPRLLICDSRVLERYLLEAKEAENVTMGCCSECSLNENITVDPDK						
	50	60	70	80	90	100	110
Human	VNFYAWKRMEVGGQQA VEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVVDKAVSGLRSLTTLRALGAQKE						
Monkey	VNFYAWKRMEVGGQQA VEVWQGLALLSEAVLRGQAVLANSSQPFEPQLQHMMDKAISGLRSITTLRALGAQ-E						
	120	130	140	150	160		
Human	AISPPDAASAAPLRTTTADTFRKLFRRVYSNFIKGLKLYTGEACRTGDR						
Monkey	AISLPDAASAAPLRTTTADTFCCKLFRVYSNFIKGLKLYTGEACRRGDR						

FIG. 10

1. AATTCTAGAAACCATGAGGGTAATAAAATA
2. CCATTATTTTATTACCCTCATGGTTTCTAG
3. ATGGCTCCGCCGCGTCTGATCTGCGAC
4. CTCGAGTCGCAGATCAGACGCGGCGGAG
5. TCGAGAGTTCTGGAACGTTACCTGCTG
6. CTTCCAGCAGGTAACGTTCCAGAACT
7. GAAGCTAAAGAAGCTGAAAACATC
8. GTGGTGATGTTTTTCAGCTTCTTTAG
9. ACCACTGGTTGTGCTGAACACTGTTC
10. CAAAGAACAGTGTTTCAGCACAAACA
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 CGAGAGTTCT GGAACGTTAC CTGCTGGAAG CTAAAGAAGC
TAGACGCTGA GCTCTCAAGA CCTTGCAATG GACGACCTTC GATTTCTTCG
6

7 9 11
TGAAAACATC ACCACTGGTT GTGCTGAACA CTGTTCTTTG AACGAAAACA
ACTTTTGTTAG TGGTGACCAA CACGACTTGT GACAAGAAAC TTGCTTTTGT
8 10

0
KpnI BamHI
TTACGGTACC G
AATGCCATGG CCTAG
12

FIG. 12

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

FIG. 13

EcoRI KpnI 1 3
 A ATTCGGTACC AGACACCAAG GTTAACTTCT ACGCTTGGA ACGTATGGAA
GCCATGG TCTGTGGTTC CAATTGAAGA TGCGAACCTT TGCATACCTT
2 4

5 7
GTTGGTCAAC AAGCAGTTGA AGTTGGCAG GGTCTGGCAC TGCTGAGCGA
CAACCACTTG TTCGTCAACT TCAAACCGTC CCAGACCGTG ACGACTCGCT
6 8

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

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

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

FIG. 15

BamHI BglIII

GA TCCAGATCTCTG
GTCTAGAGAC

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

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

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

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

FIG. 16

1. AATCAAGCTTGGATAAAAGAGCT
2. GTGGAGCTCTTTTATCCAAGCTTG
3. CCACCAAGATTGATCTGTGACTC
4. TCTCGAGTCACAGATCAATCTTG
5. GAGAGTTTTGGAAAGATACTTGTTG
6. CTTCCAACAAGTATCTTTCCAAAAC
7. GAAGCTAAAGAAGCTGAAAACATC
8. GTGGTGATGTTTTTCAGCTTCTTTAG
9. ACCACTGGTTGTGCTGAACACTGTTT
10. CAAAGAACAGTGTTTCAGCACAACCA
11. TTTGAACGAAAACATTACGGTACCG
12. GATCCGGTACCGTAATGTTTTCGTT