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United States Patent [19]
Lin

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[54] **PRODUCTION OF ERTHROPOIETIN**

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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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FIG. 1

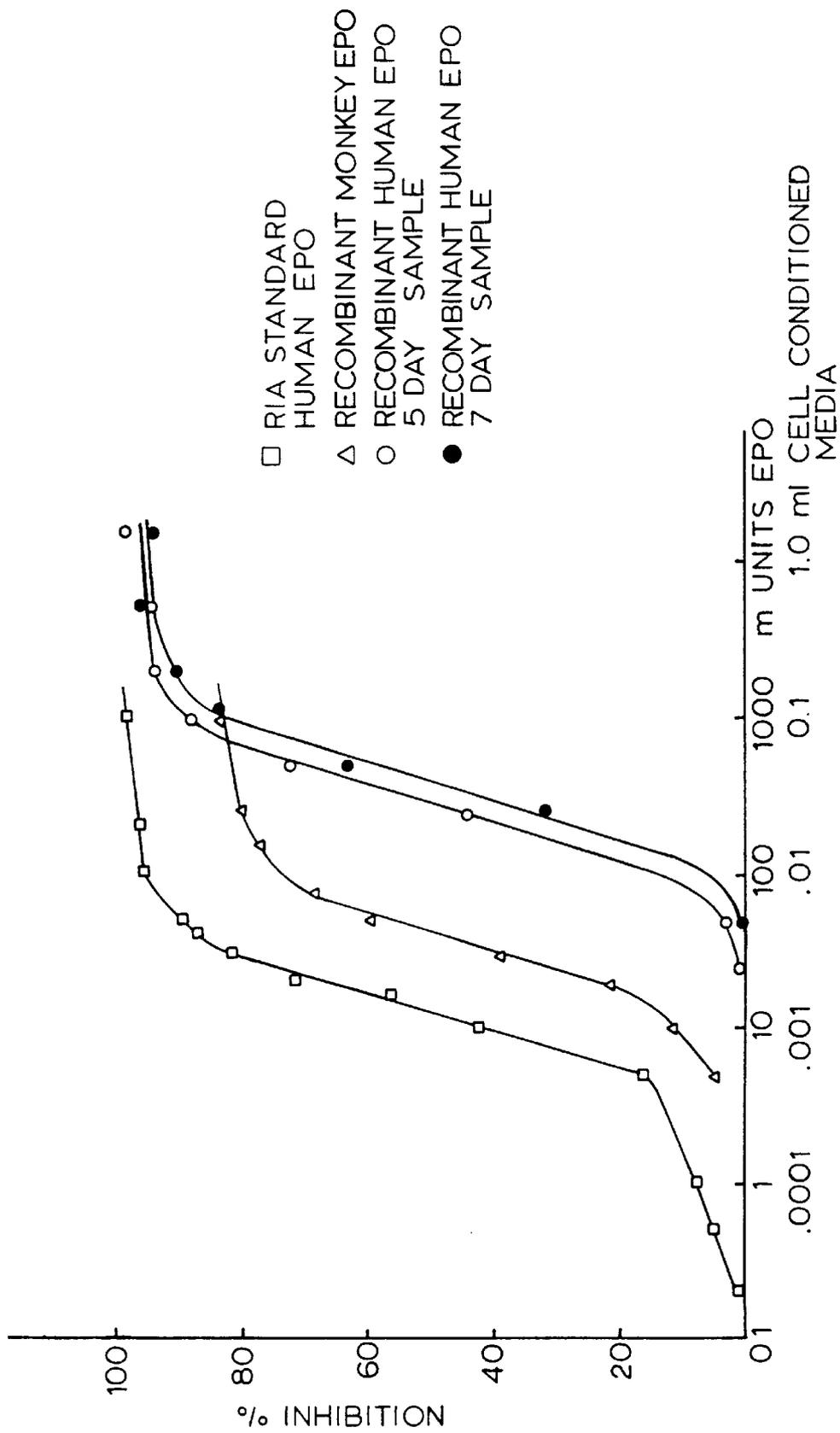


FIG. 2

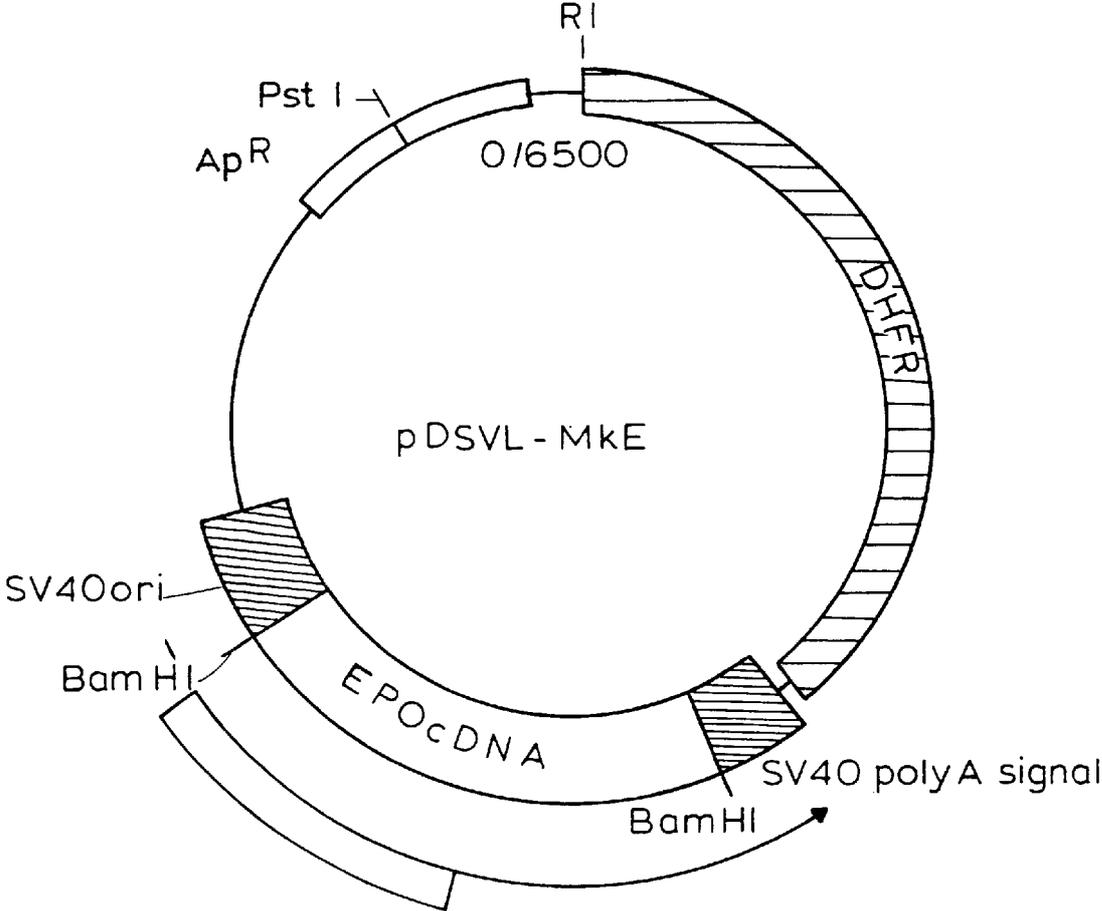


FIG. 3

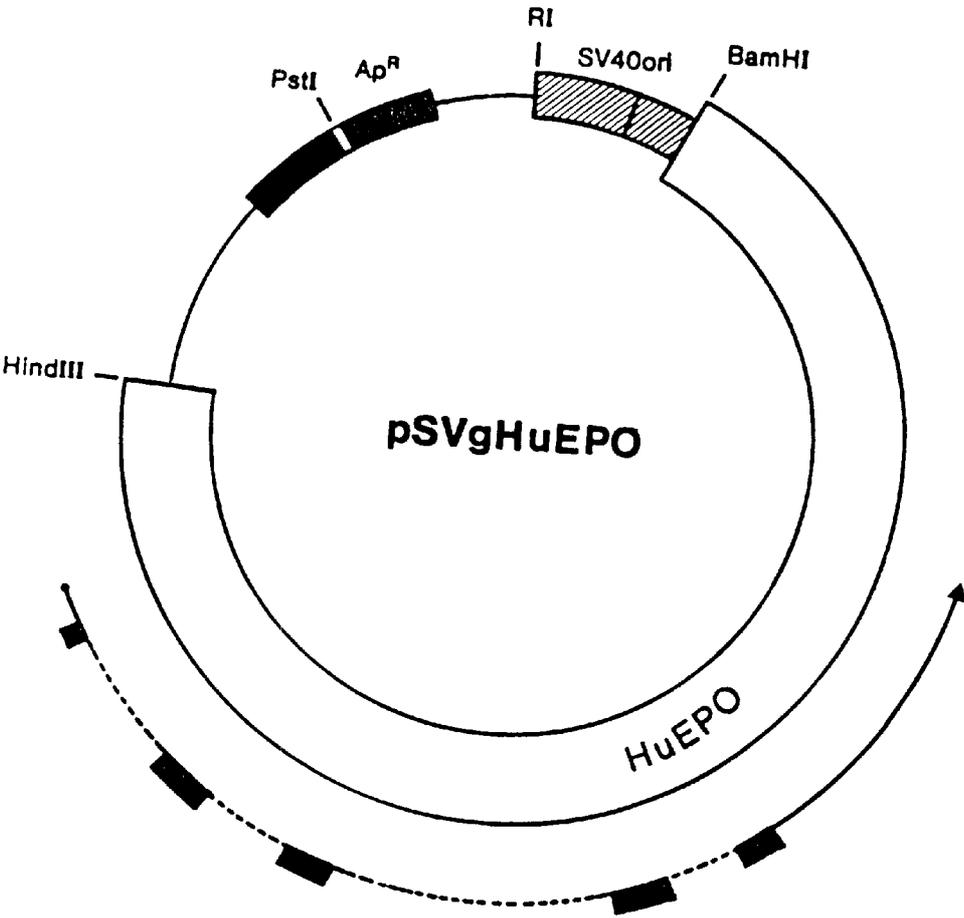


FIG. 4

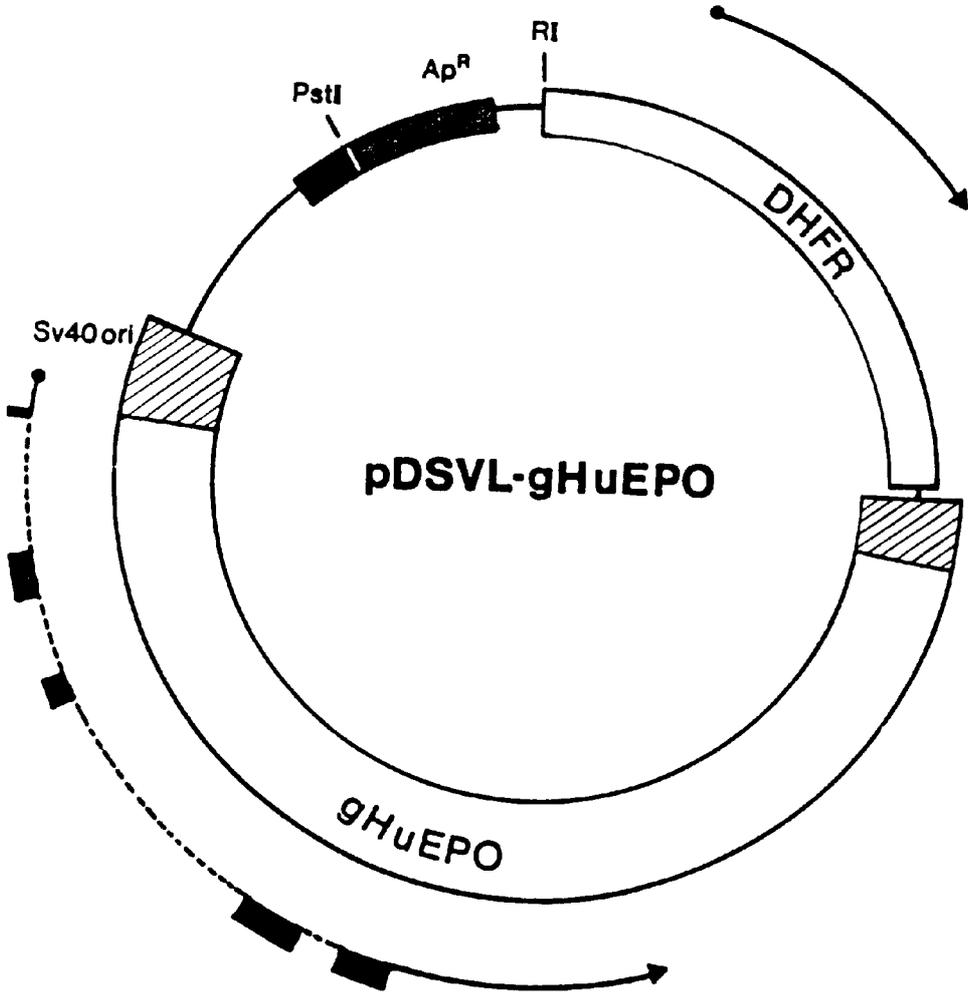


FIG. 5A

Sau3A
 GATCCCGGCCCTGGACAGCCCTCTCCTCCAGGCCCGTGGGCTGGCCCTGCC
 CGCTGAACCTCCGGGATGAGGACTCCCGGTGTGGTACCCGCGCCTAGGTCGCTGAG

-27

Met Gly Val His Glu Cys Pro Ala Trp

GGACCCCGGCCAGGCGGGAGATG GGG GTG CAC GAA TGT CCT GCC TGG

-20

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

-10

+1

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

10

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

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

40

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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 TGG AAG AGG ATG GAG GTC GGG
 60
 Gln Gln Ala Val Glu Val Trp Gln Gly GGC CTG GGC CTG Ala Leu Ser Glu
 CAG CAG GCT GTA GAA GTC TGG TGG CAG GGC CTG GGC CTG CTC TCA GAA
 70
 Ala Val Leu Arg Gly Gln Ala Val Leu Ala Asn Ser Ser Gln Pro
 GCT GTC CTG CCG GGC GGC CAG GGC 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 CAG CTG CAC ATG GAT AAA GCC ATC AGT GGC CTT
 90
 Arg Ser Ile Thr Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Glu Ala
 CGC AGC ATC ACC ACT ACT CTG CTT CGG GCG GGA GCC CAG GAA GCC
 100
 Ile Ser Leu Pro Asp Ala Ser Ala Ala Pro Leu Arg Thr Ile
 ATC TCC CTC CCA GAT GCG GCC 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 TGC AAA CTC TTC CGA GTC TAC TCC AAT TTC
 120
 130
 140

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 TAC ACG GGG GAG GCC TGC AGG AGA
 160
 Gly Asp Arg OP
 GGG GAC AGA TGA CCAGGTGCCGTCCAGCTGGGCACATCCACCACCTCCCTCACCACA
 CTGCCGTGCCACACCCCTCCCTCACCACTCCCGAACCCCATCGAGGGGCTCTCAGCTAAG
 CGCCAGCCTGTCCCATGGACACTCCAGTGCCAGCAATGACATCTCAGGGGCCAGAGGAAC
 TGTCCAGAGCACAACTCTGAGATCTAAGGATGTCGCAGGGCCAACTTGAGGGCCCCAGAGC
 AGGAAGCATTCAGAGAGCAGCCTTTAAACTCAGGAGCAGAGACAATGCAGGGGAAACACCT
 GAGCTCACTGGGCCACCTGCCAAAATTTGATGCAGGACACGCTTTGGAGGCAATTTACCTG
 TTTTGCACCTACCATCAGGGACAGGATGACTGGAGAACTTAGGTGGCAAGCTGTGACTT
 CTC AAGCCTCAGGGCCTCCCTTGGTGGCAAGAGCCCCCTTGACACTGAGAGAATATT
 TTGCAATCTGCAGCAGGAAAATACGGACAGGTTTTTGGAGGTTGGAGGGTACTTGACAG
 GTGTGTGGGGAAGCAGGGCGGTAGGGGTGGAGCTGGGATCGGAGTGAGAACCCGTGAAGAC
 AGGATGGGGCTGGCCCTCTGGTTCTCGTGGGGTCCAAGCTT HindIII

FIG.6C

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TGGTGGCCCCAAACCATACCTGAAACTAGGCAAGGAGCAAAAGCCAGCAGATCCTACGCCCTGTGGCCAGGG
                27          30
                Thr Gly Cys Ala Glu
CCAGAGCCTTCAGGGACCCCTTGACTCCCCGGGCTGTGTGCATTTTCAG   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 GTGAGTTCCTTTTFTTTTFTTTTFTTTTFTTTTGGAGAATCTCATT
TGGAGCCTGATTTTGGATGAAAGGGAGAAATGATCGGGGAAAGGTAAATGGAGCAGCAGAGATGAGGCT
GCC TGG GCG CAG AGG C T C T A T A A T C C C A G G C T G A G A T G G C C G A G A T T G C T T G A G C C C T
GGAGTTTCAGACCAACC TAGGCAGCATAGTGAGATCCCCCATCTCTACAAACATTTAAATAAATAAGTCAG
GTGAAGTGTGCATGGTGTAGTCCAGATATTTGGAAGGCTGAGGCGGGAGGATCGCTTGAGCCCCAGGAA
TTTGAGGCTGCAGTGAGCTGTGATCACACCACCTGCACCTCCAGCCTCAGTGACAGAGTGAGGCCCTGTCTCA

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FIG. 6D

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AAAAAGAAAAGAAAATAATGAGGGCTGTATGGAATACATTTCATTTACTCACTCACTCACT
CACTCATTTCATTTCATTTCATTTCAACAAGTCTTATTGCATACCCTTCTGTTTGCTCAGCTTGGTGCTTGG
GGCTGCTGAGGGGCAGGAGGGGAGGGTGACATGGGTGACATGGGTGACATGCCAGCTCCAGAGTCCACTCCCTGTAG
56          60          70          80          90
Val Gly Gln Gln Ala Val Glu Val Val Trp Gln Gly Leu Ala Leu Leu Ser Glu Ala
GTC GGG CAG CAG GCC GTA GAA GTC TGG CAG GGC CTG GCC CTG TCG 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 TTG GTC AAC TCT TCC CAG CCG TGG GAG CCC CTG

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

GAAGGGTCTTGCTAAGGAGTACAGGAACGTCCGGTATTCCTTCCCTTCTGTGGCAGTGCAGCGACCTCCT
116          120
Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala
GTTTTCCTTGGCAG 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 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 CCAGGTGTGCCACCTGGGCATATCCACCACCTCCCTCACCACAATGCTTGCCACA
 CCTCCCCGCCACTCCTGAACCCCGTCGAGGGGCTCTCAGCTCAGCGCCAGCCTGTCCCATGGACACTCC
 AGTGCCAGCAATGACATCTCAGGGGCCAGAGGAACCTGTCCAGAGAGCAACTCTGAGATCTAAGGATGTCAC
 AGGCCAACTTGAAGGCCCAGAGCAGGAAGCATTTCAGAGAGCAGCTTTAAACTCAGGGACAGAGCCCATGC
 TGGGAAGACGCCCTGAGCTCACCTCGGCACCCCTGC AAAATTGATGCCAGGACACGCTTTGGAGGCCGATTAC
 CTGTTTTCGCACCTACCATCAGGGACAGGATGACCTGGAGAACTTAGGTGGCAAGCTGTGACTTCTCCAGG
 TCTCACGGGCATGGGCACCTCCCTTGTGGCAAGAGCCCCCTTGACACCCGGGGTGGTGGGAACCATGAAGAC
 AXGATXGGGGCTGGCCCTCTGGCTCTCATGGGGTCCAAGTTTTTGTGTATTCTCAACCTATTGACAGACTGAA
 ACACAATATGAC

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FIG. 7

<u>XbaI</u>	-1	1	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	CGTGCCAGG	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
GCAAACGTGT	TCGTGTATAC	TCTAACTTCC	TGCGTGGTAA	ACTGAAACTG
CGTTTGACAA	AGCACATATG	AGATTGAAGG	ACGCACCATT	TGACTTTGAC
TATACTGGCG	AAGCATGCCG	TACTGGTGAC	CGCTAATAG	
ATATGACCGC	TTCGTACGGC	ATGACCACTG	GCGATTATCA	GCT

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FIG. 8

	-1 +1			
<u>HindIII</u>	ArgAla			
AGCTTGGATA	AAAGAGCTCC	ACCAAGATTG	ATCTGTGACT	CGAGAGTTTT
ACCTAT	TTTCTCGAGG	TGGTTCTAAC	TAGACTGA	GCTCTCAAAA
GGAAAGATAC	TTGTTGGAAG	CTAAAGAAGC	TGAAAACATC	ACCACTGGTT
CCTTTCTATG	AACAACCTTC	GATTTCTTCG	ACTTTTGTAG	TGGTGACCAA
GTGCTGAACA	CTGTTCTTTG	AACGAAAACA	TTACGGTACC	AGACACCAAG
CACGACTTGT	GACAAGAAAC	TTGCTTTTGT	AATGCCATGG	TCTGTGGTTC
GTAACTTCT	ACGCTTGGAA	ACGTATGGAA	GTTGGTCAAC	AAGCTGTTGA
CAATTGAAGA	TGCGAACCTT	TGCATACCTT	CAACCAGTTG	TTCGACAAC
AGTTTGGCAA	GGTTTGGCCT	TGTTATCTGA	AGCTGTTTTG	AGAGGTCAAG
TCAAACCGTT	CCAAACCGGA	ACAATAGACT	TCGACAAAAC	TCTCCAGTTC
CCTTGTTGGT	TAACCTTCT	CAACCATGGG	AACCATTGCA	ATTGCACGTC
GGAACAACCA	ATTGAGAAGA	GTTGGTACCC	TTGGTAAACG	TAACGTGCAG
GATAAAGCCG	TCTCTGGTTT	GAGATCTTTG	ACTACTTTGT	TGAGAGCTTT
CTATTTTCGGC	AGAGACCAA	CTCTAGAAAC	TGATGAAACA	ACTCTCGAAA
GGGTGCTCAA	AAGGAAGCCA	TTTCCCCACC	AGACGCTGCT	TCTGCCGCTC
CCCACGAGTT	TTCCTTCGGT	AAAGGGTGG	TCTGCGACGA	AGACGGCGAG
CATTGAGAAC	CATCACTGCT	GATACCTTCA	GAAAGTTATT	CAGAGTTTAC
GTAACCTTGT	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			

FIG. 9

	-20	-10	+1	10	20	30	40
Human	MGVHECPAWLWLLSLLSPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITGCAEHCSLNENITVDPDK						
Monkey	MGVHECPAWLWLLSLSVSLPLGLPVPGAPPRLICDSRVLERYLLEAKEAENVTMGCCSECSLNENITVDPDK						
	50	60	70	80	90	100	110
Human	VNFYAWKRMEVGGQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVVDKAVSGLRSLTLLRALGAQKE						
Monkey	VNFYAWKRMEVGGQAVEVWQGLALLSEAVLRGQAVLANSSQPFEPQLHMDKAISGLRSITLLRALGAQ-E						
	120	130	140	150	160		
Human	AISLPPDAASAAPLRTITADTFRKLFVSNFLRGKLLKLYTGEACRTGDR						
Monkey	AISLPPDAASAAPLRTITADTFCKLFRVSNFLRGKLLKLYTGEACRRGDR						