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[54] **PRODUCTION OF ERYTHROPOIETIN**

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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. 561,024, Dec. 13, 1983, abandoned, which is a continuation-in-part of Ser. No. 582,185, Feb. 21, 1984, abandoned, which is a continuation-in-part of Ser. No. 655,841, Sep. 28, 1984, abandoned.

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[58] **Field of Search** ..... 435/69.1, 69.4, 435/240.2, 240.22, 325, 358, 365

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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 viralborne cDNA or genomic DNA "library".

**7 Claims, 27 Drawing Sheets**

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FIG. 1

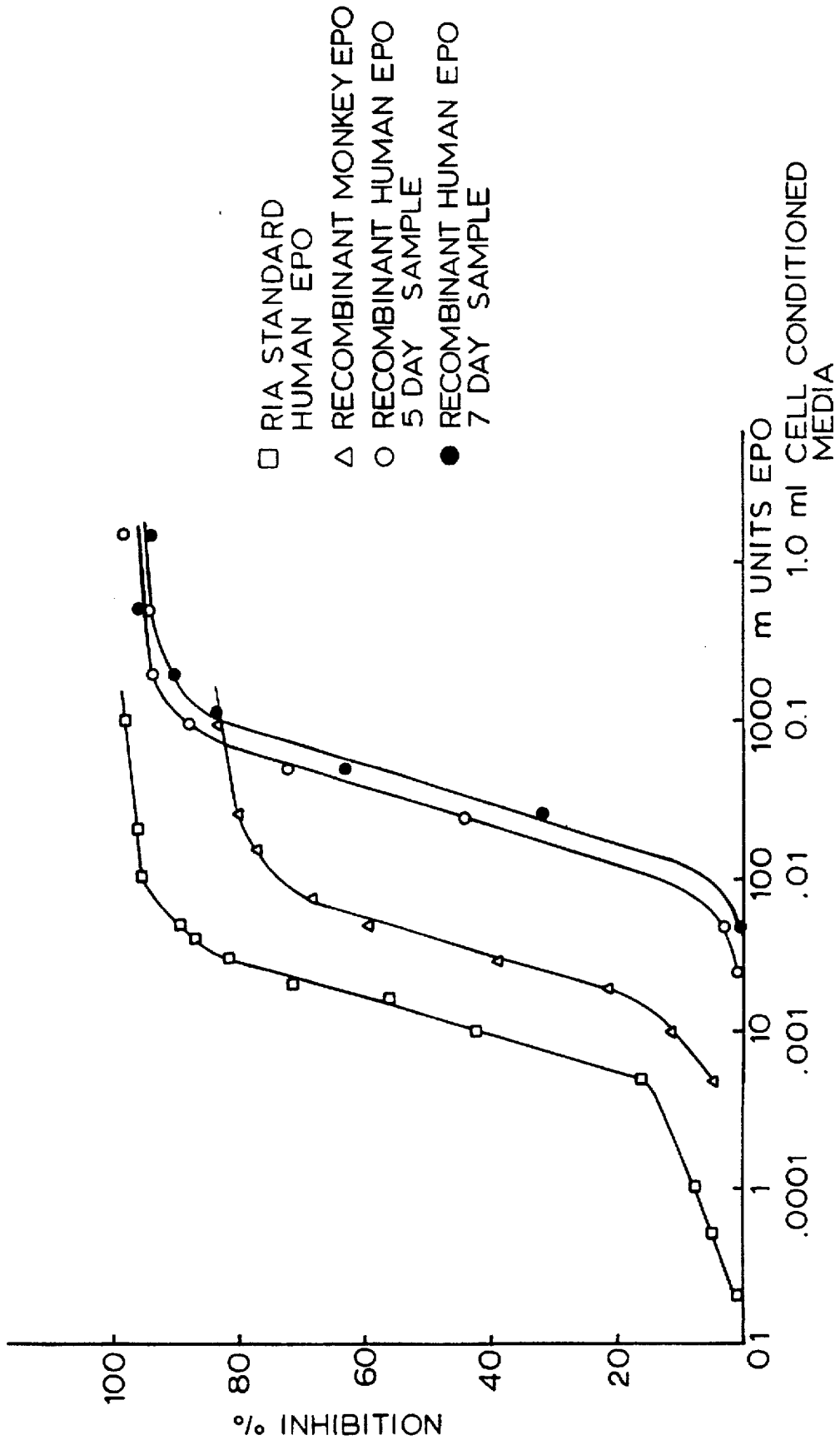


FIG. 2

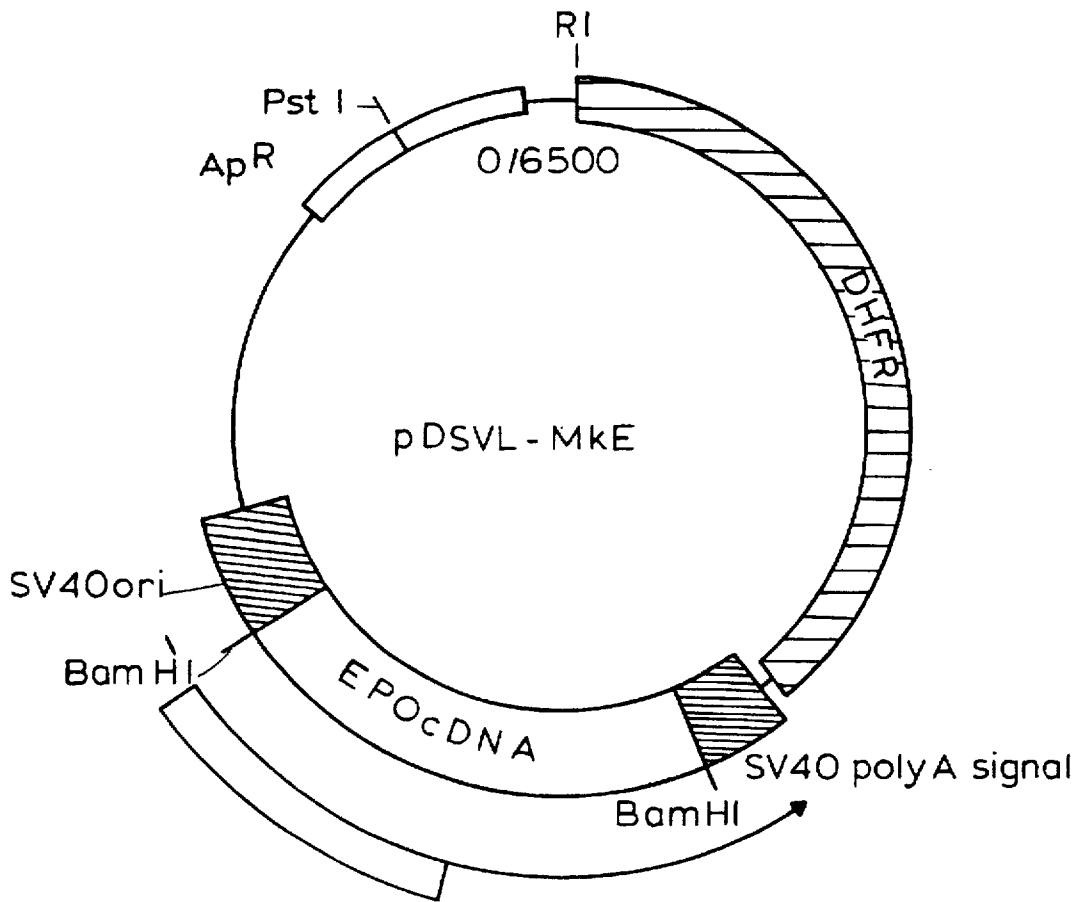


FIG. 3

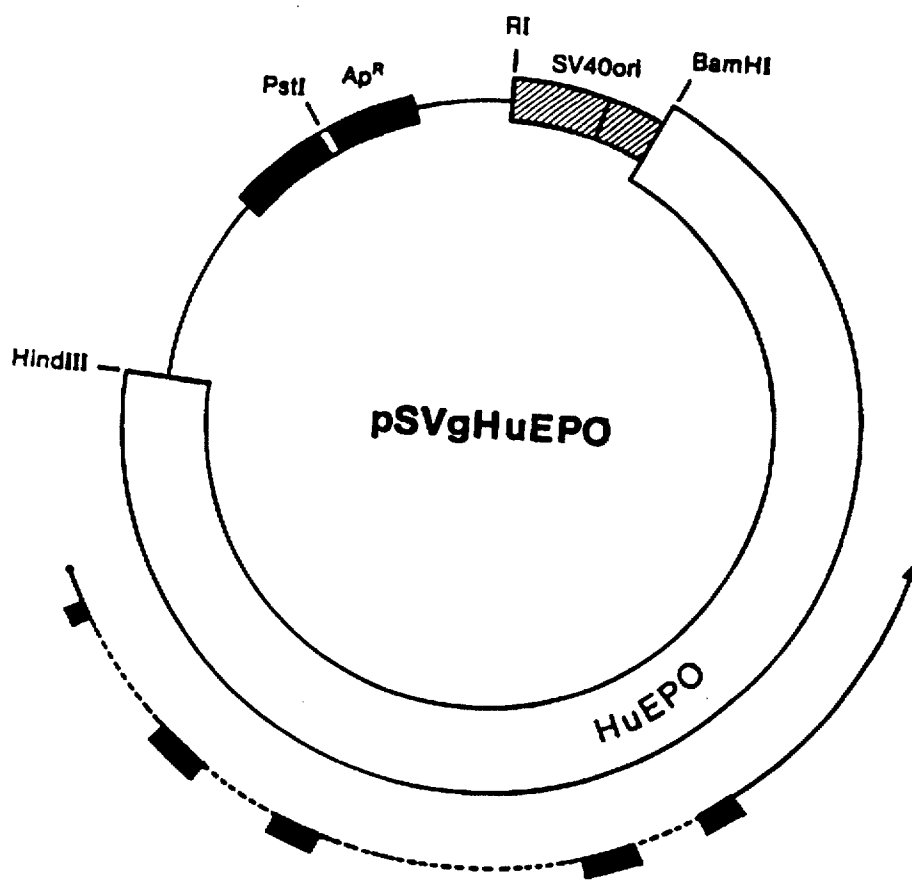




FIG. 4

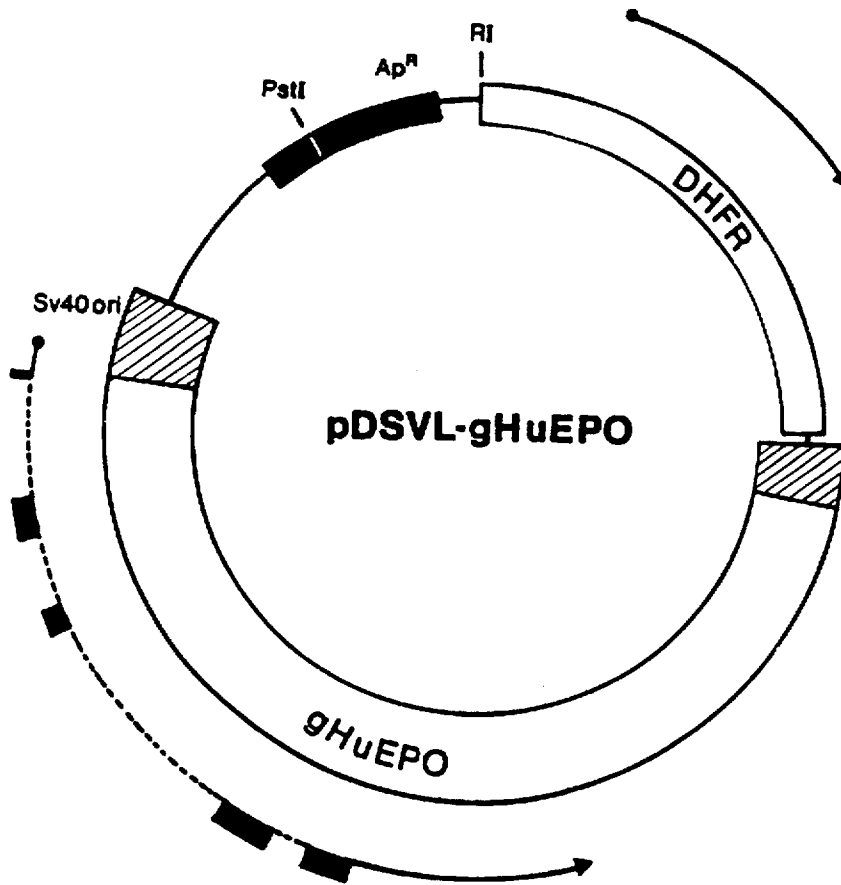


FIG. 5A

Sau3A  
 GATCCGGCCCTTGGACAGCCGCCCTCTCCTCCAGCCCGGGGGCTGGCCCTGCC  
 CGCTGAACCTCCGGGATGAGGACTCCCGGTGGTCCACCCGCGCCCTAGGTCGCTGAG  
 -27 Met Gly Val His Glu Cys Pro Ala Trp  
 GGACCCCGCCAGCGCGGAGATG GGG GTG CAC GAA TGT CCT GCC TGG  
 -20  
 Leu Trp 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  
 -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  
 -1 +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  
 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

FIG.5B

Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg Met Glu Val Gly	50	GAC ACC AAA GTT AAC TTC TAT GCC TGG AAG AGG ATG GAG GTC GGG
Gln Gln Ala Val Glu Val Trp Gln Gly GGC CTG GCC CTG CTC TCA GAA	60	CAG CAG GCT GTA GAA GTC GTC TGG TGG CAG GGC CTG GCC CTG CTC TCA GAA
Ala Val Leu Arg Gly Gln Ala Val Leu Ala Asn Ser Ser Gln Pro	70	GCT GTC CTG CTG CGG GGC CAG GCC GTG TTG GGC AAC TCT TCC CAG CCT
Phe Glu Pro Leu Gln Leu His Met Asp Lys Ala Ile Ser Gly Leu	80	TTC GAG CCC CTG CAG CTG CAC ATG GAT AAA GCC ATC AGT GGC CTT
Arg Ser Ile Thr Thr Leu Leu Thr Leu Arg Ala Leu Gly Ala Gln Glu Ala	90	CGC AGC ATC ACC ACT CTG CTG CTT CGG GCG GCG CTG GGA GCC CAG GAA GCC
Ile Ser Leu Pro Asp Ala Ser Ala Ala Pro Leu Arg Thr Ile	100	ATC TCC CTC CCA GAT GCG GCC TCG GCT GCT CCA CTC CGA ACC ATC
Thr Ala Asp Thr Phe Cys Lys Leu Phe Arg Val Tyr Ser Asn Phe	110	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 CCAGGTGGTCCAGCTGGGCACATCCACCACCTCCCTCACCAACA  
 CTGCCCTGTGCCACACCCCTCCCTCACCCTCCCGAACCCCATCGAGGGGCTCTCAGCTAAG  
 CGCCAGCCTGTCCCATGGACACTCCAGTGCAGCAATGACATCTCAGGGCCAGAGGAAC  
 TGTCCAGAGCACAACTCTGAGATCTAAGGATGTCCGAGGGCCAACTTGAGGGCCAGAGC  
 AGGAAGCATTCAGAGAGCAGCTTTTAAACTCAGGAGCAGAGACAAATGCAGGGAAAACACCT  
 GAGCTCACTCGGCCACCTGCAAAATTTGATGCAGGACACGCTTTGGAGGCAATTTACCTG  
 TTTTGGCACCTACCATCAGGGACAGGATGACTGGAGAACTTAGGTGGCAAGCTGTGACTT  
 CTC AAGGCCCTCAGGGCACTCCCTTGGTGGCAAGAGCCCCCTTGACACTGAGAGAATATT  
 TTGCAATCTGCAGCAGGAAAATAATACGGACAGGTTTTGGAGTTGGAGGGTACTTGACAG  
 GTGTGTGGGAAGCAGGGCGGTAGGGGTGGAGCTGGATCGGATGAGAACCGTGAAGAC  
 AGGATGGGGCTGGCCTCTGGTTCTCGTGGGGTCCAAGCTT  
 HindIII

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FIG. 6A

AAGCTTCTGGGCTTCCAGACCCAGCTACTTTGGGGAACCTAGCAACCCAGGCACTCTGAGTCTCCGGCCCA  
AGACCGGGATGCCCCCCAGGGGAGGTGTCCGGGAGCCCAAGCCTTTCACAGATAGCAGCTCCGCCAGTCCC  
AAGGTTGGCAACCGGCTGCACTCCCCTCCCAGCCAGGGCCCGGAGCAGCCCAATGACCCACACGC  
ACGCTGCAGACGCCCCGCTCACGCCCCCGGAGCCTCAACCCAGGGTCCCTGCCCTGCTCTGACCCCGG  
GTGGCCCTACCCCTGGCGACCCCTCACGCACACAGCCTCTCCCCACCCCAACCCGCGCACACACATG  
CAGATAACAGCCCCGACCCCGGAGAGCCGAGAGTCCCTGGGCCACCCCGGCGCTCGCCTGCCGCTG  
CGCCGACCGGCTGTCCCTCCCAGCCGACCCGGGACCCGCGCCXGCTCTGCTCCGACACCCGCGCC  
CTTGGACAGCCCCCTCTCCTTAGGCCCGTGGGCTGGCCCTGCACCCGCGAGCTTCCCAGGATGAGGXX  
-27  
-24  
Met Gly Val His  
ATG GGG GTG CAC G  
CCCCGTGACCGGCGGCCCAAGTCGCTGAGGGACCCCGGCAAGCGCGGAG  
GTGAGTACTCGGGGCTGGGCGCTCCCAGCGCCGGGTTCCTGTGAGCGGGGATTTAGCCCGCCCGGCT

FIG.6B

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ATTGCCAAGAGGTGGTTC AAGACCGCGGACTTGTCAAGGACCCCGAAGGGGGAGGGGGTGGG
GCAGCCTCCACGTGCCCGGGACTTGGGGAGTCTTGGGGATGGCAAAAACCTGGCCTGTTGAGGGGCA
CAGTTTGGGGTTGGGAGGAGTTTGGGGTTCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTGTGCTG [I.S.]
TTGCACAGCACAGATCAATAAGCCAGAGGCACACCTGAGTGTGCTGTGATGGTTGGGACAGGAAGGACGAG
CTGGGCAGAGACGTGGGGATGAAGGAAGCTGTCTTCCACAGCCACCCCTTCTCCCCCCCCCTGACTCT
CAGCCCTGGCTATCTGTTCTAG
-23
Glu Cys Pro Ala Trp Leu Trp Leu Leu Leu Ser Leu
AA TGT CCT GCC TGG CTG TGG CTT CTC CTG TCC CTG
-10
Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly Ala Pro Pro Arg Leu Ile Cys
CTG TCG CTC CCT CCT CCA GTC CTG GGC GCC CCA CCA CCA CGC CTC ATC TGT
-1 +1
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
10
20
*
26
Thr
ACG GTGAGACCCCTTCCCCAGCACATTCACAGAACTCACGCTCAGGGCTTCAGGGAACCTCCCCAGAT
CCAGGAACCTGGCACTTGGTTTGGGGTGGAGTTGGGAAGCTAGACACTGCCCCCTACATAAGAATAAGTC
    
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FIG. 6D

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AAAAAGAAAAGAAAAGAAAATAATGAGGGCTGTATGGAATACATTATTATTCACTCACTCACTCACT
CACTCACTCACTCACTCACTCACTCACTCACTCACTCACTCACTCACTCACTCACTCACTCACTCACT
GGCTGCTGAGGGGCAGGAGGGAGGGGTGACATGGGTGACACTCGACTCCAGAGTCCACTCCCTGTAG

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

Val Leu Arg Gly Gln Ala Leu Leu Val 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 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 GTGAGTAGGAGCGGACACTTCTGCTTGCCCTTCTGTAAAGAGGGGA

GAAGGGTCTTGCTAAGGAGTACAGGAACTGTCGGTATTCCTTCCCTTCTGTGGCACTGCAGCGACCTCCT

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 CCAGGTGTGCCACCTGGGCATATCCACCACCTCCCTCACCACATTTGCTTGCCACA
CCCTCCCGCCACTCCTGAACCCCGTCGAGGGGCTCTCAGCTCAGGCCAGCCTGTCCCATGGACACTCC
AGTGCCAGCAATGACATCTCAGGGCCAGAGGAACGTGCCAGAGAGCAACTCTGAGATCTAAGGATGTCAC
AGGGCCAACTTGAAGGCCCAGAGCAGGAAGCATTCAGAGAGCAGCTTTAAACTCAGGGACAGAGCCATGC
TGGGAAGACGCCCTGAGCTCACTCGGCACCCCTGCAAAATTTGATGCCAGGACACGCTTTGGAGGGGATTTAC
CTGTTTTGCACCTACCAITCAGGGACAGGATGACCTGGAGAACCTTAGGTGGCAAGCTGTGACTTCTCCAGG
TCTCACGGGCATGGGCACCTCCCTTGGTGGCAAGAGCCCCCTTGACACCCGGGTGGGAAACCATGAAGAC
AXGATXGGGGCTGGCCTCTGGCTCTCATGGGGTCCAAGTTTGTGTATTCTCAACCTATTGACAGACTGAA
ACACAATATGAC

```

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	GTTAACTTCT	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

<u>HindIII</u>	-1	+1			
	ArgAla				
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	
GTTAACTTCT	ACGCTTGGA	ACGTATGGAA	GTTGGTCAAC	AAGCTGTTGA	
CAATTGAAGA	TGCGAACCTT	TGCATACCTT	CAACCAGTTG	TTCGACAAC	
AGTTTGGCAA	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	
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	MGVHECPAWLWLLSLLSPLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPTDK						
	*****	*****	*****	*****	*****	*****	*****
Monkey	MGVHECPAWLWLLSLSPLPLGLPVPGAPPRLICDSRVLERYLLEAKEAENVTMGCSECSLNENITVPTDK						

	50	60	70	80	90	100	110
Human	VNFYAWKRMEVGGQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVVDKAVSGLRSLTLLRALGAQKE						
	*****	*****	*****	*****	*****	*****	*****
Monkey	VNFYAWKRMEVGGQAVEVWQGLALLSEAVLRGQAVLANSSQPFEPLQLHMDKAISGLRSITLLRALGAQ-E						

	120	130	140	150	160
Human	AISPPDAASAAPLRTITADTFRKLLFRVYSNFLRGKLLYTGEACRTGDR				
	***	*****	*****	*****	*****
Monkey	AISLPDAASAAPLRTITADTFCCKLFRVYSNFLRGKLLYTGEACRRGDR				

**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. CCAAACCTTCAACTGCTTGTTGACCAAC  
7. TTGGCAGGGTCTGGCACTGCTGAGCG  
8. GCCTCGCTCAGCAGTGCCAGACCCTG  
9. AGGCTGTACTGCGTGGCCAGGCA  
10. GCAGTGCCTGGCCACGCAGTACA  
11. CTGCTGGTAAACTCCTCTCAGCCGT  
12. TTCCCACGGCTGAGAGGAGTTTACCA  
13. GGAACCGCTGCAGCTGCATGTTGAC  
14. GCTTTGTCAACATGCAGCTGCAGCGG  
15. AAAGCAGTATCTGGCCTGAGATCTG  
16. GATCCAGATCTCAGGCCAGATACT

FIG. 13

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

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

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

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