

# EXHIBIT C

(part 1 of 3)

**United States Patent** [19]

[11] **Patent Number:** **5,756,349**

**Lin**

[45] **Date of Patent:** **May 26, 1998**

- [54] **PRODUCTION OF ERYTHROPOIETIN**
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- [73] **Assignee:** Amgen Inc., Thousand Oaks, Calif.
- [21] **Appl. No.:** 468,369
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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".

**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.

- [51] **Int. Cl.**<sup>6</sup> ..... **C12N 15/00**
- [52] **U.S. Cl.** ..... **435/325; 435/358; 435/365**
- [58] **Field of Search** ..... 435/69.1, 69.4, 435/240.2, 240.22, 325, 358, 365

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FIG. 1

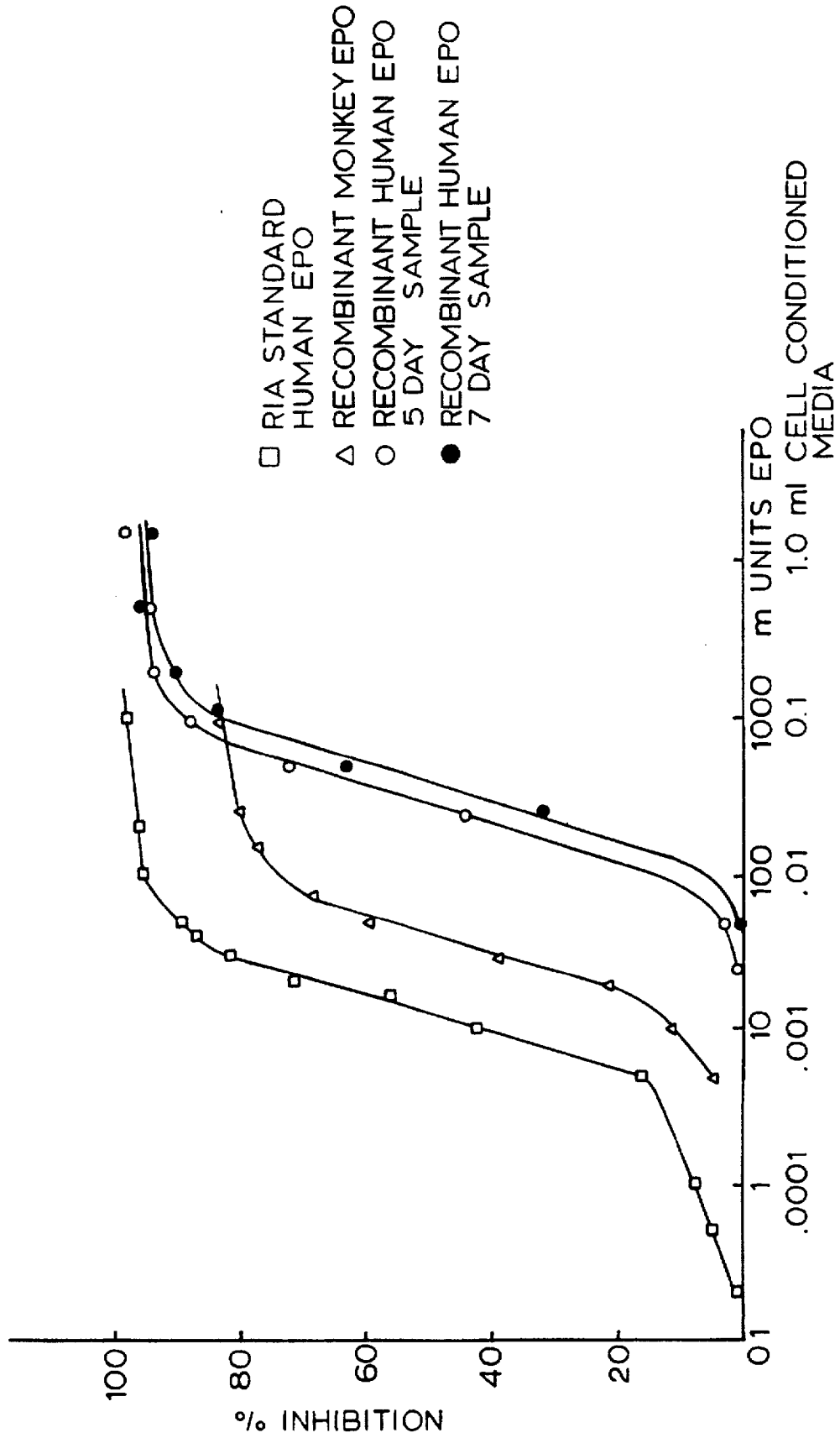


FIG. 2

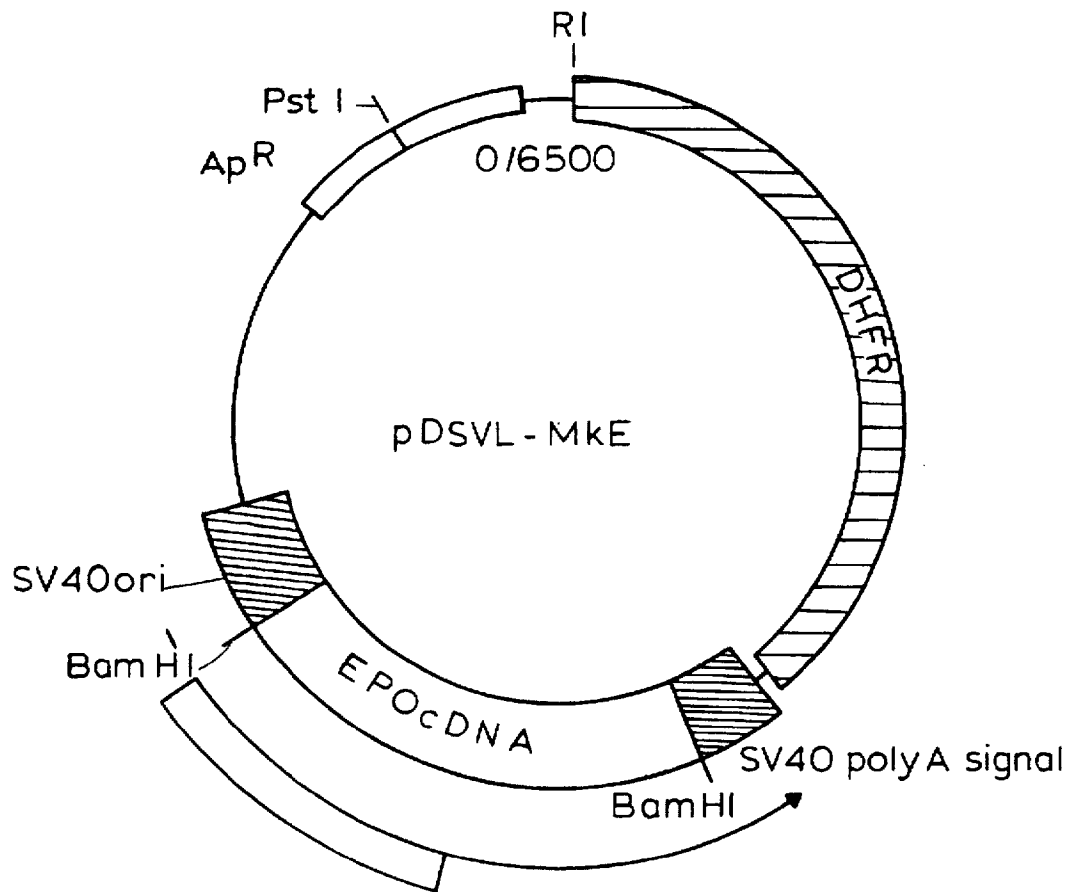


FIG. 3

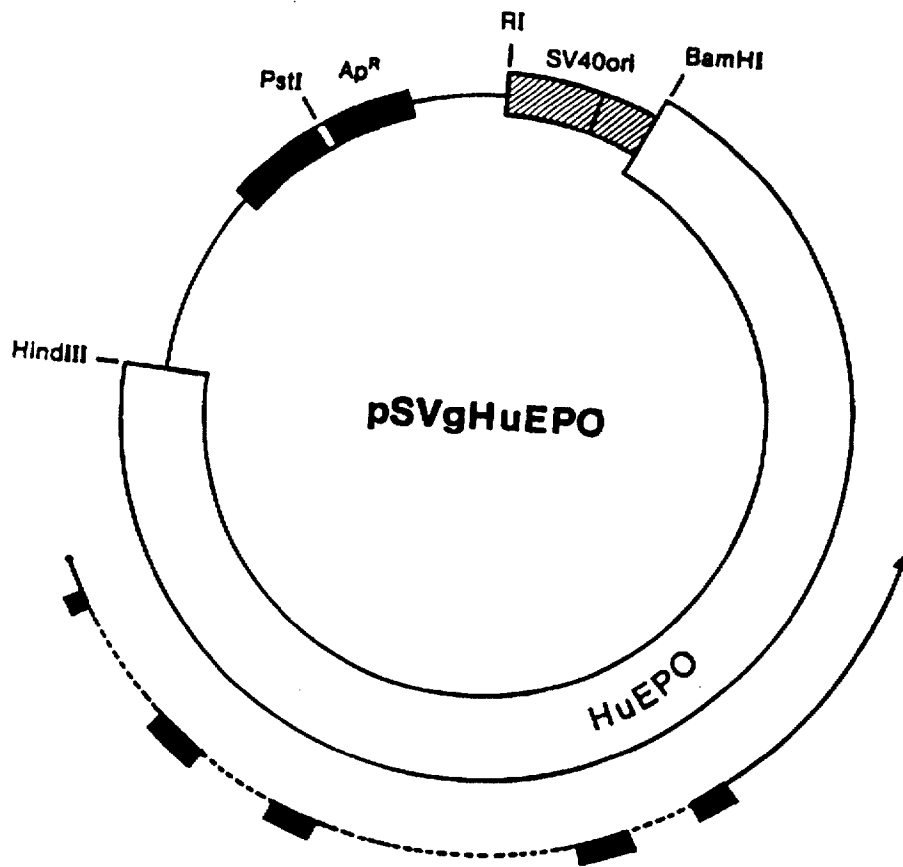


FIG. 4

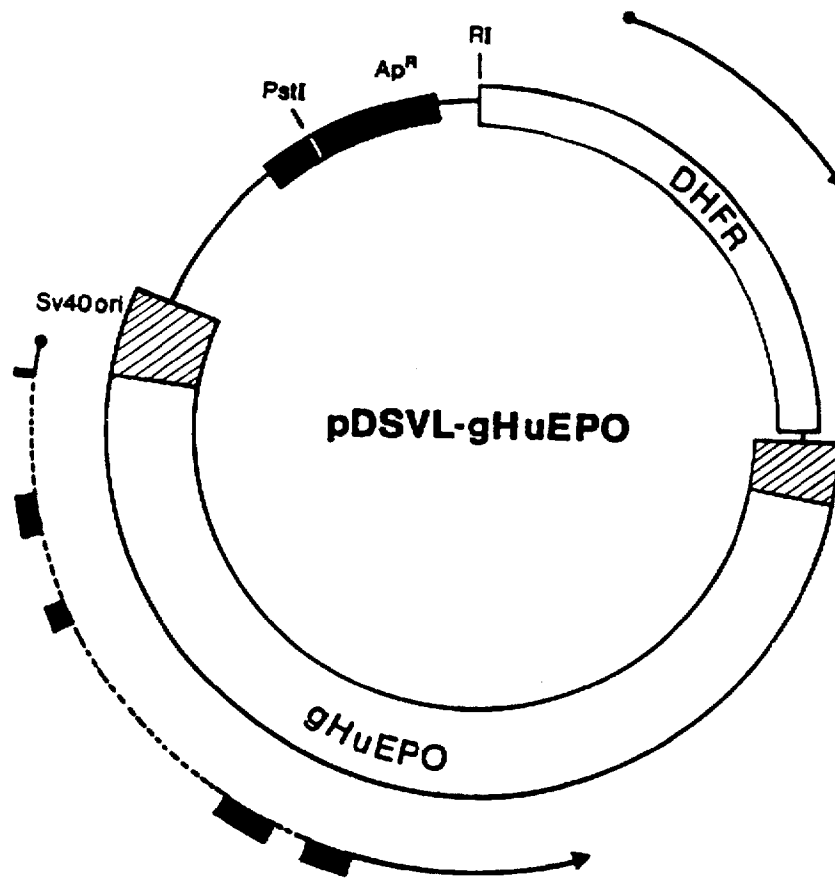




FIG. 5A

Sau3A  
 GATCCGGCCCTGGACAGCCGCCCTCTCCTCCAGGCCCGTGGGGCTGGCCCTGCCCC  
 CGCTGAACTTCCCGGATGAGGACTCCCGGTGGTCCACCGCGCCCTAGGTCGCTGAG  
 -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  
 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  
 -1 +1  
 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  
 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  
 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 AAG AGG ATG GAG GTC GGG  
 60  
 Gln Gln Ala Val Glu Val Trp Gln Gly GGC CTG GCC CTG CTC TCA GAA  
 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  
 Arg Ser Ile Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Glu Ala  
 CGC AGC ATC ACC ACT CTG CTT CGG GCG GCG CTG 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 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

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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 TAC ACG GGG GAG GCC TGC AGG AGA  
 160  
 165 Gly Asp Arg OP  
 GGG GAC AGA TGA CCAGGTGGTCCAGCTGGGCACATCCACCACCTCCCTCACCAACA  
 CTGCTGTGCCACACCCTCCCTCACCACTCCCGAACCCCATCGAGGGGCTCTCAGCTAAG  
 CGCCAGCCTGTCCCATGGACACTCCAGTCCAGCAATGACATCTCAGGGCCAGAGGAAC  
 TGTCCAGAGCACAACTCTGAGATCTAAGGATGTCCAGGGCCAACTTGAGGGCCAGAGC  
 AGGAAGCATTCAGAGAGCAGCTTTAAACTCAGGAGCAGAGACAAATGCAGGGAAAACACCT  
 GAGCTCACTCGGCCACCTGC AAAAATTTGATGCAGGACACGCTTTGGAGGCAATTTACCTG  
 TTTTGCACCTACCATCAGGGACAGGATGACTGGAGAACTTAGGTGGCAAGCTGTGACTT  
 CTC AAGGCTCACGGGCCTCCCTTGGTGGCAAGAGCCCTTGACACTGAGAGAAATATT  
 TTGCAATCTGCAGCAGGAAAATAACGGACAGGTTTTGGAGTTGGAGGGTACTTGACAG  
 GTGTGTGGGGAAGCAGGGCGGTAGGGGTGGAGCTGGATGCGAGTGAGAACCGTGAAGAC  
 AGGATGGGGCTGGCCTCTGGTTCTCGTGGGTCCAAGCTT  
 HindIII

FIG. 6A

AAGCTTCTGGGCTTCCAGACCCAGCTACTTTGGGGAACCTAGCAACCCAGGCATCTCTGAGTCTCCGCCCA  
AGACCGGATGCCCCCCCAGGGGAGGTGTCCGGGAGCCAGCCTTTCCAGATAGCACGCTCCGCCAGTCCC  
AAGGTGCGCAACCGGCTGCACTCCCCTCCCGGACCCAGGGCCCCGGGAGCAGCCCCCATGACCCACACGC  
ACGTCTGCAGACCCCGCTCACGCCCCGGGAGCCTCAACCCAGGGTCCCTGCCCTGCTCTGACCCCGG  
GTGGCCCCTACCCCTGGCGACCCCTCACGCCACACAGCCTCTCCCCCACCCCCACCCGCGCACGCACACATG  
CAGATAACAGCCCCGACCCCGCCAGAGCCGXAGATCCCTGGGCCACCCCGCCGCTCGCCTGCCGCTG  
CGCCGACCGGCTGTCCCTCCCGGAGCCGGACCCGGGGCCACCGGCCCCXGCTCTGCTCCGACACCGGCC  
CTTGGACAGCCGCCCTCTCCTCTAGGCCCGTGGGGCTGGCCCTGCACCCGCCGAGCTTCCCCGGGATGAGGXX  
  
CCCGGTGACCGGGCGGCCCAAGTCGCTGAGGGACCCCGGCCAAGCGGGAG ATG GGG GTG CAC G  
  
GTGAGTACTCGCGGGCTGGGGCTCCCGGGCCGGGTTCCTGTGAGGGGATTTAGCGCCCCCGGCT

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Met Gly Val His

FIG.6B

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ATTGGCCAAGAGGTGGTCAAGGACCCGGGACTGTCAAGGACCCCGAAGGGGGAGGGGGTGGG
GCAGCCTCCACGTGCCGGGACTTGGGGAGTCTTGGGGATGGCAAAAACCTGGCCTGTGAGGGGCA
CAGTTGGGGTTGGGAGGAGTTGGGGTTCTGCTGTGCAGTTGTGCTGTTGTCAAGTGTCTCG [I · S · ]
TTGCACACGCACAGATCAATAAGCCAGAGGCACACCTGAGTGTGTCATGGTTGGGACAGGAAGGACGAG
CTGGGGCAGAGACGTGGGGATGAAGGAGTGTCTTCCACAGCCACCCCTTCCCCCCCCCTGACTCT
-23          -20
Glu Cys Pro Ala Trp Leu Trp Leu Leu Leu Ser Leu
AA TGT CCT GCC TGG CTG TGG CTT CTC CTG TCC CTG
CAGCCTGGCTATCTGTTCTAG          -1  +1
Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Leu Ala Pro Pro Arg Leu Ile Cys
CTG TCG CTC CCT CTG GGC CTC CCA GTC CTG GGC GCC CCA CCA CGC CTC ATC TGT
-10          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 GTGAGACCCCTTCCCCAGCACATTCACAGAACTCACGCTCAGGGCTTCAGGGAACCTCTCCAGAT
CCAGGAACCTGGCACTTGGTTTGGGGTGGAGTGGGAAGCTAGACACTGCCCCCTACATAAGAATAAGTC

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FIG. 6C

TGGTGGCCCCAACCATACTGAAACTAGGCAAGGAGCAAGCCAGCAGATCCTACGCCCTGTGGGCCAGGG

CCAGAGCCTTCAGGGACCCTTGACTCCCGGGCTGTGTGCATTTCAG                                    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  
Ala Trp Lys Arg Met Glu                                    55  
GCC TGG AAG AGG ATG GAG GTGAGTTCCTTTTTTTTTTTTTTTTTTCTTTTGGAGAATCTCATT

TGCGAGCCTGATTTGGATGAAAGGGAGAATGATCGGGGAAAGGTAAAATGGAGCAGCAGAGATGAGGCT

GCCTGGGCCAGAGGCTCACGTCTATAATCCCAGGCTGAGATGGCCCGAGATGGGAGAATTGCTTGAGCCCT

GGAGTTTCAGACCAACCTAGGCAGCATAGTGAGATCCCCCATCTCTACAACATTTAAAAAAATTAGTCAG

GTGAAAGTGGTGCAATGGTGGTAGTCCCAGATAATTTGGAAGGCTGAGCGGGAGGATCGCTTGAGCCCAGGAA

TTTGAGGCTGCAGTGAGCTGTGATCACCACCACTGCACCTCCAGCTCAGTGACAGAGTGAGGCCCTGTCTCA

FIG. 6D

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AAAAAGAAAAGAAAAGAAAATAATGAGGGCTGTATGGAATACATTTCATTTCACCTCACTCACT
CACTCATTCATTTCATTTCATTCAACAAGTCTTATTGCATACCTTCTGTGTTGCTCAGCITGGTGTGG
GGCTGCTGAGGGGCAGGAGGGGACATGGGTGACATGGGTGACCTCGACTCCAGAGTCCACTCCCTGTAG

56          60          70
Val Gly 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 CTG GCC CTG TCG TCG GAA GCT

Val Leu Arg Gly Gln Ala Leu Leu Val Trp Gln 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

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
Arg Ala Leu Gly Ala Gln
CGG GCT CTG GGA GCC CAG GTGAGTAGGAGGGACACTTCTGTGCTTCCCTTCTGTGTAAGAAGGGA

110          115
GAAAGGGTCTTGCTAAGGAGTACAGGAACTGTCCGTATTCCTTCCCTTCTGTGSCACTGCAGGCCCTCCT

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 CCAGGTGTCCACCCTGGGCATATCCACCCTCCCTCACCACATTTGTTGTGCCACA  
 CCTCCCCGGCCACTCCTGAACCCCGTCGAGGGGCTCTCAGCTCAGGCCAGCCTGTCCCCATGGACACTCC  
 AGTGCCAGCAATGACATCTCAGGGCCAGAGGAACTGTCCAGAGAGCAACTCTGAGATCTAAGGATGTCCAC  
 AGGCCAACTTGAAGGGCCAGAGCAGGAAGCATTCAGAGAGCAGCTTTAAACTCAGGGACAGAGCCCATGC  
 TGGGAAGACGCCCTGAGCTCAGCTCGGCACCCCTGCAAAATTTGATGCCAGGACACCGCTTTGGAGGCCGATTAC  
 CTGTTTTCGCACCTACCATCAGGGACAGGATGACCTGGAGAACTTAGGTGGCAAGCTGTGACTTCTCCAGG  
 TCTCAGGGCATGGGCACCTCCCTTGGTGGCAAGAGCCCTTGACACCCGGGGTGGGAACCATGAAGAC  
 AXGATXGGGGCTGGCCCTCTGGCTCTCATGGGGTCCAAGTTTGTGTATTCTCAACCTATTGACAGACTGAA  
 ACACAATATGAC



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FIG. 7

<u>XbaI</u>	-1	1	<u>MetAla</u>
CTAG AAACCATGAG GGTAATAAAA TAATGGCTCC GCCGCGTCTG			
TTTGGTACTC CCATTATTTT ATTACCGAGG CGGCGCAGAC			
ATCTGCGACT CGAGAGTTCT GGAACGTTAC CTGCTGGAAG CTAAGAAGC			
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 AAATCCTCT CAGCCGTGGG			
CCGACATGAC GCACCGGTCC GTGACGACCA TTTGAGGAGA GTCGGCACCC			
AACCGCTGCA GCTGCATGTT GACAAAGCAG TATCTGGCCT GAGATCTCTG			
TTGGCGACGT CGACGTACAA CTGTTTCGTC ATAGACCGGA CTCTAGAGAC			
ACTACTCTGC TCGTGCTCT GGGTGCACAG AAAGAGGCTA TCTCTCCGCC			
TGATGAGACG ACGCACGAGA CCCACGTGTC TTTCTCCGAT AGAGAGGCGG			
GGATGCTGCA TCTGCTGCAC CGCTGCGTAC CATCACTGCT GATACCTTCC			
CCTACGACGT AGACGACGTG GCGACGCATG GTAGTGACGA CTATGGAAGG			
GCAAACCTGTT TCGTGTATAC TCTAACTTCC TCGTGGTAA 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	
GTTAACTTCT	ACGCTTGGAA	ACGTATGGAA	GTTGGTCAAC	AAGCTGTTGA	
CAATTGAAGA	TGCGAACCTT	TGCATACCTT	CAACCAGTTG	TTCGACAACT	
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	AGAGACCAA	CTCTAGAAAC	TGATGAAACA	ACTCTCGAAA	
GGGTGCTCAA	AAGGAAGCCA	TTTCCCACC	AGACGCTGCT	TCTGCCGCTC	
CCCACGAGTT	TTCCTTCGGT	AAAGGGGTGG	TCTGCGACGA	AGACGGCGAG	
CATTGAGAAC	CATCACTGCT	GATACCTTCA	GAAAGTTATT	CAGAGTTTAC	
GTAActCTTG	GTAGTGACGA	CTATGGAAGT	CTTTCAATAA	GTCTCAAATG	
TCCAActTCT	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	MGVHECPAWLWLLSLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVDPDK						
	*****	*****	*****	*****	*****	*****	*****
Monkey	MGVHECPAWLWLLSLSLPLGLPVPGAPPRLICDSRVLERYLLEAKEAENVTMGCSSECSLNENITVDPDK						

	50	60	70	80	90	100	110
Human	VNFYAWKRMEVGQQAQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSITLLRALGAQKE						
	*****	*****	*****	*****	*****	*****	*****
Monkey	VNFYAWKRMEVGQQAQAVEVWQGLALLSEAVLRGQAVLANSSQPFEPQLHMDKAISGLRSITLLRALGAQ-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.          CAAAGAACAGTGTTTCAGCACAACCA  
11.          TTTGAACGAAAACATTACGGTACCG  
12.          GATCCGGTACCGTAATGTTTTTCGTT

FIG. 11

XbaI  
EcoRI  
AATTCTAG AAACCATGAG 1 GGTAATAAAA TAATGGCTCC 3 GCCGCGTCTG  
GATC TTTGGTACTC 2 CCATTATTTT ATTACCGAGG CGGCGCAGAC 4

5  
ATCTGCGACT CGAGAGTTCT GGAACGTTAC CTGCTGGAAG CTAAAGAAGC  
TAGACGCTGA GCTCTCAAGA CCTTGCAATG GACGACCTTC GATTTCTTCG 6

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

KpnI 0 BamHI  
TTACGGTACC G  
AATGCCATGG CCTAG 12