

EXHIBIT C
PART 1 OF 2



US005547933A

United States Patent [19]

[11] **Patent Number:** **5,547,933**

Lin

[45] **Date of Patent:** **Aug. 20, 1996**

- [54] **PRODUCTION OF ERYTHROPOIETIN**
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- [21] Appl. No.: **487,774**
- [22] Filed: **Jun. 7, 1995**

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Related U.S. Application Data

- [63] Continuation of Ser. No. 202,874, Feb. 28, 1994, abandoned, which is a continuation of Ser. No. 113,178, Oct. 23, 1987, abandoned, 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, 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. 561,024, Dec. 13, 1983, abandoned.
- [51] **Int. Cl.⁶** **A61K 38/18; C12P 21/02**
- [52] **U.S. Cl.** **514/8; 435/686; 530/388.7; 530/397; 530/835**
- [58] **Field of Search** **435/69.1, 69.4, 435/69.6, 240.2, 320.1, 172.3, 13; 530/350, 380, 397, 834, 835, 23.5, 23.51, 388.7; 514/8**

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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".

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FIG. 1

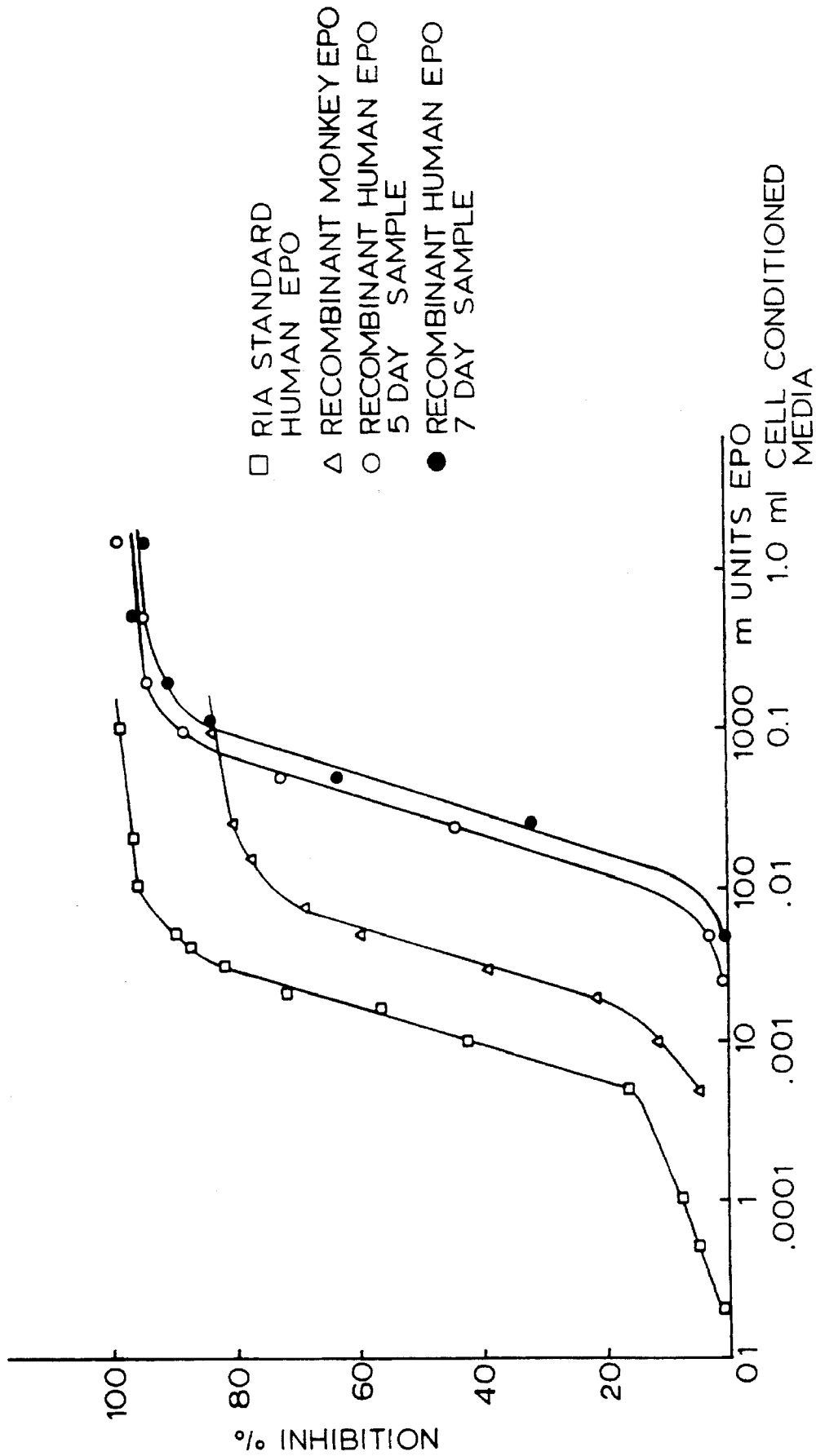


FIG. 2

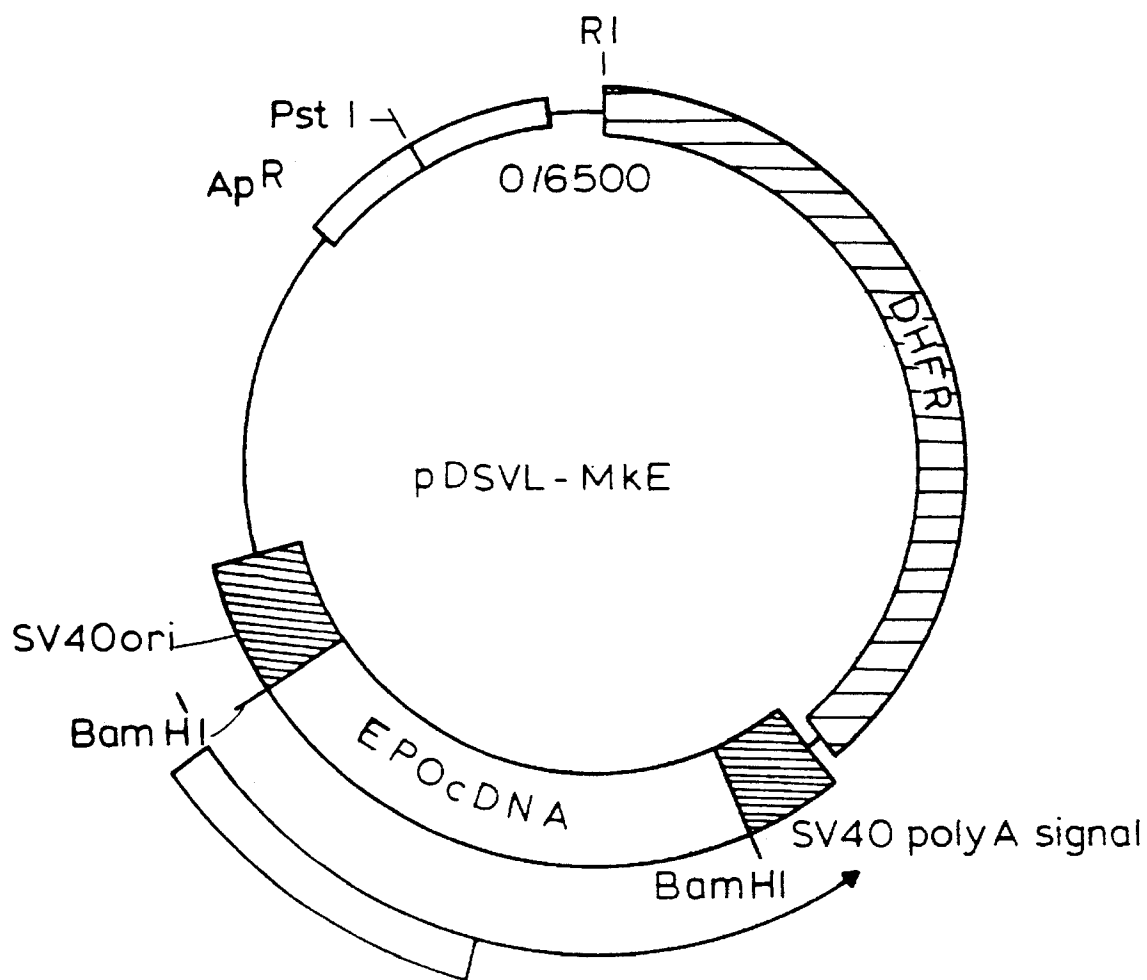


FIG. 3

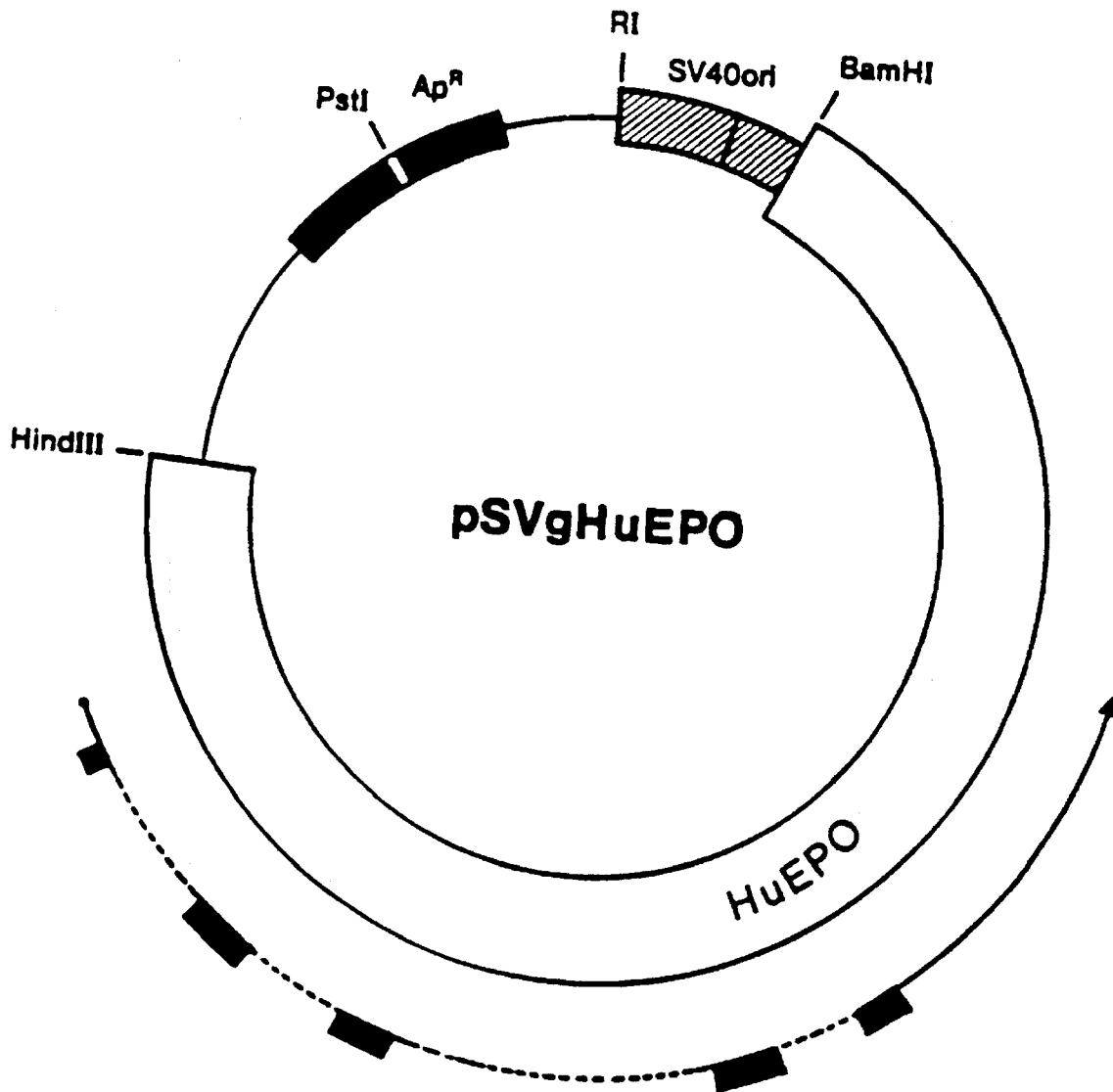


FIG. 4

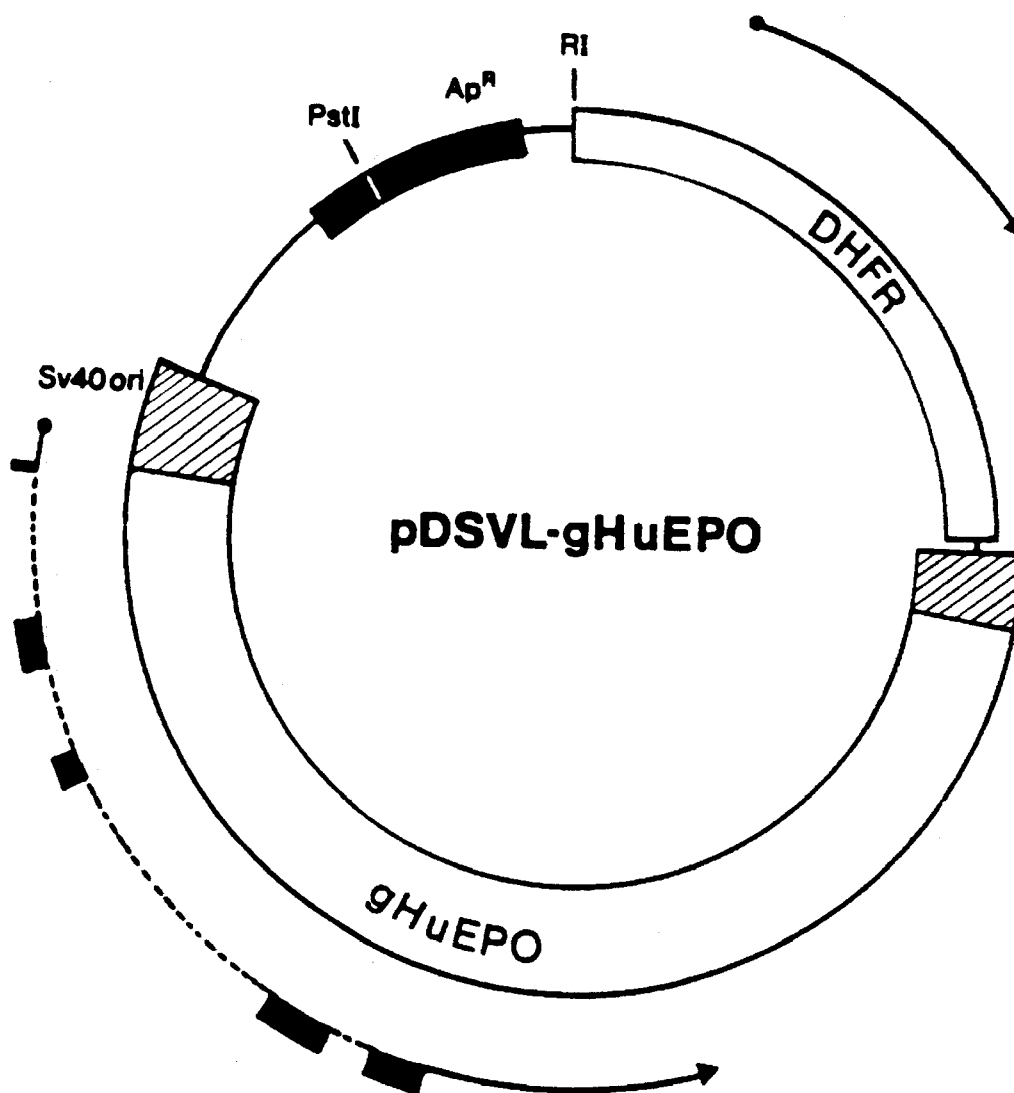


FIG. 5A

Sau3A

GATCCCGCGCCCCCTGGACAGCCGCCCTCTCCTCCAGGCCCGTGGGGCTGGCCCTGCC

CGCTGAACTTCCCGGGATGAGGACTCCCGGTGTGGTCACCGCGCGCCTAGGTCTGCTGAG

-27

-20

Met Gly Val His Glu Cys Pro Ala Trp

GGACCCCGGCCAGGCGCGGAGATG GGG GTG CAC GAA TGT CCT GCC TGG

-10

**Leu Trp Leu 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

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

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**

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												70
Gln	Gln	Ala	Val	Glu	Val	Trp	Gln	Gly	Leu	Ala	Leu	Leu	Ser	Glu	
CAG	CAG	GCT	GTA	GAA	GTC	TGG	CAG	GGC	CTG	GCC	CTG	CTC	TCA	GAA	
							80				*				
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	
			90												100
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	
							110								
Arg	Ser	Ile	Thr	Thr	Leu	Leu	Arg	Ala	Leu	Gly	Ala	Gln	Glu	Ala	
CGC	AGC	ATC	ACC	ACT	CTG	CTT	CGG	GCG	CTG	GGA	GCC	CAG	GAA	GCC	
			120												130
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	
							140								
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	

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 CCAGGTGCGTCCAGCTGGGCACATCCACCACCTCCCTCACCAACA
 CTGCCTGTGCCACACCCTCCCTCACCCTCCCGAACCCCATCGAGGGGCTCTCAGCTAAG
 CGCCAGCCTGTCCCATGGACACTCCAGTGCCAGCAATGACATCTCAGGGGCCAGAGGAAC
 TGTCCAGAGCACAACCTCTGAGATCTAAGGATGTCGCAGGGCCAACTTGAGGGCCCAGAGC
 AGGAAGCATTTCAGAGAGCAGCTTTAAACTCAGGAGCAGAGACAATGCAGGGAAAACACCT
 GAGCTCACTCGGCCACCTGC AAAATTGATGCAGGACACGCTTTGGAGGCAATTTACCTG
 TTTTTCACCTACCATCAGGGACAGGATGACTGGAGA ACTTAGGTGGCAAGCTGTGACTT
 CTC AAGGCCTCACGGGCACTCCCTTGGTGGCAAGAGCCCCCTTGACACTGAGAGAATATT
 TTGCAATCTGCAGCAGGAAAATTACGGACAGGTTTTGGAGGTTGGAGGGTACTTGACAG
 GTGfGTGGGG AAGCAGGGCGGJAGGGGTGGAGCTGGGATGCGAGfGAGAACCGTGAAGAC
 AGGATGGGGGCTGGCCTCTGGTTCTCGTGGGGTCCAAGCTT
 HindIII

FIG.6B

ATTGGCCAAGAGGTGGCTGGGTTCAAGGACCGGCGACTTGTCAAGGACCCCGGAAGGGGGAGGGGGGTGGG
GCAGCCTCCACGTGCCGCGGGGACTTGGGGGAGTTCTTGGGGATGGCAAAAACCTGGCCTGTTGAGGGGCA
CAGTTTGGGGTTGGGGAGGAGGTTTGGGGTCTGCTGTGCAGTTGTGTCGTTGTCAAGTGTCTCG [I · S ·]
TTGCACACGCACAGATCAATAAGCCAGAGGCAGCACCTGAGTGCTTGCATGGTTGGGACAGGAAGGACGAG
CTGGGGCAGAGACGTGGGGATGAAGGAAGCTGTCCTTCCACAGCCACCCTTCTCCCCCCCCGCCTGACTCT

-23 -20
CAGCCTGGCTATCTGTTCTAG **AA TGT CCT GCC TGG CTG TGG CTT CTC CTG TCC CTG**
Glu Cys Pro Ala Trp Leu Trp Leu Leu Leu Ser Leu

-10 -1 +1
Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly 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 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 GTGAGACCCTTCCCCAGCACATTCCACAGA ACTCACGCTCAGGGCTTCAGGGAACTCCTCCCAGAT
CCAGGAACCTGGCACTTGGTTTGGGGTGGAGTTGGGAAGCTAGACACTGCCCCCTACATAAGAATAAGTC

FIG.6C

TGGTGGCCCCAAACCATACCTGAAACTAGGCAAGGAGCAAAGCCAGCAGATCCTACGCCTGTGGGCCAGGG

CCAGAGCCTTCAGGGACCCTTGACTCCCCGGGCTGTGTGCATTTAG 27
Thr Gly Cys Ala Glu
ACG GGC TGT GCT GAA

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 GTGAGTTCCTTTTTTTTTTTTTTTTTCCTTCTTTTGGAGAATCTCATT

TGCGAGCCTGATTTTGGATGAAAGGGAGAATGATCGGGGGAAAGGTAAAATGGAGCAGCAGAGATGAGGCT

GCCTGGGCGCAGAGGCTCACGTCTATAATCCCAGGCTGAGATGGCCGAGATGGGAGAATTGCTTGAGCCCT

GGAGTTTCAGACCAACCTAGGCAGCATAGTGAGATCCCCCATCTCTACAAACATTTAAAAAAATTAGTCAG

GTGAAGTGGTGCATGGTGGTAGTCCCAGATATTTGGAAGGCTGAGGCGGGAGGATCGCTTGAGCCCAGGAA

TTTGAGGCTGCAGTGAGCTGTGATCACACCACTGCACTCCAGCCTCAGTGACAGAGTGAGGCCCTGTCTCA

FIG. 6D

AAAAAGAAAAGAAAAAGAAAAATAATGAGGGCTGTATGGAATACATTCATTATTTCATTCACTCACTCACT
CACTCATTTCATTTCATTTCATTTCATTCAACAAGTCTTATTGCATACCTTCTGTTTGCTCAGCTTGGTGCTTGG
GGCTGCTGAGGGGGCAGGAGGGGAGAGGGGTGACATGGGTCAGCTCGACTCCCAGAGTCCACTCCCTGTAG

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 CTG GCC CTG CTG TCG GAA GCT

80 * 90
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

100
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 GTGAGTAGGAGCGGACACTTCTGCTTGCCCTTTCTGTAAGAAGGGGA

GAAGGGTCTTGCTAAGGAGTACAGGAACTGTCCGTATTCCCTTCCCTTTCTGTGGCACTGCAGCGACCTCCT

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

FIG. 6E

130		140															
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

	150		160														
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

166

Asp Arg OP

GAC AGA TGA CCAGGTGTGTCCACCTGGGCATATCCACCACCTCCCTCACCAACATTGCTTGTGCCACA

CCCTCCCCCGCCACTCCTGAACCCCGTCGAGGGGCTCTCAGCTCAGCGCCAGCCTGTCCCATGGACACTCC

AGTGCCAGCAATGACATCTCAGGGGCCAGAGGAACTGTCCAGAGAGCAACTCTGAGATCTAAGGATGTCAC

AGGGCCAAC TTGAAGGGCCAGAGCAGGAAGCATT CAGAGAGCAGCTTTAAACTCAGGGACAGAGCCATGC

TGGGAAGACGCCTGAGCTCACTCGGCACCCTGCAAATTTGATGCCAGGACACGCTTTGGAGGCGATTTAC

CTGTTTTCGCACCTACCATCAGGGACAGGATGACCTGGAGA ACTTAGGTGGCAAGCTGTGACTTCTCCAGG

TCTCACGGGCATGGGCACTCCCTTGGTGGCAAGAGCCCCCTTGACACCGGGGTGGTGGGAACCATGAAGAC

AXGATXGGGGCTGGCCTCTGGCTCTCATGGGGTCCAAGTTTTGTGTATTCTCAACCTATTGACAGACTGAA

ACACAATATGAC

FIG. 7

<u>XbaI</u>	-1	1	<u>NotI</u>
CTAG AAACCATGAG GGTAATAAAA TAATGGCTCC GCCGCGTCTG			
TTTGGTACTC CCATTATTTT ATTACCGAGG CCGCGCAGAC			
ATCTGCGACT CGAGAGTTCT GGAACGTTAC CTGCTGGAAAG CTAAGAAGC			
TAGACGCTGA GCTCTCAAGA CCTTGCAATG GACGACCTTC GATTTCTTCG			
TGAAAACATC ACCACTGGTT GTGCTGAACA CTGTTCTTTG AACGAAAACA			
ACTTTTGTAG TGGTGACCAA CACGACTTGT GACAAGAAAC TTGCTTTTGT			
TTACGGTACC AGACACCAAG GTTAACTTCT ACGCTTGGAA ACGTATGGAA			
AATGCCATGG TCTGTGGTTC CAATTGAAGA TCGAACCTT TGCATACCTT			
GTGGTCAAC AAGCAGTTGA AGTTTGGCAG GGTCTGGCAC TGCTGAGCGA			
CAACCAAGTTG TTCGTCAACT TCAAACCGTC CCAGACCGTG ACGACTCGCT			
GGCTGTACTG CGTGGCCAGG CACTGCTGGT AAATCCTCT CAGCCGTGGG			
CCGACATGAC GCACCGGTCC GTGACGACCA TTTGAGGAGA GTCCGCACCC			
AACCCTGCA GCTGCATGTT GACAAAGCAG TATCTGGCCT GAGATCTCTG			
TTGGCGACGT CGACGTACAA CTGTTTCGTC ATAGACCGGA CTCTAGAGAC			
ACTACTCTGC TGCCTGCTCT GGGTGCACAG AAAGAGGCTA TCTCTCCGCC			
TGATGAGACG ACGCACGAGA CCCACGTGTC TTTCTCCGAT AGAGAGGCGG			
GGATGCTGCA TCTGCTGCAC CGCTGCGTAC CATCACTGCT GATACCTTCC			
CCTACGACGT AGACGACGTG GCGACGCATG GTAGTGACGA CTATGGAGG			
GCAAAGTGT TCGTGTATAC TCTAACTTCC TCGGTGGTAA ACTGAAACTG			
CGTTTGACAA AGCACATATG AGATTGAAGG ACGCACCATT TGACTTTGAC			
TATACTGGCG AAGCATGCCG TACTGGTGAC CGCTAATAG			<u>SalI</u>
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	TAGACTCTGA	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	
CCTTGTGGT	TAACTCTTCT	CAACCATGGG	AACCATTGCA	ATTGCACGTC	
GGAAACAACCA	ATTGAGAAGA	GTTGGTACCC	TTGGTAACGT	TAACGTGCAG	
GATAAAGCCG	TCTCTGGTTT	GAGATCTTTG	ACTACTTTGT	TGAGAGCTTT	
CTATTTCCGGC	AGAGACCAA	CTCTAGAAAC	TGATGAAACA	ACTCTCGAAA	
GGGTGCTCAA	AAGGAAGCCA	TTTCCCCACC	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		

SaI

ATGTAACAAA G
TACATTGTTT CAGCT

FIG. 9

	-20	-10	+1	10	20	30	40	
Human	MGVHECPAWLWLLLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITGCAEHCSLNENITVPDTK							

Monkey	MGVHECPAWLWLLLSLVSLPLGLPVPGAPPRLICDSRVLERYLLEAKEAENVTMGCSESCSLNENITVPDTK							
	50	60	70	80	90	100	110	
Human	VNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTLLRALGAQKE							

Monkey	VNFYAWKRMEVGQQAVEVWQGLALLSEAVLRGQAVLANSSQPFEPLQLHMDKAISGLRSITLLRALGAQ-E							
	120	130	140	150	160			
Human	AISPPDAASAAPLRTITADTFRKLFVYSNFLRGKLYTGEACRTGDR							

Monkey	AISLPDAASAAPLRTITADTFCKLFRVYSNFLRGKLYTGEACRRGDR							

FIG. 10

1. **AATCTAGAAACCATGAGGGTAATAAAATA**
2. **CCATTATTTTATTACCCTCATGGTTTCTAG**
3. **ATGGCTCCGCCCGCGTCTGATCTGCGAC**
4. **CTCGAGTCGCAGATCAGACGCGGGCGGAG**
5. **TCGAGAGTTCTGGAACGTTACCTGCTG**
6. **CTTCCAGCAGGTAACGTTCCAGAACT**
7. **GAAGCTAAAGAAGCTGAAAACATC**
8. **GTGGTGATGTTTTTCAGCTTCTTTAG**
9. **ACCACTGGTTGTGCTGAACACTGTTT**
10. **CAAAGAACAGTGTTTCAGCACCAACCA**
11. **TTTGAACGAAAACATTACGGTACCG**
12. **GATCCGGTACCGTAATGTTTTTCGTT**

FIG. 11

XbaI
EcoRI 1 3
AATTCTAG AAACCATGAG GGTAAATAAAA TAATGGCTCC GCCCGCTCTG
GATC TTTGGTACTC CCATTATTTT ATTACCGAGG CGGCGCAGAC
 2 4
 5
ATCTGCGACT CGAGAGTTCT GGAACGTTAC CTGCTGGAAG CTAAGAAGC
TAGACGCTGA GCTCTCAAGA CCTTGCAATG GACGACCTTC GATTTCTTCG
 6
 7 9 11
TGAAAACATC ACCACTGGTT GTGCTGAACA CTGTTCTTTG AACGAAAACA
ACTTTTGTAG TGGTGACCAA CACGACTTGT GACAAGAAAC TTGCTTTTGT
 8 10
 0
KpnI BanHI
TTACGGTACC G
AATGCCATGG CCTAG
 12

FIG. 12

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