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[54] **PRODUCTION OF ERYTHROPOIETIN**

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0117060	8/1984	European Pat. Off. .
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86/03520	6/1986	WIPO .

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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.

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[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".

14 Claims, 27 Drawing Sheets

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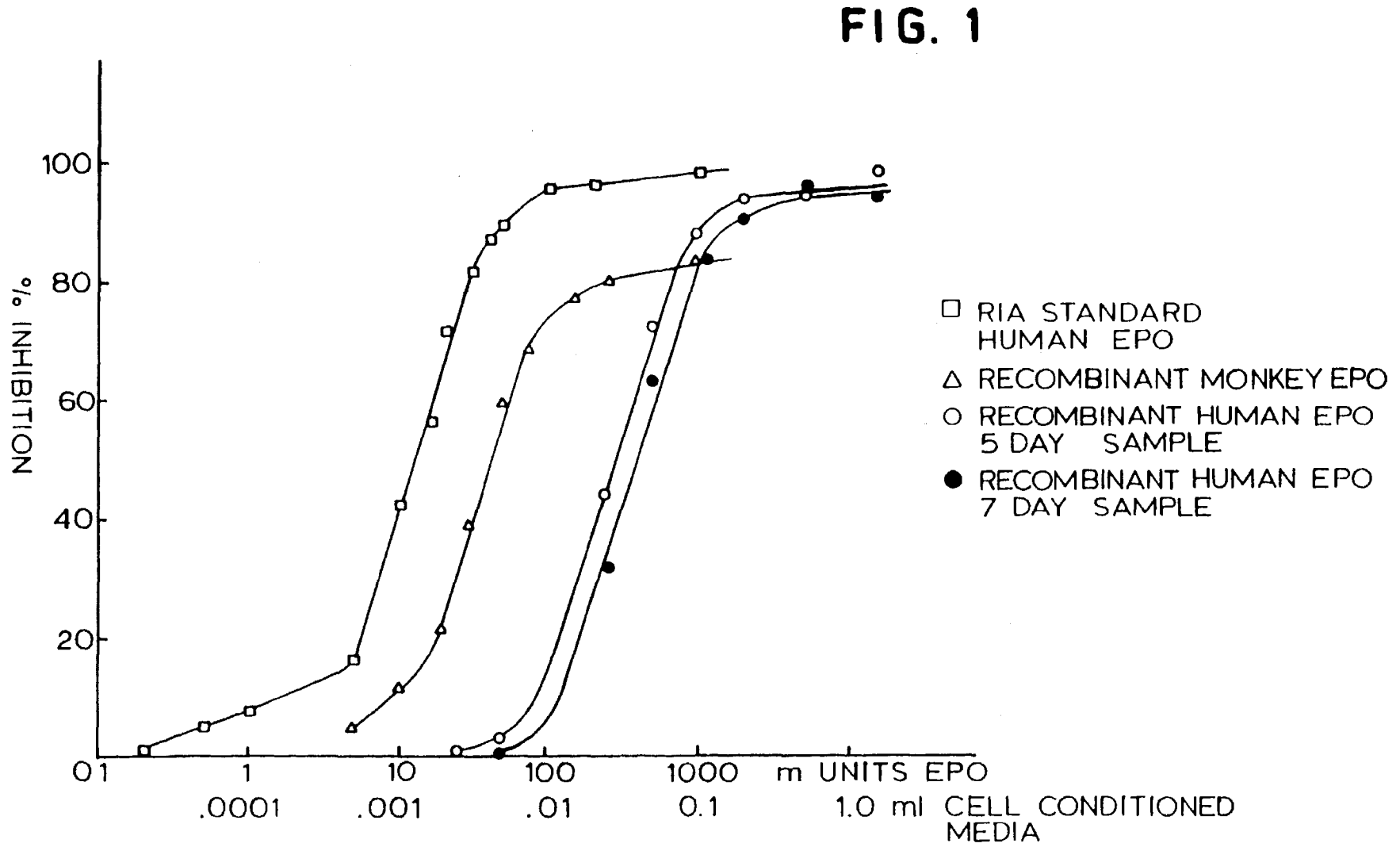


FIG. 2

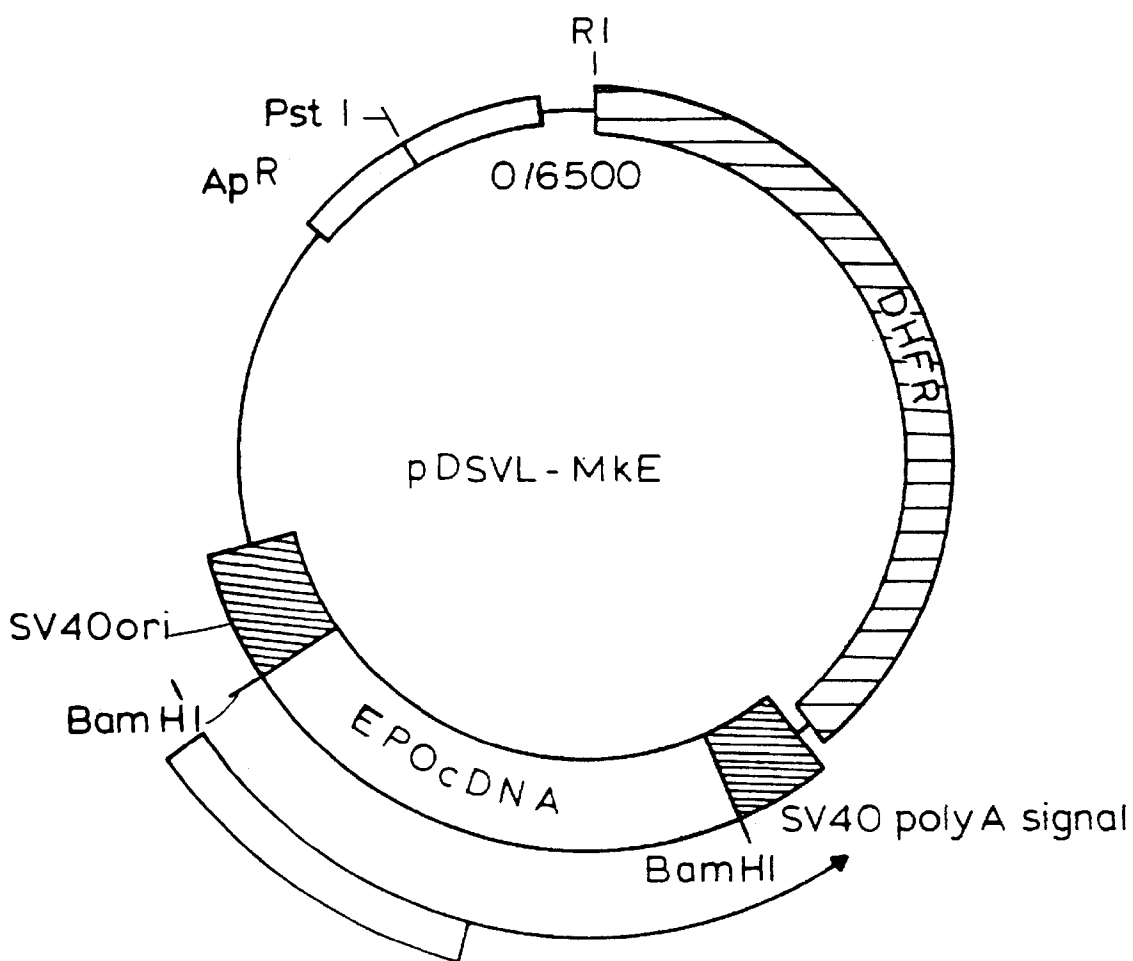


FIG. 3

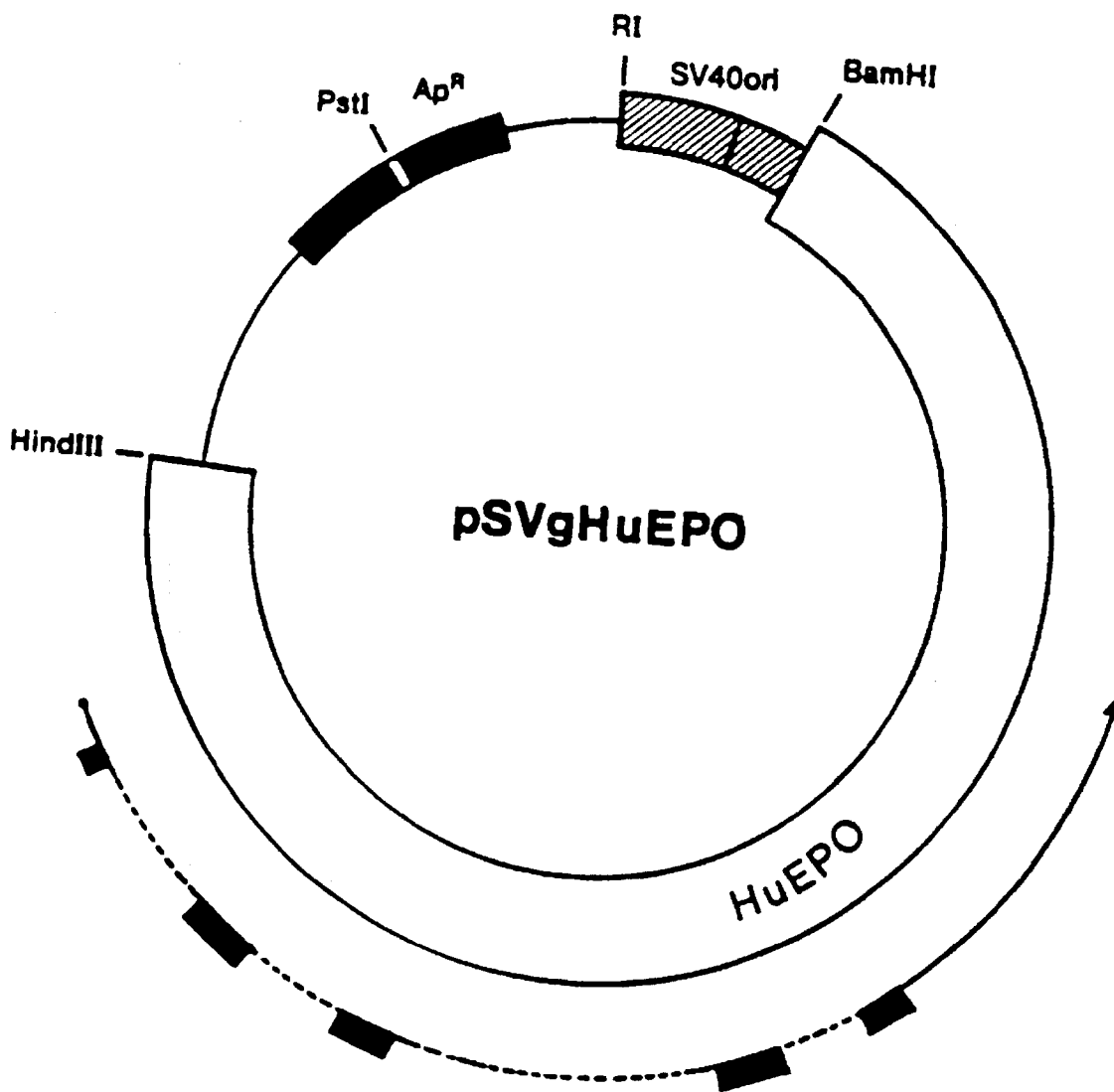
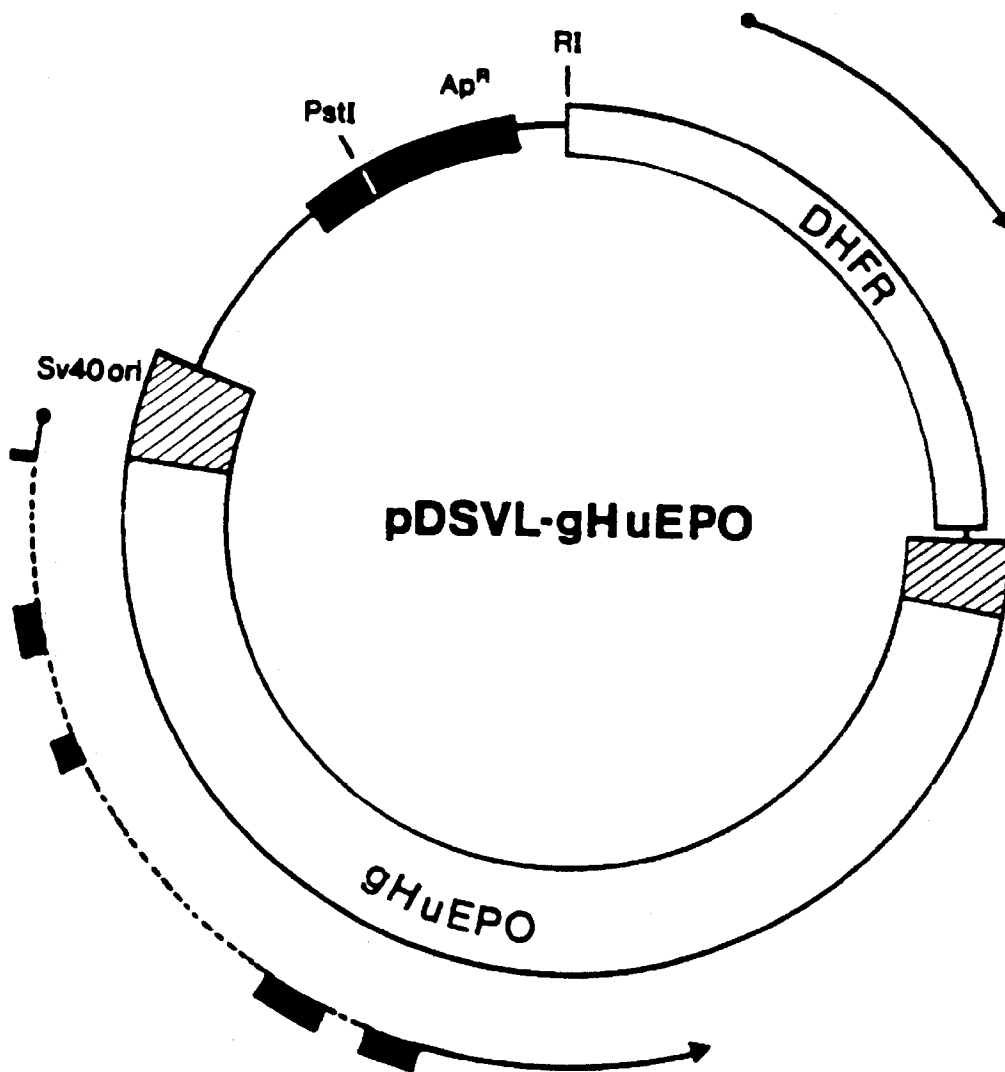


FIG. 4



**FIG. 5A**

**Sau3A**

**GATCCCGCGCCCCCTGGACAGCCGCCCTCTCCTCCAGGCCCGTGGGGCTGGCCCTGCCC**

**CGCTGAACTTCCCGGGATGAGGACTCCCGGTGTGGTCACCGCGCGCCTAGGTGCTGAG**

**-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**

							<b>50</b>										
<b>Asp</b>	<b>Thr</b>	<b>Lys</b>	<b>Val</b>	<b>Asn</b>	<b>Phe</b>	<b>Tyr</b>	<b>Ala</b>	<b>Trp</b>	<b>Lys</b>	<b>Arg</b>	<b>Met</b>	<b>Glu</b>	<b>Val</b>	<b>Gly</b>			
<b>GAC</b>	<b>ACC</b>	<b>AAA</b>	<b>GTT</b>	<b>AAC</b>	<b>TTC</b>	<b>TAT</b>	<b>GCC</b>	<b>TGG</b>	<b>AAG</b>	<b>AGG</b>	<b>ATG</b>	<b>GAG</b>	<b>GTC</b>	<b>GGG</b>			
			<b>60</b>														
<b>Gln</b>	<b>Gln</b>	<b>Ala</b>	<b>Val</b>	<b>Glu</b>	<b>Val</b>	<b>Trp</b>	<b>Gln</b>	<b>Gly</b>	<b>Leu</b>	<b>Ala</b>	<b>Leu</b>	<b>Leu</b>	<b>Ser</b>	<b>Glu</b>			
<b>CAG</b>	<b>CAG</b>	<b>GCT</b>	<b>GTA</b>	<b>GAA</b>	<b>GTC</b>	<b>TGG</b>	<b>CAG</b>	<b>GGC</b>	<b>CTG</b>	<b>GCC</b>	<b>CTG</b>	<b>CTC</b>	<b>TCA</b>	<b>GAA</b>			
							<b>80</b>										
<b>Ala</b>	<b>Val</b>	<b>Leu</b>	<b>Arg</b>	<b>Gly</b>	<b>Gln</b>	<b>Ala</b>	<b>Val</b>	<b>Leu</b>	<b>Ala</b>	<b>Asn</b>	<b>Ser</b>	<b>Ser</b>	<b>Gln</b>	<b>Pro</b>			
<b>GCT</b>	<b>GTC</b>	<b>CTG</b>	<b>CGG</b>	<b>GGC</b>	<b>CAG</b>	<b>GCC</b>	<b>GTG</b>	<b>TTG</b>	<b>GCC</b>	<b>AAC</b>	<b>TCT</b>	<b>TCC</b>	<b>CAG</b>	<b>CCT</b>			
			<b>90</b>														
<b>Phe</b>	<b>Glu</b>	<b>Pro</b>	<b>Leu</b>	<b>Gln</b>	<b>Leu</b>	<b>His</b>	<b>Met</b>	<b>Asp</b>	<b>Lys</b>	<b>Ala</b>	<b>Ile</b>	<b>Ser</b>	<b>Gly</b>	<b>Leu</b>			
<b>TTC</b>	<b>GAG</b>	<b>CCC</b>	<b>CTG</b>	<b>CAG</b>	<b>CTG</b>	<b>CAC</b>	<b>ATG</b>	<b>GAT</b>	<b>AAA</b>	<b>GCC</b>	<b>ATC</b>	<b>AGT</b>	<b>GGC</b>	<b>CTT</b>			
							<b>110</b>										
<b>Arg</b>	<b>Ser</b>	<b>Ile</b>	<b>Thr</b>	<b>Thr</b>	<b>Leu</b>	<b>Leu</b>	<b>Arg</b>	<b>Ala</b>	<b>Leu</b>	<b>Gly</b>	<b>Ala</b>	<b>Gln</b>	<b>Glu</b>	<b>Ala</b>			
<b>CGC</b>	<b>AGC</b>	<b>ATC</b>	<b>ACC</b>	<b>ACT</b>	<b>CTG</b>	<b>CTT</b>	<b>CGG</b>	<b>GCG</b>	<b>CTG</b>	<b>GGA</b>	<b>GCC</b>	<b>CAG</b>	<b>GAA</b>	<b>GCC</b>			
			<b>120</b>														
<b>Ile</b>	<b>Ser</b>	<b>Leu</b>	<b>Pro</b>	<b>Asp</b>	<b>Ala</b>	<b>Ala</b>	<b>Ser</b>	<b>Ala</b>	<b>Ala</b>	<b>Pro</b>	<b>Leu</b>	<b>Arg</b>	<b>Thr</b>	<b>Ile</b>			
<b>ATC</b>	<b>TCC</b>	<b>CTC</b>	<b>CCA</b>	<b>GAT</b>	<b>GCG</b>	<b>GCC</b>	<b>TCG</b>	<b>GCT</b>	<b>GCT</b>	<b>CCA</b>	<b>CTC</b>	<b>CGA</b>	<b>ACC</b>	<b>ATC</b>			
							<b>140</b>										
<b>Thr</b>	<b>Ala</b>	<b>Asp</b>	<b>Thr</b>	<b>Phe</b>	<b>Cys</b>	<b>Lys</b>	<b>Leu</b>	<b>Phe</b>	<b>Arg</b>	<b>Val</b>	<b>Tyr</b>	<b>Ser</b>	<b>Asn</b>	<b>Phe</b>			
<b>ACT</b>	<b>GCT</b>	<b>GAC</b>	<b>ACT</b>	<b>TTC</b>	<b>TGC</b>	<b>AAA</b>	<b>CTC</b>	<b>TTC</b>	<b>CGA</b>	<b>GTC</b>	<b>TAC</b>	<b>TCC</b>	<b>AAT</b>	<b>TTC</b>			



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 CCAGGTGCGTCCAGCTGGGCACATCCACCACCTCCCTCACCAACA  
 CTGCCTGTGCCACACCCTCCCTCACCCTCCCGAACCCCATCGAGGGGCTCTCAGCTAAG  
 CGCCAGCCTGTCCCATGGACACTCCAGTGCCAGCAATGACATCTCAGGGGCCAGAGGAAC  
 TGTCCAGAGCACAACCTCTGAGATCTAAGGATGTCGCAGGGCCAACCTTGAGGGCCCAGAGC  
 AGGAAGCATTGAGAGAGCAGCTTTAAACTCAGGAGCAGAGACAATGCAGGGAAAACACCT  
 GAGCTCACTCGGCCACCTGCAAAATTTGATGCAGGACACGCTTTGGAGGCAATTTACCTG  
 TTTTGCACCTACCATCAGGGACAGGATGACTGGAGAACTTAGGTGGCAAGCTGTGACTT  
 CTCAAGGCCTCACGGGCACTCCCTTGGTGGCAAGAGCCCCCTTGACACTGAGAGAATATT  
 TTGCAATCTGCAGCAGGAAAAATTACGGACAGGTTTTGGAGGTTGGAGGGTACTTGACAG  
 GTGfGTGGGGAAGCAGGGCCGGJAGGGGTGGAGCTGGGATGCGAGfGAGAACCCTGAAGAC  
 AGGATGGGGGCTGGCCTCTGGTTCTCGTGGGGTCCAAGCTT  
 HindIII

### FIG.6A

**AAGCTTCTGGGCTTCCAGACCCAGCTACTTTGCGGAACTCAGCAACCCAGGCATCTCTGAGTCTCCGCCCA**  
**AGACCGGGATGCCCCCAGGGGAGGTGTCCGGGAGCCCAAGCCTTTCCAGATAGCACGCTCCGCCAGTCCC**  
**AAGGGTGCAGCAACCGGCTGCACTCCCCTCCCGCGACCCAGGGCCCAGGAGCAGCCCCCATGACCCACACGC**  
**ACGTCTGCAGCAGCCCCGCTCACGCCCCGGCGAGCCTCAACCCAGGCGTCCTGCCCCCTGCTCTGACCCCGG**  
**GTGGCCCCTACCCCTGGCGACCCCTCACGCACACAGCCTCTCCCCACCCCCACCCGCGCACGCACACATG**  
**CAGATAACAGCCCCGACCCCCGGCCAGAGCCGXAGAGTCCCTGGGCCACCCCGGCCGCTCGCCTGCCGCTG**  
**CGCCGCACCGCGCTGTCTCCCGGAGCCGGACCGGGGCCACCCGCGCCCXGCTCTGCTCCGACACCGCGCCC**  
**CTTGGACAGCCGCCCTCTCCTCTAGGCCCGTGGGGCTGGCCCTGCACCCGCCGAGCTTCCCGGGATGAGGX**

**CCCGGTGACCGGCGCGCCCCAAGTCGCTGAGGGACCCCGGCCAAGCGCGGAG**

-27	-24
Met	Gly Val His
ATG	GGG GTG CAC G

**GTGAGTACTCGCGGGCTGGGCGCTCCCGCGGCCGGGTTCTGTTTGAGCGGGGATTTAGCGCCCCGGCT**

**FIG.6B**

**ATTGGCCAAGAGGTGGCTGGGTTCAAGGACCGGCGACTTGTCAAGGACCCCGGAAGGGGGAGGGGGGTGGG**  
**GCAGCCTCCACGTGCCGCGGGGACTTGGGGGAGTTCTTGGGGATGGCAAAAACCTGGCCTGTTGAGGGGCA**  
**CAGTTTGGGGTTGGGGAGGAGGTTTGGGGTTCTGCTGTGCAGTTGTGTCTGTTGTCAGTGTCTCG [ I · B · ]**  
**TTGCACACGCACAGATCAATAAGCCAGAGGCAGCACCTGAGTGCTTGCATGGTTGGGACAGGAAGGACGAG**  
**CTGGGGCAGAGACGTGGGGATGAAGGAAGCTGTCTTCCACAGCCACCCTTCTCCCCCCCCGCCTGACTCT**

-23
-20  
**CAGCCTGGCTATCTGTTCTAG**
AA
TGT
CCT
GCC
TGG
CTG
TGG
CTT
CTC
CTG
TCC
CTG

-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 GTGAGACCCCTTCCCCAGCACATTCCACAGAACTCACGCTCAGGGCTTCAGGGAACCTCCTCCAGAT**  
**CCAGGAACCTGGCACTTGGTTTGGGGTGGAGTTGGGAAGCTAGACACTGCCCCCTACATAAGAATAAGTC**

# FIG. 6C

TGGTGGCCCCAAACCATACTGAAACTAGGCAAGGAGCAAAGCCAGCAGATCCTACGCCTGTGGGCCAGGG

									27			30	
									Thr	Gly	Cys	Ala	Glu
CCAGAGCCTTCAGGGACCCTTGACTCCCCGGGCTGTGTGCATTT	CAG								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	55
Ala	Trp	Lys	Arg	Met	Glu																
GCC	TGG	AAG	AGG	ATG	GAG	GTGAGTTCCTTTTTTTTTTTTTTTTCCTTTCTTTTGGAGAATCTCATT															

TGCGAGCCTGATTTTGGATGAAAGGGAGAATGATCGGGGGAAAGGTAAAATGGAGCAGCAGAGATGAGGCT

GCCTGGGCGCAGAGGCTCACGTCTATAATCCCAGGCTGAGATGGCCGAGATGGGAGAATTGCTTGAGCCCT

GGAGTTTCAGACCAACCTAGGCAGCATAGTGAGATCCCCATCTCTACAAACATTTAAAAAATTAGTCAG

GTGAAGTGGTGCATGGTGGTAGTCCCAGATATTTGGAAGGCTGAGGCGGGAGGATCGCTTGAGCCCAGGAA

TTTGAGGCTGCAGTGAGCTGTGATCACACCACTGCACTCCAGCCTCAGTGACAGAGTGAGGCCCTGTCTCA

FIG. 6D

**AAAAAGAAAAGAAAAAAGAAAAATAATGAGGGCTGTATGGAATACATTTCATTATTCATTCACTCACTCACT**  
**CACTCATTTCATTTCATTTCATTTCATTCAACAAGTCTTATTGCATACCTTCTGTTTGCTCAGCTTGGTGCTTGG**  
**GGCTGCTGAGGGGCAGGAGGGAGAGGGGTGACATGGGTGAGCTCGACTCCCAGAGTCCACTCCCTGTAG**

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**

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 GTGAGTAGGAGCGGACACTTCTGCTTGCCCTTTCTGTAAGAAGGGGA**

**GAAGGGTCTTGCTAAGGAGTACAGGAACTGTCCGTATTCCTTCCCTTTCTGTGGCACTGCAGCGACCTCCT**

116    120  
**Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala**  
**GTTTTCTCCTTGCCAG 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  
 Asp Arg OP  
 GAC AGA TGA CCAGGTGTGTCCACCTGGGCATATCCACCACCTCCCTCACCAACATTGCTTGTGCCACA  
  
 CCCTCCCCCGCCACTCCTGAACCCCGTCGAGGGGCTCTCAGCTCAGCGCCAGCCTGTCCCATGGACACTCC  
  
 AGTGCCAGCAATGACATCTCAGGGGCCAGAGGAACTGTCCAGAGAGCAACTCTGAGATCTAAGGATGTCAC  
  
 AGGGCCAACTTGAAGGGCCCAGAGCAGGAAGCATTGAGAGAGCAGCTTTAAACTCAGGGACAGAGCCATGC  
  
 TGGGAAGACGCCTGAGCTCACTCGGCACCCTGCAAAATTTGATGCCAGGACACGCTTTGGAGGCGATTTAC  
  
 CTGTTTTCGCACCTACCATCAGGGACAGGATGACCTGGAGA ACTTAGGTGGCAAGCTGTGACTTCTCCAGG  
  
 TCTCAGGGCATGGGCACTCCCTTGGTGGCAAGAGCCCCCTTGACACCGGGGTGGTGGGAACCATGAAGAC  
  
 AXGATXGGGGCTGGCCTCTGGCTCTCATGGGGTCCAAGTTTTGTGTATTCTCAACCTATTGACAGACTGAA  
  
 ACACAATATGAC

FIG. 7

			-1	1	
	<u>XbaI</u>		MetaIa		
	CTAG	AAACCATGAG	GGTAATAAAA	TAATGGCTCC	GCCGCGTCTG
		TTGGTACTC	CCATTATTTT	ATTACCGAGG	CGGCGCAGAC
	ATCTGCCACT	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	GTCCGCACCC
	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
	GCAAACTGTT	TCGTGTATAC	TCTAACTTCC	TCCGTGGTAA	ACTGAAACTG
	CGTTTGACAA	AGCACATATG	AGATTGAAGG	ACGCACCATT	TGACTTTGAC
				<u>SalI</u>	
	TATACTGGCG	AAGCATGCCG	TACTGGTGAC	CGCTAATAG	
	ATATGACCGC	TTCGTACGGC	ATGACCACTG	GCGATTATCA	GCT

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## FIG. 8

	-1	+1		
<u>HindIII</u>			<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	TTCGACAAC
AGTTTGGCAA	GGTTTGGCCT	TGTTATCTGA	AGCTGTTTTG	AGAGGTCAAG
TCAAACCGTT	CCAAACCGGA	ACAATAGACT	TGGACAAAAC	TCTCCAGTTC
CCTTGTTGGT	TAACTCTTCT	CAACCATGGG	AACCATTGCA	ATTGCACGTC
GGAAACAACCA	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
GTAACCTTGG	GTAGTGACGA	CTATGGAAGT	CTTTCAATAA	GTCTCAAATG
TCCAACCTTCT	TGAGAGGTAA	ATTGAAGTTG	TACACCGGTG	AAGCCTGTAG
AGGTTGAAGA	ACTCTCCATT	TAACTTCAAC	ATGTGGCCAC	TTCGGACATC
AACTGGTGAC	AGATAAGCCC	GACTGATAAC	AACAGTGTAG	
TTGACCACTG	TCTATTCGGG	CTGACTATTG	TTGTCAACATC	
	<u>SalI</u>			
ATGTAACAAA	G			
TACATTGTTT	CAGCT			



FIG. 9

	-20	-10	+1	10	20	30	40	
Human	MGVHECPAWLWLLLSLLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVDPDK							
	*****							
Monkey	MGVHECPAWLWLLLSLVSLPLGLPVPGAPPRLICDSRVLERYLLEAKEAENVTMGCSESCSLNENITVDPDK							
	50	60	70	80	90	100	110	
Human	VNFYAWKRMEVGQQAQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTLLRALGAQKE							
	*****							
Monkey	VNFYAWKRMEVGQQAQAVEVWQGLALLSEAVLRGQAVLANSSQPFEPLQLHMDKAISGLRSITLLRALGAQ-E							
	120	130	140	150	160			
Human	AISPPDAASAAPLRTITADTFRKLFVYSNFLRGKLLKLYTGEACRTGDR							
	***							
Monkey	AISLPDAASAAPLRTITADTFCKLFRVYSNFLRGKLLKLYTGEACRRGDR							

**FIG. 10**

**1. AATTCTAGAAACCATGAGGGTAATAAAATA**  
**2. CCATTATTTTATTACCCTCATGGTTTCTAG**  
**3. ATGGCTCCGCCCGCTCTGATCTGCCGAC**  
**4. CTCGAGTCGCAGATCAGACGCGGCGGAG**  
**5. TCGAGAGTTCTGGAACGTTACCTGCTG**  
**6. CTTCCAGCAGGTAACGTTCCAGAACT**  
**7. GAAGCTAAAGAAGCTGAAAACATC**  
**8. GTGGTGATGTTTTTCAGCTTCTTTAG**  
**9. ACCACTGGTTGTGCTGAACACTGTTC**  
**10. CAAAGAACAGTGTTTCAGCACCAACCA**  
**11. TTTGAACGAAAACATTACGGTACCG**  
**12. GATCCGGTACCGTAATGTTTTTCGTT**



**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**

FIG. 13

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

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

9 11  
GGCTGTACTG CGTGGCCAGG CACTGCTGGT AACTCCTCT CAGCCGTGGG  
CCGACATGAC GCACCGGTCC GTGACGACCA TTTGAGGAGA GTCCGGCACCC  
10 12

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

**FIG. 14**

1. **GATCCAGATCTCTGACTACTCTGC**  
2. **ACGCAGCAGAGTAGTCAGAGATCTG**  
3. **TGCGTGCTCTGGGTGCACAGAAAGAGG**  
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**