

# **EXHIBIT 4**



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**United States Patent** [19]

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**Lin**

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[54] **PRODUCTION OF ERYTHROPOIETIN**

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[\*] **Notice:** The portion of the term of this patent subsequent to Aug. 15, 2012, has been disclaimed.

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[58] **Field of Search** ..... **435/240.2, 177.3, 435/320.1, 69.4, 240.1, 69.1, 69.6; 536/23.5, 23.51**

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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 prokaryotic 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 prokaryotic 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 end 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. 2

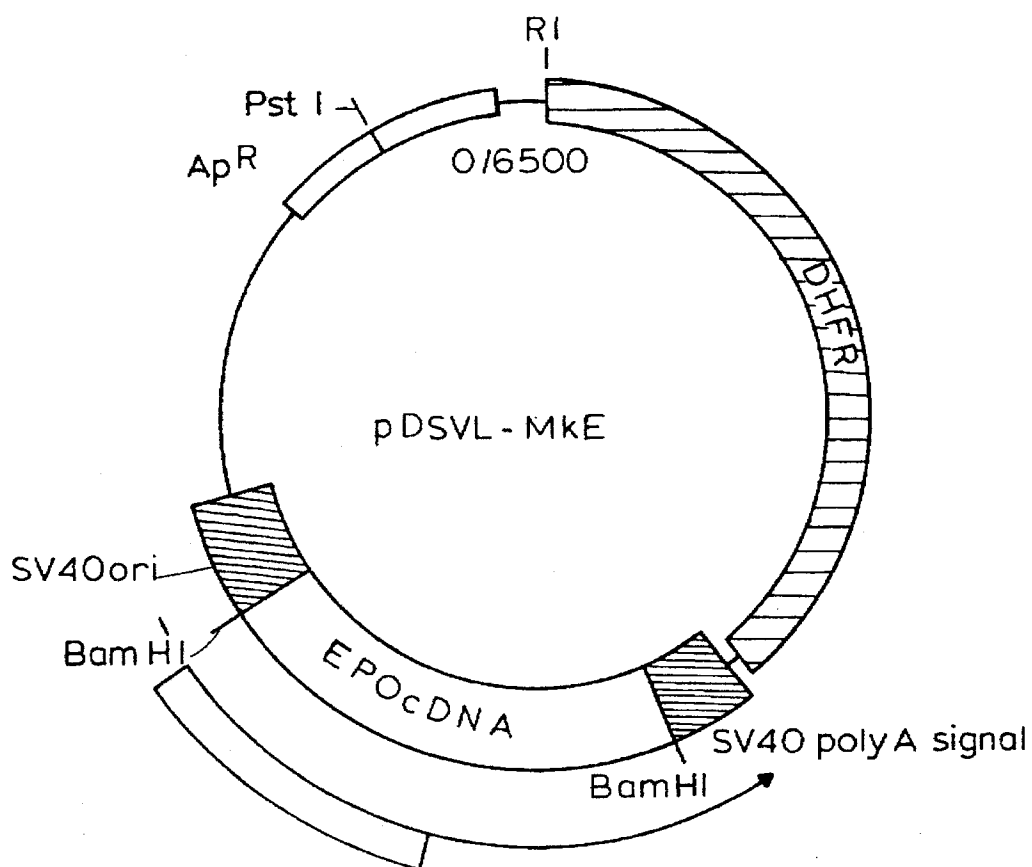


FIG. 3

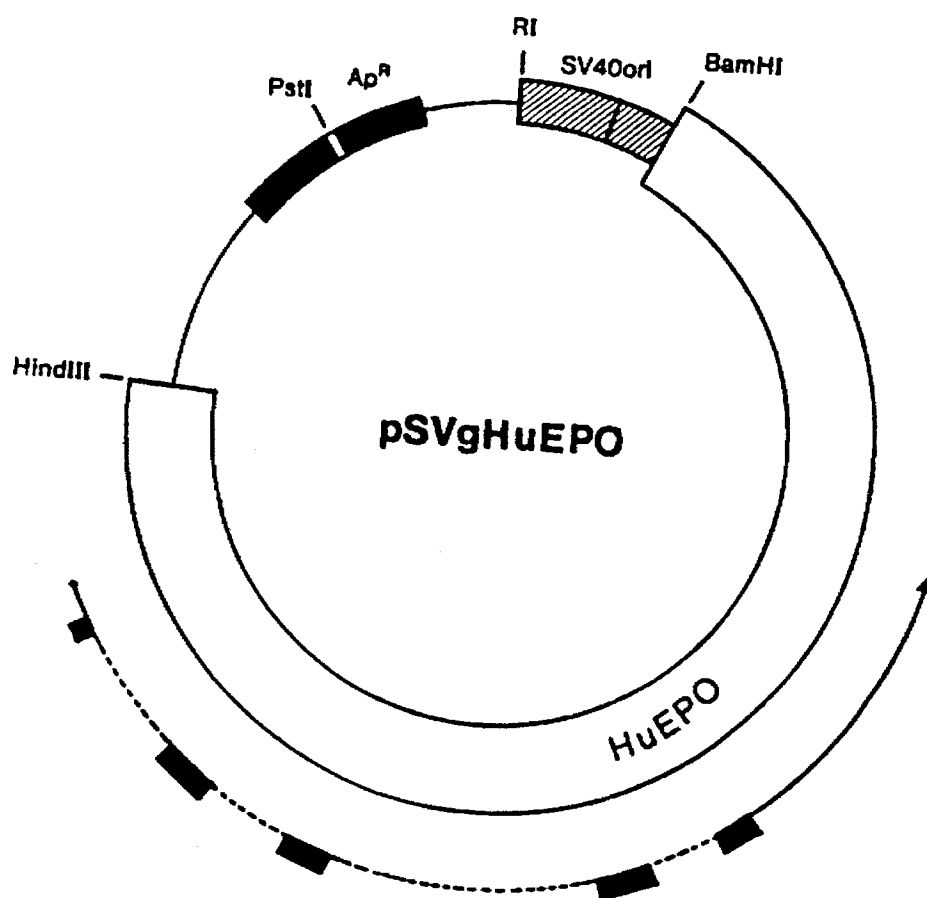


FIG. 4

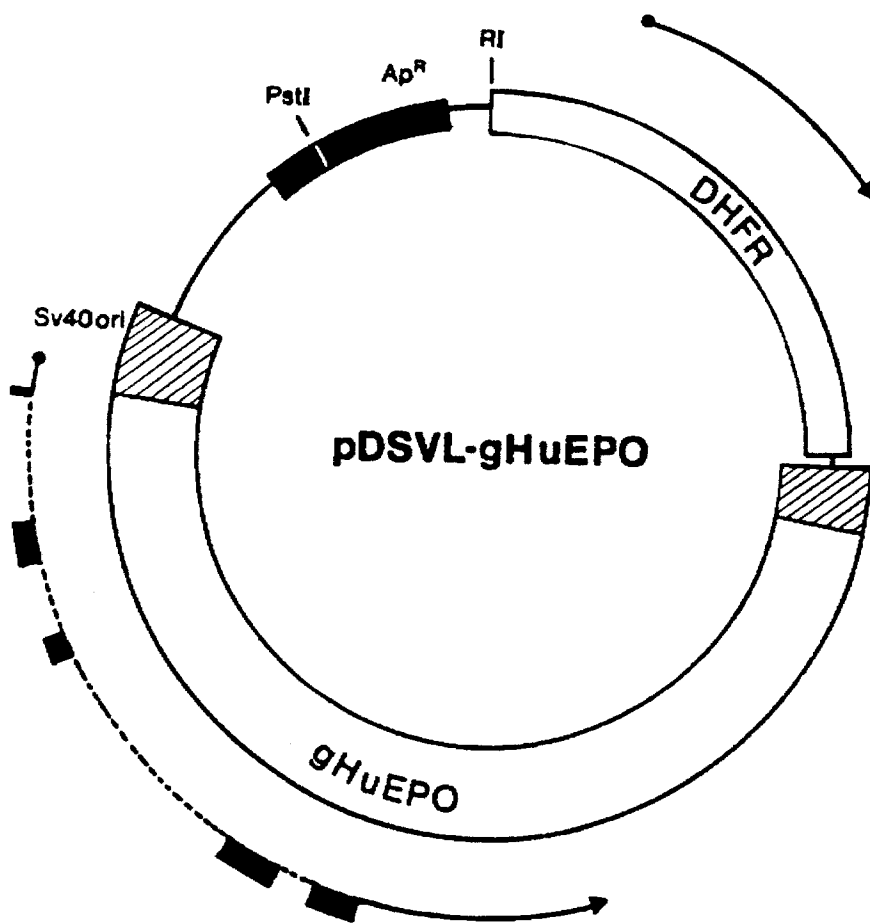




FIG. 5A

Sau3A  
 GATCCCGGCCCCCTGGACAGCCGCCCTCTCTCCAGGCCCGTGGGGCTGGCCCTGCCCC  
 CGCTGAACCTCCCGGATGAGGACTCCCGGTGTGGTCAACCGCGCCCTAGGTGCTGAG  
 -27  
 Met Gly Val His Glu Cys Pro Ala Trp  
 GGACCCCGCCAGCCGGAGATG 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 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  
 Glu Arg Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn Val Thr Met  
 GAG AGG TAC CTC TTG GAG GCC AAG GAG GGC GAG AAT GTC ACG ATG  
 20  
 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  
 30  
 \*  
 \*

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## FIG.5B

50  
 Asp Thr Lys Val Asn Phe Tyr Ala Tip Lys Arg Met Glu Val Gly  
 GAC ACC AAA GTT AAC TTC TAT TGG TGC TGG AAG AGG ATG GAG GTC GGG  
 60  
 Gln Gln Ala Val Glu Val Val Tip Gln Gln Gly GGC CTG GCC CTG Leu Ser Glu  
 CAG CAG GCT GTA GAA GTC GTC TGG TGC TGG CAG CAG GGC CTG CTC TCA GAA  
 70  
 Ala Val Leu Arg Gly Gln Ala Val Tip Ala Asn Ser Ser Gln Pro  
 GCT GTC CTG CTG CGG GGC CAG GCC GTC 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 CTG CAG CTG CAC ATG GAT AAA GCC ATC AGT GGC CTT  
 90  
 Arg Ser Ile Thr Thr Thr Leu Leu Leu Arg Ala Leu Gly Ala Gln Glu Ala  
 CGC AGC ATC ACC ACT 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 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 TTC CGA GTC TAC TCC AAT TTC  
 120  
 130  
 140