

**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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2085887 5/1982 United Kingdom .

83/04053 11/1983 WIPO .

85/01961 5/1985 WIPO .

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[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.<sup>o</sup>** ..... **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[56] **References Cited****U.S. PATENT DOCUMENTS**

3,033,753	5/1962	White et al.	530/395
3,865,801	2/1975	Chiba et al.	424/99
4,237,224	12/1980	Cohen et al.	435/69.1
4,254,095	3/1981	Fisher et al.	424/88
4,264,731	4/1981	Shine	435/91.41
4,273,875	6/1981	Manis	435/320.1
4,293,652	10/1981	Cohen	435/172.3
4,303,650	12/1981	Takezawa et al.	530/397
4,338,397	7/1982	Gilbert et al.	435/69.1
4,358,535	11/1982	Falkow et al.	435/5
4,377,513	3/1983	Sugimoto et al.	530/397
4,394,443	7/1983	Weissman et al.	435/6
4,397,840	8/1983	Takezawa et al.	424/99
4,399,216	8/1983	Axel et al.	435/6
4,411,994	10/1983	Gilbert et al.	435/69.7
4,442,205	4/1984	Hamer et al.	435/69.1
4,465,624	8/1984	Chiba et al.	530/395
4,467,016	5/1987	Lai et al.	530/380
4,468,464	8/1984	Cohen et al.	435/320.1
4,503,151	3/1985	Paddock	435/69.1
4,558,005	12/1985	Goldwasser et al.	530/380
4,558,006	12/1985	Egrie	530/380
4,568,488	2/1986	Lee-Huang	424/99
4,667,016	5/1987	Lai et al.	530/397
4,677,195	6/1987	Hewick et al.	530/397
4,695,542	9/1987	Yokata et al.	435/172.3
4,703,008	10/1987	Lin	435/240.2
4,710,473	12/1987	Morris	435/320.1
4,757,006	7/1988	Toole et al.	435/69.6

**FOREIGN PATENT DOCUMENTS**

0070685	1/1983	European Pat. Off. .
0070687	1/1983	European Pat. Off. .
0077670	4/1983	European Pat. Off. .
0093619	11/1983	European Pat. Off. .
0116446	8/1984	European Pat. Off. .
0117058	8/1984	European Pat. Off. .

Abraham et al., "Nucleotide Sequence of a Bovine Clone Encoding the Angiogenic Protein, Basic Fibroblast Growth Factor," *Science*, 233, 545-548 (Aug. 1, 1986) [F].

Adamson, "The Polycythemas: Diagnosis and Treatment," *Hosp. Practice*, 18(12), 49-57 (Dec. 1983) [A].

Aebi, "Sequence Requirements for Splicing of Higher Eukaryotic Nuclear Pre-mRNA," *Cell*, 47, 555-565 (Nov. 1986) [F].

Agarwal et al., "A General Method for Detection and Characterization of an mRNA using an Oligonucleotide Probe," *J. Biol. Chem.*, 256, 1023-1028 (Jan. 25, 1981) [F].

Anderson et al., "Isolation of a genomic clone for bovine pancreatic trypsin inhibitor by using a unique-sequence synthetic DNA probe," *P.N.A.S. (USA)*, 80, 6838-6842 (Nov. 1983) [A,F].

Baciu et al., "Erythropoietin Interaction with the Mature Red Cell Membrane," *Ann. N.Y. Acad. Sci.*, 414, 66-72 (1983) [A].

Baron et al., "Antibodies against the Chemically Synthesized Genome-Linked Protein of Poliovirus React with Native Virus-Specific Proteins," *Cell*, 28, 395-404 (Feb. 1982) [A].

(List continued on next page.)

**Primary Examiner**—James Martinell**Attorney, Agent, or Firm**—Marshall, O'Toole, Gerstein, Murray & Borun[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**

5,547,933

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## OTHER PUBLICATIONS

- Beaucage et al., "Deoxynucleoside Phosphoramidites—A new Class of Key Intermediates for Deoxypolynucleotide Synthesis," *Tetrahedron Letters*, 22(20), 1859–1862 (1981) [A,F].
- Benedum et al., "The primary structure of bovine chromogranin A: a representative of a class of acidic secretory proteins common to a variety of peptidergic cells," *EMBO J.* 5(7), 1495–1502 (1986) [F].
- Bennetzen et al., "Codon Selections in Yeast," *J. Biol. Chem.*, 257(6), 3026–3031 (Mar. 25, 1982) [A].
- Bentley et al., "Human immunoglobulin variable region genes—DNA sequences of two  $V_L$  genes and a pseudogene," *Nature*, 288, 730–733 (Dec. 1980) [F].
- Benton et al., "Screening  $\lambda$ gt Recombinant Clones by Hybridization to single Plaques in situ," *Science*, 196, 180–182 (Apr. 8, 1977) [F].
- Berzofsky et al., "Topographic Antigenic Determinants Recognized by Monoclonal Antibodies to Sperm Whale Myoglobin," *J. Biol. Chem.* 257(6), 3189–3198 (Mar. 25, 1982) [F].
- Berzofsky et al., "Properties of Monoclonal Antibodies Specific for Determinants of a Protein Antigen, Myoglobin," *J. Biol. Chem.* 255(23), 11188–11191 (Dec. 10, 1980) [F].
- Betsholtz et al., "cDNA sequence and chromosomal localization of human platelet-derived growth factor A-chain and its expression in tumour cell lines," *Nature* 320, 695–699 (Apr. 24, 1986) [F].
- Billat et al., "In Vitro and In Vivo Regulation of Hepatic Erythropoiesis by Erythropoietin and Glucocorticoids in the Rat Fetus," *Exp. Hematol.*, 10(1), 133–140 (1982) [A].
- Blattner et al., "Charon Phages: Safer Derivatives of Bacteriophage Lambda for DNA Cloning," *Science*, 196, 161–169 (Apr. 8, 1977) [A,F].
- Bos et al., "Eukaryotic Expression of Cloned cDNA Coding for Influenza Viral Glycoproteins Using an SV40 Vector: Use of Recombinant DNA Mutants to Study Structure-Function Relationships<sup>1</sup>," *Proc. Symp. Mol. Biol. Negat., Strand Viruses Meeting*, pp. 125–130, Compans et al., eds., Acad. Press (1984) [B].
- Bray et al., "Human cDNA clones for four species of  $\alpha$ -signal transduction protein," *P.N.A.S. (USA)*, 83, 8893–8897 (Dec. 1986) [F].
- Breslow et al., "Isolation and characterization of cDNA clones for human apolipoprotein A-I," *P.N.A.S. (USA)*, 79, 6861–6865 (Nov. 1982) [A,D,E,F].
- Broome et al., "Immunological screening method to detect specific translation products," *P.N.A.S. (USA)*, 75(6), 2746–2749 (Jun. 1978) [A].
- Canaani et al., "Regulated expression of human interferon  $\beta$ 1 gene after transduction into cultured mouse and rabbit cells," *P.N.A.S. (USA)*, 79, 5166–5170 (Sep. 1982) [D].
- Chan et al., "Construction and selection of recombinant plasmids containing full-length complementary DNAs corresponding to rat insulins I and II," *P.N.A.S. (USA)*, 76(10), 5036–5040 (Oct. 1979) [F].
- Chia et al., "The construction of cosmid libraries of eukaryotic DNA using the Homer series of vectors," *Nucleic Acids Res.* 10(8), 2503–2520 (1992) [F].
- Chirgwin et al., "Isolation of Biologically Active Ribonucleic Acid from Sources Enriched in Ribonuclease," *Biochemistry*, 18(24), 5294–5299 (1979) [A,F].
- Chisholm, "On the Trail of the Magic Bullet: Monoclonal antibodies promise perfectly targeted chemicals," *High Technology*, vol. 2(1), 57–63 (Jan. 1983) [A].
- Chomczynski et al., "Alkaline Transfer of DNA to Plastic Membrane," *Biochem. Biophys. Res. Commun.*, 122(1), 340–44 (1984) [A].
- Choo et al., "Molecular cloning of the gene for human anti-haemophilic factor IX," *Nature*, 299, 178–180 (Sep. 9, 1982) [A,F].
- Choppin et al., "Characterization of Erythropoietin Produced by IW32 Murine Erythroleukemia Cells," *Blood*, 64(2), 341–347 (Aug. 1984) [A,F].
- Chou et al., "Prediction of Protein Conformation," *Biochem.*, 13(2), 222–245 (1974) [A].
- Chou et al., "Prediction of the Secondary Structure of Proteins from their Amino Acid Sequence," *Advances in Enzymology*, 47, 45–47 (1978) [A].
- Chou et al., "Empirical Predictions of Protein Conformation," *Ann. Rev. Biochem.*, 47, 251–76 (1978) [A].
- Christman et al., "Amplification of expression of hepatitis B surface antigen in 3T3 cells cotransfected with a dominant-acting gene and cloned viral DNA," *P.N.A.S. (USA)*, 79, 1815–1819 (Mar. 1982) [F].
- Claus-Walker et al., "Spinal Cord Injury and Serum Erythropoietin," *Arch. Phys. Med. Rehabil.*, 65, 370–374 (Jul. 1984) [A].
- Colby et al., "Immunological Differentiation Between *E. coli* and CHO Cell-Derived Recombinant and Natural Human  $\beta$ -Interferons<sup>1</sup>," *J. Immunol.*, 133(6), 3091–3095 (1984) [B].
- Collen et al., "Biological Properties of Human Tissue-Type Plasminogen Activator Obtained by Expression of Recombinant DNA," *J. of Pharmacology and Exp. Therapeutics*, 231(1), 146–152 (1984) [B].
- Colman, "Cells that secrete foreign proteins," *TIBS*, 435–437 (Dec. 1982) [D].
- Comb et al., "Primary structure of the human Met- and Leu-enkephalin precursor and its mRNA," *Nature*, 295, 663–666 (Feb. 25, 1982) [F].
- Congote et al., "The Erythropoietins, New Erythroid Cell Stimulating Factors Extracted From Human and Bovine Fetal Tissues," Abstract 364, Proceedings 7th International Congress of Endocrinology (Quebec City, Quebec, Jul. 1–7, 1984) [A].
- Congote, "Extraction from Fetal Bovine Serum of Erythropoietin, an Erythroid Cell-Stimulating Factor," *Anal. Biochem.*, 140, 428–433 (1984) [A].
- Congote, "Regulation of Fetal Liver Erythropoiesis," *J. of Steroid Biochemistry*, 3, 423–428 (1977) [F].
- Congote, "Isolation of Two Biologically Active Peptides, Erythropoietin I and Erythropoietin II from Fetal Calf Intestine," *Biochem. Biophys. Res. Comm.*, 115(2), 477–483 (Sep. 15, 1983) [A].
- Contrera et al., "Extraction of erythropoietin from Kidneys of Hypoxic and Phenylhydrazine-treated rats," *Blood*, 25(5), 809–816 (May 1965) [D,F].
- Costantini et al., "Introduction of a Rabbit Betaglobin Gene into the Mouse Germ Line," *Nature*, 294, 92–94 (Nov. 5, 1982) [D].
- Costantini et al., "Gene Transfer into the Mouse Germ-Line," *J. Cell Physiol. Supp.* 1, 219–226 (1982) [D].
- Cotes et al., "Changes in serum immunoreactive erythropoietin during the menstrual cycle and normal pregnancy," *Brit. J. Obstet. Gyneacol.*, 90, 304–311 (April 1983) [A].

- Cotes et al., "Bio-Assay of Erythropoietin in Mice made Polycythaemic by Exposure to Air at a Reduced Pressure," *Nature*, 191, 1065-1067 (Sep. 9, 1961) [A].
- Dainiak et al., "Mechanisms of Abnormal Erythropoiesis in Malignancy," *Cancer*, 51(6), 1101-1106 (1983) [A].
- Das et al., "Use of synthetic oligonucleotide probes complementary to genes for human HLA-DR $\alpha$  and  $\beta$  as extension primers for the isolation of 5'-specific genomic clones," *P.N.A.S. (USA)*, 80, 1531-1535 (Mar. 1983) [A].
- Davis et al., "A Manual for Genetic Engineering, Advanced Bacterial Genetics", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1983), pp. 55-58 & 174-176 [A].
- Davis et al., "Active Influenza Virus Neuraminidase is Expressed in Monkey Cells from cDNA Cloned in Simian Virus 40 Vectors," *Proc. Nat'l. Acad. Sci. (USA)*, 80, 3976-3980 (1983) [B].
- Derynck et al., "Human transforming growth factor- $\beta$  complementary DNA sequence and expression in normal and transformed cells," *Nature*, 316, 701-705 (Aug. 22, 1985) [F].
- Derynck et al., "Human Transforming Growth Factor- $\alpha$ : Precursor Structure and Expression in *E. coli*," *Cell*, 38, 287-297 (Aug. 1984) [F].
- Dessypris et al., "Effect of pure erythropoietin on DNA-synthesis by human marrow day 15 erythroid burst forming units in short-term liquid culture," *Brit. J. Haematol.*, 56, 295-306 (1984) [A].
- Devos et al., "Purification of Recombinant Glycosylated Human Gamma Interferon Expressed in Transformed Chinese Hamster Ovary Cells," *J. Interferon Research*, 4, 461-468 (1984) [B].
- Docherty et al., "Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid-potentiating activity," *Nature*, 318, 66-69 (Nov. 7, 1985) [F].
- Dreesman et al., "Antibody to hepatitis B surface antigen after a single inoculation of uncoupled synthetic HBsAg peptides," *Nature*, 295, 158-160 (Jan. 14, 1982) [A].
- Dunn et al., "Erythropoietin Bioassays Using Fetal Mouse Liver Cells: Validations and Technical Improvements," *Exp. Hematol.*, 11(7), 590-600 (Aug. 1983) [A].
- Dunn, "Current Concepts in Erythropoiesis," John Wiley & Sons, Chichester, England, 1983, pp. 13, 16, 86, and 190 [A].
- Dunn et al., "Use of a computer model in the understanding of erythropoietic control mechanisms," *Chemical Abstracts*, 91, 190417r (1979) [A].
- Dunn et al., "Serum erythropoietin titers during prolonged bedrest; relevance to the anaemia of space flight," *Eur. J. Appl. Physiol.*, 52, 178-182 (1984) [A].
- Edman et al., "A Protein Sequentator," *Eur. J. Biochem.*, 1, 80-91 (1967) [F].
- Emmanouel et al., "Metabolism of pure human erythropoietin in the rat," *Am. J. Physiol.*, 247 (1 Pt 2), F168-76 (1984) [A].
- Eschbach et al., "Correction by Erythropoietin (EPO) Therapy of the Anemia of Chronic Renal Failure (CRP) in Sheep," *Clin. Res.* 29(2), 518A (1981) [F].
- Eschbach et al., "The Anemia of Chronic Renal Failure in Sheep," *J. Clin. Invest.*, 74(2), 434-441 (Aug. 1984) [A].
- Espada et al., "Purification of Human Urinary Erythropoietin," *Fed. Proc.* 41, 1159 (1982) [F].
- Fan et al., "Construction and Characterization of Moloney Murine Leukemia Virus Mutants Unable to Synthesize Glycosylated Gag Polyprotein", *Proc. Nat'l. Acad. Sci. (USA)*, 80, 5965-5969 (1983) [B].
- Farber et al., "Translation of mRNA from Human Kidneys into Biologically Active Erythropoietin Following Microinjection into *Xenopus Laevis* Oocytes," *Blood*, 62(5), Supp. No. 1, Abstract 392, 122a, (1983) [A,F].
- Farber et al., "Translation of mRNA from Anemic Baboon Kidney into Biologically Active Erythropoietin," *Exp. Hematol.*, 11, Supp. 14, Abstract 101 (1983) [A,D,E,F].
- Farber et al., "Translation of mRNA from human kidneys into biologically active erythropoietin following microinjection into *Xenopus laevis* oocytes," *J. Lab. Clin. Med.*, 102, 681 abstract [D].
- Fiddes et al., "The Gene Encoding the Common Alpha Subunit of the Four Human Glycoprotein Hormones," *J. Mol. & App. Genetics*, 1, 3-18 (1981) [A].
- Fiers et al., "The Human Fibroblast and Human Immune Interferon Genes and Their Expression in Homologous and Heterologous Cells," *Phil. Trans. R. Soc. Lond.*, B299, 29-38 (1982) [B].
- Finch, "Erythropoiesis, Erythropoietin, and Iron," *Blood*, 60(6), 1241-1246 (Dec. 1982) [A].
- Fischinger et al., "Detection of a Recombinant Murine Leukemia Virus-Related Glycoprotein on Virus-Negative Thymoma Cells," *Proc. Nat'l. Acad. Sci. (USA)*, 78(3), 1920-1924 (1981) [B].
- Fisher et al., "Effects of testosterone, cobalt & hypoxia on erythropoietin production in the isolated perfused dog kidney," *Ann. N.Y. Acad. Sci.*, 75-87 (1967) [D].
- Fisher, "Erythropoietin: Pharmacology, Biogenesis and Control of Production," *Pharmacological Review*, 24(3), 459-508 (1972) [D,E].
- Fisher et al., "Cooperative Erythropoietic Assay of Several Steroid Metabolites in Polycythemic Mice," *Steroids*, 30(6), 833-845 (Dec. 1977) [A].
- Fisher, "Control of Erythropoietin Production," *Proc. Soc. Exp. Biol. & Med.* 173, 289-305 (1983) [F].
- Garcia et al., "Immunological Neutralization of Various Erythropoietins," *Proc. Soc. Exptl. Biol. Med.*, 112, 712 and 714 (1963) [D].
- Garcia et al., "Radioimmunoassay of erythropoietin: circulating levels in normal and polycythemic human beings," *J. Lab. Clin. Med.*, 99, 624-635 (May 1982) [F].
- Garcia et al., "Radioimmunoassay of Erythropoietin," *Blood Cells* 5, 405-419 (1979) [F].
- Garoff et al., "Expression of Semliki Forest Virus Proteins from Cloned Complementary DNA. II. The Membrane-Spanning Glycoprotein E2 Is Transported to the Cell Surface Without Its Normal Cytoplasmic Domain," *J. Cell. Biol.*, 97, 652-658 (1983) [B].
- Gasser et al., "Expression of abbreviated mouse dihydrofolate reductase genes in cultured hamster cells," *P.N.A.S. (USA)*, 79, 6522-6526 (Nov. 1982) [A,D,F].
- Gene Screen, New England Nuclear, Catalog No. NEF-972 [A].
- Gething et al., "Comparison of Different Eukaryotic Vectors for the Expression of Hemagglutinin Glycoprotein of Influenza Virus," *Modern Approaches To Vaccines*, pp. 263-268, Chanock et al., eds. Cold Spring Harbor Lab (1984) [B].
- Gething et al., "Construction of influenza haemagglutinin genes that code for intracellular and secreted forms of the protein," *Nature*, 300, 598-603 (Dec. 16, 1982) [B].
- Gibson et al., "An Evaluation of Serum Erythropoietin Estimation By a Hemagglutination Inhibition assay in the Differential Diagnosis of Polycythemia," *Pathology*, 16, 155-156 (Apr. 1984) [A].

- Gluzman, "SV40-Transformed Simian Cells Support the Replication of Early SV40 Mutants," *Cell* 23, 175-182 (Jan. 1981) [F].
- Goeddel et al., "Synthesis of human fibroblast interferon by *E. coli*," *Nucleic Acids Res.*, 8(18), 4057-4074 (1980) [F].
- Goeddel et al., "Human leukocyte Interferon Produced by *E. coli* is biologically active," *Nature*, 287:411-416 (Oct. 2, 1980) [D].
- Goldwasser et al., "Purification of Erythropoietin," *P.N.A.S. (USA)*, 68(4), 697-698 (Apr. 1971) [F].
- Goldwasser et al., "Further purification of sheep plasma erythropoietin," *Bioch. Biophys. ACTA*, 64:487-496 (1962) [D].
- Goldwasser, "Some Thoughts on the Nature of Erythropoietin-Responsive Cells," *J. Cell. Physiol.*, 110 (Supp. 1), 133-135 (1982) [A].
- Goldwasser et al., "Erythropoietin and the differentiation of red blood cells," *Fed. Proc.* 34, 2285-2292 (Dec. 1975) [B,F].
- Goldwasser et al., "An Assay for Erythropoietin in Vitro at the Milliunit Level," *Endocrinology*, 97(2), 315-323 (Aug. 1975) [A,F].
- Goldwasser, "Biochemical Control of Erythroid Development," *Current Topics in Developmental Biology*, ed. A. Monroy and A. A. Noscona, 173-211, Academic Press, NY (1966) [D].
- Goldwasser et al., "Erythropoietin: Assay and Study of Its Mode of Action," *Meth. in Enzymol.*, 37, 109-121 (1975) [F].
- Goldwasser, "From Protein to Gene to Protein: The Molecular Biology of Erythropoietin," *Am. J. of Kidney Diseases*, 18(4) Supp. 1, 10-13 (Oct. 1991) [F].
- Goldwasser et al., "Progress in the purification of erythropoietin," *Ann. N.Y. Acad. Sci.*, 149:49-53 (1968) [D].
- Goldwasser et al., "The Molecular Weight of Sheep Plasma Erythropoietin," *J. of Biol. Chem.*, 247(16), 5159-5160 (Aug. 25, 1972) [F].
- Goldwasser et al., "On the mechanism of Erythropoietin-induced Differentiation," *J. of Biol. Chem.*, 249(13), 4202-4206 (Jul. 10, 1974) [B].
- Goochee et al., "Environmental Effects on Protein Glycosylation," *Biotechnology*, 8, 421-427 (May 1990) [F].
- Goochee et al., "The Oligosaccharides of Glycoproteins: Biprocess Factors Affecting Oligosaccharide Structure and their Effect on Glycoprotein Properties," *Biotechnology*, 9, 1347-1355 (Dec. 1991) [F].
- Goodman et al., "Cloning of Hormone Genes from a Mixture of cDNA Molecules," *Meth. in Enzymol.*, 68, 75-90 (1979) [F].
- Gordon et al., "A plasma extract with erythropoietic activity," *Proc. Soc. Expt. Biol. Med.*, 86:255-258 (1954) [D,E].
- Goto et al., "Production of Recombinant Human Erythropoietin in Mammalian Cells: Host-Cell Dependency of the Biological Activity of the Cloned Glycoprotein," *BioTech.* 6, 67-71 (Jan. 1988) [F].
- Gough et al., "Molecular Cloning of cDNA Encoding A Murine Haematopoietic Growth Regulator, Granulocyte-Macrophage Colony Stimulating Factor," *Nature*, 309, 763-767 (1984) [B].
- Gough et al., "Immunoprecipitation of Specific Polysomes Using *Staphylococcus aureus*: Purification of the Immunoglobulin-Chain Messenger RNA from the Mouse Myeloma MPC11," *Biochemistry* 17(25), 5560-5566 (1978) [F].
- Gouy et al., "Codon Usage in Bacteria: Correlation with Gene Expressivity," *Nucleic Acids Res.* 10, 7055-7074 (1982) [A].
- Graham et al., "A New Technique for the Assay of Infectivity of Human Adenovirus 5 DNA," *Virology* 52, 456-467 (1973) [F].
- Grantham et al., "Codon catalog usage is a genome strategy modulated for gene expressivity," *Nucleic Acids Res.* 9, r43-74 (1981) [A,F].
- Gray et al., "*Pseudomonas Aeruginosa* Secretes and Correctly Processes Human Growth Hormone," *Biotechnology*, 2, 161-165 (Feb. 1984) [A].
- Gray et al., "Expression of human immune interferon cDNA in *E. coli* and monkey cells," *Nature*, 295, 503-508 (Feb. 11, 1982) [A,D,E].
- Green et al., "Immunogenic Structure of the Influenza Virus Hemagglutinin," *Cell*, 28, 477-487 (Mar. 1982) [A].
- Grimaldi et al., "Interspersed repeated sequences in the African green monkey genome that are homologous to the human Alu family," *Nucleic Acid Research*, 9(21):5553-5568 (1981) [D].
- Groffen et al., "Isolation of Human Oncogene Sequences (V-fes Homolog) from a Cosmid Library," *Science*, 216, 1136-1138 (Jun. 4, 1982) [F].
- Grundmann et al., "Characterization of cDNA coding for human factor XIIIa," *P.N.A.S. (USA)*, 83, 8024-8028 (Nov. 1986) [F].
- Grunstein et al., "Colony Hybridization," *Meth. in Enzym.* 68, 379-389 (1979) [F].
- Grunstein et al., "Colony hybridization: A method for the isolation of cloned DNAs that contain a specific gene," *P.N.A.S. (USA)*, 72(10), 3961-3965 (Oct. 1975) [F].
- Gruss et al., "Expression of simian virus 40-rat preproinsulin recombinants in monkey kidney cells: Use of preproinsulin RNA processing signals," *P.N.A.S. (USA)*, 78(1):133-137 (Jan. 1981) [D].
- Gubler et al., "A simple and very efficient method for generating cDNA libraries," *Gene* 25, 263-269 (1983) [F].
- Haddy, "Erythropoietin in sickle cell disease," *Am. Jour. Ped. Hematol./Oncol.*, 4(2), 191-196 (Summer 1982) [A].
- Haga et al., "Plasma Erythropoietin Concentrations During the Early Anemia of Prematurity," *Acta. Paediatr. Scand.*, 72, 827-831 (1983) [A].
- Hagiwara et al., "Erythropoietin Production in a Primary Culture of Human Renal Carcinoma Cells Maintained in Nude Mice," *Blood*, 63(4), 828-835 (Apr. 1984) [A,F].
- Hamer et al., "Expression of the chromosomal mouse  $\beta^{maj}$ -globin gene cloned in SV40," *Nature*, 281:35-40 (Sep. 6, 1979) [A,D].
- Hamer et al., "A Mouse Globin Gene Promoter is Functional in SV40," *Cell*, 21:697-708 (Oct. 1980) [D].
- Hammond et al., "Production, Utilization and Excretion of Erythropoietin: I. Chronic Anemias. II. Aplastic Crisis. III. Erythropoietic Effects of Normal Plasma," *Ann. N.Y. Acad. Sci.*, 149, 516-527 (1968) [A].
- Hanahan et al., "Plasmid screening at high colony density," *Gene*, 10, 63-67 (1980) [F].
- Hartman et al., "Human Influenza Virus Hemagglutinin is Expressed in Monkey Cells Using Simian Virus 40 Vectors," *Proc. Nat'l. Acad. Sci. (USA)*, 79, 233-237 (1982) [B].
- Hauser et al., "Inducibility of human  $\beta$ -interferon in mouse L-cell clones," *Nature*, 297:650-654 (Jun. 24, 1982) [D].

5,547,933

Page 5

- Haynes et al., "Constitutive, long-term production of human interferons by hamster cells containing multiple copies of a cloned interferon gene," *Nucleic Acids Research*, 11(3), 587-706 (1983) [B].
- Haynes et al., "Production of a Glycosylated Human Protein by Recombinant DNA Technology," Humoral Factors Host Ref. [Proc. Takeda Sci. Found. Symp. Biosci. (1983)], 1st, Meeting Date 1982, 111-129 [B,D,E].
- Hellmann et al., "Familial erythrocytosis with over-production of erythropoietin," *Clin. Lab. Haemat.*, 5, 335-342 (1983) [A].
- Hewick et al., "A Gas-Liquid Solid Phase Peptide and Protein Sequenator," *J. Biol. Chem.*, 256, 7990-7997 (Aug. 1981) [A,F].
- Hirs et al., "Peptides Obtained by Tryptic Hydrolysis of Performic Acid-Oxidized Ribonuclease," *J. Biol. Chem.* 219, 623-642 (1955) [F].
- Hopp et al., "Prediction of protein antigenic determinants from amino acid sequences," *P.N.A.S. (USA)*, 78(6), 3824-3828 D-7182 (Jun. 1981) [A,F].
- Houghton et al., "The amino-terminal sequence of human fibroblast interferon as deduced from reverse transcripts obtained using synthetic oligonucleotide primers," *Nucleic Acids Res.* 8(9), 1913-1931 (1980) [F].
- Huang et al., "Identification of Human Erythropoietin Receptor," *Am. Soci. of Biological Chemists, Am. Assoc. of Immunologists, Fed. Pract. (USA)* 43(7) Abst. 2770, p. 1891 (1984) [D].
- Huang et al., "Characterization of Human Erythropoietin cDNA clones," *Am. Soc. of Biological Chemists, Am. Assoc. of Immunologists, Fed. Pract. (USA)* 43(6) Abst. 1795, p. 1724 [D].
- Itakura, et al., "Synthesis and Use of Synthetic Oligonucleotides," *Ann. Rev. Biochem.*, 53, 323-356 (1984) [F].
- Ito et al., "Solid phase synthesis of polynucleotides. VI. Further studies on polystyrene copolymers for the solid support," *Nucleic Acids Res.* 10(5), 1755-1769 (1982) [F].
- Jacobs et al., "Isolation and characterization of genomic and cDNA clones of human erythropoietin," *Nature*, 313, 806-809 (Feb. 28, 1985) [A,B,F].
- Jacobsen et al., "Relative effectiveness of phenylhydrazine treatment and hemorrhage in the production of an erythropoietic factor," *Blood*, 11:937-945 (1956) [D].
- Jacobsen et al., "Role of the kidney in erythropoiesis," *Nature*, 179:633-634 (Mar. 23, 1957) [D].
- Jaye et al., "Isolation of human anti-haemophilic factor IX cDNA clone using a unique 52-base synthetic oligonucleotide probe deduced from the amino acid sequence of bovine factor IX," *Nucleic Acids Res.* 11(8), 2325-2335 (1983) [A,F].
- Jeffreys et al., "Sequence variation and evolution of nuclear DNA in man and primates," *Phil. Trans. R. Soc. Lond.*, B 292:133-142 (1981) [D,E,F].
- Jelkman et al., "Extraction of Erythropoietin from Isolated Renal Glomeruli of Hypoxic Rats," *Exp. Hematol.*, 11(7), 581-588 (Aug. 1983) [A,F].
- Kaiser et al., "Amphiphilic Secondary Structure: Design of Peptide Hormones," *Science*, 223, 249-255 (1984) [A].
- Kajimura et al., "Cloning the Heavy Chain of Human HLA-DR Antigen Using Synthetic Oligodeoxyribonucleotides as Hybridization Probes," *DNA*, 2(3), 175-182 (1983) [F].
- Kakidani et al., "Cloning and sequence analysis of cDNA for porcine  $\beta$ -neo-endorphin/dynorphin precursor," *Nature*, 298, 245-249 (Jul. 15, 1982) [F].
- Kalmanti, "Correlation of clinical and in vitro erythropoietic responses to androgens in renal failure," *Kidney Int'l.*, 22, 383-391 (1982) [A].
- Kam et al., "Novel bacteriophage  $\lambda$  cloning vector," *P.N.A.S. (USA)*, 77, 5172-5176 (Sep. 1980) [A,F].
- Katsuoka et al., "Erythropoietin Production in Human renal Carcinoma Cells Passaged in Nude Mice and in Tissue Culture," *Gann*, 74, 534-541 (Aug. 1983) [A,F].
- Kaufman et al., "Amplification and Expression of Sequences Cotransfected with a Modular Dihydrofolate Reductase Complementary DNA Gene," *J. Mol. Biol.* 159, 601-621 (1982) [F].
- Kennell, "Principles and Practices of Nucleic Acid Hybridization," *Prog. Nucl. Acid Res. Mol. Biol.* 11, 259-301, p. 293 (1971) [A].
- Kenter et al., "Mouse Myeloma Cells That Make Short Immunoglobulin Heavy Chains: Pleiotropic Effects on Glycosylation and Chain Assembly," *J. Cell. Biol.*, 98, 2215-2221 (1984) [B].
- Kieny et al., "Expression of rabies virus glycoprotein from a recombinant vaccinia virus," *Nature*, 312, 163-166 (1984) [B].
- Kimura et al., "A frameshift addition causes silencing of the  $\delta$ -globin gene in old world monkeys, an anubis," *Nucleic Acids Res.*, 11(9):2541-2550 (1983) [D,E].
- Knopf et al., "Cloning and Expression of Multiple Protein Kinase C cDNAs," *Cell* 46, 491-502 (Aug. 15, 1986) [F].
- Kohne, "Evolution of Higher-organism DNA," *Quarterly Reviews of Biophysics*, 3, 327-375 (1970) [D,E].
- Kondor-Koch et al., "Expression of Semliki Forest Virus Proteins from Cloned Complementary DNA. I. The Fusion Activity of the Spike Glycoprotein," *J. Cell. Biol.*, 97, 644-651 (1983) [B].
- Konrad, "Applications of Genetic Engineering to the Pharmaceutical Industry," *Ann. N.Y. Acad. Sci.*, 413, 12-22 (1983) [B].
- Konwalinka et al., "A Miniaturized Agar Culture System for Cloning Humlan Erythropoietic Progenitor Cells," *Exp. Hematol.*, 12, 75-79 (1984) [A].
- Korman, "cDNA clones for the heavy chain of HLA-DR antigens obtained after immunopurification of polysomes by monoclonal antibody," *P.N.A.S. (USA)*, 79, 1844-1848 (Mar. 1982) [F].
- Kornblitt et al., "Isolation and characterization of cDNA clones for human and bovine fibronectins," *P.N.A.S. (USA)*, 80, 3218-3222 (Jun. 1983) [A,D,E].
- Kramer et al., "Comparisons of the Complete Sequences of Two Collagen Genes from *Caenorhabditis elegans*," *Cell* 30, 599-606 (Sep. 1982) [F].
- Krane, "The Role of Erythropoietin in the Anemia of Chronic Renal Failure," *Henry Ford Hosp. Med. J.*, 31(3), 177-181 (1983) [A].
- Krystal, "A Simple Microassay fro Erythropoietin Based on  $^3\text{H}$ -Thymidine Incorporation into Spleen cells from Phenylhydrazine Treated Mice," *Exp. Hematol.*, 11(7), 649-660 (Aug. 1983) [A].
- Kuhn et al., "Gene Transfer, Expression, and Molecular Cloning of the Human Transferrin Receptor Gene," *Cell*, 37, 95-103 (1984) [B].
- Kurachi et al., "Isolation and characterization of a cDNA coding for human factor IX," *P.N.A.S. (USA)*, 79, 6461-6464 (Nov. 1982) [A,F].
- Kuratowska et al., "Studies on the production of erythropoietin by isolated perfused organs," *Blood*, 18:527-534 (1961) [D].

5,547,933

Page 6

- Kurtz, "A New candidate for the regulation of erythropoiesis: Insulin-like growth factor I," *FEBS Letters*, 149(1), 105-108 (Nov. 1982) [A].
- Kyte et al., "A Simple Method for Displaying the Hydrophobic Character of a Protein," *J. Mol. Biol.*, 157, 105-132 (1982) [A,F].
- Lai, "Technical Improvements in Protein Microsequencing," *Analytica Chimica Acta*, 163, 243-248 (1984) [B,C].
- Lai et al., "Ovalbumin is synthesized in mouse cells transformed with the natural chicken ovalbumin gene," *P.N.A.S. (USA)*, 77(1), 244-248 (Jan. 1980) [D].
- Lai et al., "Structural Characterization of Human Erythropoietin," *J. of Biol. Chem.*, 261, 3116-3121 (Mar. 5, 1986) [F].
- Lange et al., "Application of erythropoietin antisera to studies of erythropoiesis," *Ann. N.Y. Acad. Sci.*, 149:281-291 (1968) [D].
- Lappin et al., "The Effect of Erythropoietin and Other Factors on DNA synthesis by Mouse Spleen Cells," *Exp. Hematol.*, 11(7), 661-666 (Aug. 1983) [A].
- Lasky et al., "Production of an HSV Subunit Vaccine by Genetically Engineered Mammalian Cell Lines," *Modern Approaches to Vaccines*, pp. 189-194, Chanock et al., eds. Cold Spring Harbor Lab. (1984) [B].
- Lathe, "Synthetic Oligonucleotide Probes Deduced from Amino Acid Sequence Data," *J. Mol. Biol.* 183, 1-12 (1985) [F].
- Laub et al., "Synthesis of Hepatitis B Surface Antigen in Mammalian Cells: Expression of the Entire Gene and the Coding Region," *J. Virol.*, 48(1), 271-280 (1983) [B].
- Laub and Ritter, "Expression of the Human Insulin Gene and cDNA in a Heterologous Mammalian System," *J. Biol. Chem.*, 258(10), 6043-6050 (May 25, 1983) [D].
- Lauffer et al., "Topology of signal recognition particle receptor in endoplasmic reticulum membrane," *Nature* 318, 334-338 (1985) [F].
- Lawn et al., "The Isolation and Characterization of Linked  $\delta$ - and  $\beta$ -Globin Genes from a Cloned Library of Human DNA," *Cell*, 15, 1157-1174 (Dec. 1978) [A,F].
- Ledeer et al., "Gangliosides: Structure, Isolation, and Analysis," *Methods in Enzymology*, 83 (Part D), 139-191 (1982) [A].
- Lee-Huang, "Cloning and Expression of Human EPO cDNA in *E. Coli*," *P.N.A.S. (USA)*, 81, 2708-2712 (May 1984) [A,D,E,F].
- Lee-Huang, "Monoclonal Antibodies to Human Erythropoietin," *Abstract No. 1463, Fed. Proc.*, 41, 520 (1982) [A,D,F].
- Lee-Huang, "A New Preparative Method for Isolation of Human Erythropoietin With Hydrophobic Interaction Chromatography," *Blood*, 56(4), 620, 622, and 624 (Oct. 1980) [A].
- Lee-Huang, "The Erythropoietin Gene," *Oncogenes, Genes and Growth Factors*, Chap. 7, pp. 199-222, ed. Gordon Garaff, John Wiley & Sons, Inc. (1987) [D].
- Lee-Huang, "Cloning of Human Erythropoietin," *Biophysical U. 45(Part 2 of 2), ABT. M-PM-A12*, p. 30a (1984) [D].
- Lerner et al., "Antibodies to Chemically Synthesized Peptides Predicted from DNA Sequences as Probes of Gene Expression," *Cell*, 23, 309-310 (Feb. 1981) [A].
- Lerner et al., "Chemically synthesized peptides predicted from the nucleotide sequence of the hepatitis B virus genome elicit antibodies reactive with the native envelope protein of Dane particles," *P.N.A.S. (USA)*, 78(6), 3403-3407 (Jun. 1981) [A].
- Lerner, "Synthetic Vaccines," *Scientific American*, 248(2), 66-74 (1983) [A].
- Lewin *Genes*, 1983, John Wiley & Sons, p. 307 [A].
- Lin et al., "Cloning and Expression of Monkey and Human Erythropoietin," *Exp. Hematol.*, 12, 357 (1984) [A].
- Lin et al., "Monkey erythropoietin gene: cloning, expression and comparison with the human erythropoietin gene," *Gene*, 44, 201-209 (1986) [A].
- Lin et al., "Cloning and expression of the human erythropoietin gene," *P.N.A.S. (USA)*, 82, 7580-7584 (Nov. 1985) [E,F].
- Lin et al., "Cloning of the Monkey EPO Gene," Abstract, *J. Cell. Biochem.*, Suppl. 8B, p. 45 (Mar. 31-Apr. 24, 1984) [A,B,C].
- Lipschitz et al., "Effect of Age on Hematopoiesis in Man," *Blood*, 63(3), 502-509 (Mar. 1983) [A].
- LKB Technical Bulletin #2217 [C].
- Maniatis et al., "The Isolation of Structural Genes from Libraries of Eucaryotic DNA," *Cell* 15, 687-701 (Oct. 1978) [F].
- Maniatis et al., *Molecular Cloning, a Laboratory Manual*, pp. 197-199, 392-393, 479-487, 493-503 Cold Spring Harbor, N.Y. (1982) [A,D,F].
- Markoff et al., "Glycosylation and Surface Expression of the Influenza Virus Neuraminidase Requires the N-Terminal Hydrophobic Region," *Molecular and Cellular Biology*, 4(1), 8-16 (1984) [B].
- Martial et al., "Human Growth Hormones: Complementary DNA Cloning and Expression in Bacteria," *Science*, 205, 602-606 (Aug. 10, 1979) [A].
- Mason et al., "Complementary DNA sequences of ovarian follicular fluid inhibin show precursor structure and homology with transforming growth factor- $\beta$ ," *Nature* 318, 659-663 (Dec. 1985) [F].
- Maurer, "Immunochemical Isolation of Prolactin Messenger RNA," *J. Biol. Chem* 255(1), 854-859 (Feb. 10, 1980) [F].
- Maxam et al., "Sequencing End-Labeled DNA with Base-Specific Chemical Cleavages," *Methods in Enzymol.* 65, 499-560 (1980) [F].
- McCormick et al., "Inducible Expression of Amplified Human Beta Interferon Genes in CHO Cells," *Molecular and Cellular Biology*, 4(1), 166-172 (1984) [B].
- McGonigle et al., "Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency," *Kidney Int'l.*, 25(2), 437-444 (1984) [A].
- Meier et al., "Alpha<sub>1</sub>- and Beta<sub>2</sub>-Adrenergic Receptors Co-Expressed on Cloned MDCK Cells are Distinct Glycoproteins," *Biochem. & Biophys. Res. Comm.*, 118(1), 73-81 (1984) [B].
- Mellon et al., "Identification of DNA Sequences Required for transcription of the human  $\alpha 1$ -Globin Gene in a New SV40 Host-Vector System," *Cell*, 27, 279-288 (Dec. 1981) [F].
- Mellor et al., "Expression of Murine H-2K<sup>2</sup> histocompatibility antigen in cells transferred with cloned H-2 genes," *Nature*, 298:529-534 (Aug. 1982) [D].
- Messing, "New M13 Vectors for Cloning," *Methods in Enzymology*, 101, 20-78 (1983) [A].
- Metcalf et al., "Effects of Purified Bacterially Synthesized Murine Multi-CSF (IL-3) on Hematopoiesis in Normal Adult Mice," *Blood*, 68(1), 46-57 (Jul. 1986) [B].
- Metcalf et al., "Quantitative Responsiveness of Murine Hemopoietic Populations in vitro and in vivo to Recombinant Multi-CSF (IL-3)," *Exp. Hematol.*, 15, 288-295 (1987) [B].

5,547,933

Page 7

- Methods in Yeast Genetics, Cold Spring Harbor Lab, Cold Spring Harbor, NY, p. 62 (1983) (not enclosed) [A].
- Miller et al., "Plasma levels of immunoreactive erythropoietin after acute blood loss in man," *Brit. J. Haematol.*, 52, 545-549 (1982) [A].
- Mirand, "Extra-renal and renal control of erythropoietin production," *Ann. N.Y. Acad. Sci.*, 149:94, 96, 98, 100, 102, 104, 106 (1968) [D].
- Mirand et al., "Current studies on the role of erythropoietin on erythropoiesis," *Ann. N.Y. Acad. Sci.*, 77:677-702 (1959) [D].
- Mladenovic et al., "Anemia of Chronic Renal Failure (CRF) in the Sheep: Response to Erythropoietin (EP) In Vivo and In Vitro," *Blood*, 58(5), Suppl. 1, 99a (1981) [F].
- Montgomery et al., "Identification and Isolation of the Yeast Cytochrome c Gene," *Cell*, 14, 67-680 (Jul. 1978) [F].
- Moriarty et al., "Expression of the Hepatitis B Virus Surface Antigen Gene in Cell Culture by using a Simian Virus 40 Vector," *P.N.A.S. (USA)*, 78(4), 2606-2610 (Apr. 1981) [D,E].
- Moriuchi et al., "Thy-1 cDNA sequence suggests a novel regulatory mechanism," *Nature*, 301, 80-82 (Jan. 1983) [F].
- Morrison, "Bioprocessing in Space—an Overview," *The World Biotech Report*, vol. 2, USA, 557-571 (1984) [A].
- Munjaal et al., "A cloned calmodulin structural gene probe is complementary to DNA sequence from diverse species," *P.N.A.S. (USA)*, 78(4), 2330-2334 (Apr. 1981) [D].
- Murphy et al., "The Role of Glycoprotein Hormones in the Regulation of Hematopoiesis," *Acta. Haematologica Japonica*, 46(7), 1380-1396 (Dec. 1983) [A].
- Myers et al., "Construction and Analysis of Simian Virus 40 Origins Defective in Tumor Antigen Binding and DNA Replication," *PNAS (USA)*, 77, 6491-6495 (Nov. 1980).
- Myklebost et al., "The Isolation and Characterization of cDNA clones for Human Apolipoprotein CII," *J. of Biol. Chem.*, 259(7), 4401-4404 (Apr. 10, 1984) [A].
- Naets, "The role of the kidney in erythropoiesis," *J. Clin. Invest.*, 39:102-110 (1960) [D].
- Nagata et al., "Synthesis in *E. Coli* of a polypeptide with human leukocyte interferon activity," *Nature*, 284, 316-320 (Mar. 27, 1980) [F].
- Nakao et al., "Erythropoiesis in anephric or kidney transplanted patients," *Israel J. Med. Sci.*, 7:986-990 (Jul.-Aug. 1971) [D].
- Nathan et al., "Erythropoietin and the Regulation of Erythropoiesis," *New Eng. J. Med.*, 308(9), 520-522 (Mar. 3, 1983) [A].
- Naughton et al., "Evidence for an Erythropoietin-Stimulating Factor in Patients with Renal and Hepatic Disease," *Acta. Haemat.*, 69, 171-179 (1983) [A].
- Naughton et al., "Evidence for a Hepatic-Renal Antagonism in the Production of Hepatic Erythropoietin," *Ann. Clin. Lab. Sci.*, 13(5), 432-438 (1983) [A].
- Nayak et al., "Characterization of Influenza Virus Glycoproteins Expressed from Cloned cDNAs in Prokaryotic and Eukaryotic Cells," *Modern Approaches To Vaccines*, pp. 165-172, Chanock et al., eds., Cold Spring Harbor Lab. (1984) [B].
- Neeser et al., "A Quantitative Determination by Capillary Gas-Liquid Chromatography of Neutral and Amino Sugars (as O-Methylxime Acetates), and a Study on Hydrolytic Conditions for Glycoproteins and Polysaccharides In Order to Increase Sugar Recoveries," *Anal. Biochem.*, 142, 58-67 (1984) [A].
- Newman et al., "Selection and Properties of a Mouse L-Cell Transformant Expressing Human Transferrin Receptor," *Nature*, 304, 643-645 (1983) [B].
- Nigg et al., "Immunofluorescent localization of the transforming protein of Rous sarcoma virus with antibodies against a synthetic src peptide," *P.N.A.S. (USA)*, 79, 5322-5326 (Sep. 1982) [A].
- Noda et al., "Primary structure of  $\alpha$ -subunit precursor of *Torpedo californica* acetylcholine receptor deduced from cDNA sequence," *Nature*, 299, 793-797 (Oct. 28, 1982) [F].
- Noda et al., "Cloning and sequence analysis of cDNA for bovine adrenal preproenkephalin," *Nature*, 295, 202-206 (Jan. 21, 1982) [F].
- Noyes et al., "Detection and partial sequence analysis of gastrin mRNA by using an oligodeoxynucleotide probe," *P.N.A.S. (USA)*, 76(4), 1770-1774 (Apr. 1979) [F].
- Nussinov, "Eukaryotic Dinucleotide Preference Rules and their Implications for Degenerate Codon Usage," *J. Mol. Biol.*, 149, 125-131 (1981) [F].
- Ogle et al., "Production of erythropoietin in vitro: a review," *In Vitro*, 14(11), 945-949 (1978) [D].
- Ohkubo et al., "Cloning and sequence analysis of cDNA for rat angiotensinogen," *P.N.A.S. (USA)*, 80, 2196-2200 (Apr. 1983) [A,F].
- Ohno et al., "Inducer-responsive expression of the cloned human interferons  $\beta$ 1 gene introduced into cultured mouse cells," *Nucleic Acids Res.*, 10(3):967-976 (1982) [D].
- Okayama et al., "High-Efficiency Cloning of Full-Length cDNA," *Mol. & Cell. Biol.*, 2(2), 161-170 (Feb. 1982) [A,F].
- Ovchinnikov et al., "The Primary Structure of *Escherichia coli* RNA Polymerase," *J. Biochem.*, 116, 621-629 (1981) [A].
- Paabo et al., "Association Between Transplantation Antigens and a Viral Membrane Protein Synthesized from a Mammalian Expression Vector," *Cell*, 35, 445-453 (1983) [B].
- Palmiter et al., "Metallothionein-Human GH Fusion Genes Stimulate Growth of Mice," *Science*, 222, 809-814 (Nov. 18, 1983) [A].
- Pankratz et al., "A Simple 3-Step Procedure for Purifying Baboon Urinary Erythropoietin to Apparent Homogeneity," *Exp. Hematol.*, 11, Suppl. 14, Abst. 102 (1983) [F].
- Parekh et al., "N-Glycosylation and in vitro Enzymatic Activity of Human Recombinant Tissue Plasminogen Activator Expressed in Chinese Hamster Ovary Cells and a murine Cell line," *Biochemistry*, 28, 7670-7679 (1989) [F].
- Pavlovic-Kentera et al., "Effects of Prostaglandin Synthetase Inhibitors, Salt Overload and Renomedullary Dissection on the Hypoxia Stimulated Erythropoietin Production in Rats," *Exp. Hematol.*, 8(Supp. 8), 283-291 (1980) [A].
- Palliger et al., "Altering Genotype and Phenotype by DNA-Mediated Gene Transfer," *Science*, 209, 1414-1422 (Sep. 19, 1980) [F].
- Pennathur-Das et al., "Evidence for the Presence of CFU-E with Increased In Vitro Sensitivity to Erythropoietin in Sickle Cell Anemia," *Blood*, 63(5), 1168-71 (May 1984) [A].
- Pennica et al., "Cloning and expression of human tissue-type plasminogen activator cDNA in *E-coli*," 301, 214-221 (Jan. 20, 1983) [B].
- Pennica et al., "Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin," *Nature*, 312, 724-728 (Dec. 27, 1984) [B,F].

5,547,933

Page 8

- Pitha et al., "Induction of human  $\beta$ -interferon synthesis with poly (rI+rC) in mouse cells transfected with cloned cDNA plasmids," *P.N.A.S. (USA)*, 79:4337-4341 (Jul. 1982) [D].
- Powell et al., "Human erythropoietin gene: High level expression in stably transfected mammalian cells and chromosome localization," *P.N.A.S. (USA)*, 83, 6465-6469 (Sep. 1986) [F].
- Prooijen-Knegt et al., "In Situ Hybridization of DNA Sequences in Human Metaphase Chromosomes Visualized by an Indirect Fluorescent Immunocytochemical Procedure," *Exp. Cell Res.*, 141, 397-407 (1982) [A].
- Ramabhadran et al., "Synthesis and Glycosylation of the Common  $\alpha$  Subunit of Human Glycoprotein Hormones in Mouse Cells," *Proc. Nat'l. Acad. Sci. (USA)*, 81, 6701-6705 (1984) [B].
- Rambach et al., "Acid Hydrolysis of Erythropoietin," *Proc. Soc. Exp. Biol.*, 99, 482-483 (1958) [F].
- Ravetech et al., "Evolutionary approach to the question of immunoglobulin heavy chain switching: Evidence from cloned human and mouse genes," *P.N.A.S. (USA)*, 77(11), 6734-6738 (Nov. 1980) [F].
- Recny et al., "Structural Characterization of Natural Human Urinary and Recombinant DNA-derived Erythropoietin," *J. Biol. Chem.*, 262(35), 17156-17163 (Dec. 15, 1987) [F].
- Reilly et al., "Use of synthetic oligonucleotides to clone genomic DNA: isolation of a tRNA<sup>Phe</sup> gene from mouse," *DNA*, 1:192 (1982) [D,E].
- Resegotti et al., "Treatment of aplastic anaemia with methenolone, stanozolol and nandrolone," *Panminerva Medica*, 23, 243-248 (1981) [A].
- Reyes et al., "Isolation of a cDNA clone for the murine transplantation antigen H-2K<sup>b</sup>," *P.N.A.S. (USA)*, 79, 3270-3274 (May 1982) [A].
- Reyes et al., "Identification of an H-2K<sup>b</sup>-Related Molecule by Molecular Cloning," *Immunogenetics*, 14, 383-392 (1981) [F].
- Riggs et al., "Synthetic DNA and Medicine," *Am. J. Hum. Genet.*, 31, 531-538 (1979) [F].
- Ringold et al., "Co-Expression and Amplification of Dihydrofolate Reductase cDNA and the *Escherichia coli* XGPR1 Gene in Chinese Hamster Ovary Cells," *J. Mol. & Appl. Genetics*, 1(3), 165-175 (1981) [F].
- Robson et al., "Polysome immunoprecipitation of phenylalanine hydroxylase mRNA from rat liver and cloning of its cDNA," *P.N.A.S. (USA)*, 79, 4701-4705 (Aug. 1982) [F].
- Rose et al., "Expression from Cloned cDNA of Cell-Surface Secreted Forms of the Glycoprotein of Vesicular Stomatitis Virus in Eucaryotic Cells," *Cell*, 30, 753-762 (1982) [B].
- Ross et al., "Phosphotyrosine-containing proteins isolated by affinity chromatography with antibodies to a synthetic hapten," *Nature*, 294, 654-656 (Dec. 17, 1981) [A].
- Roth et al., "Influenza Virus Hemagglutinin Expression Is Polarized in Cells Infected with Recombinant SV40 Viruses Carrying Cloned Hemagglutinin DNA," *Cell*, 33, 435-443 (1983) [B].
- Rothmann et al., "Erythropoietin-Dependent Erythrocytosis Associated with Hepatic Angiosarcoma," *J. Surg. Oncol.*, 20, 105-108 (1982) [A].
- Saito et al., "In Vitro Assay of Erythropoietin: Simple Determination in a Small Amount of Human Serum Samples," *Jap. J. Med.*, 23(1), 16-21 (Feb. 1984) [A].
- Saito et al., "Translation of Human Erythropoietin-mRNAs," *Exp. Hematol.*, 11(4), 228 (1983) [F].
- Sambrook et al., "Expression of Proteins on the Cell Surface Using Mammalian Vectors," *Experimental Manipulation of Gene Expression*, pp. 225-246, Acad. Press. (1983) [B].
- Sanger et al., "DNA Sequencing with chain-terminating inhibitors," *P.N.A.S. (USA)*, 74, 5463-5467 (Dec. 1977) [A,F].
- Sasaki, "Isolation of erythropoietin by monoclonal antibody," *Biomed. Biochim. Acta.*, 42(11/12), S202-206 (1983) [A,F].
- Scahill et al., "Expression and characterization of the product of a human immune interferon cDNA gene in Chinese hamster ovary cells," *Proc. Nat'l. Acad. Sci. (USA)*, 80, 4654-4658 (1983) [B].
- Schulze et al., "Identification of the cloned gene for the murine transplantation antigen H-2K<sup>b</sup> by hybridization with synthetic oligonucleotides," *Mol. & Cell Biol.*, 3(4), 750-755 (Apr. 1983) [D].
- Schwartz et al., "Severe Anemia as a Manifestation of Metastatic Jugular Paraganglioma," *Arch Otolaryngol*, 109, 269-272 (Apr. 1983) [A].
- Seeburg et al., "Synthesis of growth hormone by bacteria," *Nature*, 276, 795-798 (Dec. 1978) [F].
- Seki et al., "Isolation of a genomic clone containing structural information for the DR $\alpha$  subunit," *Fed. Proc.*, 41:365 (1982)/Chemistry and Molecular Biology of Ia/Dr Antigens Abstract 563 (1982) [D,E,F].
- Shahidi, "Androgens and Erythropoiesis," *New Eng. J. Med.*, 289, 72-80 (Jul. 12, 1973) [A].
- Sherwood et al., "A Radioimmunoassay for Erythropoietin," *Blood*, 54(4), 885-893 (Oct. 1979) [F].
- Sherwood et al., "Erythropoietin Titers in Sickle Cell Disease & Chronic Renal Failure," *Blood Suppl. 1*, 58, Abstract 105 (1981) [F].
- Sherwood et al., "Extraction of erythropoietin from normal kidneys," *Endo*, 103(3), 866-870 (1978) [D,F].
- Shiramizu et al., "Human Renal Carcinoma Cells Secreting Erythropoietin in vivo and in vitro," *Blood*, 78(10), Supp. 1 (Nov. 15, 1991) [F].
- Singer-Sam et al., "Isolation of a cDNA clone for human X-linked 3-phosphoglycerate kinase by use of a mixture of synthetic oligodeoxyribonucleotides as a detection probe," *P.N.A.S. (USA)*, 80, 802-806 (Feb. 1983) [A,D,F].
- Smith et al., "Construction and characterization of an infectious vaccinia virus recombinant that expresses the influenza hemagglutinin gene and induces resistance to influenza virus infection in hamsters," *Proc. Nat'l. Acad. Sci. (USA)*, 80, 7155-7159 (1983) [B].
- Southern et al., "Transformation of Mammalian Cells to Antibiotic Resistance with a Bacterial Gene Under Control of the SV40 Early Region Promoter," *J. Mol. Appl. Genet.*, 1(4), 327-341 (1982) [D].
- Southern, "Detection of Specific Sequences Among DNA Fragments Separated by Gel Electrophoresis," *J. Mol. Biol.*, 98, 503-517 (1975) [F].
- Spellman et al., "Carbohydrate Structure of Human Tissue Plasminogen Activator Expressed in Chinese Hamster Ovary Cells," *J. of Biol. Chem.*, 264(24), 14100-14111 (Aug. 26, 1989) [F].
- Spellman et al., "Carbohydrate Structure of Recombinant Soluble Human CD4 Expressed in Chinese Hamster Ovary Cells," *Biochemistry*, 30(9), 2395-2406 (1991) [F].
- Srinivas et al., "Membrane Association and Defective Transport of Spleen Focus-forming Virus Glycoproteins," *J. Biol. Chem.*, 258, 14718-14724 (1983) [B].



5,547,933

Page 9

- Steck et al., "Cell Surface Properties of Spontaneously Metastasizing Rat Mammary Adenocarcinoma Cell Clones," *Transplantation Proceedings*, 16, 355-360 (1984) [B].
- Storring et al., "The International Standard for Recombinant DNA Derived Erythropoietin Collaborative Study of Form Recombinant DNA Derived Erythropoietins and Two Highly Purified Human Urinary Erythropoietins," *J. of Endo.*, 134, 459-489 (1992) [F].
- Strickland, "Occurrence of Sulfate on the N-Linked Oligosaccharides of Human Erythropoietin," *J. of Cellular Biochemistry*, Suppl. 16D, Abstract No. P324 (1992) [F].
- Sue et al., "Site-specific antibodies to human erythropoietin directed toward the NH<sub>2</sub>-terminal region," *Proc. Nat. Acad. Sci. (USA)*, 80, 3651-3655 (1983) [A,E,F].
- Suggs et al., "Use of Synthetic Oligodeoxyribonucleotide for the Isolation of Specific Cloned DNA Sequences," *Developmental Biology Using Purified Genes*, 683-693 (D. Brown, Ed., 1981) [D,F].
- Suggs et al., "Use of synthetic oligonucleotides as hybridization probes: Isolation of cloned cDNA sequences for human B<sub>2</sub>-microglobulin," *P.N.A.S. (USA)*, 78, 6613-6617 (1981) [A,B,D,E,F].
- Sytowski et al., "A Novel Radioimmunoassay for Human Erythropoietin Using a Synthetic NH<sub>2</sub>-Terminal Polypeptide and Anti-Peptide Antibodies," *J. Immunol. Methods*, 69, 181-186 (1984) [A,F].
- Sytowski et al., "The Biochemistry of Erythropoietin: An Approach to its mode of Action," *Exp. Hematol.*, 8(Supp. 8), 52-63 (1980) [A].
- Szostak et al., "Hybridization with Synthetic Oligonucleotides," *Meth. in Enzymol.*, 68, 419-429 (1979) [F].
- Takeuchi et al., "Relationship between sugar chain structure and biological activity of recombinant human erythropoietin produced in Chinese hamster ovary cells," *P.N.A.S. (USA)*, 86, 7819-7822 (Oct. 1989) [F].
- Talmadge et al., "Eukaryotic Signal Sequence Transports Insulin Antigen in *Escherichia coli*," *P.N.A.S. (USA)* 77(6), 3369-3373 (Jun. 1980) [A,B,D].
- Tambourin et al., "Production of erythropoietin-like activity by a murine erythro leukemia cell line," *P.N.A.S. (USA)*, 80, 6269-6273 (1983) [A,F].
- Taub et al., "An Improved Method for Preparing Large Arrays of Bacterial Colonies Containing Plasmids for Hybridization: In Situ Purification and Stable Binding of DNA on Paper Filters," *Anal. Biochem.*, 126, 222-230 (1982) [A].
- Taub et al., "An Improved Method for preparing large arrays of bacterial colonies containing plasmids for hybridization: in situ purification and stable binding of DNA on paper filters," *Chemical Abstracts*, 97(23), 164, Abstract No. 194002y (Dec. 12, 1982) [A].
- Tong et al., "The Formation of Erythrocyte Membrane Proteins during Erythropoietin-induced Differentiation," *J. Biol. Chem.*, 256(24), 12666-12672 (Dec. 25, 1981) [A].
- Toole et al., "Molecular cloning of a cDNA encoding human antihemophilic factor," *Nature*, 312, 342-347 (Nov. 8, 1984) [F].
- Tramontano et al., "Statistical evaluation of the coding capacity of complementary DNA strands," *Nucleic Acids Research*, 12(12), 5049-5059 (1984) [A].
- Udupa et al., "Erythropoiesis in the aged mouse," *J. Lab. Clin. Med.*, 103(4), 574-580 & 581-588 (1984) [A].
- Ullrich et al., "Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity," *EMBO J.*, 5(10), 2503-2512 (1986) [F].
- Ullrich et al., "Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells," *Nature*, 309, 418-425 (May 31, 1984) [F].
- Ullrich et al., "Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes," *Nature*, 313, 756-761 (Feb. 28, 1985) [F].
- Ullrich et al., "Rat Insulin Genes: Construction of Plasmids Containing the Coding Sequences," *Science*, 196, 1313-1319 (Jun. 17, 1977) [A].
- Ullrich et al., "Isolation of the Human Insulin-like Growth Factor I Gene Using a Single Synthetic DNA Probe," *EMBO J.*, 3(2):361-364 (1984) [D,F].
- Urabe et al., "The Influence of Steroid Hormone Metabolites on the In Vitro Development of Erythroid Colonies Derived from Human Bone Marrow," *J. Exp. Med.*, 149, 1314-1325 (Jun. 1979) [A].
- Urlaub et al., "Isolation of Chinese Hamster cell mutants deficient in dihydrofolate reductase activity," *Proc. Nat. Acad. Sci. (USA)*, vol. 77(7), 4216-4220 (Jul. 1980) [A,D,F].
- Van Stone et al., "Effect of erythropoietin on anemia of peritoneally dialyzed anephric rats," *Kidney Int'l.*, 15, 370-375 (1979) [F].
- Van der Ploeg et al., "DNA Methylation in the Human  $\gamma\delta\beta$ -Globin Locus in Erythroid and Nonerythroid Tissues," *Cell*, 19, 947-958 (Apr. 1980) [F].
- Vedovato et al., "Erythropoietin Levels in Heterozygous Beta-Thalassemia," *Acta. Haematol.*, 71, 211-213 (1984) [A].
- Vichinsky et al., "Inadequate erythroid response to hypoxia in cystic fibrosis," *J. Pediatr.*, 105(1), 15-21 (Jul. 1984) [A].
- Vieira et al., "The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers," *Gene*, 19, 259-268 (1982) [F].
- Villasante et al., "Binding of microtubule protein to DNA and chromatin: possibility of simultaneous linkage of microtubule to nucleic acid and assembly of the microtubule structure," *Nucleic Acids Res*, 9(4), 895 (1981) [F].
- Walker et al., *Techniques in Molecular Biology*, Macmillan Pub. Co., N.Y., p. 280 (1983) [A].
- Wallace et al., "The use of synthetic oligonucleotides as hybridization probes. II. Hybridization of oligonucleotides of mixed sequence to rabbit  $\beta$ -globin DNA," *Nuc. Acids Res.*, 9(4), 879-894 (1981) [A,D,E,F].
- Wallace et al., "A set of synthetic oligodeoxyribonucleotide primers for DNA sequencing in the plasmid vector pBR322," *Gene*, 16, 21-26 (1981) [A].
- Wallace et al., "Directed Deletion of a Yeast Transfer RNA Intervening Sequence," *Science*, 209, 1396-1400 (Sep. 19, 1980) [D].
- Wallace et al., "Oligonucleotide Directed Mutagenesis of the Human  $\beta$ -globin gene: A General Method for Producing Specific Point Mutations in cloned DNA," *Nucleic Acids Research*, 9(15), 3647-3657 [D].
- Wallace et al., "Hybridization of synthetic oligodeoxyribonucleotides to Phi-chi 174 DNA: the effect of single base pair mismatch," *Nuc. Acids Res.*, 6(11), 3543-3557 (1979) [A,D,F].

5,547,933

Page 10

- Wallis et al., "The isolation of cDNA clones for human apolipoprotein E and the detection of apoE RNA in hepatic and extra-hepatic tissues," *EMBO J.*, 2, 2369-2373 (1983) [F].
- Walter et al., "Antibodies specific for the carboxy- and amino-terminal regions of simian virus 40 large tumor antigen," *P.N.A.S. (USA)*, 77(9), 5197-5200 (Sep. 1980) [A].
- Walter et al., "Antibodies specific for the polyoma virus middle-size tumor antigen," *P.N.A.S. (USA)*, 78, 4882-4886 (Aug. 1981) [A].
- Wang et al., "Renal and extrarenal erythropoietin production in male and female rats of various ages," *J. Lab. Clin. Med.*, 79(2), 181-186 (Feb. 1972) [D].
- Weiland et al., "In vivo Activity of Asialo-Erythropoietin in Combination with Asialo-Glycoproteins," *Blut*, 44(3), 173-175 (1982) [A].
- Weiss et al., "Studies of the pathogenesis of anemia of inflammation: Mechanism of impaired erythropoiesis," *Am. J. Vet. Res.*, 44(10), 1832-1835 (Oct. 1983) [A].
- Weissman et al., "Structure and expression of human IFN- $\alpha$  Genes," *Phil. Trans. R. Soc. Lond.*, B299, 7-28 (1982) [B].
- White et al., "Studies on Erythropoietin," *Recent Progr. Hormone Res.*, 16, 219-262 (1960) [D].
- White et al., "Haemagglutinin of influenza virus expressed from a cloned gene promotes membrane fusion," *Nature*, 300, 658-659 (1982) [B].
- Whitehead et al., "Use of a cDNA Clone for the Fourth Component of Human Complement (C4) for Analysis of a Genetic Deficiency of C4 in Guinea Pig," *P.N.A.S. (USA)*, 80, 5387-5391 (Sep. 1983) [D,E,F].
- Wiaderkiewicz et al., "Mismatch and blunt to protruding-end joining by DNA ligases," *Nucleic Acids Res.*, 15(19), 7831-7848 (1987) [F].
- Wide et al., "Molecular charge heterogeneity of human serum erythropoietin," *British J. Haemat.*, 76, 121-127 (1990) [F].
- Wiktor et al., "Protection from rabies by a vaccinia virus recombinant containing the rabies virus glycoprotein gene," *Proc. Nat'l. Acad. Sci. (USA)*, 81, 7194-7198 (1984) [B].
- Wong et al., "Synthetic peptide fragment of src gene product inhibits the src protein kinase and crossreacts immunologically with avian onc kinases and cellular phosphoproteins," *P.N.A.S. (USA)*, 78(12), 7412-7416 (Dec. 1981) [A].
- Woo, "A Sensitive and Rapid Method for Recombinant Phage Screening," *Methods in Enzymology*, 68, 389-395 (1979) [A].
- Wood et al., "Expression of active human factor VIII from recombinant DNA clones," *Nature*, 312, 330-337 (Nov. 22, 1984) [F].
- Woods et al., "Isolation of Class III cDNA Clones," *Second Meeting on Cloning of the HLA and H-2 Regions*, Abstract (Apr. 17-19, 1983) [E].
- Woods et al., "Isolation of cDNA clones for the human complement protein factor B, a class III major histocompatibility complex gene product," *P.N.A.S. (USA)* 79, 5661-5665 (Sep. 1982) [A,D,E,F].
- Woods et al., "Isolation of a cDNA Clone Corresponding to the MHC Linked Complement Protein Factor B," *Mol. Immunology*, 19, 1411 (1982) [F].
- Yanagawa et al., "Isolation of Human Erythropoietin with Monoclonal Antibodies," *J. Biol. Chem.*, 259(5), 2707-2710 (Mar. 10, 1984) [A,F].
- Yanagawa et al., "Hybridomas for Production of Monoclonal antibodies to Human Erythropoietin," *Blood*, 64(2), 357-364 (Aug. 1984) [A,F].
- Young et al., "Efficient isolation of genes by using antibody probes," *P.N.A.S.* 80, 1194-1198 (Mar. 1983) [A].
- Yuen et al., "The Spectrum of N-linked oligosaccharide structures detected by enzymic microsequencing on a recombinant soluble CD4 glycoprotein from Chinese hamster ovary cells," *Eur. J. Biochem.*, 192, 523-528 (1990) [F].
- Zinn et al., "Regulated expression of an extrachromosomal human  $\beta$ -interferon gene in mouse cells," *P.N.A.S. (USA)*, 79, 4897-4901 (Aug. 1982) [D].
- Chiba et al, *Bioch. Biophys. Res. Comm.*, 47(6), 1372-7, (1972).
- Miyake et al, *J. Biol. Chem.*, 252(15), (1977) 1.
- Takeuchi et al, *J. BiolChem.*, 263(8), 3657-63, (1988).
- Miyake et al, *J. Biol Chem.*, 252(15), 5558-64, (1977).
- Dordal et al, *Endocrin.*, 116(6), 2293-99, (1985).
- Wang et al, *Endocrin*, 116(6), 2286-92, (1985).
- Sasaki et al, *J. Biol. Chem.*, 262(25), 12059-76, (1987).
- Roh et al, *Fed. Proc.*, 29(2), 782, Abs 3030, (1970).
- Hunter et al, *Biochem. J.*, 89, 114-23 (1963).
- Papayannopoulou et al, *J. Clin. Invest.*, 51, 1179-85, (1972).
- Weiss et al, *PNAS (USA)*, 79, 5465-69, (Sep. 1982).

FIG. 1

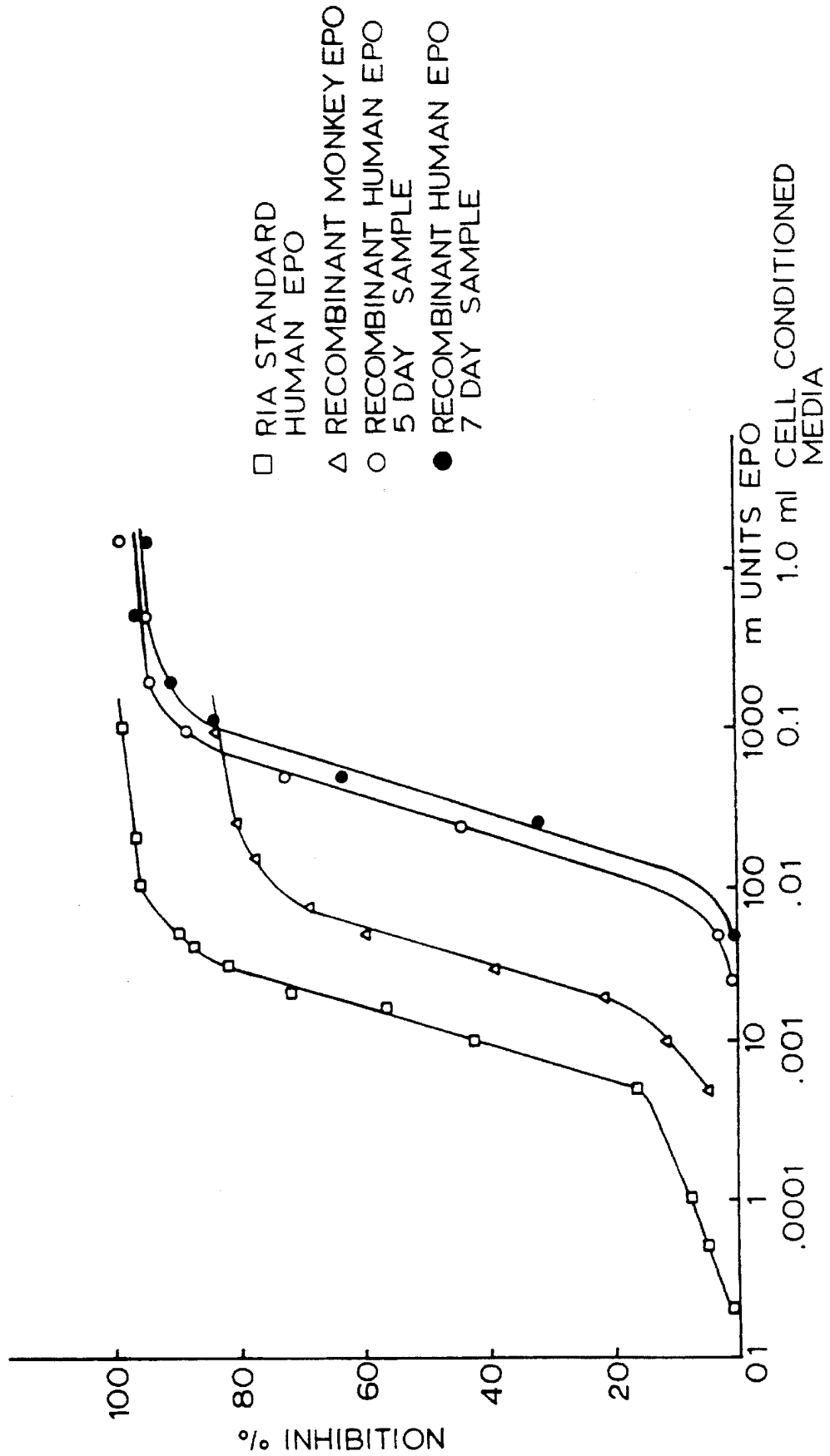


FIG. 2

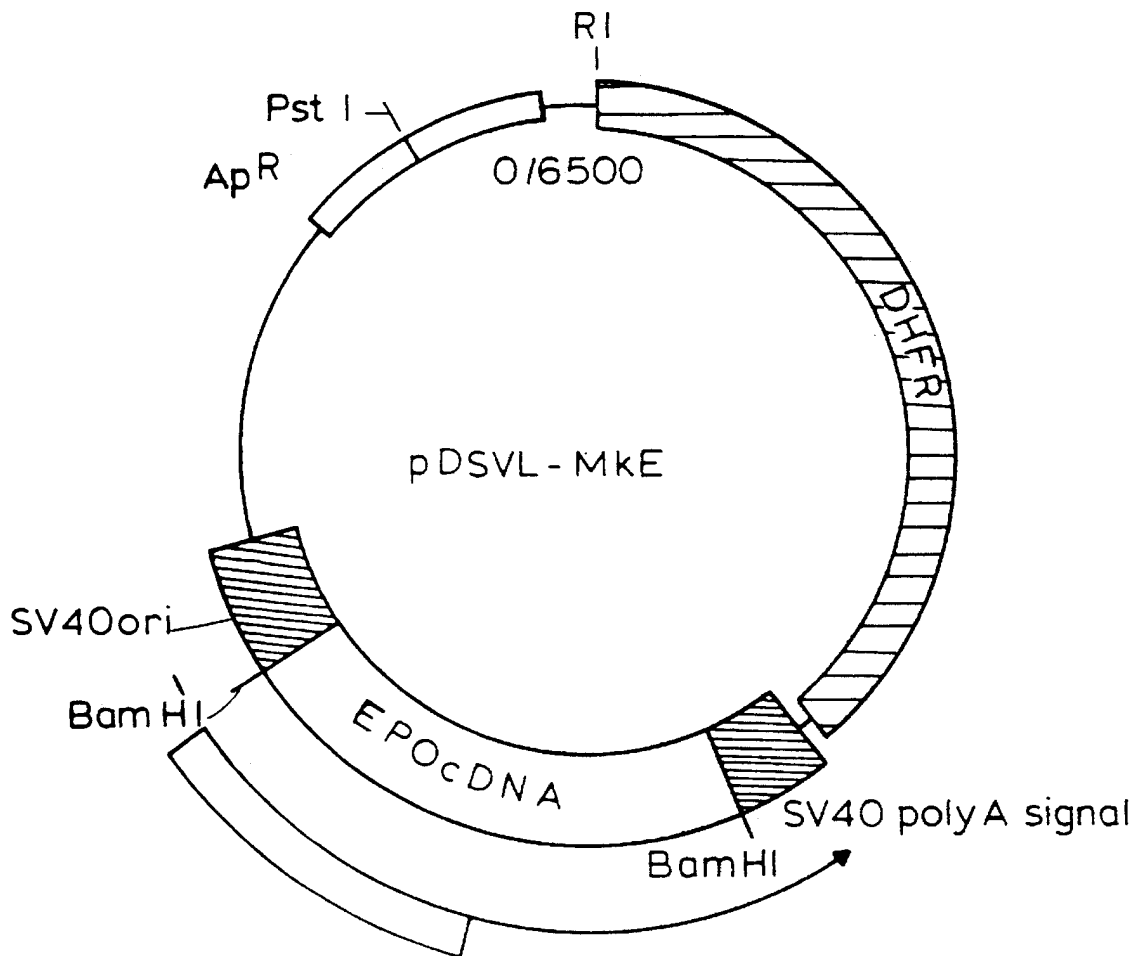


FIG. 3

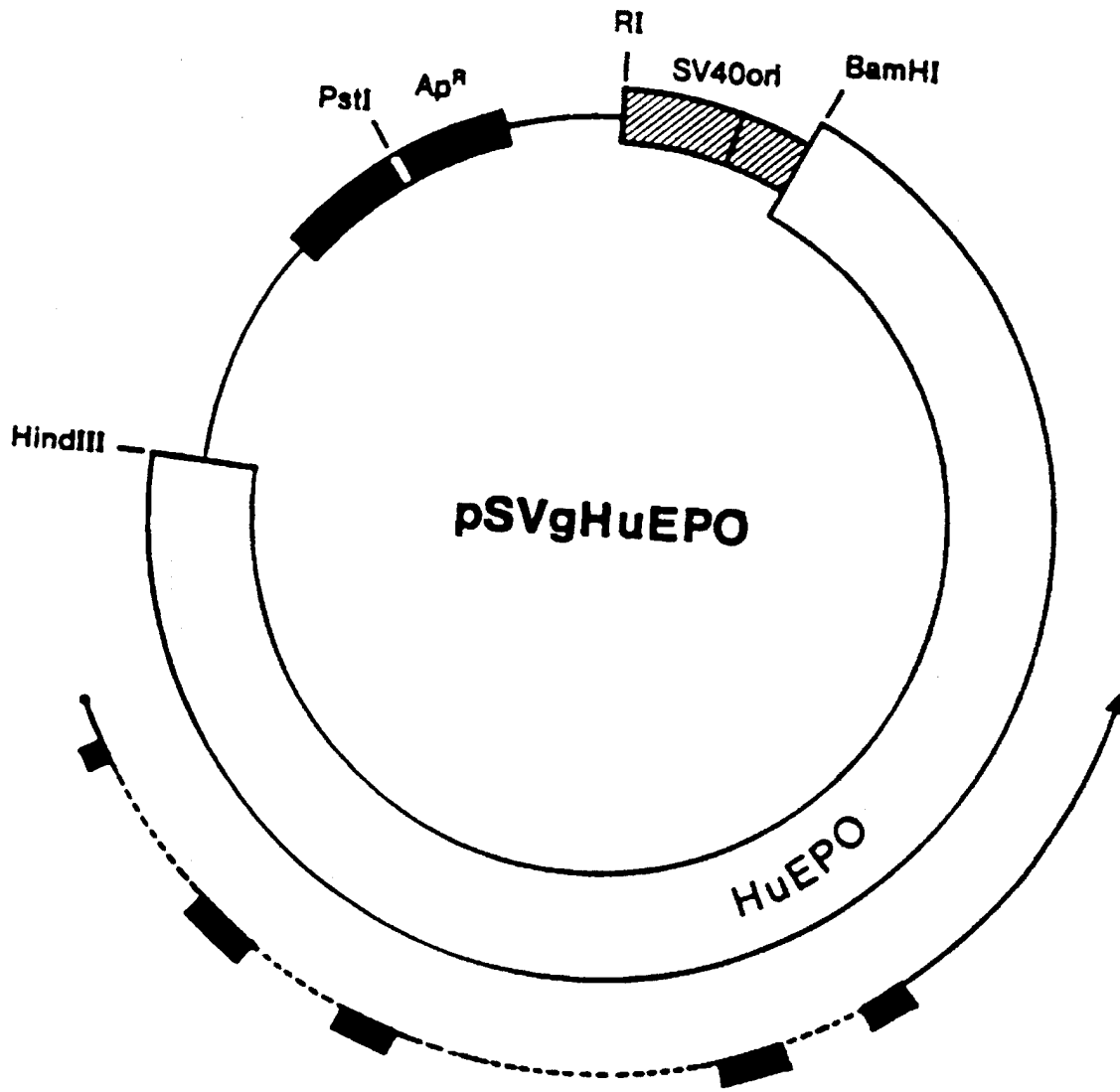


FIG. 4

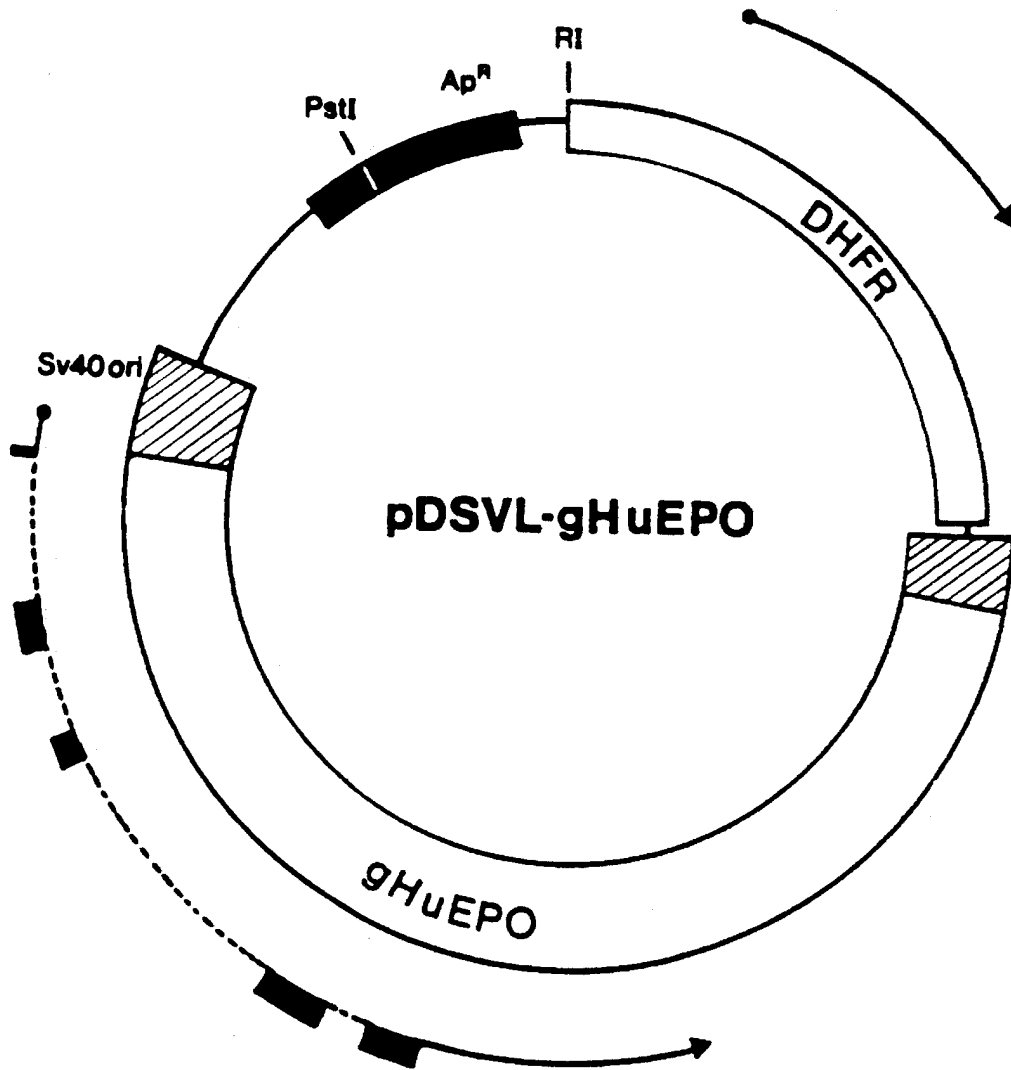


FIG. 5A

**8813A**  
**GATCCCGCCCGCCCTGGACAGCCCGCCCTCTCTCCAGCCCGCCCGTGGGCTGGCCCTGCC**  
**CGCTGAACCTTCCCGGGATGAGGACTCCCGGTGTGGTCAACCAGCCCGCTAGGTCCGCTGAG**

-27  
 Met Gly Val His Glu Cys Pro Ala Tip  
**GGACCCCGCCAGCCCGGAGATG GGG GTG CAC GAA TGT CCT GCC TGG**

-20  
 Leu Tip Leu Leu Ser Leu Val Ser Leu Pro Leu Gly Leu Pro  
**CTG TGG CTT CTC CTG TCT CTC GTG TCG CTC CCT 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 CCG CTC ATC TGT GAC AGC CGA GTC CTG**

+1  
 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  
 Gly Cys Ser Glu Ser Cys Ser Leu Asn Glu Asn Ile Thr Val Pro  
**GCC TGT TCC GAA AGC TGC AGC TTG AAT GAG AAT ATC ACC GTC CCA**

30  
 \*  
 \* 40

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 Val Trp Gln Gln Gly Leu Ala Leu Leu Ser Glu  
 CAG CAG GCT GTA GAA GTC GTC TGG CAG GCC CTG GCC CTG CTC TCA GAA  
 70  
 80 \*  
 Ala Val Leu Arg Gly Gln Ala Val Leu Ala Asn Ser Ser Gln Pro  
 GCT GTC CTG CCG CGG GGC CAG GCC GTC TGG TTC GCC AAC TCT TCC CAG CCT  
 90  
 Phe Glu Pro Leu Gln Leu His Met Asp Lys Ala Ile Ser Gly Leu  
 TTC GAG CCC ACC CTG CAG CTG CAC ATG GAT AAA GCC ATC AGT GGC CTT  
 100  
 110  
 Arg Ser Ile Thr Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Glu Ala  
 CGC AGC ATC ACC ACT ACT CTG CTT CCG GCG GCG GGA GCC CAG GAA GCC  
 120  
 Ile Ser Leu Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile  
 ATC TCC CTC CCA GAT GAT GCG GCC TCG GCT GCT CCA CTC CGA ACC ATC  
 130  
 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 TTC CGA GTC TAC TCC AAT TTC





FIG. 6A

AAGCTTCTGGGCTTCCAGACCCAGCTACTTTGGGAACTCAGCAACCCAGGCATCTCTGAGTCTCCGCCCCA  
 AGACCGGAGTCCCGGATCCCGGAGGGTGTCCCGGAGCCCGAGCCCTTTCCCAAGATAGCAAGCTCCGCCCCAGTCCC  
 AAGGGTCCGCAACCGGCTGCCTCCCTCCCGGACCCAGGGGCCCCGAGCAGCCCCCACTGACCCCAACAGCC  
 ACGTCTCAGCAAGCCCCGCTCAGCCCCCGGAGGCCCTCAACCCAGGGGCTCCCTGCCCCCTGCTGTAACCCCGG  
 GTGGCCCTAACCCCTGGCGAACCCCTCAAGCCACAGCCCTCTCCCGGACCCCGGCAAGCCGCAACACATG  
 CAGATAACAGCCCCGACCCCGGCGAGGCCGXAAGTCCCTGGGCCACCCCGGCTCGCCCTGCCCCGCTG  
 CGCCGACCGGCTGTCTCCCGGAGCCCGGACCCCGGCGGACCCCGGCGCTGCTCCGACACCCGCGCC  
 CTTGGACAGCCGCTCTCTCTAGGCCCGTGGGCTGGCCCTGCACCCGCGAGCTTCCCGGATGAGGIX  
  
 CCCGGTACCGGCGCCCGCAAGTCCCTAGGGGACCCCGGCGGAG ATG GGG GTG CAC G  
 GTGAGTACTCGCGGCTGGGCTCCCGGCGGCGGTTCTCTGAGCGGGGATTTAGCGCCCCCGGCT

-27      -24  
 Not Gly Val His

FIG. 6B

ATTGCCAAGAGGGTGGCTGGGTTCAAGGACCCGGGACTTGTCAAGGACCCCGAAGGGGGAGGGGGGTGGG  
 GCAGCCTCCACGGTCCCGGGGACTTGGGGGAGTTCTTGGGGATGGCAAAAACCTGGCCCTGTTGAGGGGCCA  
 CAGTTGGGGTGGGAGGGAGGTTTGGGGTCTGCTGTGCAAGTTGTGTGCTGTTGTCAAGTGTCTCG [ I . B . ]  
 TTGCACAGCCACAGATCAATAAGCCAGAGGCCACCTGAGTGTGCTTGCATGGTTGGGACAGGAAAGGACGGAG  
 CTGGGGCAGAGACGTGGGGATGAGGAGCTGTCTCTCCACAGCCACTTCTCCCCCCCCCTGACTCT  
 CAGCCTGGCTATCTGTCTAG  
 -23 -20  
 Glu Cys Pro Ala Trp Leu Trp Leu Leu Leu Leu Ser Leu  
 AA TGT CCT GCC TGG CTG TGG CTT CTC CTG TCC CTG  
 -1 +1  
 Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly Ala Pro Pro Arg Leu Ile Cys  
 CTG TCG CTC CCT CCT CTG GGC CTC CCA GTC CTG GCC 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 GTGAGACCCCTTCCCCAGCACATTCACAGAACTCACGGGCTTCAGGGAACTCCCTCCAGAT  
 CCAGGAACTGGCAGTGGTTGGGGTGGAGTGGGAAAGCTAGACACTGCCCCCTACATAAGAAATAGTC

FIG. 6C

TGGTGGCCCCAACCATACTGAACTAGCCAAAGGAGCAAGCCAGCAGATCCTACGCCCTGTGGGCCACAGGG

CCAGAGCCCTTCAGGGACCCTTGACTCCCGGGCTGTGTGCATTTTCAG 27 Thr Gly Cys Ala Glu 30  
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  
GCC TGG AAG AGG ATG GAG GTGAGTTCCTTTT TTTTTCCTTTCTTTTGGGAAATCTCATT

TGGAGCCCTGATTTGGATGAAAGGGGAAATGATCGGGGAAAGGTAAATGGAGCAGCAGAGATGAGGCT  
GCCTGGGCGCAGAGGCTCAGTCTATAATCCAGGCTGAGATGGCCGAGATGGGAGAAATTGCTTGAGCCCT

GGAGTTTCAGACCAACCTAGGCAGCATAGTGAGATCCCCCATCTCTACAAACATTTAAAAAATTAGTCA  
GTGAAAGTGGTGCATGGTGGTAGTCCCGAGTATTGGAAAGGCTGAGGCGGAGGATCGCTTGAGCCCAAGAA

TTTGAGGCTGCAGTGAGCTGTGATCACACCACCTGCACCTCCAGCCTCAGTGACAGAGTGAGGCCCTGTCTCA



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 ACT GCT GAC ACT TTC CGC AAA CTC TTC CGA GTC TAC TCC  
140  
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 AAG ACA GGG  
150  
166 Asp Arg OP  
GAC AGA TGA CCAGGTGTGTCCACCTGGGGCATATCCACCCTCCCTCACCAACATTGCTTGTGCCACA  
CCCTCCCCGGCACTCCTGAAACCCCGTCCGAGGGGCTCTCAGCTCAGCGGCCAGCCCTGTCCCATGGACACTCC  
AGTCCAGCAATGACATCTCAGGGGCCAGAGGAACTGTCCAGAGAGCACTCTGAGATCTAAGGATGTCCAC  
AGGGCCAACTTGAAGGGCCCAAGCAGGAAAGCATTCCAGAGAGCCAGCTTTAAACTCAGGGACAGGCCATTGC  
TGGGAAAGACGCCCTGAGCTCAGCTCCGGCACCCCTGCAAAATTTGATGCCAGGACACCGCTTTGGAGGCCGATTTAC  
CTGTTTCCACCTACCATCAGGGACAGGATGACCTGGAGAACTTAGGTGGCAAGCTGTGACTTCTCCAGG  
TCTCACGGGCGCATGGGCACCTCCCTTGGTGGCAAGAGGCCCCCTTGCACCCGGGGTGGTGGAAACCATGAAAGAC  
AAGATXGGGGCTGGCCCTGTGGCTCTCATGGGGTCCAAAGTTTGTGTATTTCTCAACCTATTGACAGACTGAA  
ACACAATATGAC

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## FIG. 7

-1 1  
XbaI NotAla

CTAG AAACCATGAG GGTAAATAAAA TAATGGCTCC GCCCGCTCTG  
TTGGTACTC CCATTATTTT ATTACCGAGG CCGCGCAGAC

ATCTGCCACT CGAGAGTTCT GGAACGTTAC CTGCTGGAAG CTAAGAAGC  
TAGACGCTGA GCTCTCAAGA CCTTGCAATG GACGACCTTC GATTTCTTCG

TGAAAACATC ACCACTGGTT GTGCTGAACA CTGTTCTTTG AACGAAAACA  
ACTTTTGTAG TGGTGACCAA CACGACTTGT GACAAGAAC TTGCTTTTGT

TTACGGTACC AGACACCAAG GTTAACTTCT ACGCTTGGAA ACGTATGGAA  
AATGCCATGG TCTGTGGTTC CAATTGAAGA TCGAACCTT 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 TCGTGCTCT GGGTGCACAG AAAGAGGCTA TCTCTCCGCC  
TGATGAGACG ACGCACGAGA CCCACGTGTC TTTCTCCGAT AGAGAGGCGG

GGATGCTGCA TCTGCTGCAC CGCTGCGTAC CATCACTGCT GATACCTTCC  
CCTACGACGT AGACGACGTG GCGACGCATG GTAGTGACGA CTATGGAAGG

GCAAACTGTT TCGTGTATAC TCTAACTTCC TCGGTGGTAA ACTGAAACTG  
CGTTTGACAA AGCACATATG AGATTGAAGG ACGCACCATT TGACTTTGAC

SalI

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	TGCACAAAAC	TCTCCAGTTC
CCTTGTTGGT	TAACTCTTCT	CAACCATGGG	AACCATTGCA	ATTGCACGTC
GGAACAACCA	ATTGAGAAGA	GTTGGTACCC	TTGGTAACGT	TAACGTGCAG
GATAAAGCCG	TCTCTGGTTT	GAGATCTTTG	ACTACTTTGT	TGAGAGCTTT
CTATTTCCGGC	AGAGACCAA	CTCTAGAAAC	TGATGAACA	ACTCTCGAAA
GGGTGCTCAA	AAGGAAGCCA	TTTCCCACC	AGACGCTGCT	TCTGCCGCTC
CCCACGAGTT	TTCCTTCGGT	AAAGGGGTGG	TCTGCGACGA	AGACGGCGAG
CATTGAGAAC	CATCACTGCT	GATACCTTCA	GAAAGTTATT	CAGAGTTTAC
GTAACCTTG	GTAGTGACGA	CTATGGAAGT	CTTTCAATA	GTCTCAAATG
TCCAACCTTCT	TGAGAGGTAA	ATTGAAGTTG	TACACCGGTG	AAGCCTGTAG
AGGTTGAAGA	ACTCTCCATT	TAACTTCAAC	ATGTGGCCAC	TTCGGACATC
AACTGGTGAC	AGATAAGCCC	GACTGATAAC	AACAGTGTAG	
TTGACCACTG	TCTATTCGGG	CTGACTATTG	TTGTCAATC	
	<u>SalI</u>			
ATGTAACAAA	G			
TACATTGTTT	CAGCT			



FIG. 9

	-20	-10	+1	10	20	30	40
Human	MGVHECPAWLWLLLSLPLGLPVLGAPPRLICDSRVLERYLLEAKEAENITGCAEHCSLNENITVDPDK						
Monkey	MGVHECPAWLWLLLSLPLGLPVPGAPPRLICDSRVLERYLLEAKEAENVTMGCSESCSLNENITVDPDK						

	50	60	70	80	90	100	110
Human	VNFYAWKRMEVGGQAVEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTLLRALGAQKE						
Monkey	VNFYAWKRMEVGGQAVEVWQGLALLSEAVLRGQAVLANSQPFEPQLHMDKAI SGLRSLTLLRALGAQ-E						

	120	130	140	150	160
Human	AISPPDAASAAPLRTITADTFRKLFRVYSNFRGKLLKLYTGEACRTGDR				
Monkey	AISLPDAASAAPLRTITADTFCKLFRVYSNFRGKLLKLYTGEACRRGDR				

**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

EGORI KPHI <sup>1</sup>  
 A ATTCGGTACC AGACACCAAG GTTAACTTCT ACCCTTGGAA ACGTATGGAA <sup>3</sup>  
 GCCATGG TCTGTGGTTC CAATTGAAGA TCCGAACCTT TGCATACCTT <sup>4</sup>  
<sup>2</sup>

<sup>5</sup> GTTGGTCAAC AAGCAGTTGA AGTTTGGCAG GGTCTGGCAC TGCTGAGCGA  
 CAACCACTTG TTCGTCAACT TCAAAACCGTC CCAAGCCGTG ACGACTCGCT <sup>8</sup>  
<sup>6</sup>

<sup>9</sup> GGCTGTACTG CGTGGCCAGG CACTGCTGGT AAATCCTCT CAGCCGTGGG  
 CCGACNTGAC GCACCCGGTCC GTGACGACCA TTTGAGGAGA GTCCGGCACCC <sup>12</sup>  
<sup>10</sup>

<sup>13</sup> AACCGCTGCA GCTGCATGTT GACAAAGCAG TATCTGGCCT GAGATCTG  
 TTGGCGACGT CGACGTACAA CTGTTTCGTC ATAGACCGGA CTCTAGACCTAC <sup>16</sup>  
<sup>14</sup>

EGIIII BPHI <sup>15</sup>  
<sup>15</sup>

**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**

FIG. 15

BamHI BglII

GA TCCAGATCTCTG

GTCTAGAGAC

<u>1</u>		<u>3</u>		<u>5</u>
<u>ACTACTCTGC</u>	<u>TGCGTCTCT</u>	<u>GGGTGCACAG</u>	<u>AAAGAGGCTA</u>	<u>TCTCTCCGCC</u>
<u>TGATGAGACG</u>	<u>ACGCACGAGA</u>	<u>CCCACGTGTC</u>	<u>TTTCTCCGAT</u>	<u>AGAGAGGCGG</u>
<u>2</u>		<u>4</u>		

		<u>7</u>		<u>9</u>
<u>GGATGCTGCA</u>	<u>TCTGCTGCAC</u>	<u>CGCTGCGTAC</u>	<u>CATCACTGCT</u>	<u>GATACCTTCC</u>
<u>CCTACGACGT</u>	<u>AGACGACGTG</u>	<u>GCGACGCATG</u>	<u>GTAGTGACGA</u>	<u>CTATGGAAGG</u>
<u>6</u>		<u>8</u>		

		<u>11</u>		<u>13</u>
<u>GCAAACGTGT</u>	<u>TCGTGTATAC</u>	<u>TCTAACTTCC</u>	<u>TGCGTGGTAA</u>	<u>ACTGAAACTG</u>
<u>CGTTTGACAA</u>	<u>AGCACATATG</u>	<u>AGATTGAAGG</u>	<u>ACGCACCATT</u>	<u>TGACTTTGAC</u>
<u>10</u>		<u>12</u>		

		<u>15</u>		<u>Sa</u> II
<u>TATACTGGCG</u>	<u>AAGCATGCCG</u>	<u>TACTGGTGAC</u>	<u>CGCTAATAG</u>	
<u>ATATGACCGC</u>	<u>TTCGTACGGC</u>	<u>ATGACCACTG</u>	<u>GCGATTATC</u>	<u>AGCT</u>
<u>14</u>		<u>16</u>		

**FIG. 16**

**1. AATCAAGCTTGGATAAAAGAGCT**  
**2. GTGGAGCTCTTTTATCCAAGCTTG**  
**3. CCACCAAGATTGATCTGTGACTC**  
**4. TCTCGAGTCACAGATCAATCTTG**  
**5. GAGAGTTTTGGAAAGATACTTGTTG**  
**6. CTTCCAACAAGTATCTTTCAAAAC**  
**7. GAAGCTAAAGAAGCTGAAAACATC**  
**8. GTGGTGATGTTTTTCAGCTTCTTTAG**  
**9. ACCACTGGTTGTGCTGAACACTGTTT**  
**10. CAAAGAACAGTGTTTCAGCACAACCA**  
**11. TTTGAACGAAAACATTACGGTACCG**  
**12. GATCCGGTACCGTAATGTTTTTCGTT**