



EXHIBIT 2  
Part 1

# United States Patent [19]

[11] Patent Number: **5,618,698**

Lin

[45] Date of Patent: **\*Apr. 8, 1997**

[54] **PRODUCTION OF ERYTHROPOIETIN**

[75] Inventor: **Fu-Kuen Lin**, Thousand Oaks, Calif.

[73] Assignee: **Kirin-Amgen, Inc.**, Thousand Oaks, Calif.

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

[21] Appl. No.: **468,381**

[22] Filed: **Jun. 6, 1995**

0117058 8/1984 European Pat. Off. .  
 0117059 8/1984 European Pat. Off. .  
 0117060 8/1984 European Pat. Off. .  
 0123294 10/1984 European Pat. Off. .  
 0136490 4/1985 European Pat. Off. .  
 3316297A1 11/1983 Germany .  
 3348298C2 11/1983 Germany .  
 2085887 5/1982 United Kingdom .  
 83/04053 11/1983 WIPO .  
 85/01961 5/1985 WIPO .  
 85/03079 7/1985 WIPO .  
 85/04419 10/1985 WIPO .  
 86/03520 5/1986 WIPO .

### OTHER PUBLICATIONS

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

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

Aebi et al., "Sequence Requirements for Splicing of Higher Eukaryotic Nuclear Pre-mRNA," *Cell*, 47, 555-565 (Nov. 21, 1986).

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).

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).

Autonsson et al., "Posttranslational Modifications of Fibronectin," *J. Biol. Chem.*, 266(25), 16859-16861 (1991).

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

(List continued on next page.)

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

### [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 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".

9 Claims, 27 Drawing Sheets

### Related U.S. Application Data

[63] Continuation of Ser. No. 113,179, Oct. 23, 1987, Pat. No. 5,441,868, which is a continuation of Ser. No. 675,298, Nov. 20, 1984, Pat. No. 4,703,008, which is a continuation-in-part of Ser. No. 655,841, Sep. 28, 1984, which is a continuation in part of Ser. No. 582,783, Feb. 21, 1984, abandoned, which is a continuation in part of Ser. No. 561,024, Dec. 13, 1983.

[51] Int. Cl.<sup>6</sup> ..... **C12P 71/02; C12N 15/27**

[52] U.S. Cl. .... **435/69.4; 435/69.6; 435/325; 536/23.51**

[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

### References Cited

#### U.S. PATENT DOCUMENTS

3,033,753 5/1962 White et al. .... 536/365  
 3,865,801 2/1975 Chiba et al. .... 536/357  
 4,237,224 12/1980 Cohen et al. .... 435/61.1  
 4,254,095 3/1981 Fisher et al. .... 436/513  
 4,264,731 4/1981 Shine ..... 435/91.41  
 4,273,875 6/1981 Masris ..... 435/91.4  
 4,293,652 10/1981 Cohen ..... 435/172.3  
 4,303,650 12/1981 Takezawa et al. .... 603/391  
 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. .... 527/395  
 4,394,143 7/1983 Weissman et al. .... 435/6  
 4,397,840 8/1983 Takezawa et al. .... 361/399  
 4,399,216 8/1983 Asel et al. .... 435/6  
 4,411,994 10/1983 Gilbert et al. .... 435/69.7  
 4,442,205 4/1984 Hamer et al. .... 435/69.3  
 4,465,624 8/1984 Chiba et al. .... 530/325  
 4,468,464 8/1984 Cohen et al. .... 435/320.1  
 4,503,151 4/1985 Paddock ..... 435/69.1  
 4,558,005 12/1985 Goldwasser et al. .... 435/7.92  
 4,558,006 12/1985 Egrie ..... 435/7.94  
 4,568,488 2/1986 Lee-Huang ..... 562/397  
 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. .... 435/69.4  
 4,703,008 13/1987 Lin ..... 435/240.2  
 4,710,473 12/1987 Morris ..... 435/320.1  
 4,757,006 7/1988 Toole et al. .... 435/61.6

#### 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 5/1984 European Pat. Off. .

5,618,698

Page 2

## OTHER PUBLICATIONS

- 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).
- Beaucage et al., "Deoxynucleoside Phosphoramidites—A new Class of Key Intermediates for Deoxypolynucleotide Synthesis," *Tetrahedron Letters*, 22(20), 1859-1862 (1981).
- 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.* 7(7), 1495-1502 (1986).
- Bennetzen et al., "Codon Selection in Yeast," *J. Biol. Chem.*, 257(6), 3026-3031 (Mar. 25, 1982).
- Bentley et al., "Human immunoglobulin variable region genes: DNA sequences of two V<sub>H</sub> genes and a pseudogene," *Nature* 288, 730-733 (Dec. 1980).
- Benton et al., "Screening  $\lambda$ gt10 Recombinant Clones by Hybridization to single Plaques in situ," *Science* 196, 180-182 (Apr. 8, 1977).
- Berzofsky et al., "Topographic Antigenic Determinants Recognized by Monoclonal Antibodies to Sperm Whale Myoglobin," *J. Biol. Chem.* 257(6), 3189-3198 (Mar. 25, 1982).
- 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).
- Betscholtz 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).
- 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).
- Blattner et al., "Charon Phages: Safer Derivatives of Bacteriophage Lambda for DNA Cloning," *Science*, 196, 161-169 (Apr. 8, 1977).
- 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," *Proc. Symp. Mol. Biol. Negat. Strand Viruses Meeting*, pp. 125-130, Companis et al., eds., Acad. Press (1984).
- Bray et al., "Human cDNA clones for four species of G $\alpha$  signal transduction protein," *P.N.A.S. (USA)*, 83, 8893-8897 (Dec. 1986).
- Breslow et al., "Isolation and characterization of cDNA clones for human apolipoprotein A I," *P.N.A.S. (USA)*, 79, 6861-6865 (Nov. 1982).
- Broume et al., "Immunological screening method to detect specific translation products," *P.N.A.S. (USA)*, 75(6), 2746-2749 (Jun 1978).
- Browne, et al., "Erythropoietin: Gene Cloning, Protein Structure, and Biological Properties," *Cold Spring Harbor Symposia on Quantitative Biology*, L1, 693-702 (1986).
- 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).
- 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).
- Chia et al., "The construction of cosmid libraries of eukaryotic DNA using the Homer series of vectors," *Nucleic Acids Res.* 10(8), 2503-2520 (1982).
- Chiba et al., "Stabilization of Urinary Erythropoietin," *Biochem. and Biophys. Res. Commun.*, 47(6), 1372-1377 (1972).
- Chirgwin et al., "Isolation of Biologically Active Ribonucleic Acid from Sources Enriched in Ribonuclease," *Biochemistry*, 18(24), 5294-5299 (1979).
- Chisfollm, "On the Trail of the Magic Bullet: Monoclonal antibodies promise perfectly targeted chemicals," *High Technology*, vol. 2(1), 57-63 (Jan. 1983).
- Chomczynski et al., "Alkaline Transfer of DNA to Plastic Membrane," *Biochem. Biophys. Res. Commun.*, 122(1), 340-344 (Jul. 18, 1984).
- Choo et al., "Molecular cloning of the gene for human anti-haemophilic factor IX," *Nature*, 299, 178-180 (Sep. 9, 1982).
- Choppin et al., "Characterization of Erythropoietin Produced by IW32 Murine Erythroleukemia Cells," *Blood*, 64(2), 341-347 (Aug. 1984).
- Chou et al., "Prediction of the Secondary Structure of Proteins from their Amino Acid Sequence," *Advances in Enzymology*, 47, 45-47 (1978).
- Chou et al., "Empirical Predictions of Protein Conformation," *Ann. Rev. Biochem.*, 47, 251-276 (1978).
- Chou et al., "Prediction of Protein Conformation," *Biochem.*, 13(2), 222-245 (1974).
- 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.* 79, 1815-1819 (Mar. 1982).
- Claus-Walker et al., "Spinal Cord Injury and Serum Erythropoietin," *Arch. Phys. Med. Rehabil.* 65, 370-374 (Jul. 1984).
- Colby et al., "Immunological Differentiation Between *E. coli* and CHO Cell Derived Recombinant and Natural Human  $\beta$ -Interferons," *J. Immunol.* 133(6) 3091-3095 (1984).
- Collen et al., "Biological Properties of Human Tissue-Type Plasminogen Activator Obtained by Expression of Recombinant DNA in Mammalian Cells," *J. of Pharmacology and Exp. Therapeutics*, 231 (1), 146-152 (1984).
- Colman, "Cells that secrete foreign proteins," *TIBS*, 435-437 (Dec. 1982).
- Comis et al., "Primary structure of the human Met and Leu-enkephalin precursor and its mRNA," *Nature*, 295, 663-666 (Feb. 25, 1982).
- Congote, "Regulation of Fetal Liver Erythropoiesis," *J. of Steroid Biochemistry*, 3, 423-428 (1977).
- Congote, "Extraction from Fetal Bovine Serum of Erythropoietin, an Erythroid Cell Stimulating Factor," *Anal. Biochem.*, 140, 428-433 (1984).
- 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).
- 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, Ju., 1-7, 1984).
- Contrera et al., "Extraction of erythropoietin from Kidneys of Hypoxic and Phenylhydrazine-treated rats," *Blood*, 25(5), 809-816 (May 1965).
- Costantini et al., "Introduction of a Rabbit  $\beta$ 2-microglobulin Gene into the Mouse Gem Line," *Nature*, 294, 92-94 (Nov. 5, 1981).

5,618,698

Page 3

- Costantini et al., "Gene Transfer into the Mouse Germ-Line," *J. Cell Physiol. Suppl.* 1, 219-226 (1982).
- Cotes et al., "Changes in serum immunoreactive erythropoietin during the menstrual cycle and normal pregnancy," *Brit. J. Obstet. Gynaecol.*, 90, 304-311 (Apr. 1983).
- 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).
- Dainiak et al., "Mechanisms of Abnormal Erythropoiesis in Malignancy," *Cancer*, 51(6), 1101-1106 (1983).
- Das et al., "Use of synthetic oligonucleotide probes complementary to genes for human III<sub>A</sub>-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).
- 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.
- 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).
- 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).
- Derynck et al., "Human Transforming Growth Factor- $\alpha$ : Precursor Structure and Expression in *E. coli*," *Cell*, 38, 287-297 (Aug. 1984).
- 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).
- Devos et al., "Purification of Recombinant Glycosylated Human Gamma Interferon Expressed in Transformed Chinese Hamster Ovary Cells," *J. Interferon Research*, 4,461-468 (1984).
- Doeherty et al., "Sequence of human tissue inhibitor of metalloproteinases and its identity to erythroid-potentiating activity," *Nature*, 318, 66-69 (Nov. 7, 1985).
- Dordal et al., "Function and Composition of the Carbohydrate Portion of Human Urinary Erythropoietin," *Experimental Hematology*, 10, Supp. 11, p. 133, Abstract No. 222 (1982).
- Dordal et al., "The Role of Carbohydrate in Erythropoietin Action," *Endocrinology*, 116(6), 2293-2299 (1985).
- 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).
- Dunn et al., "Use of a computer model in the understanding of erythropoietic control mechanisms," *Chemical Abstracts*, 91, 90417r (1979).
- Dunn, "Current Concepts in Erythropoiesis", John Wiley & Sons, Chichester, England, 1983.
- Dunn et al., "Serum erythropoietin titers during prolonged bedrest: relevance to the anaemia of space flight," *Eur. J. Appl. Physiol.*, 52, 178-182 (1984).
- Dunn et al., "Erythropoietin Bioassays Using Fetal Mouse Liver Cells: Validations and Technical Improvements," *Exp. Hematol.*, 11 (7), 590-600 (Aug. 1983).
- Edman et al., "A Protein Sequenator," *Eur. J. Biochem.* 1, 80-91 (1967).
- Emmanuel et al., "Metabolism of pure human erythropoietin in the rat," *Am. J. Physiol.*, 247 (1 Pt 2), F168-76 (1984).
- Eschbach et al., "Correction by Erythropoietin (EPO) Therapy of the Anemia of Chronic Renal Failure (CRF) in Sheep," *Clin. Res.* 29(2), 518A (1981).
- Eschbach et al., "The Anemia of Chronic Renal Failure in Sheep," *J. Clin. Invest.* 74(2), 434-441 (Aug. 1984).
- Espada et al., "Purification of Human Urinary Erythropoietin," *Fed. Proc.* 41, 1159 (1982).
- 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).
- 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 (Nov. 1983).
- Farber et al., "Translation of mRNA from Anemic Baboon Kidney into Biologically Active Erythropoietin," *Exp. Hematol.*, 11, Supp. 14, Abstract 101 (1983).
- Farber, "Translation of RNA from Human Kidneys into Biologically Active Erythropoietin Following Microinjection into *Xenopus Laevis* Oocytes," *Clin. Res.* 31 (4), 769A (Nov. 1983).
- 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).
- Fiddes et al., "The Gene Encoding the Common Alpha Subunit of the Four Human Glycoprotein Hormones," *J. Mol. & App. Genetics*, 1, 3-18 (1981).
- Fiers et al., "The Human Fibroblast and Human Immune Interferon Genes and Their Expression in Homologous and Heterologous Cells," *Phil. Trans. R. Soc. Lond.*, 3299, 29-38 (1982).
- Finch, "Erythropoiesis, Erythropoietin, and Iron," *Blood*, 60(6), 1241-1246 (Dec. 1982).
- 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).
- Fisher et al., "Cooperative Erythropoietic Assay of Several Steroid Metabolites in Polycythemic Mice," *Steroids*, 30(6), 833-845 (Dec. 1977).
- Fisher, "Erythropoietin: Pharmacology, Biogenesis and Control of Production," *Pharmacological Reviews*, 24(3), 459-508 (1972).
- Fisher, "Control of Erythropoietin Production," *Proc. Soc. Exp. Biol. & Med.* 173, 289-305 (1983).
- 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).
- Garcia et al., "Radioimmunoassay of erythropoietin: circulating levels in normal and polycythemic human beings," *J. Lab. Clin. Med.*, 99, 624-635 (May 1982).
- Garcia et al., "Radioimmunoassay of Erythropoietin," *Blood Cells* 5, 405-419 (1979).
- Garcia et al., "Immunological Neutralization of Various Erythropoietins," *Proc. Soc. Exptl. Biol. Med.*, 112, 712-714 (1962).
- 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).

5,618,698

Page 4

- Gasser et al., "Expression of abbreviated mouse dihydrofolate reductase genes in cultured hamster cells," *P.N.A.S. (USA)*, 79, 6522-6526 (Nov. 1982).
- Gene Screen, New England Nuclear, Catalog No. NEB-972.
- 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).
- Gething et al., "Construction of influenza haemagglutinin genes that code for intracellular and secrete forms of the protein," *Nature*, 300, 598-603 (Dec. 16, 1982).
- Gething et al., "Cell-surface expression of influenza haemagglutinin from a cloned DNA copy of the RNA gene," *Nature*, 293, 620-625 (22 Oct. 1981).
- 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).
- Gluzman, "SV40-Transformed Simian Cells Support the Replication of Early SV40 Mutants," *Cell* 23, 175-182 (Jan. 1981).
- Goedde et al., "Synthesis of human fibronectin by *E. coli*," *Nucleic Acids Res.*, 8(18), 4057-4074 (1980).
- Goedde et al., "Human leukocyte interferon produced by *E. coli* is biologically active," *Nature*, 287:411-416 (Oct. 2, 1980).
- Goldwasser et al., "Erythropoietin: Assay and Study of Its Mode of Action," *Meth. in Enzymol.*, 37, 109-121 (1975).
- 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).
- Goldwasser, "Biochemical Control of Erythroid Development," *Current Topics in Developmental Biology*, ed. A. Monroy and A.A. Noscona, 173-211, Academic Press, NY (1966).
- Goldwasser et al., "The Molecular Weight of Sheep Plasma Erythropoietin," *J. of Biol. Chem.* 247(16), 5159-60 (Aug. 25, 1972).
- Goldwasser et al., "Progress in the purification of erythropoietin," *Ann. N.Y. Acad. Sci.*, 149:49-53 (1968).
- Goldwasser et al., "On the mechanism of Erythropoietin-induced Differentiation," *J. of Biol. Chem.*, 249(13), 4202-4206 (Jul. 10, 1974).
- Goldwasser et al., "Purification of Erythropoietin," *P.N.A.S. (USA)*, 68(4), 697-698 (Apr. 1971).
- Goldwasser et al., "Further purification of sheep plasma erythropoietin," *Bioch. Biophys. Acta*, 64, 487-496 (1962).
- Goldwasser, "Some Thoughts on the Nature of Erythropoietin-Responsive Cells," *J. Cell. Physiol.* 110 (Supp. 1), 133-135 (1982).
- Goldwasser et al., "An Assay for Erythropoietin in Vitro at the Milliuin Level," *Endocrinology*, 97(2), 315-323 (Aug. 1975).
- Goldwasser et al., "Erythropoietin and the differentiation of red blood cells," *Fed. Proc.* 34, 2285-2292 (Dec. 1975).
- Goochee et al., "Environmental Effects on Protein Glycosylation," *Biotechnology*, 8, 421-427 (May 1990).
- Goochee et al., "The Oligosaccharides of Glycoproteins: Bioprocess Factors Affecting Oligosaccharide Structure and their Effect on Glycoprotein Properties," *Biotechnology*, 9, 1347-1355 (Dec. 1991).
- Goodman et al., "Cloning of Human Gene- from a Mixture of cDNA Molecules," *Meth. in Enzymol.* 68, 75-90 (1979).
- Gordon et al., "A plasma extract with erythropoietic activity," *Proc. Soc. Expt. Biol. Med.*, 86:255-258 (1954).
- 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).
- 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).
- Gough et al., "Molecular Cloning of cDNA Encoding a Murine Haematopoietic Growth Regulator, Granulocyte Macrophage Colony Stimulating Factor," *Nature*, 309, 763-767 (1984).
- Gouy et al., "Codon Usage in Bacteria: Correlation with Gene Expressivity," *Nucleic Acids Rev.* 10, 7055-7074 (1982).
- Graham et al., "A New Technique for the Assay of Infectivity of Human Adenovirus 5 DNA," *Virology* 52, 456-467 (1973).
- Granham et al., "Codon catalog usage is a genome strategy modulated for gene expressivity," *Nucleic Acids Rev.* 9, r43-74 (1981).
- Gray et al., "*Pseudomonas aeruginosa* Secretes and Correctly Processes Human Growth Hormone," *Biotechnology*, 2, 161-165 (Feb. 1984).
- Gray et al., "Expression of human immune interferon cDNA in *E. coli* and monkey cells," *Nature*, 295, 503-508 (Feb. 11, 1982).
- Green et al., "Immunogenic Structure of the Influenza Virus Hemagglutinin," *Cell*, 28, 477-487 (Mar. 1982).
- Greenwood et al., "The Preparation of <sup>125</sup>I-Labelled Human Growth Hormone of High Specific Radioactivity," *Biochem. J.* 89, 114-123 (1963).
- 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).
- Groffen et al., "Isolation of Human Oncogene Sequences (v-fes Homolog) from a Cosmid Library," *Science*, 216, 1136-1138 (Jun. 4, 1982).
- Grundmann et al., "Characterization of cDNA coding for human factor XIIIa," *P.N.A.S. (USA)*, 83, 8024-8028 (Nov. 1986).
- Grunstein et al., "Colony Hybridization," *Meth. in Enzymol.* 68, 379-389 (1979).
- 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).
- Gross 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).
- Gubier et al., "A simple and very efficient method for generating cDNA libraries," *Gene* 25, 263-269 (1983).
- Haddy, "Erythropoietin in sickle cell disease," *Am. Jour. Ped. Hematol/Oncol.* 4(2), 191-196 (Summer 1982).
- Haga et al., "Plasma Erythropoietin Concentrations During the Early Anemia of Prematurity," *Acta. Paediatr. Scand.* 72, 827-831 (1983).
- 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).
- Hamer et al., "Expression of the chromosomal mouse  $\beta^{H2k}$  globin gene cloned in SV40," *Nature*, 281, 35-40 (Sep. 6, 1979).

5,618,698

Page 5

- Hamer et al., "A Mouse Globin Gene Promoter is Functional in SV40." *Cell*, 21, 697-708 (Oct. 1980).
- 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).
- Hanahan et al., "Plasmid screening at high colony density." *Gene*, 10, 63-67 (1980).
- 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).
- Hauser et al., "Inducibility of human  $\beta$  interferon in mouse L-cell clones." *Nature*, 297, 650-654 (Jun. 24, 1982).
- 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), 587-607 (1983).
- 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, 11-29.
- Hellmann et al., "Familial erythrocytosis with over production of erythropoietin." *Chn. Lab. Haematol.*, 5, 335-342 (1983).
- Hewick et al., "A Gas-Liquid Solid Phase Peptide and Protein Sequenator." *J. Biol. Chem.*, 256, 7990-7997 (Aug. 1981).
- Higashi et al., "Structure and Expression of a Cloned cDNA for Mouse Interferon- $\beta$ ." *J. Biol. Chem.*, 258(15):9522-9529 (1983).
- Higashi et al., "Characterization of N-Glycolyneuraminic Acid-containing Gangliosides as Tumor-associated Hanganulzin-Deicher Antigen in Human Colon Cancer." *Cancer Research*, 45, 3796-3802 (1985).
- Hirs et al., "Peptides Obtained by Tryptic Hydrolysis of Performic Acid-Oxidized Ribonuclease." *J. Biol. Chem.*, 219, 623-642 (1955).
- Hokke et al., "Sialylated carbohydrate chains of recombinant human glycoproteins expressed in Chinese hamster ovary cells contain traces of N-glycolyneuraminic acid." *FEBS Letters*, 275, 9-14 (1990).
- 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).
- 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).
- Huang et al., "Identification of Human Erythropoietin Receptor." *Am. Soc. of Biological Chemists, Am. Assoc. of Immunologists, Fed. Pract. (USA)* 43(7) Abst. 2770, p. 1891 (1984).
- 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.
- Imai et al., "Physicochemical and Biological Comparison of Recombinant Human Erythropoietin with Human Urinary Erythropoietin." *J. Biochem.*, 107, 352-359 (1990).
- Itakura, et al., "Synthesis and Use of Synthetic Oligonucleotides." *Ann. Rev. Biochem.*, 53, 323-356 (1984).
- 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).
- Jacobs et al., "Isolation and characterization of genomic and cDNA clones of human erythropoietin." *Nature*, 313, 806-809 (Feb. 28, 1985).
- Jacobsen et al., "Relative effectiveness of phenylhydrazine treatment and hemorrhage in the production of an erythropoietic factor." *Blood*, 11:937-945 (1956).
- Jacobson et al., "Role of the kidney in erythropoiesis." *Nature*, 179:633-634 (Mar. 23, 1957).
- 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).
- Jellreys et al., "Sequence variation and evolution of nuclear DNA in man and the primates." *Phil. Trans. R. Soc. Lond., B* 292, 133-142 (1981).
- Jelkman et al., "Extraction of Erythropoietin from Isolated Renal Glomeruli of Hypoxic Rats." *Exp. Hematol.*, 11(7), 581-588 (Aug. 1983).
- Kaiser et al., "Amphiphilic Secondary Structure: Design of Peptide Hormones." *Science*, 223, 249-255 (1984).
- Kajimura et al., "Cloning the Heavy Chain of Human HLA-DR Antigen Using Synthetic Oligodeoxyribonucleotides as Hybridization Probes." *DNA*, 2(3), 175-182 (1983).
- Kakidani et al., "Cloning and sequence analysis of cDNA for porcine  $\beta$ -neo-endorphin/dynorphin precursor." *Nature*, 298, 245-249 (Jul. 15, 1982).
- Kalmani, "Correlation of clinical and in vitro erythropoietic responses to androgens in renal failure." *Kidney Int'l.*, 22, 383-391 (1982).
- Kam et al., "Novel bacteriophage  $\lambda$  cloning vector." *P.N.A.S. (USA)*, 77, 5172-5176 (Sep. 1980).
- Katsuoaka et al., "Erythropoietin Production in Human renal Carcinoma Cells Passaged in Nude Mice and in Tissue Culture." *Gann*, 74, 534-541 (Aug. 1983).
- Kaufman et al., "Amplification and Expression of Sequences Cotransfected with a Modular Dihydrofolate Reductase Complementary DNA Gene." *J. Mol. Biol.* 159, 601-621 (1982).
- Kaufman et al., "Expression and Amplification of DNA Introduced into Mammalian Cells." *Gene Amplification*. RT Schimke ed., Cold Spring Harbor, New York, 245-250 (1982).
- Kennell, "Principles and Practices of Nucleic Acid Hybridization." *Prog. Nucl. Acid Res. Mol. Biol.* 13, 259-301 (1971).
- Keriter et al., "Mouse Myeloma Cells That Make Short Immunoglobulin Heavy Chains: Pleiotropic Effects on Glycosylation and Chain Assembly." *J. Cell. Biol.*, 98, 215-222 (1984).
- Kieny et al., "Expression of rabies virus glycoprotein from a recombinant vaccinia virus." *Nature*, 312, 163-166 (1984).
- 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).
- Knopf et al., "Cloning and Expression of Multiple Protein Kinase C cDNAs." *Cell* 46, 491-502 (Aug. 15, 1986).
- Koehn, "Evolution of Higher organism DNA." *Quarterly Reviews of Biophysics*, 3:327-375 (1970).
- Kondor-Koch et al., "Expression of Semliki Forest Virus Proteins from Cloned Complementary DNA 1. The Fusion Activity of the Spike Glycoprotein." *J. Cell. Biol.*, 97, 644-651 (1983).

5,618,698

Page 6

- Konrad, "Applications of Genetic Engineering to the Pharmaceutical Industry," *Ann. N.Y. Acad. Sci.*, 413, 12-22 (1982).
- Konwalinka et al., "A Miniaturized Agar Culture System for Cloning Human Erythropoietic Progenitor Cells," *Exp. Hematol.*, 12, 75-79 (1984).
- Korman, "cDNA clones for the heavy chain of HLA DR antigen, obtained after immunopurification of polysomes by monoclonal antibody," *P.N.A.S. (USA)*, 79, 1844-1848 (Mar. 1982).
- Kornblith et al., "Isolation and characterization of cDNA clones for human and bovine fibronectins," *P.N.A.S. (USA)*, 80, 3218-3222 (Jun. 1983).
- Kramer et al., "Comparisons of the Complete Sequences of Two Collagen Genes from *Caenorhabditis elegans*," *Cell* 30, 599-605 (Sep. 1982).
- Krane, "The Role of Erythropoietin in the Anemia of Chronic Renal Failure," *Henry Ford Hosp. Med. J.*, 31(3), 177-181 (1983).
- Krystal, "A Simple Microassay for Erythropoietin Based on <sup>3</sup>H-Thymidine Incorporation into Spleen cells from Phenylhydrazine Treated Mice," *Exp. Hematol.*, 11(7), 649-660 (Aug. 1983).
- Kuhn et al., "Gene Transfer, Expression, and Molecular Cloning of the Human Transferrin Receptor Gene," *Cell*, 37, 95-103 (1984).
- Kurachi et al., "Isolation and characterization of a cDNA coding for human factor IX," *P.N.A.S. (USA)*, 79, 6461-6464 (Nov. 1982).
- Kuratowska et al., "Studies on the production of erythropoietin by isolated perfused organs," *Blood*, 18:527-534 (1961).
- Kurtz, "A New candidate for the regulation of erythropoiesis: insulin like growth factor 1," *FEBS Letters*, 149(1), 105-108 (Nov. 1982).
- Kyte et al., "A Simple Method for Displaying the Hydrophobic Character of a Protein," *J. Mol. Biol.*, 157, 105-132 (1982).
- 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).
- Lai et al., "Structural Characterization of Human Erythropoietin," *J. of Biol. Chem.*, 261, 3116-3121 (Mar. 5, 1986).
- Lai, "Technical Improvements in Protein Microsequencing," *Analytica Chimica Acta*, 163, 253-248 (1984).
- Lange et al., "Application of erythropoietin antisera to studies of erythropoiesis," *Ann. N.Y. Acad. Sci.*, 149:281-291 (1968).
- 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).
- 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).
- Lathe, "Synthetic Oligonucleotide Probes Deducible from Amino Acid Sequence Data," *J. Mol. Biol.*, 183, 1-12 (1985).
- 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).
- 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).
- Lauffer et al., "Topology of signal recognition particle receptor in endoplasmic reticulum membrane," *Nature*, 318, 334-338 (Nov. 28, 1985).
- Lawn et al., "The Isolation and Characterization of Linked  $\delta$  and  $\beta$ -Globin Genes from a Cloned Library of Human DNA," *Cell*, 5, 1157-1174 (Dec. 1978).
- Ledeen et al., "Gangliosides: Structure, Isolation, and Analysis," *Methods in Enzymology*, 83 (Part D), 139-191 (1982).
- Lee Huang, "The Erythropoietin Gene," *Oncogenes, Genes and Growth Factors*, Chap. 7, pp. 199-222, ed. Gordon Garall, John Wiley & Sons, Inc. (1987).
- Lee-Huang, "Cloning of Human Erythropoietin," *Biophysical J.*, 45(Part 2 of 2), ABT. M-PM-A12, p. 30a (1984).
- Lee Huang, "Monoclonal Antibodies to Human Erythropoietin," *Abstract No. 1463, Fed. Proc.*, 41:526 (1982).
- Lee Huang, "A New Preparative Method for Isolation of Human Erythropoietin With Hydrophobic Interaction Chromatography," *Blood*, 56(4), 620-624 (Oct. 1980).
- Lee-Huang, "Cloning and Expression of Human EPO cDNA in *E. Coli*," *P.N.A.S. (USA)*, 81, 2708-2712 (May 1984).
- 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).
- Lerner, "Synthetic Vaccines," *Scientific American*, 248(2), 66-74 (1983).
- Lerner et al., "Antibodies to Chemically Synthesized Peptides Predicted from DNA Sequences as Probes of Gene Expression," *Cell*, 23, 309-310 (Feb. 1981).
- Lewin *Genes*, 1983, John Wiley & Sons, p. 307.
- Lin et al., "Cloning and expression of the human erythropoietin gene," *P.N.A.S. (USA)*, 82, 7580-7584 (Nov. 1985).
- Lin et al., "Monkey erythropoietin gene: cloning, expression and comparison with the human erythropoietin gene," *Gene*, 44, 201-209 (1986).
- Lin et al., "Cloning of the Monkey EPO Gene," *Abstract, J. Cell. Biochem., Suppl.* 8B, p. 45 (Mar. 31-Apr. 24, 1984).
- Lin et al., "Cloning and Expression of Monkey and Human Erythropoietin," *Exp. Hematol.*, 12, 357 (1984).
- Lipschitz et al., "Effect of Age on Hematopoiesis in Man," *Blood*, 63(3), 502-509 (Mar. 1983).
- LKB Technical Bulletin #2217.
- Maniatis et al., "The Isolation of Structural Genes from Libraries of Eucaryotic DNA," *Cell* 15, 687-701 (Oct. 1978).
- Maniatis et al., "Molecular Cloning, a Laboratory Manual", pp. 5, 197-199, 392-393, 479-487, 493-503 Cold Springs Harbor, N.Y. (1982).
- 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).
- Marshall, "Glycoproteins," *Annual Review of Biochemistry*, Sneli et al. eds., vol. 41, pp. 673-702, Annual Reviews Inc., Palo Alto, California (1972).
- Martial et al., "Human Growth Hormones: Complementary DNA Cloning and Expression in Bacteria," *Science*, 205, 602-606 (Aug. 10, 1979).
- 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).

5,618,698

Page 7

- Maurer, "Immunoenzymological Isolation of Prolactin Messenger RNA," *J. Biol. Chem.* 255(1), 854-859 (Feb. 10, 1980).
- Maxam et al., "Sequencing End-Labeled DNA with Base-Specific Chemical Cleavages," *Methods in Enzymol.* 65, 499-560 (1980).
- McCormick et al., "Regulated Expression of Human Interferon Genes In Chinese Hamster Ovary Cells," *DNA*, 2(1): 86 Abst 86 (1983).
- McCormick et al., "Inducible Expression of Amplified Human Beta Interferon Genes in CHO Cells," *Mol. Cell. Biol.*, 4(1):166-172 (Jan. 1984).
- McGinagle et al., "Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency," *Kidney Int'l.*, 25(2), 437-444 (1984).
- Meier et al., "Alpha<sub>v</sub> and Beta<sub>3</sub>-Adhesive Receptors Co-Expressed on Cloned MDCK Cells are Distinct Glycoproteins," *Biochem. & Biophys. Res. Comm.*, 118(1), 73-81 (1984).
- Mellen 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).
- Meilor et al., "Expression of Murine H-2K<sup>b</sup> histocompatibility antigen in cells transferred with cloned H-2 genes," *Nature*, 298:529-534 (Aug. 1982).
- Messing, "New M13 Vectors for Cloning," *Methods in Enzymology*, 101, 20-78 (1983).
- 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).
- 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).
- Methods in Yeast Genetics. Cold Spring Harbor Lab, Cold Spring Harbor, NY, p. 62 (1983).
- Miller et al., "Plasma levels of immunoreactive erythropoietin after acute blood loss in man," *Brit. J. Haematol.*, 52, 545-549 (1982).
- Mirand, "Extra-renal and renal control of erythropoietin production," *Ann. N.Y. Acad. Sci.*, 149:94-106 (1968).
- Mirand et al., "Current studies on the role of erythropoietin on erythropoiesis," *Ann. N.Y. Acad. Sci.*, 77:677-702 (1959).
- Miyake et al., "Purification of Human Erythropoietin," *J. Biol. Chem.*, vol. 252(15), 5558-5564 (Aug. 1977).
- 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).
- Montgomery et al., "Identification and Isolation of the Yeast Cytochrome c Gene," *Cell*, 14, 673-680 (Jul. 1978).
- 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):2606-10 (Apr. 1981).
- Moriuchi et al., "Tny-1 cDNA sequence suggests a novel regulatory mechanism," *Nature*, 301, 80-82 (Jan. 1983).
- Morrison, "Bioprocessing in Space: an Overview," *The World Biotech Report*, vol. 2:USA, 557-571 (1984).
- 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).
- Murphy et al., "The Role of Glycoprotein Hormones in the Regulation of Hematopoiesis," *Acta. Haematologica Japonica*, 46(7), 1380-1396 (Dec. 1983).
- Myers et al., "Construction and Analysis of Simian Virus 40 Origins Defective in Tumor Antigen Binding and DNA Replication," *P.N.A.S. (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).
- Nacts, "The role of the kidney in erythropoiesis," *J. Clin. Invest.*, 39:102-110 (1960).
- Nagata et al., "Synthesis in *E. Coli* of a polypeptide with human leukocyte interferon activity," *Nature*, 284, 316-320 (Mar. 27, 1980).
- Nakao et al., "Erythropoiesis in anephric or kidney transplanted patients," *Israel J. Med. Sci.*, 7:986-989 (Jul.-Aug. 1971).
- Nathan et al., "Erythropoietin and the Regulation of Erythropoiesis," *New Eng. J. Med.*, 308(9), 520-522 (Mar. 3, 1983).
- Naughton et al., "Evidence for an Erythropoietin Stimulating Factor in Patients with Renal and Hepatic Disease," *Acta. Haemat.*, 69, 171-179 (1983).
- Naughton et al., "Evidence for a Hepatic-Renal Antagonism in the Production of Hepatic Erythropoietin," *Ann. Clin. Lab. Sci.*, 13(5), 432-438 (1983).
- Nayak et al., "Characterization of Influenza Virus Glycoproteins Expressed from Cloned cDNAs in Prokaryotic and Eukaryotic Cells," *Modern Approaches To Vaccines*, pp. 165-172, Charock et al., eds., Cold Spring Harbor Lab. (1984).
- 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).
- Newman et al., "Selection and Properties of a Mouse L-Cell Transformant Expressing Human Transferrin Receptor," *Nature*, 304, 643-645 (1983).
- 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).
- Nimtz et al., "Structures of sialylated oligosaccharides of human erythropoietin expressed in recombinant BHK-21 cells," *Eur. J. Biochem.*, 213, 39-56 (1993).
- Noda et al., "Primary structure of c $\alpha$ -subunit precursor of *Torpedo californica* acetylcholine receptor deduced from cDNA sequence," *Nature*, 299, 793-797 (Oct. 28, 1982).
- Noda et al., "Cloning and sequence analysis of cDNA for bovine adrenal prepronkephalin," *Nature*, 295, 202-206 (Jan. 21, 1982).
- 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).
- Nussinov, "Eukaryotic Dinucleotide Preference Rules and their Implications for Degenerate Codon Usage," *J. Mol. Biol.*, 149, 125-131 (1981).
- Ogle et al., "Production of erythropoietin in vitro: a review," *In Vitro*, 14(11), 945-949 (1978).
- Ohkubo et al., "Cloning and sequence analysis of cDNA for rat angiotensinogen," *P.N.A.S. (USA)*, 80, 2196-2200 (Apr. 1983).
- 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).

5,618,698

Page 8

- Okayama et al., "High-Efficiency Cloning of Full Length cDNA," *Mol. & Cell. Biol.*, 2(2), 161-170 (Feb. 1982).
- Ovchinnikov et al., "The Primary Structure of *Escherichia coli* RNA Polymerase," *J. Biochem.*, 116, 621-629 (1981).
- Paabo et al., "Association Between Transplantation Antigens and a Viral Membrane Protein Synthesized from a Mammalian Expression Vector," *Cell*, 35, 445-453 (1982).
- Palmiter et al., "Metallothionein-Human GH Fusion Genes Stimulate Growth of Mice," *Science*, 222, 809-814 (Nov. 18, 1983).
- Pankratz et al., "A Simple 3-Step Procedure for Purifying Baboon Urinary Erythropoietin to Apparent Homogeneity," *Exp. Hematol.*, 11, Supp. 14, Abst. 102 (1983).
- Papayannopoulos et al., "On the In Vivo Action of Erythropoietin: A Quantitative Analysis," *J. of Clin. Investigation*, 51, 1179-1185 (1972).
- 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).
- Pavlovic-Kemera 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).
- Pellicer et al., "Altering Genotype and Phenotype by DNA-Mediated Gene Transfer," *Science*, 209, 1414-1422 (Sep. 19, 1980).
- 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).
- Pennica et al., "Cloning and expression of human tissue-type plasminogen activator cDNA in E-coli," *Nature*, 301, 214-221 (Jan. 20, 1983).
- Pennica et al., "Human tumour necrosis factor: precursor structure, expression and homology to lymphotaxin," *Nature* 312, 724-728 (Dec. 27, 1984).
- Pitha et al., "Induction of human B-interferon synthesis with poly (rIrrC) in mouse cells transfected with cloned cDNA plasmids," *P.N.A.S. (USA)*, 79, 4337-4341 (Jul. 1982).
- "Points to Consider in the Characterization of Cell Lines Used to Produce Biologics," Jun. 1, 1984. Office of Biologics Research Review, Center for Drugs & Biologics, U.S. Food & Drug Administration (Section A, Part 2).
- 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).
- Prooijen-Knegt, "In Situ Hybridization of DNA Sequences in Human Metaphase Chromosomes Visualized by an Indirect Fluorescent Immunocytochemical Procedure," *Exp. Cell Res.*, 141, 397-407 (1982).
- 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).
- Rambach et al., "Acid Hydrolysis of Erythropoietin," *Proc. Soc. Exp. Biol.*, 99, 482-483 (1958).
- Ravetch 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).
- Reeny et al., "Structural Characterization of Natural Human Urinary and Recombinant DNA-derived Erythropoietin," *J. Biol. Chem.*, 262(35), 17156-17163 (Dec. 15, 1987).
- Reilly et al., "Use of synthetic oligonucleotides to clone genomic DNA: isolation of a (RNA<sup>pol</sup>) gene from mouse," *DNA*, 1:192 (1982).
- Resegotti et al., "Treatment of aplastic anaemia with methenolone, stanozolol and nandrolone," *Panminerva medica*, 23, 243-248 (1981).
- Reyes et al., "Identification of an H-2K<sup>b</sup> Related Molecule by Molecular Cloning," *Immunogenetics*, 14, 383-392 (1981).
- 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).
- Rigby, "Expression of cloned genes in eukaryotic cells using vector systems derived from viral replicons," *Genetic Engineering*, R. Williamson, ed., 3:83-140, Academic Press, London (1982).
- Riggs et al., "Synthetic DNA and Medicine," *Am. J. Hum. Genet.*, 31, 531-538 (1979).
- 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).
- 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).
- Roh et al., "Plasma Disappearance of <sup>125</sup>I-labeled Human Urinary Erythropoietin in Rabbits," *Fed. Proc.*, 29(2), 782-783 (1970).
- 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).
- Ross et al., "Phosphotyrosine-containing proteins isolated by affinity chromatography with antibodies to a synthetic hapten," *Nature*, 294, 654-656 (Dec. 17, 1981).
- 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).
- Rothmann et al., "Erythropoietin-Dependent Erythrocytosis Associated with Hepatic Angiosarcoma," *J. Surg. Oncol.*, 20, 105-108 (1982).
- Saito et al., "Translation of Human Erythropoietin mRNAs," *Exp. Hematol.*, 11(14), 228 (1983).
- 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).
- Sambrook et al., "Expression of Proteins on the Cell Surface Using Mammalian Vectors," *Experimental Manipulation of Gene Expression*, pp. 225-246, Acad. Press. (1983).
- Sanger et al., "DNA Sequencing with chain terminating inhibitors," *P.N.A.S. (USA)*, 74, 5463-5467 (Dec. 1977).
- Sasaki, "Carbohydrate Structure of Erythropoietin Expressed in Chinese Hamster Ovary Cells by a Human Erythropoietin cDNA," *J. Biol. Chem.*, 262(25), 12059-12070 (Sep. 5, 1987).
- Sasaki, "Isolation of erythropoietin by monoclonal antibody," *Biomed. Biochim. Acta*, 42(11/12), S202-S206 (1983).
- Seahill 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).



5,618,698

Page 9

- 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).
- Schwartz et al., "Severe Anemia as a Manifestation of Metastatic Jugular Paraganglioma," *Arch Otolaryngol.* 109, 269-272 (Apr. 1983).
- Seeburg et al., "Synthesis of growth hormone by bacteria," *Nature*, 276, 795-798 (Dec. 1978).
- 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/D: Antigens Abstract: 563 (1982).
- Shanidi, "Androgens and Erythropoiesis," *New Eng. J. Med.*, 289, 72-80 (Jul. 12, 1973).
- Sherwood et al., "Erythropoietin Titers in Sickle Cell Disease & Chronic Renal Failure," *Blood Suppl.*, 1, 58, Abstract 105 (1981).
- Sherwood et al., "Extraction of erythropoietin from normal kidneys," *Endocrinology*, 103(3), 866-870 (1978).
- Sherwood et al., "A Radioimmunoassay for Erythropoietin," *Blood*, 54(4), 885-893 (Oct. 1979).
- Shiramizu et al., "Human Renal Carcinoma Cells Secreting Erythropoietin *in vivo* and *in vitro*," *Blood*, 78(10), Supp. 1 (Nov. 15, 1991).
- 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).
- 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).
- 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).
- Southern, "Detection of Specific Sequences Among DNA Fragments Separated by Gel Electrophoresis," *J. Mol. Biol.*, 98, 503-517 (1975).
- Spellman et al., "Carbohydrate Structure of Recombinant Soluble Human CD4 Expressed in Chinese Hamster Ovary Cells," *Biochemistry*, 30(9), 2395-2406 (1991).
- 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).
- Srinivas et al., "Membrane Association and Defective Transport of Spleen Focus Forming Virus Glycoproteins," *J. Biol. Chem.*, 258, 14718-14724 (1983).
- Stanley, "Surface Carbohydrate Alterations of Mutant Mammalian Cells Selected for Resistance to Plant Lectins," *The Biochemistry of Glycoproteins and Proteoglycans, Chapter 4*: 161-189, Lennarz ed., Plenum Press (1980).
- Steck et al., "Cell Surface Properties of Spontaneously Metastasizing Rat Mammary Adenocarcinoma Cell Clones," *Transplantation Proceedings*, 16, 355-360 (1984).
- Storring et al., "The International Standard for Recombinant DNA derived Erythropoietin: Collaborative Study of four recombinant DNA derived erythropoietins and two highly purified human urinary erythropoietins," *J. of Endo.*, 134, 459-84 (1992).
- Strickland, "Occurrence of Sulfate on the N-Linked Oligosaccharides of Human Erythropoietin," *J. of Cellular Biochemistry, Suppl.* 16D, Abstract No. P324 (1992).
- 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).
- 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).
- 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).
- Sveda et al., "Functional expression in primate cells of cloned DNA coding for the hemagglutinin surface glycoprotein of influenza virus," *Proc. Nat'l. Acad. Sci. (USA)*, 78(10):5488-5492 (Sep. 1981).
- Sytowski et al., "The Biochemistry of Erythropoietin: An Approach to its mode of Action," *Exp. Hematol.*, 8(Supp. 8), 52-63 (1980).
- 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).
- Szostak et al., "Hybridization with Synthetic Oligonucleotides," *Meth. in Enzymol.*, 68, 419-428 (1979).
- 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).
- Takeuchi, "Comparative Study of the Asparagine-linked Sugar Chains of Human Erythropoietin Purified from Urine and the Culture Medium of Recombinant Chinese Hamster Ovary Cells," *J. Biol. Chem.*, 263(8), 3657-3663 (Mar. 15, 1988).
- Talmadge et al., "Eukaryotic Signal Sequence Transports Insulin Antigen in *Escherichia coli*," *P.N.A.S. USA* 77(6), 3369-3373 (Jun. 1980).
- Tambourin et al., "Production of erythropoietin-like activity by a murine erythroleukemia cell line," *P.N.A.S. (USA)*, 80, 6269-6273 (1983).
- Taniguchi et al., "Structure and expression of a cloned cDNA coding for the hemagglutinin surface glycoprotein of influenza virus," *Nature*, 302:305-310 (24 Mar. 1983).
- 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).
- 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).
- Testa et al., "Role of Purified Erythropoietin in the Amplification of the Erythroid Compartment," *Exp. Hematol.*, 8(Supp. 8), 144-152 (1980).
- Tong et al., "The Formation of Erythrocyte Membrane Proteins during Erythropoietin-induced Differentiation," *J. Biol. Chem.*, 256(24), 12666-12672 (Dec. 25, 1981).
- Toole et al., "Molecular cloning of a cDNA encoding human antihemophilic factor," *Nature*, 312, 342-347 (Nov. 8, 1984).

5,618,698

Page 10

- Tramontario et al., "Statistical evaluation of the coding capacity of complementary DNA strands." *Nucleic Acids Research*, 12(12), 5049-5059 (1984).
- Tsuda et al., "Comparative Structural Study of N-Linked Oligosaccharides of Urinary and Recombinant Erythropoietins." *J. Amer. Chem. Soc.*, 27(15), 5646-5654 (1988).
- Udupa et al., "Erythropoiesis in the aged mouse." *J. Lab. Clin. Med.*, 103(4), 574-580 & 581-588 (1984).
- Ullrich et al., "Rat Insulin Genes: Construction of Plasmids Containing the Coding Sequences." *Science*, 196, 1313-1319 (Jun. 17, 1977).
- 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).
- Ullrich et al., "Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells." *Nature* 309, 118-125 (May 31, 1984).
- 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).
- Ullrich et al., "Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes." *Nature*, 313, 756-761 (Feb. 28, 1985).
- 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).
- 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).
- Van der Ploeg et al., "DNA Methylation in the Human  $\delta\beta$ -Globin Locus in Erythroid and Nonerythroid Tissues." *Cell*, 19, 947-958 (Apr. 1980).
- Van Stone et al., "Effects of erythropoietin on anemia of peritoneally dialyzed anephric rats." *Kidney Int'l.*, 15, 370-375 (1979).
- Varki, "Diversity in the sialic acids." Oxford University Press, 25-40, (1992).
- Vedovato et al., "Erythropoietin Levels in Heterozygous Beta-Thalassemia." *Acta Haematol.*, 71, 211-213 (1984).
- Vichinsky et al., "Inadequate erythroid response to hypoxia in cystic fibrosis." *J. Pediatr.*, 105(1), 15-21 (Jul. 1984).
- Vieira et al., "The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers." *Gene*, 19, 259-268 (1982).
- 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).
- Walker et al., *Techniques in Molecular Biology*; Macmillan Pub. Co., N.Y., p. 280 (1983).
- 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).
- Wallace et al., "Directed Deletion of a Yeast Transfer RNA Intervening Sequence." *Science*, 209:1396-1400 (Sep. 19, 1980).
- 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 (1981).
- 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).
- Wallace et al., "A set of synthetic oligodeoxyribonucleotide primers for DNA sequencing in the plasmid vector pBR322." *Gene*, 16, 21-26 (1981).
- 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).
- 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).
- Walter et al., "Antibodies specific for the polyoma virus middle-size tumor antigen." *P.N.A.S. (USA)*, 78, 4882-4886 (Aug. 1981).
- Wang et al., "Some Chemical Properties of Human Erythropoietin." *Endocrinology*, 116(6), 2286-2292 (1985).
- 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).
- Welland et al., "In vivo Activity of Asialo-Erythropoietin in Combination with Asialo-Glycoproteins." *Blut*, 44(3), 173-175 (1982).
- Weiss et al., "Characterization of a monoclonal antibody to human erythropoietin." *P.N.A.S. (USA)*, 79, 5465-5469 (1982).
- Weiss et al., "Studies of the pathogenesis of anemia of inflammation: Mechanism of impaired erythropoiesis." *Am. J. Vet. Res.*, 44(10), 1832-1835 (Oct. 1982).
- Weissman et al., "Structure and expression of human IFN- $\alpha$  Genes." *Phil. Trans. R. Soc. Lond.*, B299, 7-28 (1982).
- White et al., "Studies on Erythropoietin." *Recent Progr. Hormone Res.*, 16:219-262 (1960).
- White et al., "Haemagglutinin of influenza virus expressed from a cloned gene promotes membrane fusion." *Nature*, 300, 658-659 (1982).
- 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." *PNAS (USA)*, 80:5387-5391 (Sep. 1983).
- Wiaderkiewicz et al., "Mismatch and blunt to protruding end joining by DNA ligases." *Nucleic Acids Res.* 15(19), 7831-7848 (1987).
- Wickens et al., "Expression of a chicken chromosomal ovalbumin gene injected into frog oocyte nuclei." *Nature* 285:628-634 (26 Jun. 1980).
- Wide et al., "Molecular charge heterogeneity of human serum erythropoietin." *British J. Haematol.*, 76, 121-127 (1990).
- 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).
- 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).
- Wong et al., "Interferon  $\gamma$  Induces Enhanced Expression of Ia And H 2 Antigens on B Lymphoid, Macrophage, and Myeloid Cell Lines." *J. Immunol.*, 131 (2):788-793 (Aug. 1983).
- Woo, "A Sensitive and Rapid Method for Recombinant Phage Screening." *Methods in Enzymology*, 68, 389-395 (1979).

5,618,698

Page 11

Wood et al., "Expression of active human factor VIII from recombinant DNA clones." *Nature*, 312, 330-336 (Nov. 22, 1984).

Woods et al., "Isolation of a cDNA Clone Corresponding to the MHC Linked Complement Protein Factor B." *Mol. Immunology*, 19, 1411 (1982).

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).

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)*.

Yanagawa et al., "Hybridomas for Production of Monoclonal antibodies to Human Erythropoietin." *Blood*, 64(2), 357-364 (Aug. 1984).

Yanagawa et al., "Isolation of Human Erythropoietin with Monoclonal Antibodies." *J. Biol. Chem.*, 259(5), 2707-2710 (Mar. 10, 1984).

Yanagi, "Recombinant Human Erythropoietin Produced by Namalwa Cells," *DNA*, 8(6), 419-427 (1989).

Young et al., "Efficient isolation of genes by using antibody probes," *P.N.A.S.* 80, 1194-1198 (Mar. 1983).

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).

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).



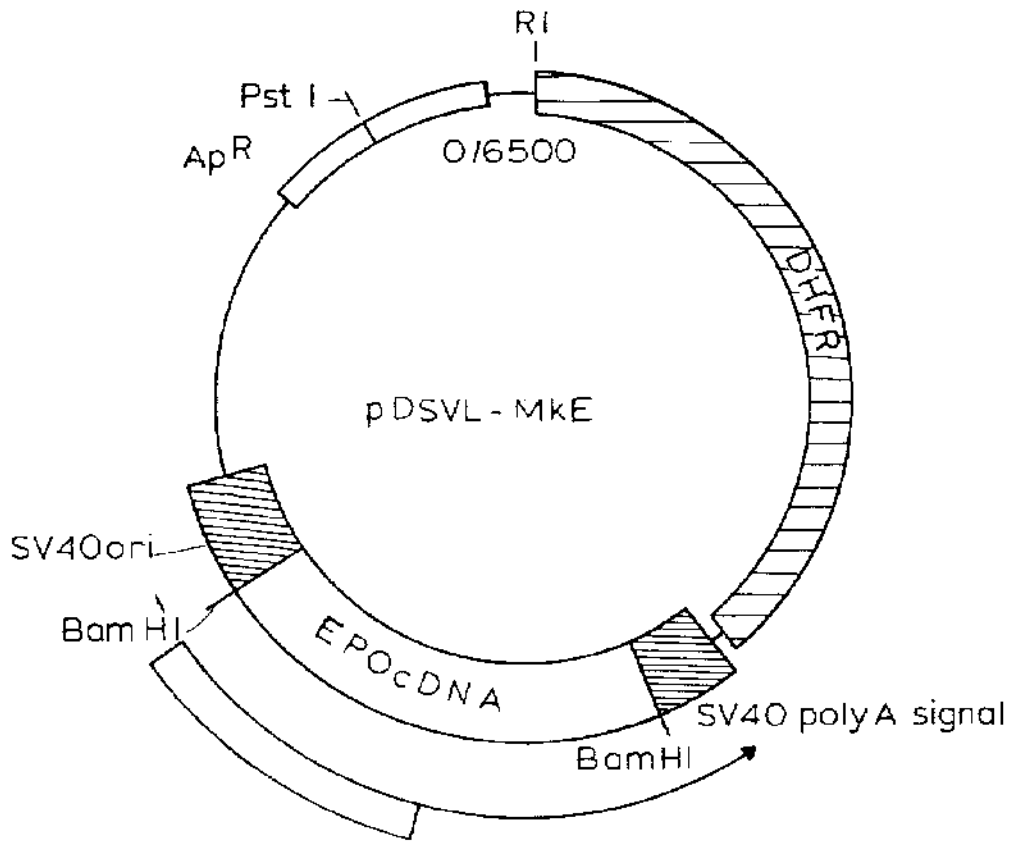
U.S. Patent

Apr. 8, 1997

Sheet 2 of 27

5,618,698

FIG. 2



**U.S. Patent**

Apr. 8, 1997

Sheet 3 of 27

**5,618,698**

*FIG. 3*

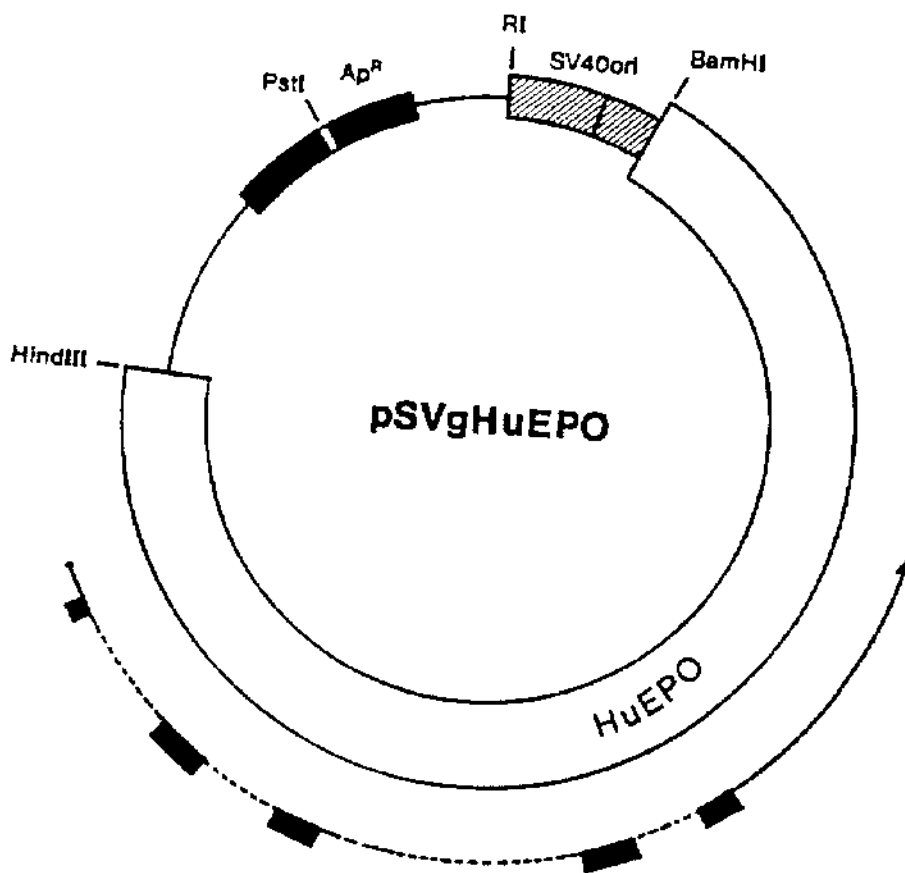


FIG. 4

