APPENDIX A

Statement Regarding Prior Art	Specification Cite[1]
1. The prior art to the patents-in-suit is "rich in patent and literature publications relating to 'recombinant DNA' methodologies for the isolation, synthesis, purification and amplification of genetic materials for use in transformation of selected host organisms."	Col. 2, lns. 39-59.
2. "Manufacture of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is known."	Col. 3, lns. 22-24.
3. An approach to microbiological processing known in the prior art to the patents-in-suit is "a gene that specifies the structure of a desired polypeptide product is either isolated from a 'donor organism' or chemically synthesized and then stably introduced into another organism which is preferably a self-replicating unicellular organism such as bacteria, yeast or mammalian cells in culture. Once this is done, the existing machinery for gene expression in the 'transformed' or 'transfected' microbial host cells operates to construct the desired product, using the exogenous DNA as a template for transcription of mRNA which is then translated into a continuous sequence of amino acid	Col. 2, lns. 27-38.

[1] All specifications cited refer to U.S. Patent No. 5,441,868.

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residues."	
4. DNA manufacturing procedures taught in the prior art to the patents-in-suit "provide a superior means for accomplishing such highly desirable results as" ease in assembly of expression vectors capable of providing high levels of microbial expression.	Col. 3, lns. 22-47.
5. DNA manufacturing procedures of the prior art to the patents-in-suit "provide a superior means for accomplishing such highly desirable results as: … providing for ready insertion of the DNA in convenient expression vectors in association with desired promoter/regulator and terminator sequences.…"	Col. 3, lns. 22-47.
6. "Among the more significant recent advances in hybridization procedures [in the prior art to the patents-in-suit] for the screening of recombinant clones is the use of labeled mixed synthetic oligonucleotide probes, each of which is potentially the complete complement of a specific DNA sequence in the hybridization sample including a heterogeneous mixture of single stranded DNAs or RNAs. These procedures are acknowledged to be especially useful in the detection of cDNA clones derived from sources which provide extremely low amounts of mRNA	Col. 4, lns. 22-32.

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sequences for the polypeptide of interest."	
7. "In general, the mixed probe procedures [of the prior art to the patents-in-suit] have been expanded upon by various workers to the point where reliable results have reportedly been obtained in a cDNA clone isolation using a 32 member mixed "pool" of 16-base-long (16-mer) oligonucleotide probes of uniformly, varying DNA sequences together with a single 11-mer to effect a two-site 'positive' confirmation of the presence of cDNA of interest."	Col. 4, lns. 44-54.
8. "[R]eliable procedures exist[ing] for developing phage-borne libraries of genomic DNA of human and other mammalian species origins" are described in the prior art to the patents-in-suit.	Col. 4, lns. 61-64.
9. "[Prior art to the patents-in-suit] report the successful isolation of a gene coding for the alpha subunit of the human pituitary glycoprotein hormones from the Maniatis Library through use of a "full length" probe including a complete 621 base pair fragment of a previously-isolated cDNA sequence for the alpha subunit."	Col. 5, lns. 7-14.
10. "[Prior art to the patents-in-suit] report isolation of human genomic clones for human HLA-DR using a 175 base pair	Col. 5, lns. 14-17.

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synthetic oligonucleotide."	
11. "[Prior art to the patents-in-suit] report the isolation of genomic clone for bovine pancreatic trypsin inhibitor (BPTI) using a single probe 86 base pairs in length and constructed according to the known amino acid sequence of BPTI."	Col. 5, lns. 17-22.
12. The prior art to the patents-in-suit taught "[e]rythropoiesis, the production of red blood cells, occurs continuously throughout the human life span to offset cell destruction. Erythropoiesis is a very precisely controlled physiological mechanism enabling sufficient numbers of red blood cells to be available in the blood for proper tissue oxygenation, but not so many that the cells would impede circulation. The formation of red blood cells occurs in the bone marrow and is under the control of the hormone, erythropoietin."	Col. 5, lns. 58-66.
13. The prior art to the patents-in-suit taught "[e]rythropoietin, an acidic glycoprotein of approximately 34,000 dalton molecular weight, may occur in three forms: α, β and asialo. The α and β forms differ slightly in carbohydrate components[, but] have the same potency, biological activity and molecular weight. The asialo form is an α or	Cols. 5-6, lns. 67-11.

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α form with the terminal carbohydrate (sialic acid) removed. Erythropoietin is present in very low concentrations in plasma when the body is in a healthy state wherein tissues receive sufficient oxygenation from the existing number of erythrocytes. This normal low concentration is enough to stimulate replacement of red blood cells which are lost normally through aging."	
14. The prior art to the patents-in-suit taught that "[b]ecause erythropoietin is essential in the process of red blood cell formation, the hormone has potential useful application in both the diagnosis and the treatment of blood disorders characterized by low or defective red blood cell production."	Col. 6, lns. 42-46.
15. "[Prior art to the patents-in-suit described] a therapeutic regimen for uremic sheep based on in vivo response to erythropoietin-rich plasma infusions and proposing a dosage of 10 U EPO/kg per day for 15-40 days as corrective of anemia of the type associated with chronic renal failure."	Col. 6, lns. 46-57.
16. "[Prior art to the patents-in-suit] describes a method for partially purifying erythropoietin from sheep blood plasma which provides low yields of a crude solid extract	Col. 7, lns. 23-25.

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containing erythropoietin."	
17. Prior art to the patents-in-suit taught a method of purifying human erythropoietin from urine of patients with aplastic anemia, which is described in Miyake, et al., J. Biol.Chem., Vol. 252, No. 15 Aug. 10, 1977), pp. 5558-5564. "This seven-step procedure includes ion exchange chromatography, ethanol precipitation, gel filtration, and adsorption chromatography, and yields a pure erythropoietin preparation with a potency of 70,400 units/mg of protein in 21% yield."	Col. 7, lns. 35-42.
18. "[Prior art to the patents-in-suit] describes a process for the production of hybrid human lymphoblastoid cells, reporting production levels ranging from 3 to 420 Units of erythropoietin per ml of suspension of cells (distributed into the cultures after mammalian host propagation containing) up to 10^7 cells per ml. At the highest production levels asserted to have been obtained, the rate of erythropoietin production could be calculated to be from 40 to about 4,000 units/10^6 cells/48 hours in in vitro culture following transfer of cells from in vivo propagation systems."	Col. 7, lns. 49-60.
19. A detailed description of the preparation and use of a	Col. 8, lns. 22-44.

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monoclonal, anti-erythropoietin antibody appears in the prior art to the patents-in-suit.	
20. "[In the prior art to the patents-in-suit, the] polypeptide sequence [of the] first twenty amino acid residues of mature human erythropoietin isolated according to the method of Miyake, et al., J.Biol.Chem., 252, 5558-5564 (1977) and upon which amino acid analysis was performed by the gas phase sequencer (Applied Biosystems, Inc.) according to the procedure of Hewick, M., et al., J.Biol.Chem., 256, 7990-7997 (1981)."	Col. 9, lns. 17-25.
21. With respect to the prior art to the patents-in-suit "[w]hile polyclonal and monoclonal antibodies as described above provide highly useful materials for use in immunoassays for detection and quantification of erythropoietin and can be useful in the affinity purification of erythropoietin, it appears unlikely that these materials can readily provide for large scale isolation of quantities of erythropoietin from mammalian sources sufficient for further analysis...."	Col. 9, lns. 31-38.
22. The prior art to the patents-in-suit "reported the in vitro translation of human kidney mRNA by frog oocytes. The resultant translation product mixture was estimated to include on the order of 220 mU of a translation product	Col. 10, lns. 18-31

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having the activity of erythropoietin per microgram of injected mRNA. While such levels of in vitro translation of exogenous mRNA coding for erythropoietin were acknowledged to be quite low (compared even to the prior reported levels of baboon mRNA translation into the sought-for product) it was held that the results confirm the human kidney as a site of erythropoietin expression, allowing for the construction of an enriched human kidney cDNA library from which the desired gene might be isolated.	
23. "[The prior art to the patents-in-suit described] expression systems employing Chinese hamster ovary (CHO) DHFR cells and the selectable marker, DHFR."	Col. 26, lns. 59-65.
24. "CHO DHFR cells (DuX-B11) CHO K1 cells, … lack[ing] the enzyme dihydrofolate reductase (DHFR) due to mutations in the structural genes and therefore requir[ing] the presence of glycine, hypoxanthine, and thymidine in the culture media" were described in the prior art to the patents-in-suit.	Cols. 26-27, lns. 66-3.