

# **EXHIBIT 2**

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3/19/87

PATENT

IN THE UNITED STATES PATENT  
AND TRADEMARK OFFICE

Application of:	)	"Production of
FU-KUEN LIN	)	Erythropoietin"
Serial No: 675,298	)	Group Art Unit 127
Filed: November 30, 1984	)	Examiner - A. Tanenholtz

APPLICANT'S AMENDMENT AND REPLY  
UNDER 37 C.F.R. §1.111 AND §1.115

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GROUP 120

Hon. Commissioner of Patents  
and Trademarks  
Washington, D.C. 20231

Sir:

This is in response to the Office Action dated February 5, 1987 in the above-identified application wherein substantially all prior rejections of pending claims 14, 15, 17-36, 58 and 61-72 were withdrawn, but wherein new grounds for rejection were advanced under 35 U.S.C. §§102, 103 and 112, and wherein all claims were "provisionally" rejected under 35 U.S.C. §101. Reconsideration and allowance of all claims is respectfully requested in view of the following amendments and remarks.

AMENDMENT

IN THE CLAIMS

Please cancel claims 14, 15, 17-36, 58 and 61-72 without prejudice and enter the following new claims 73-103.

31  
--73. A purified and isolated DNA sequence for use in securing expression in a procaryotic or eucaryotic

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*Cont'd*

host cell of a polypeptide product having at least a part of the primary structural conformation and having a therapeutic activity of naturally-occurring erythropoietin, said DNA sequence selected from the group consisting of:

- (a) the DNA sequences set out in Figures 5 and 6 or their complementary strands;
- (b) DNA sequences which hybridize under stringent conditions to the DNA sequences defined in (a); and
- (c) DNA sequences which, but for the degeneracy of the genetic code, would hybridize under stringent conditions to the DNA sequences defined in (a) and (b).

74. A procaryotic or eucaryotic host cell transformed or transfected with a DNA sequence according to claim 73 in a manner allowing the host cell to express said polypeptide product.

75. A biologically functional circular plasmid or viral DNA vector including a DNA sequence according to claim 73.

76. A procaryotic or eucaryotic host cell stably transformed or transfected with a DNA vector according to claim 75.

77. A purified and isolated DNA sequence coding for procaryotic or eucaryotic host expression of a polypeptide having part or all of the primary structural conformation and having a therapeutic activity of erythropoietin.

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Amended*

78. A cDNA sequence according to claim 77.

79. A monkey species erythropoietin coding DNA sequence according to claim 78.

80. A DNA sequence according to claim 79 and including the protein coding region set forth in Figure 5.

81. A genomic DNA sequence according to claim <sup>79</sup> 77.

82. A human species erythropoietin coding DNA sequence according to claim 81.

83. A DNA sequence according to claim 82 and including the protein coding region set forth in Figure 6.

84. A manufactured DNA sequence according to claim 77.

85. A manufactured DNA sequence according to claim 84 and including one or more codons preferred for expression in E.coli cells.

86. A manufactured DNA sequence according to claim 85, coding for expression of human species erythropoietin.

87. A manufactured DNA sequence according to claim 86 including the protein coding region set forth in Figure 7.

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*Cont'd*

88. A manufactured DNA sequence according to claim 84 and including one or more codons preferred for expression in yeast cells.

89. A manufactured DNA sequence according to claim 88, coding for expression of human species erythropoietin..

90. A manufactured DNA sequence according to claim 89 including the protein coding region set forth in Figure 8.

91. A DNA sequence according to claim 77 covalently associated with a detectable label substance.

92. A DNA sequence according to claim 91 wherein the detectable label is a radiolabel.

93. A single-strand DNA sequence according to claim 91.

94. A biologically functional circular plasmid or viral DNA vector including a DNA sequence according to claim 77.

95. A procaryotic or eucaryotic host cell stably transformed or transfected with a DNA vector according to claim 94.

96. A purified and isolated DNA sequence coding for a polypeptide fragment or polypeptide analog of

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naturally-occurring erythropoietin having a therapeutic activity of erythropoietin.

97. A biologically functional circular plasmid or viral DNA vector including a DNA sequence according to claim 96.

98. A procaryotic or eucaryotic host cell stably transformed or transfected with a DNA vector according to claim 97.

99. A DNA sequence according to claim 96 which is a manufactured sequence.

100. A DNA sequence coding for [Phe<sup>15</sup>]hEPO, [Phe<sup>49</sup>]hEPO, [Phe<sup>145</sup>]hEPO, [His<sup>7</sup>]hEPO, [Asn<sup>2</sup> des-Pro<sup>2</sup> through Ile<sup>6</sup>]hEPO, [des-Thr<sup>163</sup> through Arg<sup>166</sup>]hEPO, or [ $\Delta$ 27-55]hEPO.

101. A biologically functional circular plasmid or viral DNA vector including a DNA sequence according to claim 100.

102. A procaryotic or eucaryotic host cell stably transformed or transfected with a DNA vector according to claim 101.

103. A purified and isolated DNA sequence as set out in Figures 5 or 6 or the complementary strand of such a sequence.--

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REMARKS

Applicant acknowledges with thanks the interview kindly granted to his counsel, Mr. Borun and Mr. Odre, on March 4, 1987.

Upon entry of the above-requested amendments to the claims, claims 73 through 103 will remain in the application and will be correlated to prior claims 14, 15, 17-36, 58 and 61-72 in the manner indicated in the following Table.

<u>Prior Claim</u>	<u>New Claim</u>	<u>Prior Claim</u>	<u>New Claim</u>
14	73	30	90
15	74	31	91
61	75	32	92
62	76	33	93
69	--	63	94
17	77	64	95
18	78	70	--
19	79	34	96
20	80	65	97
21	81	66	98
22	82	71	--
23	83	36	99
24	84	35	100
25	85	67	101
26	86	68	102
27	87	72	--
28	88	58	103
29	89		

Applicant notes that none of the claims whose entry is sought correspond to prior claims 69-72. Applicant specifically reserves his right to pursue claims of the same or similar scope in a duly filed continuing application.

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A. The Claimed Subject Matter

As related in Applicant's communication dated October 3, 1986, the claims remaining in this application relate to DNA sequences, DNA vectors, transformed and transfected host cells useful in the preparation of erythropoietin products including, e.g., polypeptide analogs of erythropoietin.

Independent claim 73 is thus directed to purified and isolated DNA sequences generally defined by reference to the DNA sequences revealed in Figures 5 and 6 (previously Tables V and VI). Dependent claims 74-76 respectively relate to host cells transformed or transfected with DNA of claim 73, vectors including the DNA of claim 73, and hosts transformed with such vectors.

Independent claim 77 is directed generally to DNA sequences which code for procaryotic or eucaryotic host polypeptides having erythropoietin amino acid sequences and having one or more of erythropoietin's biological activities. Dependent claims 78-95 are directed to presently preferred forms of DNA sequences, vectors, and transformed or transfected hosts based on the claim 77 DNA sequences.

Independent claim 96 is generally directed to DNA sequences of the invention which encode polypeptide fragments and analogs of erythropoietin and dependent claims 97-99 are likewise directed to preferred forms of sequences, vectors, and transformed and transfected hosts. Independent claim 100 and dependent claims 101 and 102 relate to specific erythropoietin analog DNA sequences.

Finally, independent claim 103 is directed to the specific human and monkey erythropoietin-encoding purified and isolated DNA sequences as revealed in Figures 5 and 6.

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B. The Outstanding Office Action, The Rejections of the Claims and Applicant's Responses Thereto

In a communication dated October 3, 1986 responding to the Action of July 3, 1986, Applicant submitted the full text of the Chirgwin et al. reference (Ref. C8) to complete the Information Disclosure Statement filed on April 24, 1986 and also submitted a Supplemental Information Disclosure Statement directing the Patent Office's attention to references B15, B16, C135 and C136. Applicant respectfully solicits the Examiner's acknowledgement of receipt and consideration of the same and notation of such consideration on the previously submitted Forms PTO-1449.

Applicant understands that the amendments and remarks set out in his communication dated October 3, 1986 have resulted in the reconsideration and withdrawal of the following rejections propounded under Sections 101, 102, 103 and 112 in the Action dated July 3, 1986:

1. The Section 112 (first paragraph) rejection of claims 14, 15, 17-36, 58 and 61-72 on grounds relating to permanence of A.T.C.C. Budapest Treaty deposits;

2. The Section 112 (second paragraph) rejection of claims 14, 15, 17-36, 58 and 61-72 based on,

- (a) alleged indefiniteness of the term "pro-caryotic or eucaryotic",
- (b) alleged indefiniteness based on failure to specify a "fragment" size,
- (c) alleged indefiniteness of the term "biological properties",
- (d) an instance of improper Markush group language, and,

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(e) an instance of improper characterization of dependence for one of the claims;

3. The Section 101 rejection of claims 14, 24, 34 and 36 as being drawn to naturally-occurring subject matter;

4. The Section 102(b) and/or 103 rejection of claims 14, 24, 34 and 36 based on the Sugimoto et al. reference;

5. The Section 102(b) and/or 103 rejection of claims 14, 15, 17, 18, 20, 24-27, 33, 34, 61-66, 69, 70 and 71 based on the Lee-Huang (P.N.A.S.) reference;

6. The Section 102(a) and/or 103 rejection of claims 14, 15, 17-20, 24, 33, 34, 36, 58, 61-66, 69-70 and 71 based on Applicant's publication [J.Cell.Bioch., Suppl. 8B, p. 45 (1984)]; and,

7. The Section 103 rejection of all claims based variously on the Sugimoto et al., Cohen et al., Paddock, Farber et al., Bennetzen et al. Gouy et al., and Lewin references.

Due to the number and variety of new objections and rejections set forth in the recently received Action dated February 5, 1987, Applicant once again submits that the issues raised therein are best treated by means of responses which "track" the order of their appearance in the Action.

1. The Objection to the Disclosure Based On Figures 5-8 May Properly Be Withdrawn

The Examiner asserts that new drawing Figures 5-8 did not accompany Applicant's communication of October 3, 1986. Applicant attaches hereto as Exhibit No. 1 an envelope containing another set of Figures 5-8 and requests

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that the outstanding objection to the specification be withdrawn.

2. The Rejection of Prior Claims 20, 23, 27 and 30 Under The Second Paragraph of Section 112 Is Inapplicable To Corresponding New Claims 80, 83, 87 and 90

Partially reiterating a prior position taken by the Patent Office, the Examiner has objected to Applicant's reference in certain claims to DNA sequence information contained in Figures of the drawing, stating: "Ordinarily claims should not refer to drawings particularly as here where the material referred to can be easily described without resort to drawings". Applicant respectfully disagrees with the Examiner's position and reiterates the remarks set out at pages 11 and 12 of his prior communication responding to the prior Patent Office notation that the DNA sequences of the Figures could "adequately be expressed in words". Applicant again relies on the authority of the decisions of In re Faust, 86 U.S.P.Q. 114, 115 (1943) and Ex parte Squires, 133 U.S.P.Q. 598, 600 (Bd. App. 1961) in support of his position.

Responding to the prior Patent Office suggestion, Applicant has wholly reconstituted Tables V, VI, XIV and XXI as drawing Figures 5-8. The subject DNA sequences thus appear twice in the application as it is presently constituted. As the Examiner will note, Figure 5 spans three full pages, Figure 6 covers five pages, and Figures 7 and 8 each comprehend a full page of information. Applicant respectfully disagrees that this sequence information "can be easily described without resort to drawings" as suggested by the Examiner. It simply cannot be reasonably suggested that there might be a violation of the standards of definiteness

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of Section 112 (second paragraph) in Applicant's refraining from setting out the voluminous DNA sequences in the claims, when this same information already appear twice in the specification. Applicant therefore submits that the outstanding rejection of prior claims 20, 23, 27 and 30 may not properly be applied to corresponding new claims 80, 83, 87 and 90.

3. The "Provisional" Rejection of  
All Claims Under 35 U.S.C. §101  
May Not Properly Be Maintained

Reiterating a prior Patent Office position, the Examiner has lodged a "provisional" rejection of all claims under Section 101 based on the presentation of claims of similar scope in Applicant's "parent" patent applications Serial Nos. 582,185 and 655,841. Applicant previously acknowledged with thanks this notation of potential nonconformity with Section 101 and did not contend (as suggested by the Examiner) that the statute would not bar allowance of the same claims in more than one application. Applicant further notes that Serial No. 582,185 has been expressly abandoned and that a provisional election of prosecution of non-overlapping claim 48 is being concurrently made in Serial No. 655,841, with a corresponding cancellation of claims 1-47 therein. Applicant continues to submit, however, that the provisional notation does not provide a present basis for rejection of the claims. It is thus submitted that the outstanding "provisional" rejection be withdrawn.

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- 4. The Rejection of Prior Claims 14, 15, 17-19, 21, 23, 24, 25, 28, 31-34, 36, 58, 61-66 and 69-71 Under The First Paragraph of Section 112 May Not Properly Be Applied to Corresponding New Claims 73, 74, 77-79, 81, 83, 84, 85, 88, 91, 92, 93, 96 and 103

It was the Examiner's position that Applicant's use of the term "fragments thereof" in reference to claimed DNA sequence portions and/or his use of the term, "having at least a part of the primary structural conformation and one or more of the biological activities of naturally-occurring erythropoietin" in reference to polypeptides encoded by claimed DNA sequences was not "enabled" by the specification. The Examiner argued that either of these terms allows the claims "to read on proteins and peptides completely unrelated to erythropoietin" and that "those unrelated proteins could possess the common biological activity of being an antigen". Based on this argument, a Section 112 rejection was lodged against 14, 15, 17-19, 21, 22, 24, 25, 28, 31-34, 36, 58, 61-66 and 69-71. Applicant respectfully traverses the Examiner's rejection on such grounds.

Consistent with the substance of the discussions with the Examiner at the interview of March 4, 1987, wherein it was noted by the Examiner that the term "fragments" appeared to introduce a redundancy in claim 14, and notwithstanding Applicant's traversal, Applicant has herewith sought amendment to delete reference to "fragments" from prior independent claims 14 and 58. Corresponding new claims 73 and 103 no longer contain this term. Furthermore, Applicant has sought amendment of prior claims 14 and 17 referring to "biological activity" in a manner providing for recitation of "therapeutic activity" and similar recitation was added to prior claim 34. Corresponding new independent

*amendment about claim 96*

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claims 73, 77 and 96 thus refer to "therapeutic activity" rather than "biological activity".

These amendments have been sought for the purpose of advancing prosecution of the application and without waiver of Applicant's right to pursue claims of the form previously advanced in a duly filed continuing application.

Amendment of the claims is believed to moot the issues raised in the rejection and no rejection may properly be lodged against new claims 73, 74, 77-79, 81, 83, 84, 85, 88, 91, 92, 93, 96 and 103.

5. The Rejection of Prior Claim 35 Under 35 U.S.C. §112, Second Paragraph May Not Properly Be Applied to Corresponding Claim 100

The Examiner has rejected claim 35 with the notation that "use of brackets for indicating different species and strains of erythropoietin DNA sequence is improper since brackets in claims designates excluding the bracketed material". Applicant respectfully disagrees with the Examiner's position and proposes that brackets may properly be a part of a claim and are improper only when used for purposes of an amendment designating the deletion of a portion of a claims which already properly contains bracketed subject matter. In support of this position, applicant cites to 37 C.F.R. §1.121 which provides in pertinent part:

"(d) Where underlining or brackets are intended to appear in the printed patent or are properly part of the claimed material and are not intended as symbolic of changes in the particular claim, amendment by rewriting in accordance with paragraph (b) of this section shall be prohibited."

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It should be apparent from the above, that the brackets present in claim 100 are not "intended as symbolic of changes" and their use is thus in keeping with all "definiteness" requirements of 35 U.S.C. §112 and no proper basis for rejection exists.

6. The Rejection of Prior Claims 14, 15, 61, 62 and 69 Under 35 U.S.C. §112, First Paragraph, May Not Properly Be Applied To Corresponding Claims 73-76

It was the Examiner's position, based on the disclosures of Walker et al., Techniques In Mol. Biology, Macmillan Pub. Co., N.Y., p. 280 (1983) and Kennell et al., Progr.Nucl.Acid.Res.Mol.Biol., 11, 259-301 (1971)\*, that claims 14, 15, 61 and 69 (which refer to DNA hybridization) may be rejected because "the disclosure is enabling only for claims limited [to] the conditions of hybridization". Applicant respectfully disagrees and submits that a reading of the claims in light of the specification reveals that the reference to hybridization is not at all unduly broad. As noted at specification page 22, lines 5-7 and again at specification page 94, lines 19-24, the context of the hybridization event referred to in the claims is specifically correlated to the hybridization conditions illustrated in the specification with respect to the initial isolation of monkey and human erythropoietin-encoding DNA, or more stringent conditions.

Notwithstanding this position, in keeping with the discussions with the Examiner at the interview of March 4, 1987, Applicant has amended claim 73 to refer to hybridiza-

\* Applicant was provided a copy of pages 259 and 293 of this reference. If other portions are relied upon, advice of same is requested.

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tion "under stringent conditions". Applicant therefore submits that claims 73-76 are not properly subject to rejection.

7. The Rejection of Prior Claims 14, 17, 18, 21-24, 26, 27, 31-36, 58 and 61-68 Under 35 U.S.C. §103 Based on Sue et al. Considered with Breslow et al. or Woods et al. References May Not Properly be Applied to Corresponding Claims 73, 77, 78, 81-84, 86, 87, 91-93, 96, 99-100, 103, 75, 76, 94, 95, 97, 98, 101 and 102

It was the Examiner's position that the disclosures of the Sue et al. reference (P.N.A.S., 80) taken together with the publications by Breslow et al. [P.N.A.S. (USA), 79, pp. 6861-6865 (1982)] and Woods et al. [P.N.A.S. (USA), 79, pp. 5661-5665 (1982)] render the claimed subject matter obvious. The Examiner noted that the Sue et al. publication discloses what were "believed to be" the first 26 amino terminal residues of human erythropoietin and that the Breslow et al. and Woods et al. references disclose cDNA isolation using mixed probe sequences deduced from known amino acid data of blood protein. Acknowledging that the Sue et al. reference incorrectly designates the presence of an asparagine residue rather than a cysteine residue at position 7, the Examiner nonetheless concludes that:

"It would be "obvious to isolate the human erythropoietin cDNA sequences by utilizing the Sue et al. erythropoietin amino acid sequence data to devise oligonucleotide probes for use in sequencing a cDNA liver library in the manner taught by Breslow et al. or Woods et al. The fact that the erythropoietin 26 amino acid amino terminal peptide sequence of Sue et al. differs from that of erythropoietin by designating Asn instead of Cys at the seven position is patentably irrelevant since it would not interfere with the preparation of oligonucleotide probes."

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Applicant respectfully disagrees with the Examiner's position, submits that the Examiner's conclusions concerning preparation of probes based on the Sue et al. reference are in error, and submits, in turn, that the combination of references falls far short of existing legal standards for support of a conclusion of obviousness.

Briefly stated, Applicant did not do what the Examiner suggests could have been done based on the cited references. More significantly, Applicant would not have been able to do what the Examiner suggests could have been done based on the cited references, i.e., prepare a small number of oligonucleotides and probe for erythropoietin-encoding DNA within a relatively small DNA library.

As the Examiner will recall, Applicant succeeded in his discovery of DNA encoding erythropoietin using screening procedures which are themselves submitted to involve patentable advances in the art of DNA hybridization (as set forth in original claim 60 of the application). More specifically, Applicant employed two distinct sets of mixed probes to find the human genomic sequence. A first set consisted of a mixture of 128 20-mers (see specification Table II). The amino acid sequence which formed the basis for construction of the first set of probes is now known to correspond to residues 46-52 of human erythropoietin. Applicant used both the set of 128 20-mers of Table II and a second set of 128 17-mers (See specification Table III, relating to the sequence now known to correspond to erythropoietin residues 86-91) to jointly probe 1,500,000 phage plaques of human genomic library for the human sequence. Three positive clones were isolated. The set of 128 20-mers was thereafter used to successfully screen a 200,000 colony

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monkey kidney cDNA library, with only seven positive clones being isolated from the 200,000 screened. Applicant's use of mixed probes for screening a DNA library (and especially a mammalian genomic library) where the message sought was present in low abundance had been projected as being "impractical" shortly before applicant's successful work. See, Anderson et al., Reference C2, and specification page 8, line 29 through page 9, line 20 and page 96, lines 2-13. As noted at specification page 9, the Anderson et al. reference states in pertinent part:

"More generally, mixed-sequence oligodeoxy-nucleotide probes have been used to isolate protein genes of unknown sequence from cDNA libraries. Such probes are typically mixtures of 8-32 oligonucleotides, 14-17 nucleotides in length, representing every possible codon combination for a small stretch (5-6 residues) of amino acid sequence. Under stringent hybridization conditions that discriminate against incorrectly base-paired probes, these mixtures are capable of locating specific gene sequences in clone libraries of low-to-moderate complexity. Nevertheless, because of their short length and heterogeneity, mixed probes often lack the specificity required for probing sequences as complex as a mammalian genome. This makes such a method impractical for the isolation of mammalian protein genes when the corresponding mRNAs are unavailable." (Citations omitted.)

Turning now to the "secondary" references, in both the Woods et al. and Breslow et al. procedures, an opportunity to develop multiple probes suitable for use in screening a cDNA (human liver) library arose as the result of prior knowledge of the certain identity of regions of amino acid residues which were specified by relatively "unambiguous" codons. Thus, in the Breslow reference, the mixture of oligonucleotide probes was synthesized which corresponded to a specific Apo-I protein sequence (Gln-Lys-

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Lys-Trp-Gln) known to be present in the polypeptide whose encoding DNA was sought. Only a total of 16 different oligonucleotides was needed in order to develop a complete set of 14-mer probes for use in the cDNA library probing procedures. See Figure 1 on page 6862 of the reference. This low number was due to the relative lack of degeneracy among codons for tryptophane (no degeneracy), lysine (2-fold degeneracy), and glutamine (2-fold degeneracy) residues which made up the known sequence. Screening a cDNA library of only 10,000 colonies (provided by the authors of the Woods, et al. reference) Breslow et al. were able to isolate twenty positive clones. It is noteworthy that Apo-I DNA containing clones were thus conspicuously in relatively large abundance in the library. Nonetheless, the reported screening procedure failed to allow isolation of any clone including the full sequence of the Apo-I gene.

In the "two probe", Woods et al. reference, a total of 32 17-mers were needed to develop one complete set of probes corresponding to amino acids 9-14 of the protein sought, and an additional total of 48 17-mers were needed to ensure complete consonance of at least one probe to the DNA sequence encoding residues 78-83 of the desired protein. See Figure 2 on page 5662 of the reference. The ability of Woods, et al. to isolate 32 positive clones from a total of only 50,000 clones screened when hybridizing with one set of probes, and then to isolate 19 of the 32 using the second set, testifies to the relatively high abundance of the message in the library screened. (Once again, no full sequence clone is stated as having been isolated.)

Turning now to the correct sequence of amino acids within the first 26 residues of human erythropoietin, it is

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revealed that no opportunity exists (similar to that presented to Woods et al. and Breslow et al.) to effectively employ such relatively small numbers of probes to isolate genomic encoding human erythropoietin. The correct first 26 residues of human erythropoietin corresponding to the following indicated levels of codon degeneracy: Ala(4), Pro(4), Pro(4), Arg(6), Leu(6), Ile(3), Cys(2), Asp(2), Ser(6), Arg(6), Val(4), Leu(6), Glu(2), Arg(6), Tyr(2), Leu(6), Leu(6), Glu(2), Ala(4), Lys(2), Glu(2), Ala(4), Glu(2), Asn(2), Ile(3) and Thr(4). [A preliminary estimate of the number of oligonucleotides needed for a complete set of mixed probes can be made by simply multiplying the degree(s) of degeneracy within the sequence. As an example, to guarantee that one 17-mer oligonucleotide probe which is an exact replica of the true erythropoietin sequence will be present in a set of mixed probes corresponding to the bases encoding the first five amino acids plus the first two bases of the sixth amino acid, one would need to make  $4 \times 4 \times 4 \times 6 \times 6$ , or 2304, probes].

An array of the potential oligonucleotides based on the correct erythropoietin sequence is set forth below in Table I below.

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TABLE I

<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>
<u>Ala</u>	<u>Pro</u>	<u>Pro</u>	<u>Arg</u>	<u>Leu</u>	<u>Ile</u>	<u>Cys</u>	<u>Asp</u>	<u>Ser</u>	<u>Arg</u>
T	G	G	T	T	T		T	T	G
GCC	CCA	CCA	CGC	CTC	ATC	TGT	GAC	AGC	CGA
G	T	T	G	G	A	C			T
A	C	C	A	A					C
			AGG	TTG				G	
			A	A				TCA	AGG
								T	A
								C	
<u>11</u>	<u>12</u>	<u>13</u>	<u>14</u>	<u>15</u>	<u>16</u>	<u>17</u>	<u>18</u>	<u>19</u>	<u>20</u>
<u>Val</u>	<u>Leu</u>	<u>Glu</u>	<u>Arg</u>	<u>Tyr</u>	<u>Leu</u>	<u>Leu</u>	<u>Glu</u>	<u>Ala</u>	<u>Lys</u>
T	C				T			T	
GTC	CTG	GAG	AGG	TAT	CTC	TTG	GAG	GCC	AAG
G	A	A	A	C	G	A	A	G	A
A	T				A			A	
			G			G			
	TTG		CGA		TTG	CTA			
	A		T		A	T			
			C			C			
<u>21</u>	<u>22</u>	<u>23</u>	<u>24</u>	<u>25</u>	<u>26</u>				
<u>Glu</u>	<u>Ala</u>	<u>Glu</u>	<u>Asn</u>	<u>Ile</u>	<u>Thr</u>				
	T			T	C				
GAG	GCC	GAG	AAT	ATC	ACG				
A	G	A	C	A	A				
	A			A	T				

An array of potential oligonucleotides based on the incorrect sequence of Sue et al. is set out in Table II below. Note well that the sequence includes two errors. Not only is the seventh residue incorrect, the twenty-fourth residue is incorrectly specified as lysine rather than asparagine.

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TABLE II

1	2	3	4	5	6	7	8	9	10
<u>Ala</u>	<u>Pro</u>	<u>Pro</u>	<u>Arg</u>	<u>Leu</u>	<u>Ile</u>	<u>Asn</u>	<u>Asp</u>	<u>Ser</u>	<u>Arg</u>
T GCC G G	G CCA T T	G CCA T T	T CGC G G	T CTC G G	T ATC A	T AAT C	T GAC	T AGC	G CGA T C
			AGG A	TTG A				G TCA T C	AGG A
11	12	13	14	15	16	17	18	19	20
<u>Val</u>	<u>Leu</u>	<u>Glu</u>	<u>Arg</u>	<u>Tyr</u>	<u>Leu</u>	<u>Leu</u>	<u>Glu</u>	<u>Ala</u>	<u>Lys</u>
T GTC G A	C CTG A T	GAG A	AGG A	TAT C	T CTC G A	TTG A	GAG A	T GCC G A	AAG A
	TTG A		G CGA T C		TTG A	G CTA T C			
21	22	23	24	25	26				
<u>Glu</u>	<u>Ala</u>	<u>Glu</u>	<u>Lys</u>	<u>Ile</u>	<u>Thr</u>				
GAG A	T GCC G A	GAG A	AAA G	T ATC A	C ACG A T				

The practical consequence of the two errors in the Sue et al. sequence is that no probe mixture comprehending either of the two incorrectly projected residues (Asn<sup>7</sup> and Lys<sup>24</sup>) could have been employed to detect the subject erythropoietin gene.

To illustrate the problem of incorrect sequence information by Sue et al., one notes that if the Applicant had used the amino acid information in the reference and even gone beyond the notation of Woods et al. by generating a 64-member mixture of 17-mer probes based on the sequence spanning the entire codon for lysine at position 20 through

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the first two, non-degenerate bases of the codon for isoleucine at position 25, none of the 64 probes would have been a duplicate of the erythropoietin gene sequence due to the presence of the error in identifying the residue at position 24. Applicant, of course, would have had no way of knowing in advance that such work would be fruitless.

During the interview of March 4, 1987, the Examiner advanced a suggestion that notwithstanding the two errors in the Sue et al. human erythropoietin amino acid sequence, and notwithstanding numerous instances of 4- and 6-fold degeneracy of potential codons within the "correct" portions of the Sue et al. sequences, and notwithstanding the above-quoted notations of the Anderson et al. reference, the "correct" portions of the Sue et al. sequence appeared to provide opportunities to construct relatively small sets of mixed oligonucleotides for use in probing a human genomic library for the human erythropoietin gene. Reference was made to the amino acid residues immediately preceding the incorrectly noted 24th residue and the Examiner suggested that 12-mer or 14-mer mixtures might have been usefully applied in the manner of the 14-mer probes of Breslow et al. to find the human erythropoietin gene. Applicant's counsel acknowledged that mixtures of probes of this length could be made and that such mixtures would include small numbers of oligonucleotides on the order of those used in the Breslow et al. and Woods et al. systems, but traversed the Examiner's suggestion that any substantial likelihood of success would be expected to attend using probes of this size to isolate an erythropoietin gene from a human genomic library. This traversal was based on the relatively straightforward probabilities associated with a hybridiza-

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tion "hunt" for a DNA sequence that is a specific match to a given probe sequence. The Examiner is invited to consider the following:

1. It is clear that one could construct a set of 32 14-mer probes based on the potential codons for the "correct" 18th through 21st erythropoietin residues (Glu-Ala-Lys-Glu) plus the first two non-degenerate bases of residue 22 (Ala). One could also construct a set of 64 14-mers based on the potential codons for the "correct" 19th through 22nd residues (Ala-Lys-Glu-Ala) plus the first two non-degenerate bases of residue 23 (Glu).

2. The simple random probability of finding any specific string of five amino acid-specifying codons (i.e., 15 bases in a row) is one in  $21^5$ , or one in  $4.08 \times 10^6$  amino acid triplet codons.

3. The human genome comprises approximately  $3.3 \times 10^9$  base pairs, corresponding to  $1.1 \times 10^9$  triplets.

4. Thus, the simple probabilities are that the human genome will contain approximately 270 copies of any particular string of five amino acid-specifying codons. This means that a single 15-mer sequence probe will likely result in about 270 "positive" hybridizations under rigorously stringent conditions. When 14-mer mixed sequence probes are employed, stringency conditions must be relaxed and many fold more non-specific hybridizations will occur, rendering the mixed probe system essentially useless for identifying an erythropoietin gene in the human genome.

5. As one example of the non-specificity of a mixed probe system as described in paragraph 1 above, Applicant notes that the amino acid sequence Ala-Lys-Glu-Ala-Glu appears as residues 1190-1194 in the beta chain of

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an E. coli DNA directed RNA polymerase [See, Ovchinnikov et al., European Journal of Biochemistry, 116, 621-629 (1981) attached as Exhibit No. 2 hereto]. To the extent that E. coli DNA is present in the E. coli cell lawn in which phage DNA plaques of a human genomic library reside, probes for the Ala-Lys-Glu-Ala-Glu sequence would hybridize to E. coli DNA as well as to the human DNA encoding erythropoietin and other duplicate DNA sequences in the human, viral and bacterial genomes.

In hindsight, and with knowledge of the precise errors in the Sue et al. sequence, a "best case" set of 17-mer mixed probes could be designed to include a single oligonucleotide probe exactly corresponding to the true erythropoietin DNA sequence. Such a mixture would span the bases making up the codons beginning with lysine at position 19 through the non-degenerate first two bases of the codon for either asparagine or lysine at position 24. The mixture, however, would have had to have included 128 different oligonucleotides. This number of probes is far in excess of 16 or 32 or 48 component mixtures of the Breslow et al. or Woods et al. references.

To Applicant's knowledge, 128 mixed probes had never before been successfully employed in screening a cDNA library, much less a human genomic library which is approximately one hundred times more complex than a cDNA library. More significantly, in the absence of knowledge of the entire DNA sequence of a monkey kidney cDNA library or of a human genome, one cannot determine whether this sequence is unique to erythropoietin DNA. Indeed, it is only in hindsight (based on sequence data first provided herein) that one sees that in the amino terminal encoding region there is

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complete homology between monkey and human DNA sequences. Attached hereto as Exhibit No. 3 is Applicant's paper describing the monkey erythropoietin gene isolation [Lin et al., Gene, 44, 201-209 (1986)]. While Applicant's 20-mer mixture of probes corresponding to human sequence residues 46-52 was useful in isolating the monkey gene, the 17-mer mixture corresponding to residues 86-91 could not be used due to the divergence of homology between the human and monkey amino acid sequences. See Exhibit No. 3 and especially the "Conclusions" spanning pages 206-208.

It should then be noted that, as set forth in Example 4, two sets of probes were needed to isolate 3 positive human erythropoietin genomic clones in a screen of 1,500,000 phage plaques. No useful second set of 17-mer or 20-mer probes comprehending this same amino terminal region could be made without substantial amplification of the number of required oligonucleotides beyond 128 required for the first probe set.

Because Applicant could not have used the Sue et al. reference information to follow the Breslow et al. and Woods et al. procedures to screen a human genomic library or a monkey cDNA library without substantially departing from the quite simple procedures disclosed in the references, it cannot properly be argued that the claimed subject matter would have been obvious to a person of ordinary skill in the art at the time Applicant's invention was made. The outstanding rejection of claims 14, 17, 18, 21-24, 26, 27, 31-36, 58 and 61-68 may not therefore properly be applied to corresponding claims 73, 77, 78, 81-84, 86, 87, 91-93, 96, 99-100, 103 and 75, 76, 94, 95, 97, 98, 101 and 102.

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8. The "Subsidiary" Rejections of Prior  
Claims 15, 69-72, 19, 20, 25 and 28-30  
Under 35 U.S.C. §103 Over the Sue et al.  
And Other References May Not Properly  
Be Applied to Corresponding Claims  
74, 79, 80, 85 and 88-90

In three paragraphs spanning pages 5 and 6 of the Office Action dated February 5, 1987, the Examiner lodged rejections of certain claims as being unpatentable over the combination of the Sue et al., Breslow et al. and Woods et al. references (as discussed immediately above) in further view of certain additional secondary references. More specifically, claims 15 and 69 through 72 were rejected in view of Talmadge et al. [P.N.A.S. (USA), 77, pp. 3369-3373 (1980)] which deals with expression of a rat proinsulin in E.coli. Claims 19 and 20 were rejected in further view of Farber et al. (apparently either reference C32 or reference C33) which discloses primate erythropoietin messenger RNA isolation. Finally, claims 25 and 28-30 were rejected in view of the additional references to Gouy et al. (PTO Reference "U") or Bennetzen et al. (PTO Reference "R") concerning preference codons for E.coli and yeast.

Because the primary rejection of claims based on the Sue et al. reference taken together with Breslow et al. or Woods et al. has been demonstrated to be without proper foundation, Applicant submits that the "subsidiary" rejections of claims 15, 69-72, 19, 20, 25, and 28-30 may not properly be applied to corresponding claims.

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- 9. The Rejection of Prio Claims 14, 15, 17-19, 21, 22, 24, 25, 28, 31-34, 36, 58 and 61-72 Under 35 U.S.C. §102(b) in View Of Talmadge et al. May Not Properly Be Applied to Corresponding Claims 73, 74, 77-79, 81-82, 84-85, 88, 91-93, 96, 99, 103 75, 76, 94, 95, 96, 98, 101 and 102

Cross-referencing to the outstanding Section 112 rejection of claims 14, 15, 17-19, 21, 22, 24, 25, 28, 31-34, 36, 58 and 61-72 based on alleged undue breadth of the terms "fragments thereof" and "having at least...activities", the Examiner has interposed a Section 102(b) rejection of these claims based on the Talmadge et al. reference. More specifically, the Examiner argues that the claims are "deemed to embrace the DNA sequences and protein expression method of Talmadge et al."

Applicant respectfully disagrees with the Examiner's position but submits that the issue is mooted by the amendments to the claims discussed, infra, with respect to Section 112 issues.

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- 10. The Rejection of Claims 69-72 Under 35 U.S. §103 Based on Talmadge et al. Is Mooted By Cancellation of These Claims

Citing to the authority of the decisions of In re Durden and In re Albertson\*, the Examiner rejected claims 69-72 as being directed to obvious methods in view of the DNA expression systems described in Talmadge, et al.

As previously noted Applicant has sought cancellation without prejudice of prior claims 69-72 and has not inserted claims corresponding thereto in this amendment. The issues raised by the rejection are no longer present in the application.

\* The Examiner appears to have inadvertently mis-cited "In re Larsen" rather than In re Albertson.

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CONCLUSION

The foregoing remarks are believed to establish that claims 73-103 are in condition for allowance and an early notice under 37 C.F.R. §1.311 is solicited.

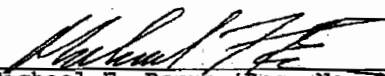
Consistent with Applicant's position, enclosed herewith pursuant to 37 C.F.R. §1.311(b) is an "Authorization to Charge Issue Fee to Deposit Account".

Applicant further notes that this case has been made special by the Commissioner (see Notice dated May 2, 1986) and, consistent with MPEP §1309, Applicant requests that this application be suitably "tagged" upon allowance of the claims to allow for priority in printing.

Respectfully submitted,

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