

EXHIBIT E

Claims of U.S. Patent Application No. 06/675,298, Grouped According To PTO's**July 3, 1986 Restriction Requirement**

Restriction Group	Claims of '298 Application
Group I: "Polypeptide"	<ol style="list-style-type: none"> 1. A purified and isolated polypeptide having part or all of the primary structural conformation and one or more of the biological properties of naturally-occurring erythropoietin and characterized by being the product of prokaryotic or eukaryotic expression of an exogenous DNA sequence. 2. A polypeptide according to claim 1 further characterized by being free of association with any mammalian protein. 3. A polypeptide according to claim 1 wherein the exogenous DNA sequence is a cDNA sequence. 4. A polypeptide according to claim 1 wherein the exogenous DNA sequence is a manufactured DNA sequence. 5. A polypeptide according to claim 1 wherein the exogenous DNA sequence is a genomic DNA sequence. 6. A polypeptide according to claim 1 wherein the exogenous DNA sequence is carried on an autonomous replicating circular DNA plasmid or viral vector. 7. A polypeptide according to claim 1 possessing part or all of the primary structural conformation of human erythropoietin as set forth in Table VI or any naturally occurring allelic variant thereof. 8. A polypeptide according to claim 1 possessing part or all of the primary structural conformation of monkey erythropoietin as set forth in Table V or any naturally occurring allelic variant thereof. 9. A polypeptide according to claim 1 which has the immunological properties of naturally-occurring erythropoietin. 10. A polypeptide according to claim 1 which has the <i>in vivo</i> biological activity of naturally-occurring erythropoietin. 11. A polypeptide according to claim 1 which has the <i>in vitro</i> biological activity of naturally-occurring erythropoietin. 12. A polypeptide according to claim 1 further characterized by being covalently associated with a detectable label substance. 13. A polypeptide according to claim 12 wherein said detectable label is a radiolabel. 16. A polypeptide product of the expression of a DNA sequence of claim 14 in a prokaryotic or eukaryotic host. 39. A polypeptide product of the expression in a prokaryotic or eukaryotic host cell of a DNA sequence according to claims 17 or 34. 40. A glycoprotein product having a primary structural conformation sufficiently duplicative of that of a naturally-occurring erythropoietin to allow possession of one or more of the biological properties thereof and having an average carbohydrate

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(Group I: "Polypeptide," cont.)	<p>composition which differs from that of naturally-occurring erythropoietin.</p> <p>41. A glycoprotein product having a primary structural conformation sufficiently duplicative of that of a naturally-occurring human erythropoietin to allow possession of one or more of the biological properties thereof and having an average carbohydrate composition which differs from that of naturally-occurring human erythropoietin.</p> <p>47. A synthetic polypeptide having part of all of the amino acid sequence as set forth in Table V and having one or more of the <i>in vivo</i> or <i>in vitro</i> biological activities of naturally-occurring monkey erythropoietin.</p> <p>48. A synthetic polypeptide having part of all of the amino acid sequence as set forth in Table VI, other than a sequence of residues entirely within the sequence numbered 1 through 20, and having a biological property of naturally-occurring human erythropoietin.</p> <p>49. A synthetic polypeptide having part of all of the secondary conformation of part or all of the amino acid sequence set forth in Table VI, other than a sequence of residues entirely within the sequence numbered 1 through 20, having a biological property of naturally-occurring human erythropoietin.</p> <p>50. A process for the production of a polypeptide having part of all of the primary structural conformation and one or more of the biological properties of naturally-occurring erythropoietin, said process comprising: growing, under suitable nutrient conditions, prokaryotic or eukaryotic host cells transformed or transfected with a DNA vector according to claim 37, and isolating desired polypeptide products of the expression of DNA sequences in said vector.</p> <p>51. An antibody substance characterized by immunoreactivity with erythropoietin and with a synthetic polypeptide having a primary structural conformation substantially duplicative of a continuous sequence of amino acid residues extant in naturally-occurring erythropoietin except for any polypeptide comprising a sequence of amino acid residues entirely comprehended within sequence, A-P-P-R-L-I-C-D-S-R-V-L-E-R-Y-L-L-E-A-K.</p> <p>52. An antibody according to claim 51, which is a monoclonal antibody.</p> <p>53. An antibody according to claim 51, which is a polyclonal antibody.</p> <p>54. An antibody according to claim 51, which is immunoreactive with erythropoietin and a synthetic polypeptide having the sequence selected from the sequences: V-P-D-T-K-V-N-F-Y-A-W-K-R-M-E-V-G; K-E-A-I-S-P-P-D-A-A-S-A-A; V-Y-S-N-F-L-R-G-K-L-K-L-Y-T-G-E-A-C-R-T-G-D-R.</p> <p>59. A polypeptide product of the expression of a DNA sequence according to claim 58 in a prokaryotic or eukaryotic host cell.</p>

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Group II: "DNA"	<p>14. A DNA sequence for use in securing expression in a prokaryotic or eukaryotic host cell of a polypeptide product having at least a part of the primary structural conformation and one or more of the biological properties of naturally-occurring erythropoietin, said DNA sequence selected from among: (a) the DNA sequence set out in Tables V and VI or their complementary strands; (b) DNA sequences which hybridize to the DNA sequences defined in (a) or fragments thereof; and (c) DNA sequences which, but for the degeneracy of the genetic code, would hybridize to the DNA sequences defined in (a) or (b).</p> <p>15. A prokaryotic or eukaryotic host cell transformed or transfected with a DNA sequence according to claim 14 in a manner allowing the host cell to express said polypeptide product.</p> <p>17. A purified and isolated DNA sequence coding for prokaryotic or eukaryotic host expression of a polypeptide having part or all of the primary structural conformation and one or more of the biological activities of erythropoietin.</p> <p>18. A cDNA sequence according to claim 17.</p> <p>19. A monkey species erythropoietin coding DNA sequence according to claim 18.</p> <p>20. A DNA sequence according to claim 19 and including the protein coding region set forth in Figure 5.</p> <p>21. A genomic DNA sequence according to claim 17.</p> <p>22. A human species erythropoietin coding DNA sequence according to claim 21.</p> <p>23. A DNA sequence according to claim 22 and including the protein coding region set forth in Figure 6.</p> <p>24. A manufactured DNA sequence according to claim 17.</p> <p>25. A manufactured DNA sequence according to claim 24 and including one or more codons preferred for expression in <i>E. coli</i> cells.</p> <p>26. A manufactured DNA sequence according to claim 25, coding for expression of human species erythropoietin.</p> <p>27. A manufactured DNA sequence according to claim 26 including the protein coding region set forth in Figure 7.</p> <p>28. A manufactured DNA sequence according to claim 24 and including one or more codons preferred for expression in yeast cells.</p> <p>29. A manufactured DNA sequence according to claim 28, coding for expression of human species erythropoietin.</p> <p>30. A manufactured DNA sequence according to claim 29 including the protein coding region set forth in Figure 8.</p>

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(Group II: "DNA," cont.)	<p>31. A DNA sequence according to claim 17 covalently associated with a detectable label substance.</p> <p>32. A DNA sequence according to claim 31 wherein the detectable label is a radiolabel.</p> <p>33. A single-strand DNA sequence according to claim 31.</p> <p>34. A purified and isolated DNA sequence coding for a polypeptide fragment or polypeptide analog of naturally-occurring erythropoietin.</p> <p>35. A DNA sequence coding for [Phe¹⁵]_{hEPO}, [Phe⁴⁹]_{hEPO}, [Phe¹⁴⁵]_{hEPO}, [His⁷]_{hEPO}, [Asn² des- Pro² through Ile⁶]_{hEPO}, [des-Thr¹⁶³ through Arg¹⁶⁶]_{hEPO}, or [Δ27-55]_{hEPO}.</p> <p>36. A DNA sequence according to claim 34 which is a manufactured sequence.</p> <p>58. A purified and isolated DNA sequence as set out in Figure 5 or 6 or a fragment thereof or the complementary strand of such a sequence or fragment.</p> <p>61. A biologically functional circular plasmid or viral DNA vector including a DNA sequence according to claim 14.</p> <p>62. A prokaryotic or eukaryotic host cell stably transformed or transfected with a DNA vector according to claim 61.</p> <p>63. A biologically functional circular plasmid or viral DNA vector including a DNA sequence according to claim 17.</p> <p>64. A prokaryotic or eukaryotic host cell stably transformed or transfected with a DNA vector according to claim 63.</p> <p>65. A biologically functional circular plasmid or viral DNA vector including a DNA sequence according to claim 34.</p> <p>66. A prokaryotic or eukaryotic host cell stably transformed or transfected with a DNA vector according to claim 65.</p> <p>67. A biologically functional circular plasmid or viral DNA vector including a DNA sequence according to claim 35.</p> <p>68. A prokaryotic or eukaryotic host cell stably transformed or transfected with a DNA vector according to claim 67.</p> <p>69. A process for the production of a polypeptide having part or all of the primary structural conformation and one or more of the biological activities of naturally-occurring erythropoietin, said process comprising: growing, under suitable nutrient conditions, prokaryotic or eukaryotic host cells transformed or transfected with a DNA vector according to claim 62, and isolating desired polypeptide products of the expression of DNA sequences in said vector.</p> <p>70. A process for the production of a polypeptide having part or all of the primary structural conformation and one or more of the biological activities of naturally-</p>

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(Group II: "DNA," cont.)	<p>occurring erythropoietin, said process comprising: growing, under suitable nutrient conditions, prokaryotic or eukaryotic host cells transformed or transfected with a DNA vector according to claim 63, and isolating desired polypeptide products of the expression of DNA sequences in said vector.</p> <p>71. A process for the production of a polypeptide having part or all of the primary structural conformation and one or more of the biological activities of naturally-occurring erythropoietin, said process comprising: growing, under suitable nutrient conditions, prokaryotic or eukaryotic host cells transformed or transfected with a DNA vector according to claim 65, and isolating desired polypeptide products of the expression of DNA sequences in said vector.</p> <p>72. A process for the production of a polypeptide having part or all of the primary structural conformation and one or more of the biological activities of naturally-occurring erythropoietin, said process comprising: growing, under suitable nutrient conditions, prokaryotic or eukaryotic host cells transformed or transfected with a DNA vector according to claim 67, and isolating desired polypeptide products of the expression of DNA sequences in said vector.</p>
Group III: "Plasmid"	<p>37. A biologically functional circular plasmid or viral DNA vector including a DNA sequence according to either of claims 14, 17, 34 or 35.</p> <p>38. A prokaryotic or eukaryotic host cell stably transformed or transfected with a DNA vector according to claim 37.</p>
Group IV: "Cells"	<p>42. Vertebrate cells which can be propagated <i>in vitro</i> continuously and which upon growth in culture are capable of producing in the medium of their growth in excess of 100 U of erythropoietin per 10^6 cells in 48 hours as determined by radioimmunoassay.</p> <p>43. Vertebrate cells according to claim 42 capable of producing in excess of 500 U erythropoietin per 10^6 cells in 48 hours.</p> <p>44. Vertebrate cells according to claim 42 capable of producing in excess of 1,000 U erythropoietin per 10^6 cells in 48 hours.</p> <p>45. Vertebrate cells according to claim 42 which are mammalian or avian cells.</p> <p>46. Vertebrate cells according to claim 45 which are COS-1 cells or CHO cells.</p>
Group V: "Pharmaceutical Composition"	<p>55. A pharmaceutical composition comprising an effective amount of a polypeptide according to claims 1, 16, 39, 40 or 41 and a pharmaceutically acceptable diluent, adjuvant or carrier.</p> <p>56. A method for providing erythropoietin therapy to a mammal comprising administering an effective amount of a polypeptide according to claims 1, 16, 39, 40 or 41.</p> <p>57. A method according to claim 56 wherein the therapy comprises enhancing hematocrit levels.</p>

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Group VI: "Assay"	<p>60. An improvement in the method for detection of a specific single stranded polynucleotide of unknown sequence in a heterogeneous cellular or viral sample including multiple single-stranded polynucleotides wherein:</p> <p>(a) a mixture of labeled single-stranded polynucleotide probes is prepared having uniformly varying sequences of bases, each of said probes being potentially specifically complementary to a sequence of bases which is putatively unique to the polynucleotide to be detected;</p> <p>(b) the sample is fixed to a solid substrate;</p> <p>(c) the substrate having the sample fixed thereto is treated to diminish further binding of polynucleotides thereto except by way of hybridization to polynucleotides in said sample;</p> <p>(d) the treated substrate having the sample fixed thereto is transitorily contacted with said mixture of labeled probes under conditions facilitative of hybridization only between totally complementary polynucleotides; and</p> <p>(e) the specific polynucleotide is detected by monitoring for the presence of a hybridization reaction between it and a totally complementary probe within said mixture of labeled probes, as evidenced by the presence of a higher density of labeled material on the substrate at the locus of the specific polynucleotide in comparison to a background density of labeled material resulting from non-specific binding of labeled probes to the substrate,</p> <p>said improvement comprising using in excess of 32 mixed probes and performance of one or more of the following:</p> <p>(1) employing a nylon-based paper as said solid substrate;</p> <p>(2) treating with a protease in step (c);</p> <p>(3) employing individual labeled probe concentrations of approximately 0.025 picomoles; and</p> <p>(4) employing as one of the hybridization conditions in step (d) stringent temperatures approaching to with 4°C away from the lowest calculated Td of any of the probes employed.</p>