# APPENDIX B
# (U.S. Patent No. 5,547,933)
# Part 1 of 4

US005547933A

# United States Patent [19]

Lin

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[54] **PRODUCTION OF ERYTHROPOIETIN**

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[57] **ABSTRACT**

Disclosed are novel polypeptides possessing part or all of the primary structural conformation and one or more of the biological properties of mammalian erythropoietin ("EPO") which are characterized in preferred forms by being the product of procaryotic or eucaryotic host expression of an exogenous DNA sequence. Illustratively, genomic DNA, cDNA and manufactured DNA sequences coding for part or all of the sequence of amino acid residues of EPO or for analogs thereof are incorporated into autonomously replicating plasmid or viral vectors employed to transform or transfect suitable procaryotic or eucaryotic host cells such as bacteria, yeast or vertebrate cells in culture. Upon isolation from culture media or cellular lysates or fragments, products of expression of the DNA sequences display, e.g., the immunological properties and in vitro and in vivo biological activities of EPO of human or monkey species origins. Disclosed also are chemically synthesized polypeptides sharing the biochemical and immunological properties of EPO. Also disclosed are improved methods for the detection of specific single stranded polynucleotides in a heterologous cellular or viral sample prepared from, e.g., DNA present in a plasmid or viral-borne cDNA or genomic DNA "library".

**14 Claims, 27 Drawing Sheets**

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FIG. 1

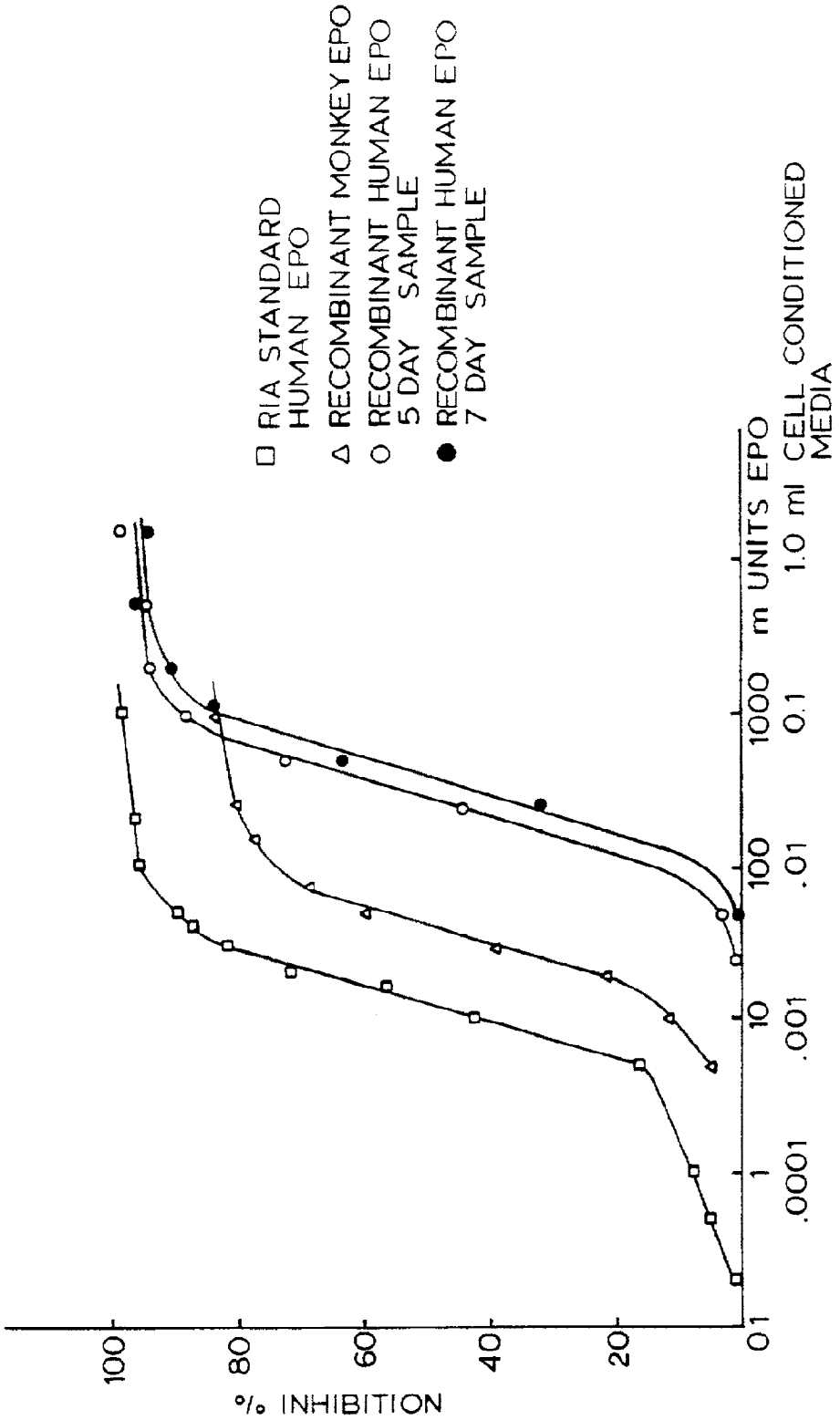

FIG. 2

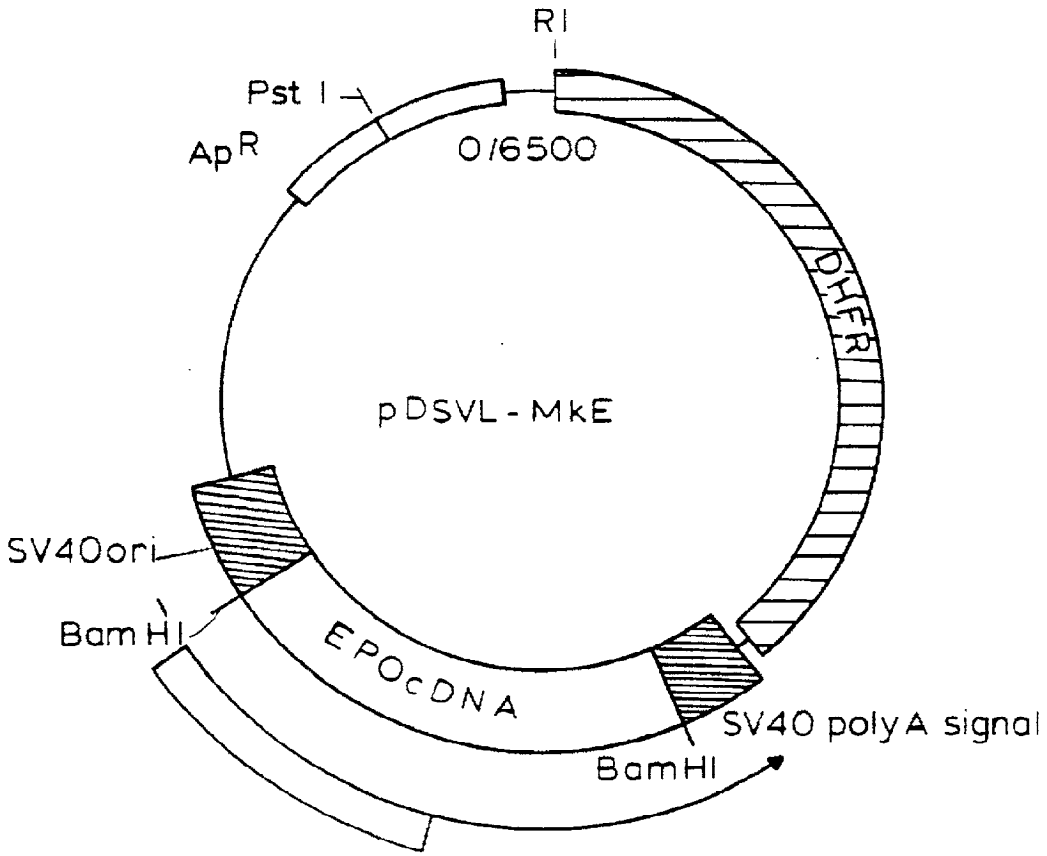

FIG. 3

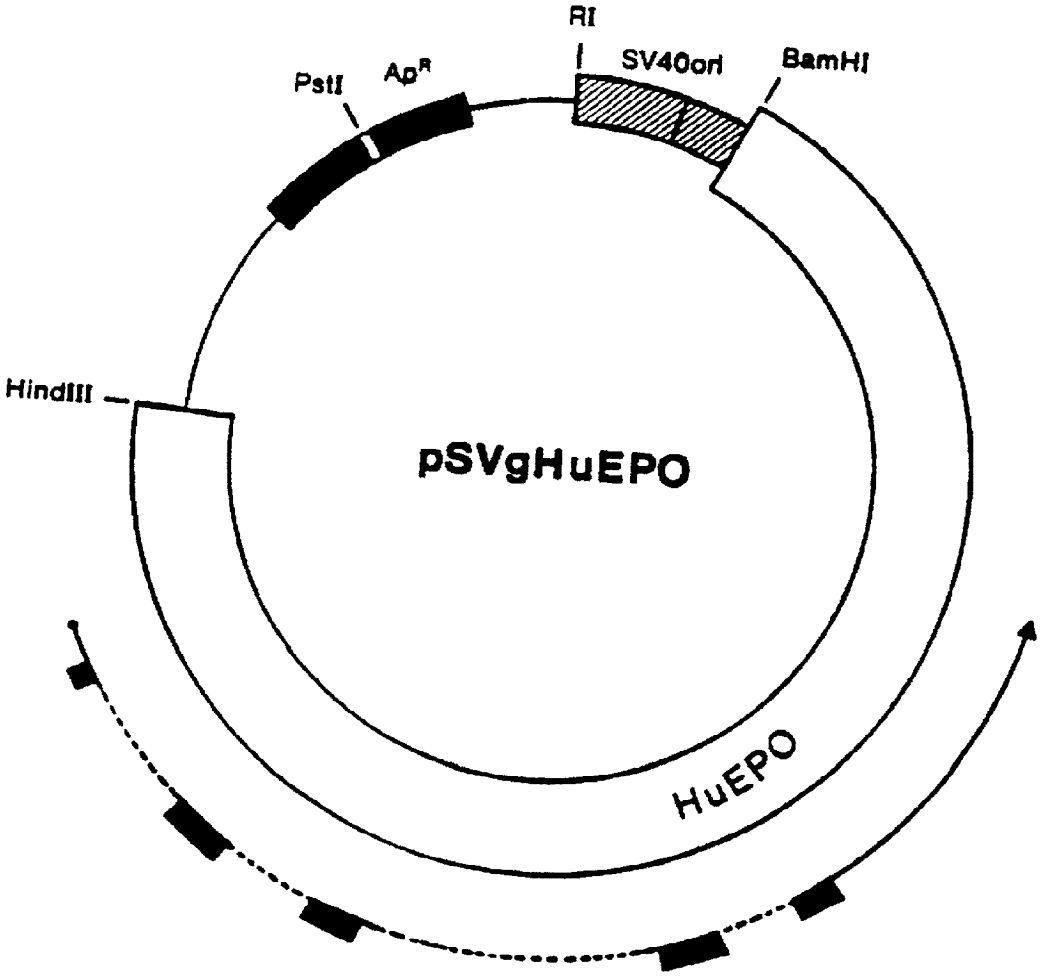

FIG. 4

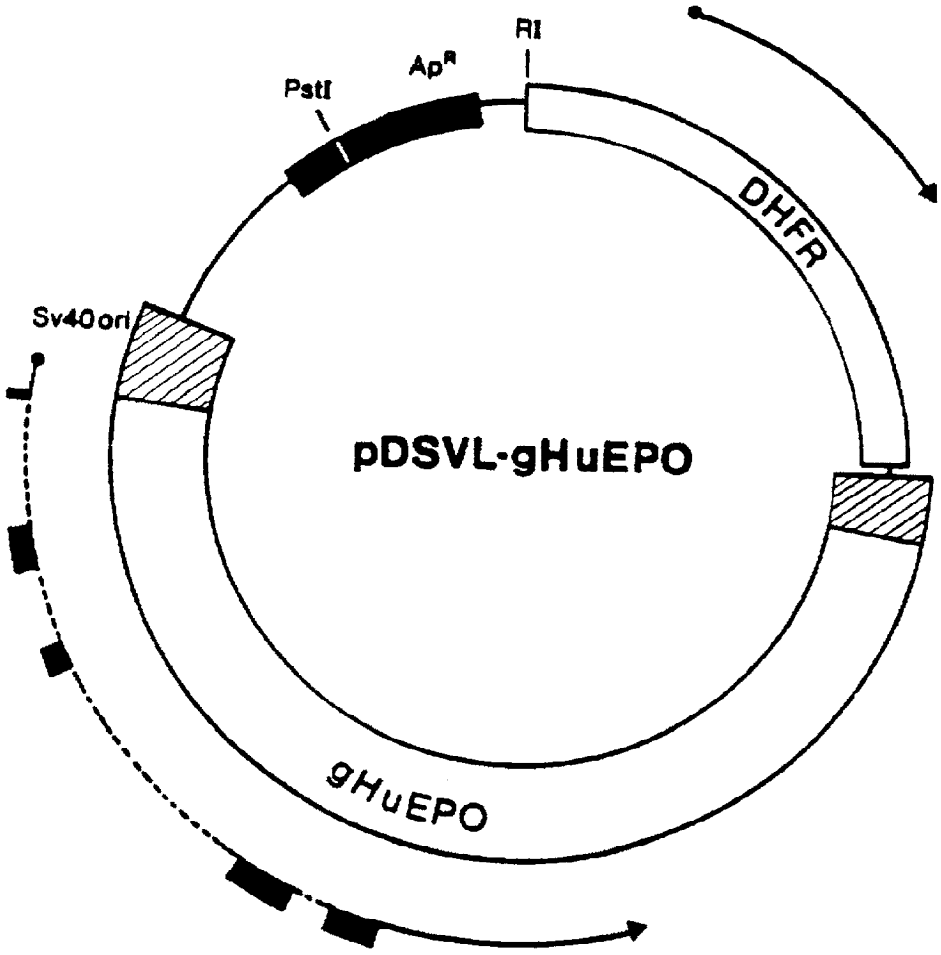

FIG. 5A

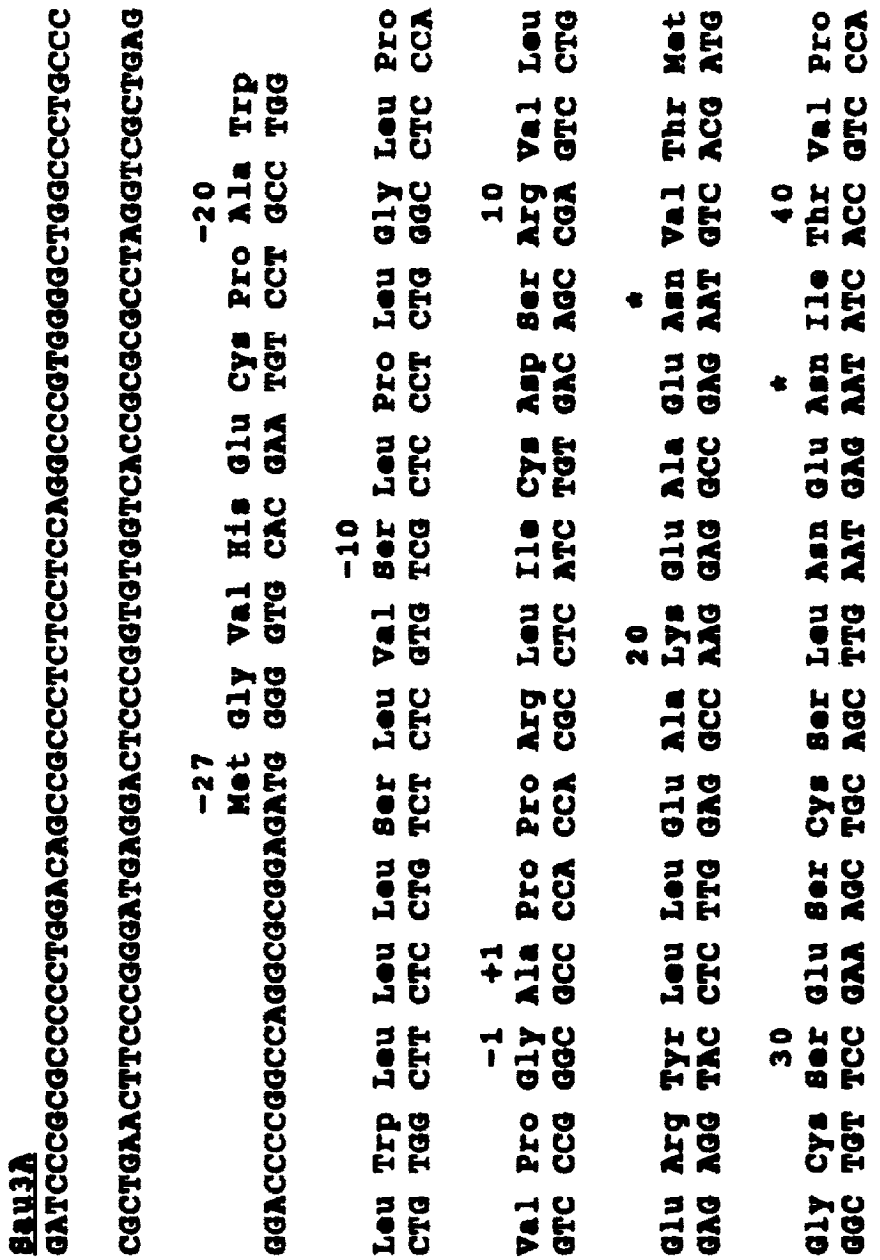

FIG.5B

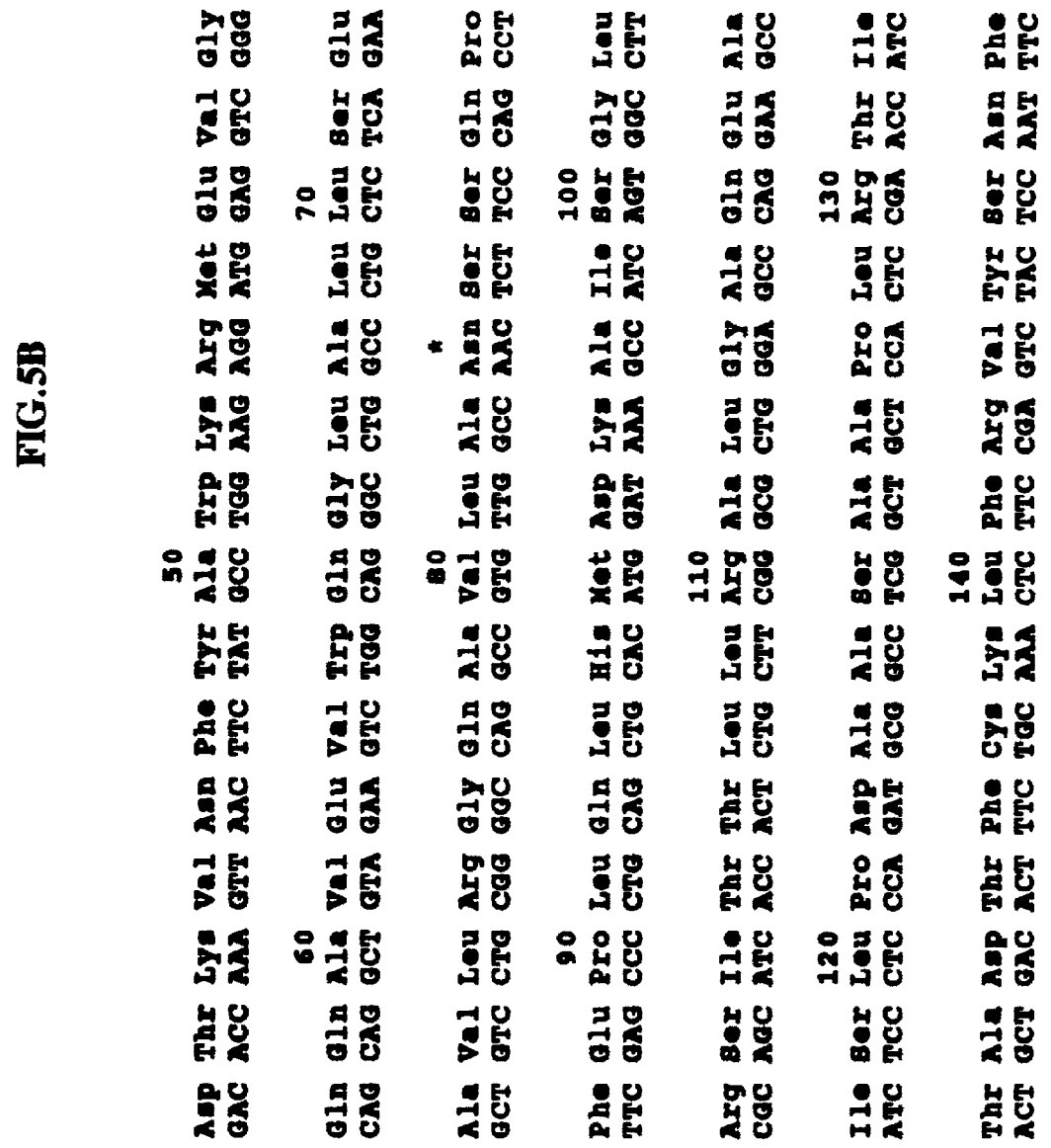

FIG. 5C

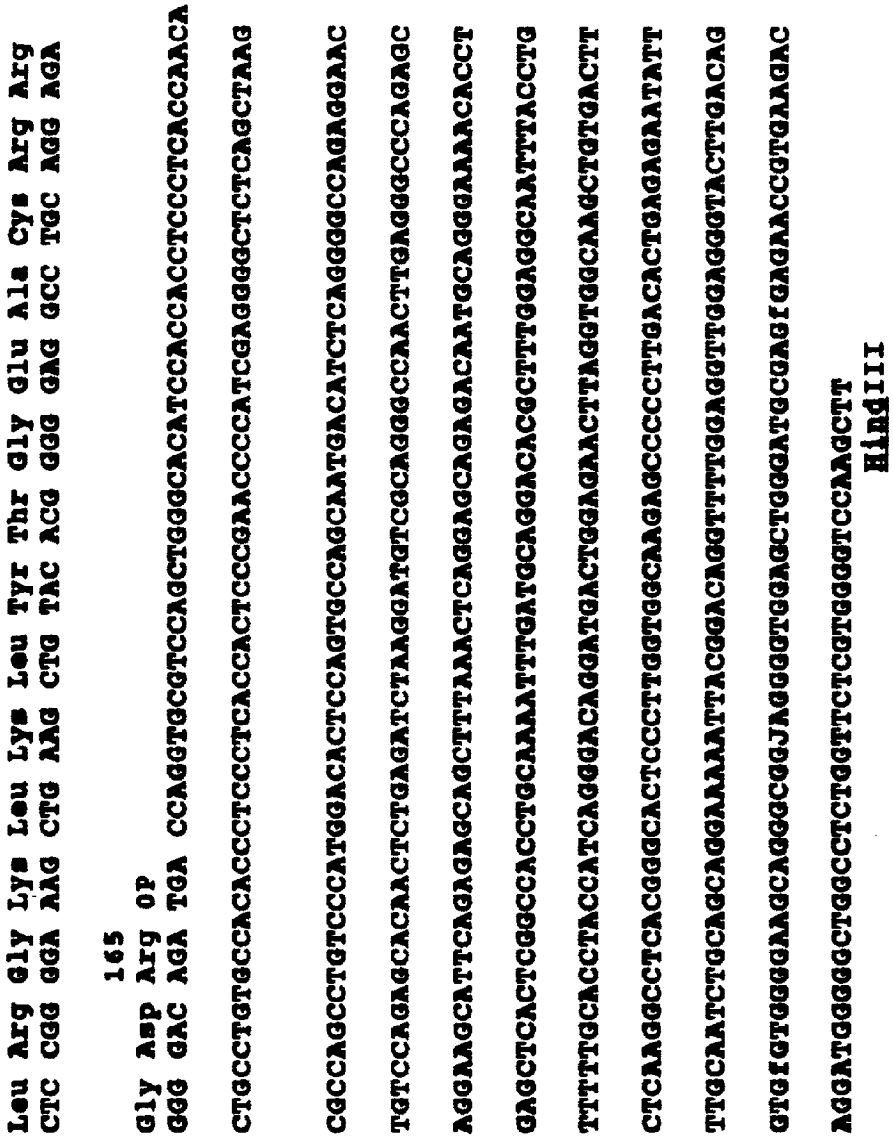

FIG.6A