EXHIBIT 2

US005621080A

United States Patent [19]

Lin

[11] **Patent Number: 5,621,080**

[45] **Date of Patent: *Apr. 15, 1997**

[54] **PRODUCTION OF ERYTHROPOIETIN**

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[*] Notice: The portion of the term of this patent subsequent to Aug. 20, 2013, has been disclaimed.

[21] Appl. No.: **468,556**

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

Related U.S. Application Data

[63] Continuation of Ser. No. 202,874, Feb. 28, 1994, abandoned, which is a continuation of Ser. No. 113,178, Oct. 23, 1987, abandoned, which is a continuation of Ser. No. 675,298, Nov. 30, 1984, Pat. No. 4,703,008, which is a continuation-in-part of Ser. No. 655,841, Sep. 28, 1984, abandoned, which is a continuation-in-part of Ser. No. 582,185, Feb. 21, 1984, abandoned, which is a continuation-in-part of Ser. No. 561,024, Dec. 13, 1983, abandoned.

[51] **Int. Cl.**[6] **C07K 14/505**; A61K 38/00

[52] **U.S. Cl.** **530/350**; 530/380; 530/399

[58] **Field of Search** 435/240.2, 172.3, 435/320.1, 69.4, 240.1, 69.1, 69.6; 536/23.5, 23.51; 530/350, 380, 399; 514/12

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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".

7 Claims, 27 Drawing Sheets

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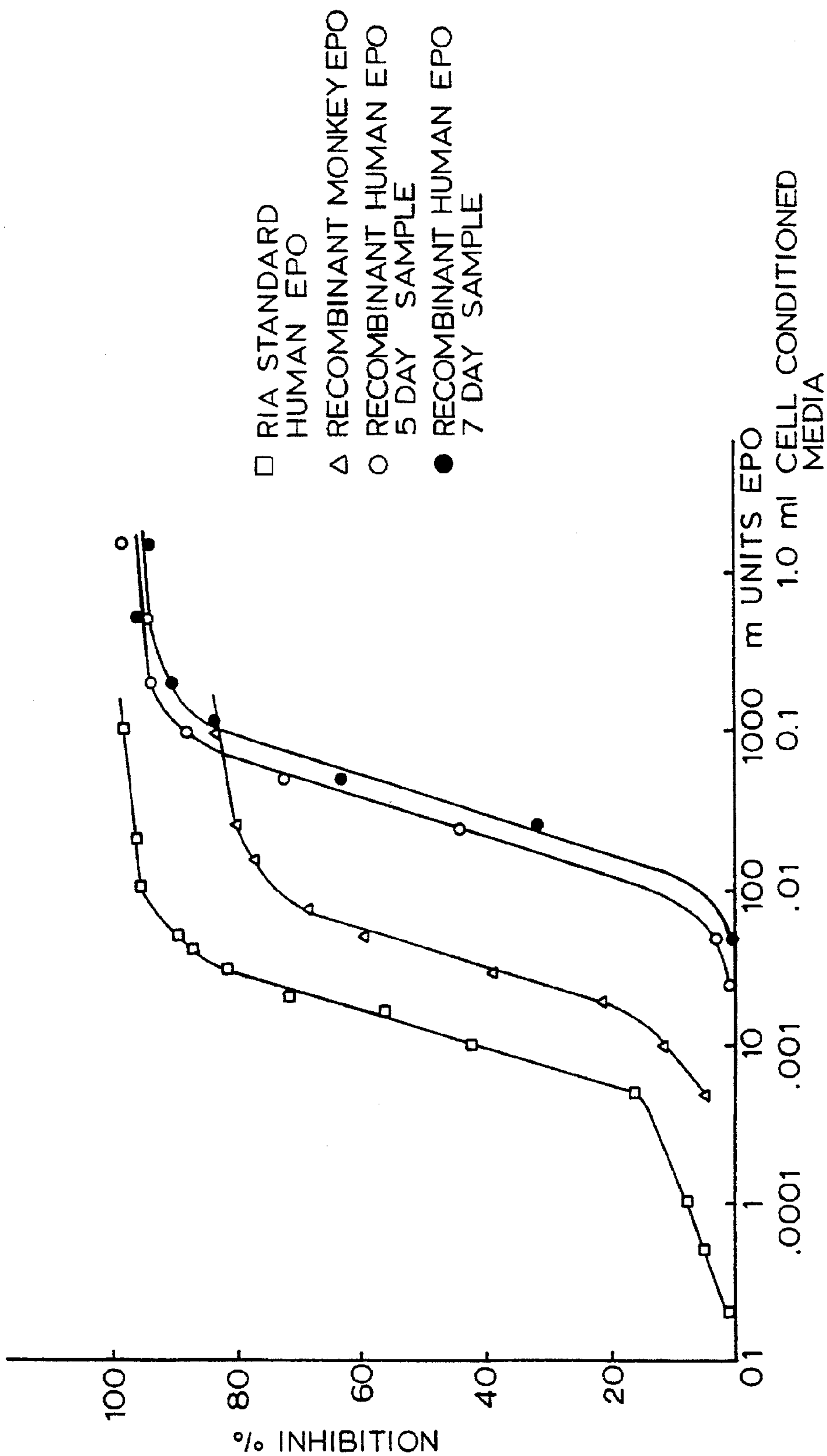
FIG. 1



□ RIA STANDARD HUMAN EPO
△ RECOMBINANT MONKEY EPO
○ RECOMBINANT HUMAN EPO 5 DAY SAMPLE
● RECOMBINANT HUMAN EPO 7 DAY SAMPLE
% INHIBITION
100
80
60
40
20
0
m UNITS EPO
1.0 ml CELL CONDITIONED MEDIA
1
.0001
10
.001
100
.01
1000
0.1

FIG. 2

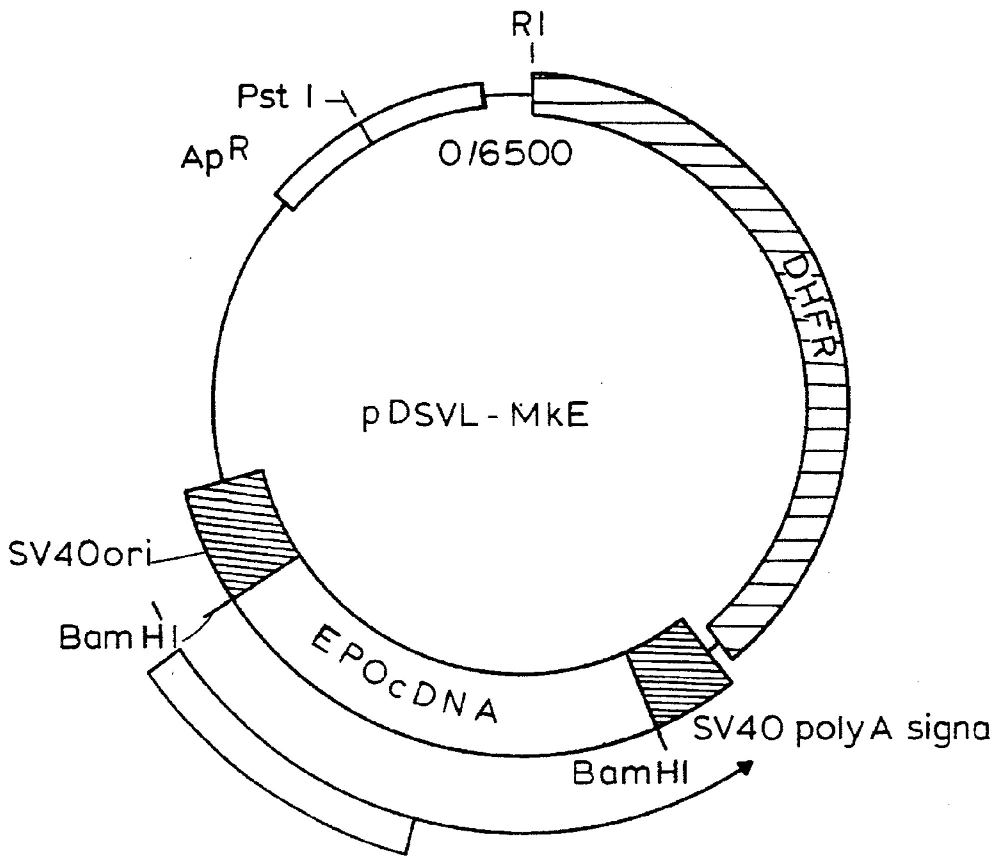

RI
Pst I
Ap^R
0/6500
DHFR
pDSVL-MkE
SV40ori
Bam HI
EPOcDNA
SV40 polyA signal
BamHI

FIG.3

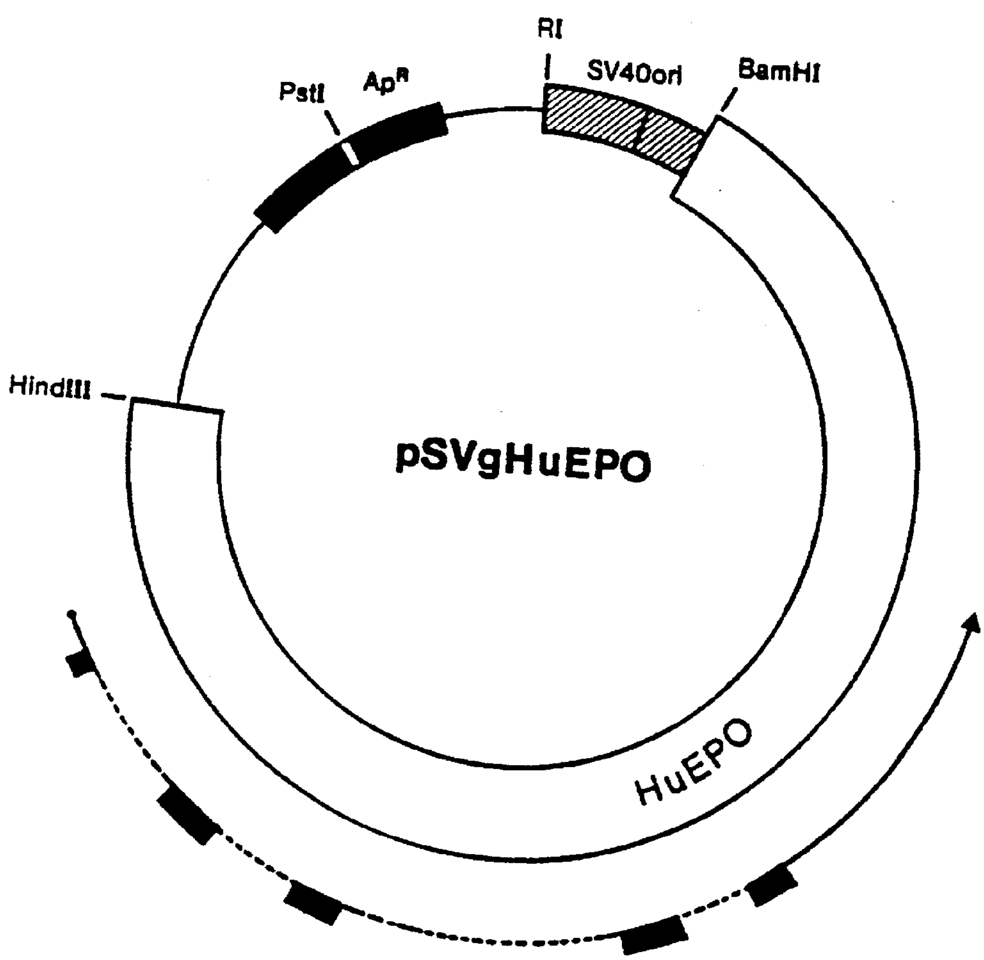


FIG. 4

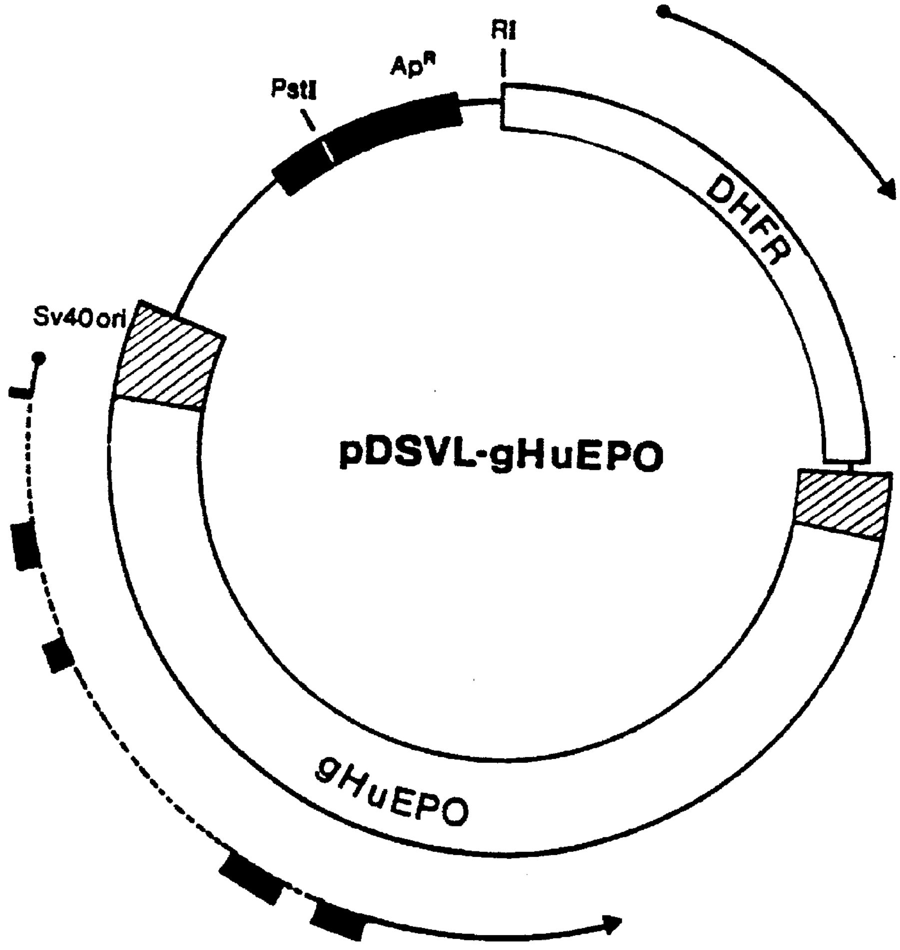


FIG. 5A

```
Sau3A
GATCCCGCGCCCCCTGGACAGCCGCCCTCTCCTCCAGGCCCGTGGGGCTGGCCCTGCCC

CGCTGAACTTCCCGGGATGAGGACTCCCGGTGTGGTCACCGCGCGCCTAGGTCGCTGAG

                     -27                           -20
                     Met Gly Val His Glu Cys Pro Ala Trp
GGACCCCGGCCAGGCGCGGAGATG GGG GTG CAC GAA TGT CCT GCC TGG

                                    -10
Leu Trp Leu Leu Leu Ser Leu Val Ser Leu Pro Leu Gly Leu Pro
CTG TGG CTT CTC CTG TCT CTC GTG TCG CTC CCT CTG GGC CTC CCA

         -1  +1                                 10
Val Pro Gly Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu
GTC CCG GGC GCC CCA CCA CGC CTC ATC TGT GAC AGC CGA GTC CTG

                             20                  *
Glu Arg Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn Val Thr Met
GAG AGG TAC CTC TTG GAG GCC AAG GAG GCC GAG AAT GTC ACG ATG

         30                                  *       40
Gly Cys Ser Glu Ser Cys Ser Leu Asn Glu Asn Ile Thr Val Pro
GGC TGT TCC GAA AGC TGC AGC TTG AAT GAG AAT ATC ACC GTC CCA
```

FIG.5B

```
                             50
Asp Thr Lys Val Asn Phe Tyr Ala Trp Lys Arg Met Glu Val Gly
GAC ACC AAA GTT AAC TTC TAT GCC TGG AAG AGG ATG GAG GTC GGG

         60                                     70
Gln Gln Ala Val Glu Val Trp Gln Gly Leu Ala Leu Leu Ser Glu
CAG CAG GCT GTA GAA GTC TGG CAG GGC CTG GCC CTG CTC TCA GAA

                             80          *
Ala Val Leu Arg Gly Gln Ala Val Leu Ala Asn Ser Ser Gln Pro
GCT GTC CTG CGG GGC CAG GCC GTG TTG GCC AAC TCT TCC CAG CCT

         90                                     100
Phe Glu Pro Leu Gln Leu His Met Asp Lys Ala Ile Ser Gly Leu
TTC GAG CCC CTG CAG CTG CAC ATG GAT AAA GCC ATC AGT GGC CTT

                             110
Arg Ser Ile Thr Thr Leu Leu Arg Ala Leu Gly Ala Gln Glu Ala
CGC AGC ATC ACC ACT CTG CTT CGG GCG CTG GGA GCC CAG GAA GCC

        120                                     130
Ile Ser Leu Pro Asp Ala Ala Ser Ala Ala Pro Leu Arg Thr Ile
ATC TCC CTC CCA GAT GCG GCC TCG GCT GCT CCA CTC CGA ACC ATC

                            140
Thr Ala Asp Thr Phe Cys Lys Leu Phe Arg Val Tyr Ser Asn Phe
ACT GCT GAC ACT TTC TGC AAA CTC TTC CGA GTC TAC TCC AAT TTC
```

FIG. 5C

```
        150                                     160
Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala Cys Arg Arg
CTC CGG GGA AAG CTG AAG CTG TAC ACG GGG GAG GCC TGC AGG AGA

        165
Gly Asp Arg OP
GGG GAC AGA TGA CCAGGTGCGTCCAGCTGGGCACATCCACCACCTCCCTCACCAACA

CTGCCTGTGCCACACCCTCCCTCACCACTCCCGAACCCCATCGAGGGGCTCTCAGCTAAG

CGCCAGCCTGTCCCATGGACACTCCAGTGCCAGCAATGACATCTCAGGGGCCAGAGGAAC

TGTCCAGAGCACAACTCTGAGATCTAAGGATGTCGCAGGGCCAACTTGAGGGCCCAGAGC

AGGAAGCATTCAGAGAGCAGCTTTAAACTCAGGAGCAGAGACAATGCAGGGAAAACACCT

GAGCTCACTCGGCCACCTGCAAAATTTGATGCAGGACACGCTTTGGAGGCAATTTACCTG

TTTTTGCACCTACCATCAGGGACAGGATGACTGGAGAACTTAGGTGGCAAGCTGTGACTT

CTCAAGGCCTCACGGGCACTCCCTTGGTGGCAAGAGCCCCCTTGACACTGAGAGAATATT

TTGCAATCTGCAGCAGGAAAAATTACGGACAGGTTTTGGAGGTTGGAGGGTACTTGACAG

GTGfGTGGGGAAGCAGGGCGGJAGGGGTGGAGCTGGGATGCGAGfGAGAACCGTGAAGAC

AGGATGGGGGCTGGCCTCTGGTTCTCGTGGGGTCCAAGCTT
                                     HindIII
```

FIG.6A

```
AAGCTTCTGGGCTTCCAGACCCAGCTACTTTGCGGAACTCAGCAACCCAGGCATCTCTGAGTCTCCGCCCA

AGACCGGGATGCCCCCCAGGGGAGGTGTCCGGGAGCCCAGCCTTTCCCAGATAGCACGCTCCGCCAGTCCC

AAGGGTGCGCAACCGGCTGCACTCCCCTCCCGCGACCCAGGGCCCGGGAGCAGCCCCCATGACCCACACGC

ACGTCTGCAGCAGCCCCGCTCACGCCCCGGCGAGCCTCAACCCAGGCGTCCTGCCCCTGCTCTGACCCCGG

GTGGCCCCTACCCCTGGCGACCCCTCACGCACACAGCCTCTCCCCCACCCCCACCCGCGCACGCACACATG

CAGATAACAGCCCCGACCCCCGGCCAGAGCCGXAGAGTCCCTGGGCCACCCCGGCCGCTCGCCTGCCGCTG

CGCCGCACCGCGCTGTCCTCCCGGAGCCGGACCGGGGCCACCGCGCCCXGCTCTGCTCCGACACCGCGCCC

CTTGGACAGCCGCCCTCTCCTCTAGGCCCGTGGGGCTGGCCCTGCACCGCCGAGCTTCCCGGGATGAGGXX

                                                   -27         -24
                                                   Met Gly Val His
CCCGGTGACCGGCGCGCCCCAAGTCGCTGAGGGACCCCGGCCAAGCGCGGAG ATG GGG GTG CAC G

GTGAGTACTCGCGGGCTGGGCGCTCCCGGCGGCCGGGTTCCTGTTTGAGCGGGGATTTAGCGCCCCGGCT
```

FIG.6B

```
ATTGGCCAAGAGGTGGCTGGGTTCAAGGACCGGCGACTTGTCAAGGACCCCGGAAGGGGGAGGGGGGTGGG

GCAGCCTCCACGTGCCGCGGGGACTTGGGGGAGTTCTTGGGGATGGCAAAAACCTGGCCTGTTGAGGGGCA

CAGTTTGGGGTTGGGGAGGAGGTTTGGGGTTCTGCTGTGCAGTTGTGTCGTTGTCAGTGTCTCG[I·S·]

TTGCACACGCACAGATCAATAAGCCAGAGGCAGCACCTGAGTGCTTGCATGGTTGGGACAGGAAGGACGAG

CTGGGGCAGAGACGTGGGGATGAAGGAAGCTGTCCTTCCACAGCCACCCTTCTCCCCCCCCGCCTGACTCT

                        -23         -20
                        Glu Cys Pro Ala Trp Leu Trp Leu Leu Leu Ser Leu
CAGCCTGGCTATCTGTTCTAG    AA TGT CCT GCC TGG CTG TGG CTT CTC CTG TCC CTG

    -10                                     -1  +1
Leu Ser Leu Pro Leu Gly Leu Pro Val Leu Gly Ala Pro Pro Arg Leu Ile Cys
CTG TCG CTC CCT CTG GGC CTC CCA GTC CTG GGC GCC CCA CCA CGC CTC ATC TGT

         10                                 20                  *
Asp Ser Arg Val Leu Glu Arg Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn Ile
GAC AGC CGA GTC CTG GAG AGG TAC CTC TTG GAG GCC AAG GAG GCC GAG AAT ATC

 26
Thr
ACG GTGAGACCCCTTCCCCAGCACATTCCACAGAACTCACGCTCAGGGCTTCAGGGAACTCCTCCCAGAT

CCAGGAACCTGGCACTTGGTTTGGGGTGGAGTTGGGAAGCTAGACACTGCCCCCCTACATAAGAATAAGTC
```