pDRIVE5s-hEndoglin

A plasmid with a native tissue specific human endoglin promoter

Catalog # pdrive5s-hendoglin

For research use only

Version # 09F17-MM

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT116 *E. coli* bacteria transformed by a pDRIVE5s plasmid.
- GT116 genotype is: *F-, mcrA, Δ(mrr-hsdRMS-mcrBC), Ø80lacZΔM15, ΔlacX74, recA1, endA1 Δdcm ΔsbcC-sbcD.*
- 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar)

Shipping and storage:

- Products are shipped at room temperature.
- Transformed bacteria should be stored at -20˚C. Bacteria are stable up to one year when properly stored.
- Store *E. coli* Fast-Media® Zeo at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Bacteria have been lyophilized, and their viability upon resuspension has been verified.

GENERAL PRODUCT USE

pDRIVE5s is an expression plasmid containing a native or composite promoter of interest. **pDRIVE5s** may be used to:

- Subclone a promoter of interest into another vector. Unique restriction sites are present at each end of the promoter allowing convenient excision. The 5' sites are *Sda* I, and *Spe* I. *Sda* I is compatible with *Nsi* I and *Pst* I. *Spe* I is compatible with *Avr* II, *Nhe* I and *Xba* I. The 3' restriction site is *Nco* I which includes the ATG start codon, and is compatible with *BspH* I and *BspLU11* I.

- Compare the activity of different promoters in transient transfection experiments. Each pDRIVE5s promoter drives the expression of the SEAP reporter gene which allows for testing of the promoter's activity in transient transfection experiments. Furthermore, the SEAP gene is flanked by unique restriction sites (*Nco* I and *Nhe* I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS

Human endoglin promoter

Complete Promoter size: 888 bp

Specificity: Endothelial cells

Endoglin (CD105) is a transforming growth factor-beta (TGF-β) coreceptor expressed mainly on endothelial cells and preferentially in the neovasculature associated with hypoxia such as ischemic tissues and tumors. The endoglin promoter lacks consensus TATA and CAAT boxes but contains Sp1, NF-κB and TGF-β binding sites[1]. These sites are located in a -400/+341 fragment upstream of the endoglin gene which is sufficient to confer endothelial specificity. Regulation of endoglin occurs at the transcriptional level; basal endoglin transcription is highly dependent on Sp1 and is stimulated by TGF-β and hypoxia[2]. The endoglin promoter has been used to target gene expression to the vasculature in transgenic animals[3].

1. Rius C. et al., 1998. Cloning of the promoter region of human endoglin, the target gene for hereditary hemorrhagic telangiectasia type 1. Blood. 92(12):4677-90. **2. Sanchez-Elsner T. et al., 2002.** Endoglin expression is regulated by transcriptional cooperation between the hypoxia and transforming growth factor-beta pathways. J Biol Chem. 277(46):43799-808. **3. Cowan PJ. et al., 2003.** Targeting gene expression to endothelium in transgenic animals: a comparison of the human ICAM-2, PECAM-1 and endoglin promoters. Xenotransplantation. 10(3):223-31.

PLASMID FEATURES

• **SEAP gene** encodes an engineered secreted embryonic alkaline phosphatase. The levels of SEAP in the culture medium of transfected cells expressing the reporter gene can be assayed with chromogenic or luminescent methods

• **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.

• **pMB1 Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.

• **EM2K** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli.*

• ***Zeo*** gene confers zeocin resistance therefore allowing the selection of transformed *E. coli* carrying a pDRIVE5s plasmid.

Note: *Stable transfection of clones cannot be performed due to the absence of an eukaryotic promoter upstream of the Sh ble gene.*

METHODS

Growth of pDRIVE5s-transformed bacteria:

Use sterile conditions to do the following:

1- Resuspend the lyophilized *E. coli* by adding 1 ml of LB medium in the tube containing the disk. Let sit for 5 minutes. Mix gently by inverting the tube several times.

2- Streak bacteria taken from this suspension on a zeocin LB agar plate prepared with the *E. coli* Fast-Media® Zeo agar provided (see below).

3- Place the plate in an incubator at 37˚C overnight.

4- Isolate a single colony and grow the bacteria in TB supplemented with zeocin using the Fast-Media® Zeo liquid provided (see below).

5- Extract the pDRIVE5s plasmid DNA using the method of your choice.

Selection of bacteria with *E. coli* Fast-Media Zeo:

E. coli Fast-Media® Zeo is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* Fast-Media® Zeo is a TB (liquid) or LB (solid) based medium with zeocin, and contains stabilizers.

E. coli Fast-Media® Zeo can be ordered separately (catalog code fas-zn-l, fas-zn-s).

Method:

1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.

2- Add 200 ml of distilled water to the flask

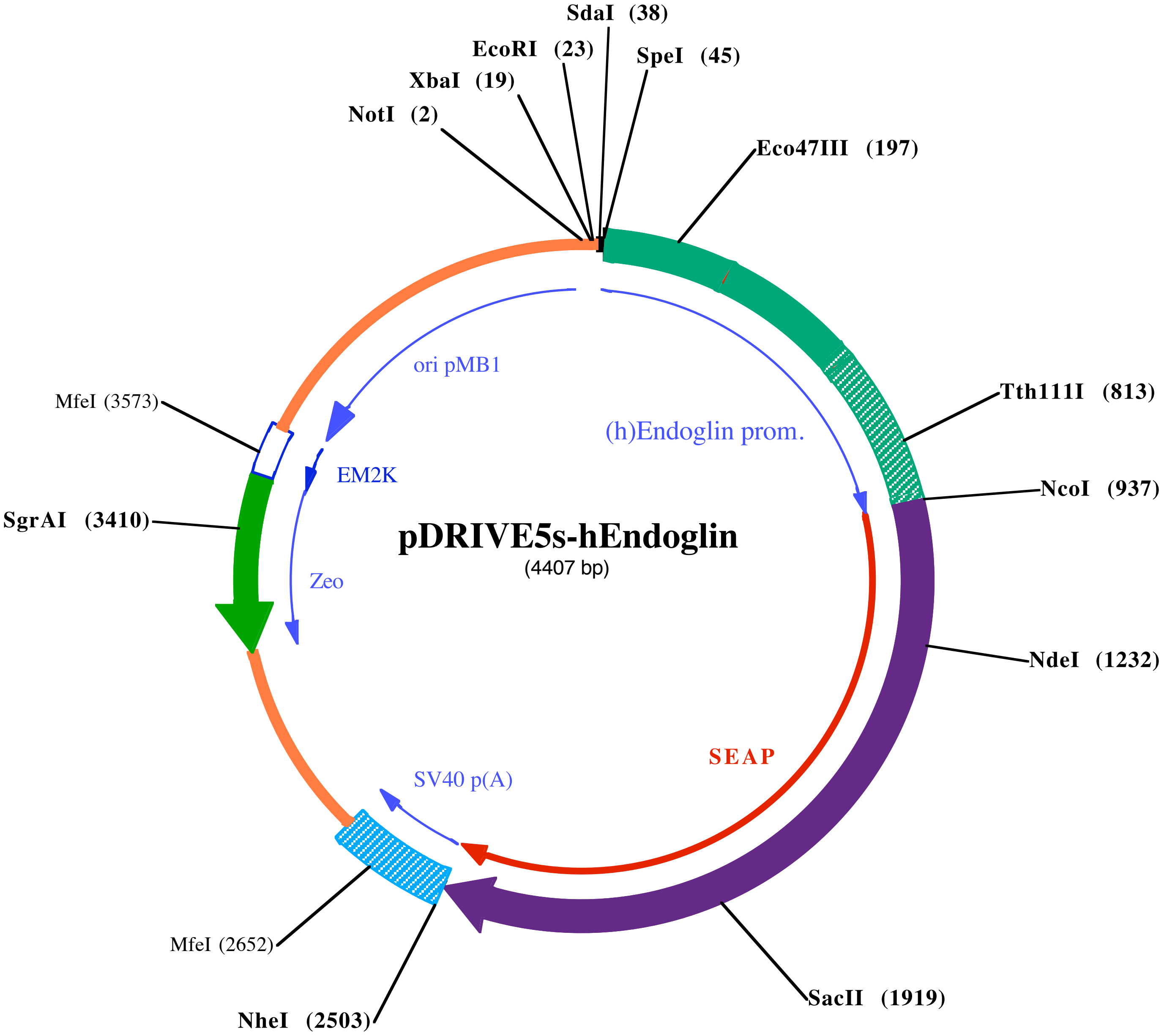
3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**

4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**

5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.

6- Let agar medium cool to 45˚C before pouring plates. Let liquid media cool to 37˚C before seeding bacteria.

Note: *Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.*


pDRIVE5s-hEndoglin
(4407 bp)
SdaI (38)
EcoRI (23)
SpeI (45)
XbaI (19)
NotI (2)
Eco47III (197)
Tth111I (813)
NcoI (937)
NdeI (1232)
SacII (1919)
NheI (2503)
MfeI (2652)
SgrAI (3410)
MfeI (3573)
ori pMB1
(h)Endoglin prom.
EM2K
Zeo
SEAP
SV40 p(A)

InvivoGen

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                           EcoRI  (23)
     NotI  (2)         XbaI  (19)    SdaI  (38)  SpeI  (45)
   1 GCGGCCGCGTCGACGATATCTAGAATTCGGATCCTGCAGGGCCCACTAGTCGCCTTGCTGTGCCACTTTGGGACTTCCCTCCCTAGCCTGAGCTTCAGTT

                                                                                                   Eco47III
 101 TTCCTGCCTGTTAGGCAGCCCCATGTCAACTGCACTTAGTAGGCCGGGTTTGATGCCCGACAAGACGTGAAGTGGTGGAGGTGGGCAGGATCCCAGCGCT

 201 ACCATCTTCTTGAACCAGTGATCTCAACACATCGGATTTCTGTTTCCTCATCTGCAAAATGGGATCAGTGAGCTCAGGTGGGTCACAAATTCTACAGGAA

 301 CTACTTTAGCCAAGCCCGGCCCCCTGAAAGTTCCCCTCGGTGGGCTGTTAGGGTGATTGTTTTCATCTGTGGGGCTCCCTGATGCGTCCCACCCACCAGC

 401 CTTGGAGAGGGTGGGATGGGAGGGTGGGGTGCTTGGGGAGACAAGCCTAGAGCCTGGGCCCTCCCACCCCACTGCCTCCCCCCATCCCAGGGCCCCCCAC

 501 CCAGTGACAAAGCCCGTGGCACTTCCTCTACCCGGTTGGCAGGCGGCCTGGCCCAGCCCCTTCTCTAAGGAAGCGCATTTCCTGCCTCCCTGGGCCGGCC

 601 GGGCTGGATGAGCCGGGAGCTCCCTGCTGCCGGTCATACCACAGCCTTCATCTGCGCCCTGGGGCCAGGACTGCTGCTGTCACTGCCATCCATTGGAGCC

 701 CAGCACCCCCTCCCCGCCCATCCTTCGGACAGCAACTCCAGCCCAGCCCCGCGTCCCTGTGTCCACTTCTCCTGACCCCTCGGCCGCCACCCCAGAAGGC

              Tth111I  (813)
 801 TGGAGCAGGGACGCCGTCGCTCCGGCCGCCTGCTCCCCTCGGGTCCCCGTGCGAGCCCACGCCGGCCCCGGTGCCCGCCCGCAGCCCTGCCACTGGACAC

                                         NcoI  (937)
 901 AGGATAAGGCCCAGCGCACAGGCCCCCACGTGGACAcCATGGTTCTGGGGCCCTGCATGCTGCTGCTGCTGCTGCTGCTGGGCCTGAGGCTACAGCTCTC
                                          1▸M  V  L  G  P  C  M  L  L  L  L  L  L  L  G  L  R  L  Q  L  S
1001 CCTGGGCATCATCCCAGTTGAGGAGGAGAACCCGGACTTCTGGAACCGCGAGGCAGCCGAGGCCCTGGGTGCCGCCAAGAAGCTGCAGCCTGCACAGACA
 21▸   L  G  I  I  P  V  E  E  E  N  P  D  F  W  N  R  E  A  A  E  A  L  G  A  A  K  K  L  Q  P  A  Q  T
1101 GCCGCCAAGAACCTCATCATCTTCCTGGGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCCTAAAAGGGCAGAAGAAGGACAAACTGGGGC
 55▸  A  A  K  N  L  I  I  F  L  G  D  G  M  G  V  S  T  V  T  A  A  R  I  L  K  G  Q  K  K  D  K  L  G
                                   NdeI  (1232)
1201 CTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCACGGC
 88▸ P  E  I  P  L  A  M  D  R  F  P  Y  V  A  L  S  K  T  Y  N  V  D  K  H  V  P  D  S  G  A  T  A  T  A
1301 CTACCTGTGCGGGGTCAAGGGCAACTTCCAGACCATTGGCTTGAGTGCAGCCGCCCGCTTTAACCAGTGCAACACGACACGCGGCAACGAGGTCATCTCC
121▸   Y  L  C  G  V  K  G  N  F  Q  T  I  G  L  S  A  A  A  R  F  N  Q  C  N  T  T  R  G  N  E  V  I  S
1401 GTGATGAATCGGGCCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACCACCACACGAGTGCAGCACGCCTCGCCAGCCGGCACCTACGCCCACACGGTGA
155▸  V  M  N  R  A  K  K  A  G  K  S  V  G  V  V  T  T  T  R  V  Q  H  A  S  P  A  G  T  Y  A  H  T  V
1501 ACCGCAACTGGTACTCGGACGCCGACGTGCCTGCCTCGGCCCGCCAGGAGGGGTGCCAGGACATCGCTACGCAGCTCATCTCCAACATGGACATTGATGT
188▸ N  R  N  W  Y  S  D  A  D  V  P  A  S  A  R  Q  E  G  C  Q  D  I  A  T  Q  L  I  S  N  M  D  I  D  V
1601 GATCCTGGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCCAGACCCTGAGTACCCAGATGACTACAGCCAAGGTGGGACCAGGCTGGACGGGAAG
221▸   I  L  G  G  G  R  K  Y  M  F  R  M  G  T  P  D  P  E  Y  P  D  D  Y  S  Q  G  G  T  R  L  D  G  K
1701 AATCTGGTGCAGGAATGGCTGGCGAAGCGCCAGGGTGCCCGGTATGTGTGGAACCGCACTGAGCTCATGCAGGCTTCCCTGGACCCGTCTGTGACCCATC
255▸  N  L  V  Q  E  W  L  A  K  R  Q  G  A  R  Y  V  W  N  R  T  E  L  M  Q  A  S  L  D  P  S  V  T  H
1801 TCATGGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCCT
288▸ L  M  G  L  F  E  P  G  D  M  K  Y  E  I  H  R  D  S  T  L  D  P  S  L  M  E  M  T  E  A  A  L  R  L
                    SacII  (1919)
1901 GCTGAGCAGGAACCCCCGCGGCTTCTTCCTCTTCGTGGAGGGTGGTCGCATCGACCACGGTCATCACGAAAGCAGGGCTTACCGGGCACTGACTGAGACG
321▸   L  S  R  N  P  R  G  F  F  L  F  V  E  G  G  R  I  D  H  G  H  H  E  S  R  A  Y  R  A  L  T  E  T
2001 ATCATGTTCGACGACGCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGGACACGCTGAGCCTCGTCACTGCCGACCACTCCCACGTCTTCTCCTTCG
355▸  I  M  F  D  D  A  I  E  R  A  G  Q  L  T  S  E  E  D  T  L  S  L  V  T  A  D  H  S  H  V  F  S  F
2101 GAGGCTACCCCCTGCGAGGGAGCTCCATCTTCGGGCTGGCCCCTGGCAAGGCCCGGGACAGGAAGGCCTACACGGTCCTCCTATACGGAAACGGTCCAGG
388▸ G  G  Y  P  L  R  G  S  S  I  F  G  L  A  P  G  K  A  R  D  R  K  A  Y  T  V  L  L  Y  G  N  G  P  G
2201 CTATGTGCTCAAGGACGGCGCCCGGCCGGATGTTACCGAGAGCGAGAGCGGGAGCCCCGAGTATCGGCAGCAGTCAGCAGTGCCCCTGGACGAAGAGACC
421▸   Y  V  L  K  D  G  A  R  P  D  V  T  E  S  E  S  G  S  P  E  Y  R  Q  Q  S  A  V  P  L  D  E  E  T
2301 CACGCAGGCGAGGACGTGGCGGTGTTCGCGCGCGGCCCGCAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGACCTTCATAGCGCACGTCATGGCCTTCG
455▸  H  A  G  E  D  V  A  V  F  A  R  G  P  Q  A  H  L  V  H  G  V  Q  E  Q  T  F  I  A  H  V  M  A  F
2401 CCGCCTGCCTGGAGCCCTACACCGCCTGCGACCTGGCGCCCCCCGCCGGCACCACCGACGCCGCGCACCCGGGGCGGTCCCGGTCCAAGCGTCTGGATTG
488▸ A  A  C  L  E  P  Y  T  A  C  D  L  A  P  P  A  G  T  T  D  A  A  H  P  G  R  S  R  S  K  R  L  D  •
       NheI  (2503)
2501 AAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGC
521▸

                                                        MfeI (2652)
2601 TATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTT

2701 TTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCC

2801 TTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTG
2901 TATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAA
3001 TACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAAC
3101 AAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGG
                                                       125◂ •  D  Q  E  E  A  V  F  H  V  C  N  G  A  P
3201 TCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACT
109◂ D  R  L  A  F  E  R  G  W  P  Q  E  G  I  E  T  M  A  P  G  S  A  D  R  F  N  T  S  V  V  E  S  W  E
3301 CGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTC
 76◂   A  Y  L  E  D  L  G  R  V  W  V  W  A  L  T  N  D  P  V  V  Q  D  Q  V  A  S  I  F  L  T  V  D  D
             SgrAI  (3410)
3401 CCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGC
 43◂  R  V  V  G  A  F  D  D  E  V  F  D  R  S  F  G  L  R  D  T  W  F  E  V  A  G  A  V  D  R  A  T  L
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                                                                                        MfeI (3573)
3501 ACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCCTGTCAGGAGAGGAAAGAGAAGAAGGTTAGTACAATTGCTATAGTGAGTTGTATTATACT
   9 V  P  V  A  S  T  L  K  A  M
3601 ATGCAGATATACTATGCCAATGATTAATTGTCAAACTAGGGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAA

3701 AGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATA

3801 AAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGC

3901 GTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACC

4001 GCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAG

4101 GTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACC

4201 TTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAG

4301 GATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATT

4401 TAAATCA
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