

pDRIVE5s-mPGK

A plasmid with the native ubiquitous murine phosphoglycerate kinase promoter

Catalog # pDRIVE5s-mpgk

For research use only

Version # 11D20-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*), Δ *080lacZ* Δ *M15*, Δ *lacX74*, *rspL* (*StrA*), *recA1*, *endA1* Δ *dem* Δ *sbcC-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' site is *Sda* I. *Sda* I is compatible with *Nsi* I and *Pst* I. The 3' restriction site is *Bsp*H I. *Bsp*H I is compatible with *Nco* I and *Bsp*LU11 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 (*Bsp* HI and *Nhe* I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS

Murine PGK-1 (gene : Phosphoglycerate Kinase) promoter

Complete Promoter size: 1440bp

Specificity: Ubiquitous

Pgk-1 is an X-linked gene encoding 3-phosphoglycerate kinase, an enzyme necessary in every cell for glycolysis. The promoter region of the pgk-1 gene is rich in G and C nucleotides and contains five copies of the hexadeoxynucleotide, GGGCGG, a potential binding site for the Sp1 transcription factor, a CCAAT sequence, but no TATA box¹. This promoter can efficiently drive high levels of expression of reporter genes (i.e. SEAP, LacZ and GFP) and therapeutic genes, such as tumor-associated antigens^{2, 3}. Furthermore, in contrast to the CMV promoter, the PGK promoter yields sustained expression².

1. Adra CN. et al. 1987. Cloning and expression of the mouse pgk-1 gene and the nucleotide sequence of its promoter. *Gene* 60(1):65-74.
2. Gerolami R. et al. 2000. Gene transfer to hepatocellular carcinoma: transduction efficacy and transgene expression kinetics by using retroviral and lentiviral vectors. *Cancer Gene Ther* 7(9):1286-92.
3. Lizee G. et al., 2004. Lentivirus vector-mediated expression of tumor-associated epitopes by human antigen presenting cells. *Hum Gene Ther*. 15(4):393-404.

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.

TECHNICAL SUPPORT

Toll free (US): 888-457-5873

Outside US: (+1) 858-457-5873

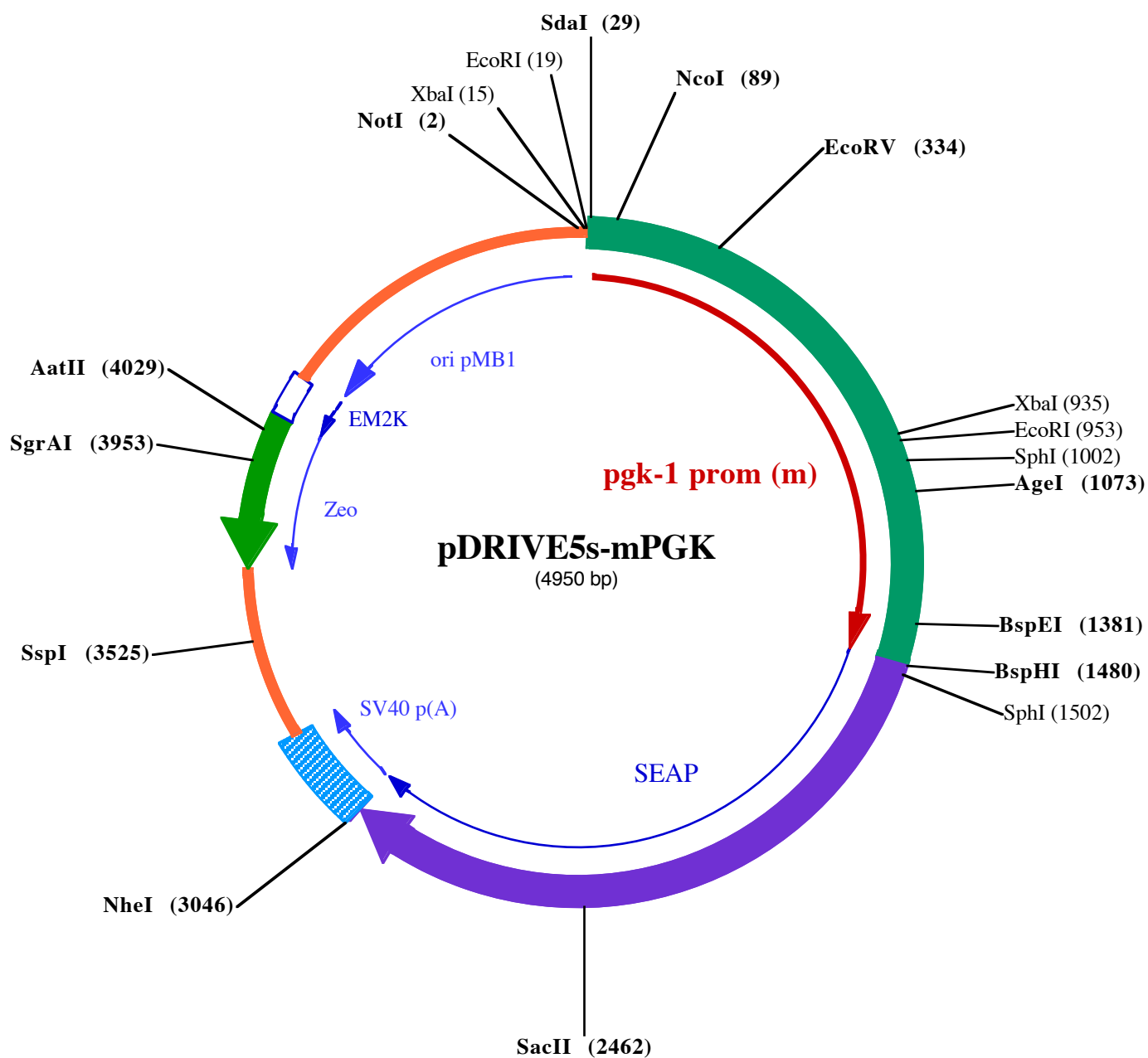
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EcoRI (19)
NotI (2) XbaI (15) SdaI (29) NcoI (89)
1 GCGGCCGCTATGCATCTAGAATTCCTGCAGGGCCACTAGGTCGGTTCATGTATCCGATTCAGACTGCACACTGCTATTGGATAACCATGGGGCTC
101 TCAGCATTCTCAGCTCTTTGTCTCTCCATGTCACGTGGCTCCTGTTACCCAGTTCCTTTTCCGTCCTCTGTTCTTAAAACTGTTTCCTTTCTCGG
202 ACCTGTCTCTCTCCATGTATATGCTTATATAAAAAGCTGCATAGGATAGAAAACACATGGTATTGTCTTTCTGAGTTACTTTCACATAATATAGTAAC
EcoRV (334)
302 TTCCAGAGGTGCTTTGGCCAGTCACTCTGGATATCTGCCAGTGAGAGAGGTGGAAAGAAAAACCAGGAGAGTGAACAAGGGCTTCCATTTCTCATGTG
401 CCCTTCAATTTCTAACTAGTTGCTCTCAGACCGTCAGGCAAGCACTTTACCCTGAGCACCGTCCTTAGCCCAAATGAGTGTCAGTAGAGATTTA
501 AAGTTTTGTTTTTTTTTAAACAGTCTGGA GAT T G G A T C C A C G G C C T C TGGCCCGCATTTTACCCTGAGCTACACTCCCAAAGCAGTCAAAAT
598 ACAGTGGCCAGGATTGAAATGATCACTTAGATGCTTTGACGTCTTGATAAGACACTAAATCTTTGTCTATCAGTTACTTTCATCTTTAATAACAGAAGCT
698 ACTTAGGAATTTTATGAGCATTGTTAGTTAGCATGACATGCTATATGTATTCGTCATTATGAATAATGTAACCACAGCAATTACATTGTACTTTTTAT
798 TATAAAAAGGGGGAGGGGAAGCCCTGTCCTTTTTTAACTTCTGAGAGTTCGATTACTAAGTAAGACCTTATGTAGACTCCATTTGGGAGCTGAGAAA
XbaI (935) EcoRI (953) SphI (1002)
899 GCAGAGGATCCAAAAGGGGATGACATTTGCAAAGGCTAGAAAAGGCGCTGGGAATTCTACCGGTAGGGGAGGGCCTTTTCCAAAGCAGTCTGGAGC
AgeI (1073)
1000 ATGCGCTTTAGCAGCCCCGCTGGCAGCTTGGCGCTACACAAGTGGCCTTGGCCTCGCACACATCCACATCCACCGGTAGCGCCAACCGGCTCCGTTCC
1100 TTTGGTGGCCCTTCGCGCCACTTCTACTCTCCCTAGTCAGGAAGTTC C C C C C G C C C G C A G C T C G C G T C G T G C A G G A C G T G A C A A A T G G A A G T A
1199 GCACGTCTCACTAGTCTCGTGACATGGACAGCACCGCTGAGCAATGGAAGCGGTAGGCCTTTGGGCGAGCGCCAAATAGCAGCTTTGCTCCTTCGCTT
BspEI (1381)
1299 TCTGGGCTCAGAGGCTGGGAAGGGTGGTCCGGGGGGGCTCAGGGGGGCTCAGGGGGGGGCGGGGGGGCCCGAAGGCTCCCGGAGGCCCGGCATT
BspHI (1480) SphI (1502)
1399 CTGCACGCTTCAAAGCGCACGTCTGTCGCGCTGTTCTCTCTCTCATCTCCGGGCTTTGACCTCACGGTGTGCGCATCATGATTCTGGGGCCCTG
1499 CATGCTGCTGCTGCTGCTGCTGGGCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGAACCCTGGAACCGCGAGGCGAG
6 M L L L L L L L L L L G L R L Q L S L G I I P V E E E N P D F W N R E A
1600 CCGAGGCCCTGGGTGCCGCAAGAAGCTGCAGCTGCACAGACAGCCGCAAGAACCCTCATCATCTCTGGGCGATGGGATGGGGGTCTACGGTGACA
40 A E A L G A A K K L Q P A Q T A A K N L I I F L G D G M G V S T V T
1701 GCTGCCAGGATCCTAAAAGGGCAGAAGAAGGACAACTGGGCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGGCTCTGTCAAAGACATACAA
74 A A R I L K G Q K K D K L G P E I P L A M D R F P Y V A L S K T Y N
1802 TGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCAGCCCTACCTGTGCGGGGTCAAGGGCAACTCCAGACATTGGCTTGAGTGACGCCCGCCGCT
107 V D K H V P D S G A T A T A Y L C G V K G N F Q T I G L S A A A R
1903 TTAACAGTGAACACACACGCGGCAACGAGGTCTCCGTGATGAATCGGGCAAGAAGCAGGGAAGTCAGTGGGAGTGGTAACCACCACACGAGTG
141 F N Q C N T T R G N E V I S V M N R A K K A G K S V G V V T T T R V
2004 CAGCAGCCCTCGCCAGCCGACCTACGCCACACCGGTGAACCGCAACTGGTACTGGACGCCGACGTGCCTGCCTCGGCCCGCAGGAGGGGTGCCAGGA
175 Q H A S P A G T Y A H T V N R N W Y S D A D V P A S A R Q E G C Q D
2105 CATCGCTACGAGCTCATCTCAAACATGGACATTGATGTATCTGGTGGAGCCGAAAGTACATGTTTCGCATGGGAACCCAGACCTGAGTACCCAG
208 I A T Q L I S N M D I D V I L G G G R K Y M F R M G T P D P E Y P
2206 ATGACTACAGCAAGGTGGACCGGCTGGACGGGAAGAACTGGTGGAGGATGGCTGGCAGGCGCCAGGTCGCCGGTATGTGTCCAGCCAGCTGAG
242 D D Y S Q G G T R L D G K N L V Q E W L A K R Q G A R Y V W N R T E
2307 CTATGAGGCTTCCCTGGACCGCTGTGACCCATCTCATGGTCTCTTGGAGCTGGAGACATGAAATACGAGATCCACCGAGACTCCACTGGACCC
276 L M Q A S L D P S V T H L M G L F E P G D M K Y E I H R D S T L D P
SacII (2462)
2408 CTCCTGATGGAGATGACAGAGGCTGCCCTGCGCTGCTGAGCAGGAACCCCGCGGCTTCTCTCTCTCGTGGAGGGTGGTGCATCGACACCGGTGCATC
309 S L M E M T E A A L R L L S R N P R G F F L F V E G R I D H G H
2509 ACGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTTCGAGCAGCCATTGAGAGGGGGGGCCAGCTCACCGAGGAGGACACGCTGAGCCCTC
343 H E S R A Y R A L T E T I M F D D A I E R A G Q L T S E E D T L S L
2610 GTCAGTCGCGACCTCCACGCTCTCTCTCGGAGGCTACCCCTGCGAGGGAGCTCCATCTTCCGGGCTGGCCCTGGCAAGGCCCGGACAGGAAGGC
377 V T A D H S H V F S F G Y P L R G S S I F G L A P G K A R D R K A
2711 CTACAGGTCCTCTATACGGAACCGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGCGGATGTTACCGAGAGCGAGAGCGGGAGCCCGAGTATCGGC
410 Y T V L L Y G N G P G Y V L K D G A R P D V T E S E S G S P E Y R
2812 AGCAGTCAGCAGTCCCTGGACGAGAGACCACGAGGAGGACGCTGGTGGAGGCTGTCGCGCGCGGCCAGGCGCACCTGTTTACGCGGCGCAGGAG
444 Q Q S A V P L D E E T H A G E D V A V F A R G P Q A H L V H G V Q E
2913 CAGACCTTATAGCGCACGTGATGCCCTTCGCGCCTGCTGGAGCCCTACACCGCTGCGACCTGGCGCCCGCCCGGACACCCAGCAGCCGCGCACCC
478 Q T F I A H V M A F A A C L E P Y T A C D L A P P A G T T D A A H P
NheI (3046)
3014 GGGGCGGTCCCGTCCAAGCGTCTGGATTGAAGCTAGCTGGCCAGACATGATAAGATAATTGATGAGTTTGACAAACCACAAC TAGAATGCAGTGA
511 G R S R S K R L D
3115 AAATGCTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTAAGAAGTCAATAAACAAGTTAACAACAACAATTGCATTTATTTATGTT
TCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAATTAATTTCAAATACAGCATAGCAAACCTTTAAC
3317 CTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCAATGTGCATTAGCTGTTTGCAGCCTCACCT
3418 TCTTTTCATGGAGTTAAGATATAGTGTATTTTCCAAAGGTTTGAAGTCTTCTATTCTTTATGTTTAAATGCAGTACCTCCACATTCCTTTTAA
SspI (3525)
3519 GTAAATATTTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCA
3620 GTTTAGTAGTTGACTTAGGGAACAAAGGAACCTTAATAGAAATTGGACAGCAAGAAGCGAGCTTCTAGCTTATCCCTCAGTCTGCTCCTCTGCCACAA
1254 D Q E E A V F
3721 AGTGACCGCAGTTGCGCGCCGGTGCAGGCGGAACCTCCCGCCCCACGGCTGCTCGCCGATCTCGGTGATGGCGGCGCCGAGGCGTCCCGGAAGTTC
1174 H V C N G A P 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
3822 GTGGACAGCAGCTCCGACCACTCGGCTACAGCTGCTCAGGGCCGCGCACCCACCCAGGCCAGGGTGTGTCGCGCACCACTGGTCTGAGCCCGCT
834 T S V V E S W E 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
SgrAI (3953)
3923 GATGAACAGGGTACGCTGCTCCGGACACACCGGGCAAGTCTGCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCCGAGAACTCGACCGCTCCGG
504 I F L T V D D 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

AatII (4029)

4024 CGACGTCGCGCGGGTGAGCACGGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCCTGTCAGGAGAGAAAGAGAAGAAGGTTAGTACAATTGCTA
16 V D R A T L V P V A S T L K A M
4125 TAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAAGCTAGGGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAA
4226 AGGCCAGGAACCGTAAAAAGGCCGCTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGA
4327 AACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCCTCCTGTTCCGACCTGCCGTTACCGGATACCTGTCCGCCTT
4428 TCTCCCTTCGGGAAGCGTGGCGCTTTCATAGCTCACGCTGTAGGTATCTCAGTTCGGGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCC
4529 CCGTTCAGCCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGG
4630 ATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCCTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCT
4731 GAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGCAACAAACCACCGCTGGTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACGC
4832 GCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGT
4933 TAATTAACATTTAAATCA