Product usage

Before using this product, please read the Limited Use statement below

Important Limited Use information for pTiGer2-eGFP

The purchase of the pTiGer2-eGFP vector conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes.

Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic, or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research.

If the purchaser is unwilling to accept the limitations of this limited use statement, InvivoGen is willing to accept return of the product with a full refund. The product must be returned in resaleable condition. For information on purchasing a license to this product for purposes other than research, contact us at outlicensing@invivogen.com.

pTiGer2-eGFP

A multigenic plasmid for inducible enhanced green fluorescent protein expression, selectable with Zeocin®

Catalog code: ptg2-gfp

https://www.invivogen.com/tet-on-ptiger-reporter

For research use only

Version 24A16-NJ

PRODUCT INFORMATION

Contents:

- **20 µg of pTiGer2-eGFP** provided as lyophilized DNA
- **1 ml of Zeocin®** (100 mg/ml)

Storage and Stability:

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20 °C.
- Resuspended DNA should be stored at -20 °C and is stable for up to 1 year.
- Store Zeocin® at 4 °C or at -20 °C. The expiry date is specified on the product label.

Quality control

- Plasmid construct is confirmed by restriction analysis and full-length open reading frame (ORF) sequencing.
- After purification by ion exchange chromatography, predominant supercoiled conformation is verified by electrophoresis.

PRODUCT DESCRIPTION

InvivoGen provides a family of plasmids featuring a tetracycline-inducible reporter gene. The **pTiGer2-eGFP** plasmid encodes the enhanced green fluorescent protein (eGFP) and the Zeocin® resistance marker for selection in both mammalian cells and bacteria. This plasmid can be used as a transfection control for plasmids of the pTiGer-mcs family.

The eGFP expression is only possible upon transfection of cells featuring the tetracycline repressor (TetR) protein[1], such as InvivoGen's HEK-RepTor™ or A549-RepTor™ cells. These cells express TetR constitutively in the nucleus, where it binds to tetracycline operator (tetO) sequences and represses gene transcription. Upon incubation with doxycycline (a synthetic tetracycline derivative), TetR is released from the tetO sequences and the eGFP-encoding gene is transcribed.

pTiGer-SEAP and pTiGer-Lucia plasmids are also available.

PLASMID FEATURES

SEAP expression cassette

- **hCMV enh/ hEF1 prom:** This composite promoter combines the human cytomegalovirus (HCMV) enhancer and the core promoter of the human elongation factor-1α (EF-1α)[2].
- **tetOtetO:** This sequence is also known as TRE (Tetracycline Response Element). It is a repeat of the 19-nucleotide sequence of the tetracycline operator (tetO)[1].
- **eGFP (enhanced green fluorescent protein)** is a synthetic variant of the wild-type GFP. It features mutations that allow for better folding efficiency at 37°C and higher-intensity emission, compared to GFP. Moreover its codon sequence is optimized for expression in mammalian cells. The eGFP excitation peak is at ~ 490 nm, and its emission peak at ~ 509 nm.
- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[3].

Zeocin® antibiotic selection cassette

- **pMB1 Ori:** This minimal *E. coli* origin of replication with the same activity as the longer Ori.
- **hAldA enh/ hFerH prom:** This composite promoter combines the human aldehyde dehydrogenase (aldA) enhancer and the core promoter of the human ferritin heavy chain gene (FerH).
- **EM7**: This bacterial promoter enables the constitutive expression of the *Sh ble* gene in *E. coli*.
- ***Sh ble*:** The resistance to Zeocin® is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The *Sh ble* gene is driven by the human AldA/FerH promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.
- **mEF1 5'UTR:** The 5'UTR (untranslated region) of the murine EF-1α enhances *Sh ble*-encoding mRNA stability and protein translation.
- **hβGlo pAn:** The human β-Globin pAn is a strong polyadenylation signal placed downstream of *Sh ble*[4].

1. Hillen, W., Wissmann, A. (1989). Tet repressor-tet operator interaction. Protein-Nucleic Acid Interaction. DOI: 10.1007/978-1-349-09871-2_7. 2. Kim DW. *et al.*, 1990. Use of the human elongation factor 1α promoter as a versatile and efficient expression system. Gene 91(2):217-23. 3. Carswell S. & Alwine JC., 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site:effects of upstream sequences. Mol Cell Biol. 9(10):4248-58. 4. Yu J. & Russell JE., 2001. Structural and functional analysis of an mRNP complex that mediates the highstability of human β-globin mRNA. Mol Cell Biol. 21(17):5879-88.

METHODS

- **Plasmid resuspension**

- Quickly spin the tube to pellet the DNA.
- To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile water.
- Store the resuspended plasmid at -20°C.

- **Plasmid amplification and cloning**

Plasmid amplification and cloning can be performed in *E. coli* GT115 or other commonly used laboratory *E. coli* strains, such as DH5α.

- **Zeocin® usage**

Zeocin® can be used at 25 µg/ml in *E. coli* in liquid or solid media and at 50-200 µg/ml to select Zeocin®-resistant mammalian cells.

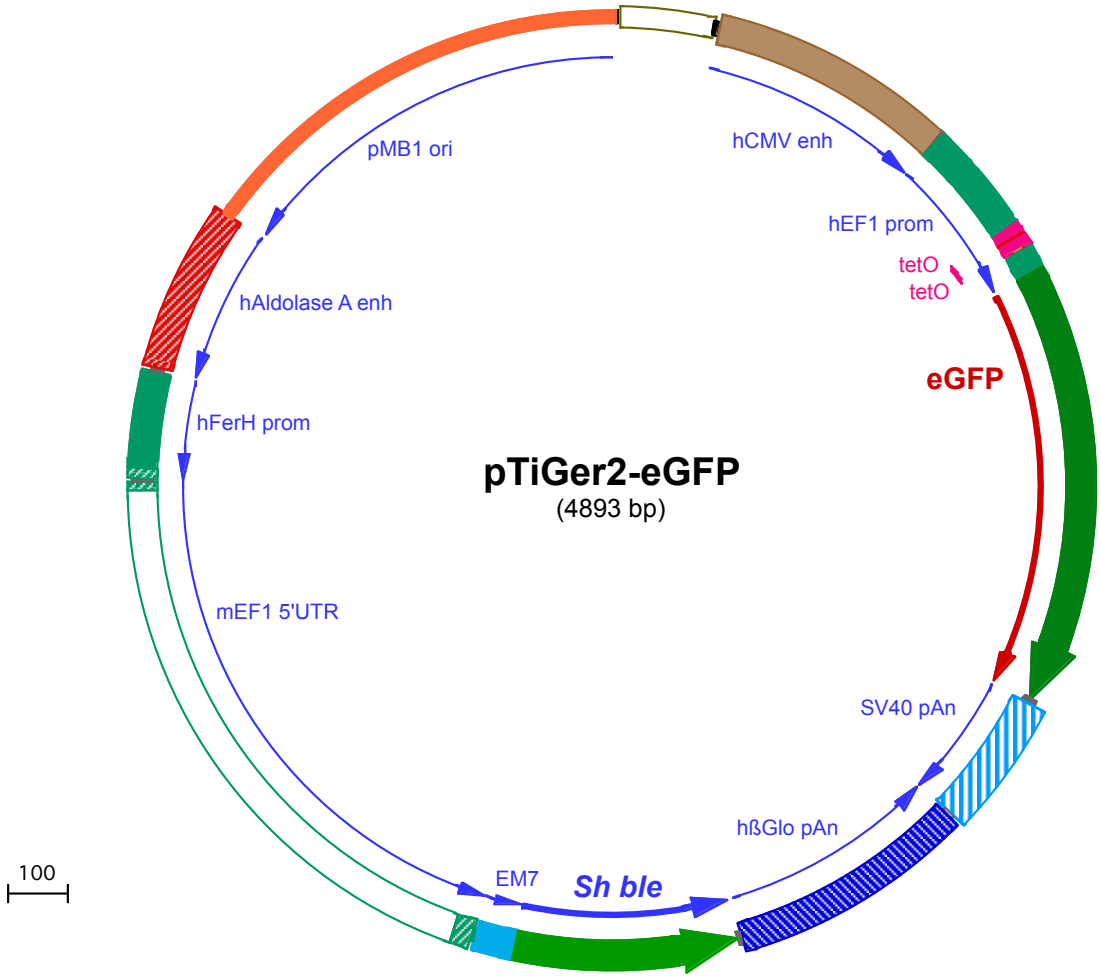
- **Generation of Tet-inducible expression cells**

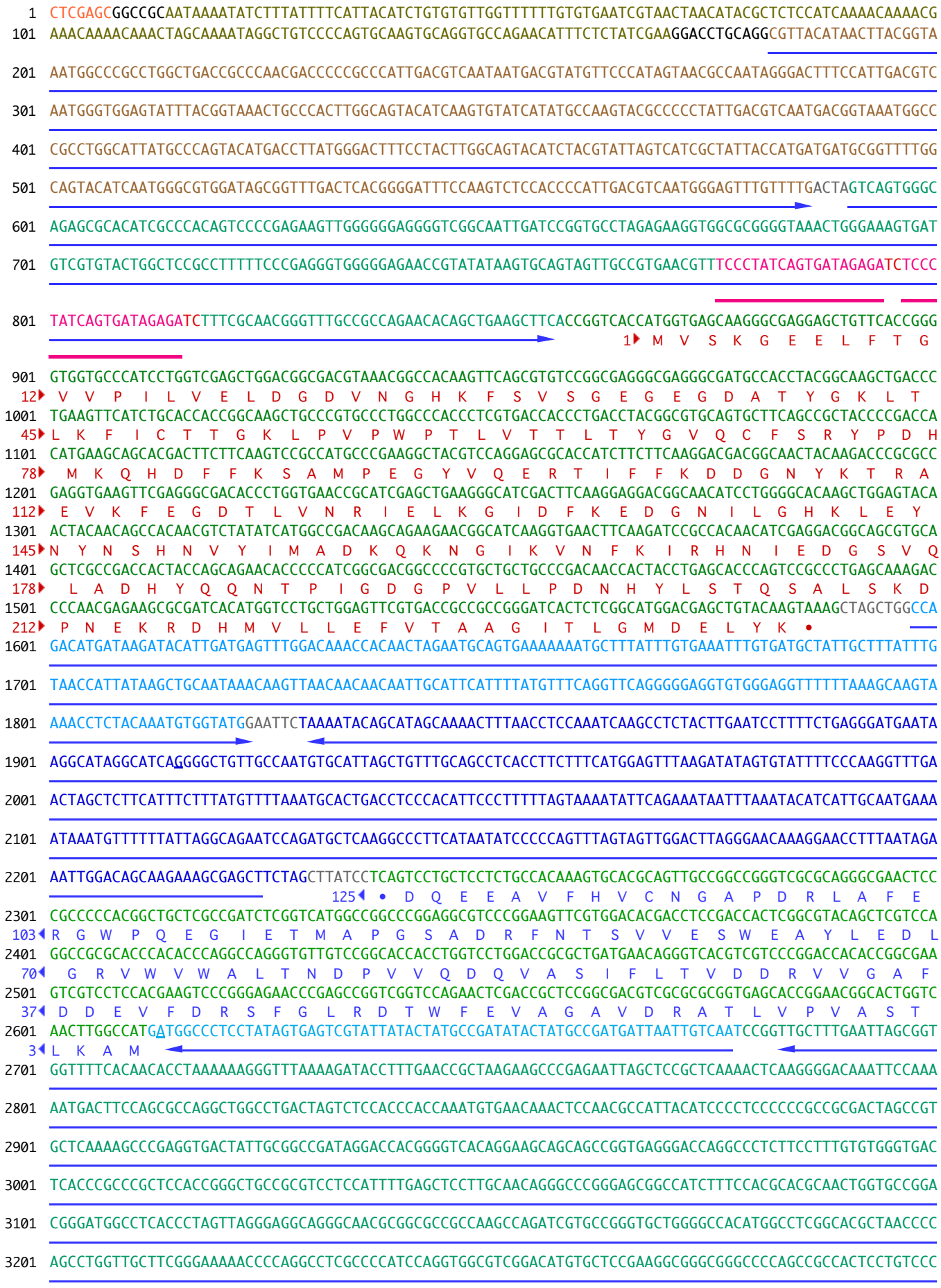
For a general procedure using InvivoGen's RepTor™ cell lines, please visit: https://www.invivogen.com/reptor-cells.

RELATED PRODUCTS

Product	Description	Cat. Code
Zeocin®	Selection antibiotic	ant-zn-1
HEK-RepTor™ cells	TetR-expressing cells	hk-rtor

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com


pTiGer2-eGFP
(4893 bp)
pMB1 ori
hCMV enh
hEF1 prom
tetO
tetO
eGFP
hAldolase A enh
hFerH prom
mEF1 5'UTR
SV40 pAn
hßGlo pAn
EM7
Sh ble
100


1 CTCGAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACG
101 AAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGACCTGCAGGCGTTACATAACTTACGGTA
201 AATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC
301 AATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC
401 CGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTTGG
501 CAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGACTAGTCAGTGGGC
601 AGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGATCCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGAT
701 GTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTTGCCGTGAACGTTTCCCTATCAGTGATAGAGATCTCCC
801 TATCAGTGATAGAGATCTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCACCGGTCACCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGG
1 M V S K G E E L F T G
901 GTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCC
12 V V P I L V E L D G D V N G H K F S V S G E G E G D A T Y G K L T
1001 TGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCA
45 L K F I C T T G K L P V P W P T L V T T L T Y G V Q C F S R Y P D H
1101 CATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCC
78 M K Q H D F F K S A M P E G Y V Q E R T I F F K D D G N Y K T R A
1201 GAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACA
112 E V K F E G D T L V N R I E L K G I D F K E D G N I L G H K L E Y
1301 ACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCA
145 N Y N S H N V Y I M A D K Q K N G I K V N F K I R H N I E D G S V Q
1401 GCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGAC
178 L A D H Y Q Q N T P I G D G P V L L P D N H Y L S T Q S A L S K D
1501 CCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAAGCTAGCTGGCCA
212 P N E K R D H M V L L E F V T A A G I T L G M D E L Y K •
1601 GACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTG
1701 TAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTA
1801 AAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATA
1901 AGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGA
2001 ACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAA
2101 ATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGA
2201 AATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCC
125 • D Q E E A V F H V C N G A P D R L A F E
2301 CGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCA
103 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 A Y L E D L
2401 GGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAA
70 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 R V V G A F
2501 GTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTC
37 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 V P V A S T
2601 AACTTGGCCATGATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAATCCGGTTGCTTTGAATTAGCGGT
3 L K A M
2701 GGTTTTCACAACACCTAAAAAAGGGTTTAAAAGATACCTTTGAACCGCTAAGAAGCCCGAGAATTAGCTCCGCTCAAAACTCAAGGGGACAAATTCCAAA
2801 AATGACTTCCAGCGCCAGGCTGGCCTGACTAGTCTCCACCCACCAAATGTGAACAAACTCCAACGCCATTACATCCCCTCCCCCCGCCGCGACTAGCCGT
2901 GCTCAAAAGCCCGAGGTGACTATTGCGGCCGATAGGACCACGGGGTCACAGGAAGCAGCAGCCGGTGAGGGACCAGGCCCTCTTCCTTTGTGTGGGTGAC
3001 TCACCCGCCCGCTCCACCGGGCTGCCGCGTCCTCCATTTTGAGCTCCTTGCAACAGGGCCCGGGAGCGGCCATCTTTCCACGCACGCAACTGGTGCCGGA
3101 CGGGATGGCCTCACCCTAGTTAGGGAGGCAGGGCAACGCGGCGCCGCCAAGCCAGATCGTGCCGGGTGCTGGGGCCACATGGCCTCGGCACGCTAACCCC
3201 AGCCTGGTTGCTTCGGGAAAAACCCCAGGCCTCGCCCCATCCAGGTGGCGTCGGACATGTGCTCCGAAGGCGGGCGGGCCCCAGCCGCCACTCCTGTCCC


3301 TCCATTCCTCCCCAACCATGACCTCTCCGGGCTCCGGGCGAGCAAGCCCCGCACCCTCCCTTTGTTAGCCCCTATTGCTGAACGGCAATCGAAGGCAGCA
3401 GGGCAACAACAACAAAAAAAAAAAAAGACCAGAGTGCGGCCGGAGTAGCACGCGGCGGCGGCGCGGACACCACGCTAGGCCTCAAGCCGGACACGAGGCGA
3501 GGCTACGGGGTTGCCGCTAGGCCTCGCACTCTGCCTCCCGCGCCGCCCGCAACTCGAAGCGGGAATGCTCGCAGCTAATCCCCGCCGACGACAGCGGGGC
3601 CCGGCCCGCTCGGAGCAGGACCTCCAGCTCGGCGGCCCGCGGAAGCCACACCCGCCCCTCACCTGCGTTCTGACGGCAAGCTTCGGCGAAGAACGTCTGG
3701 CCCTGCGGGTCGCTTGTGGTCTCTTATAGCCGCGTCGGCGTCAGGCCCGCCCCGGCCAATCAGCGCCGCCCGCCCCGAGCCCGCTCCTTCCGGTGGGCGC
3801 GGGACCCCGCCCCTGCTGTGGGGGAGGGGCGGCCGCTGGAGGCCCTCGCGCGCTCTGGCGGAACTAGTCGACGGACTGGGCTACGGGCCGCCCCCGAGAG
3901 GCGCAGCCCAGAGGCCGCGCTAGGAAGGGGCGGGCGCCGAGAACACGATCCCTCCCCACCCCCCTCGGACGTGACTCGGACCACATCCCGCGGGTCGCTA
4001 GGGCCCTCCCTTCTGCTCCTTTCCCCCAGCCTGGCGCGCTCTGGGGCGCCGTGACTCAGCCAGAATGTTGGCAATGGGGGAGGGCGGAACGGGGAAGTGG
4101 AGGACGCGGATGGAAAAGTCGGAAACGAAGGAAGCTGAGTTTCGCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGT
4201 AAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGAC
4301 TATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGG
4401 AAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCC
4501 GACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAG
4601 CGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGT
4701 TACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAA
4801 AAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGT