

Product usage

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

Important Limited Use information for pTiGer3-mcs

The purchase of the pTiGer3-mcs 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.

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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.

TECHNICAL SUPPORT

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pTiGer3-mcs

A multigenic cloning plasmid for inducible expression, selectable with Hygromycin

Catalog code: ptg3-mcs

<https://www.invivogen.com/tet-on-ptiger-mcs>

For research use only

Version 24A15-NJ

PRODUCT INFORMATION

Contents:

- 20 µg of pTiGer3-mcs provided as lyophilized DNA
- 1 ml of Hygromycin (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 Hygromycin 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 multiple cloning site for the tetracycline-inducible expression of a gene of interest (GOI). The pTiGer3-mcs plasmid contains the Hygromycin resistance marker for selection in both mammalian cells and bacteria.

The expression of the GOI is only possible upon transfection of cells featuring the tetracycline repressor (TetR) protein¹, 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 GOI is transcribed.

pTiGer2-mcs and pTiGer4-mcs plasmids, selectable using Zeocin® and Puromycin, respectively, are also available.

PLASMID FEATURES

Mutli-cloning site 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.
- **MCS:** The multiple cloning site comprises the following restriction sites: AgeI, BstEII, NcoI, BamHI, Acc65I, XbaI, NsiI, EcoRV, and NheI.
- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.

Hygromycin 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 *hph* gene in *E. coli*.
- **hph:** The resistance to Hygromycin is conferred by the *hph* gene. The *hph* 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 *hph*-encoding mRNA stability and protein translation.
- **hβGlo pAn:** The human β-Globin pAn is a strong polyadenylation signal placed downstream of *hph*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 high stability 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.

• **Hygromycin usage**

Hygromycin can be used at 50-100 µg/ml in *E. coli* in liquid or solid media and at 50-500 µg/ml to select Hygromycin-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/tet-on-cell-lines>.

RELATED PRODUCTS

Product	Description	Cat. Code
Hygromycin	Selection antibiotic	ant-hg-1
ChemiComp GT115	Competent <i>E. coli</i>	gt115-1
HEK-RepTor™ cells	TetR-expressing cells	hk-rtor

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

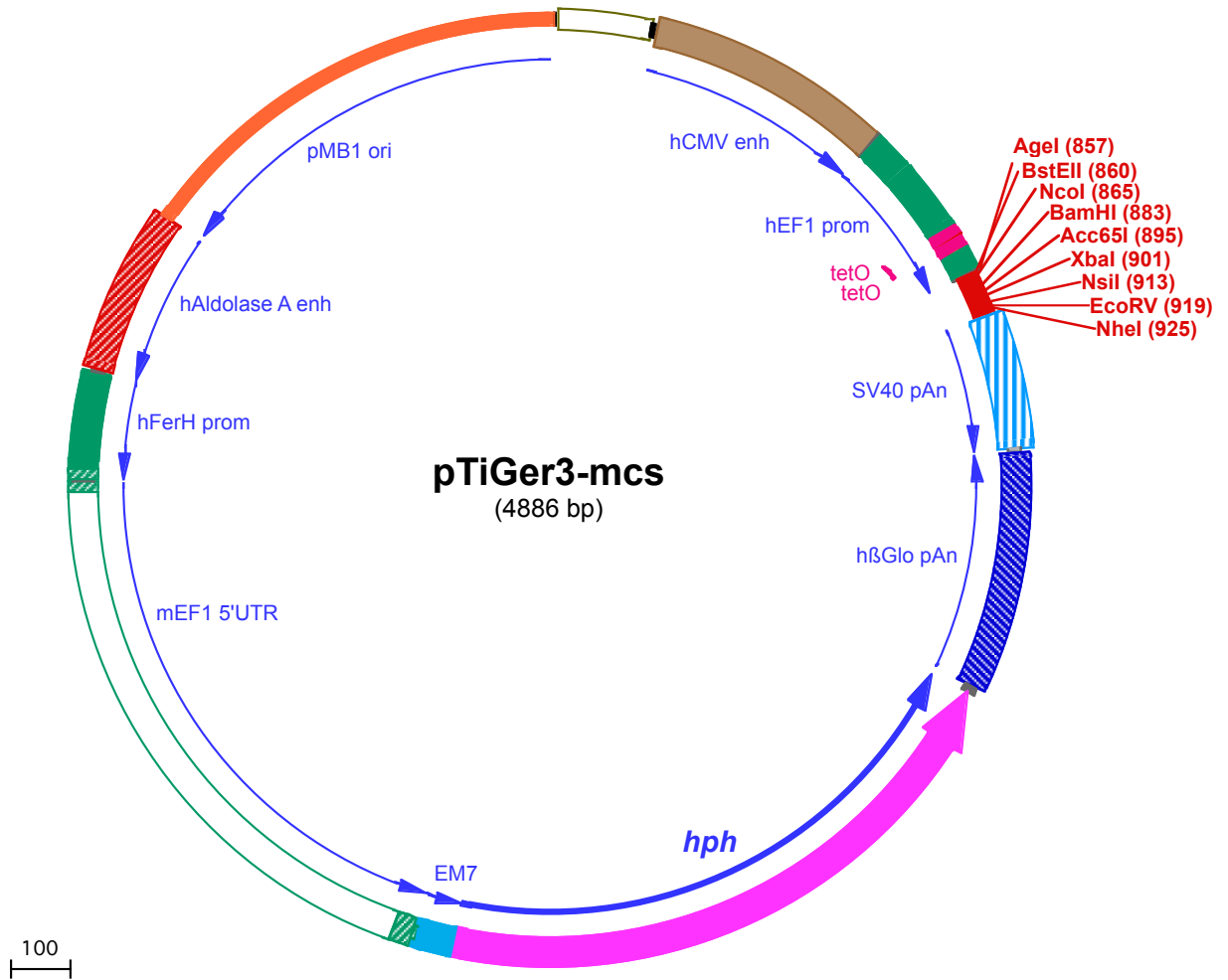
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1 CTCGAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACG
101 AAACAAAACAAACTAGCAAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGACCTGCAGGCGTTACATAACTTACGGTA
201 AATGGCCCCGCTGGGTGACCCGCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC
301 AATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC
401 CGCCTGGCATTATGCCAGTACATGACCTTATGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGTATTACCATGATGATGCGGTTTTGG
501 CAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTGACTAGTCAGTGGCC
601 AGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGGAGGGTTCGGCAATTGATCCGGTGCCTAGAGAAGTGGCGCGGGTAAACTGGGAAAGTGAT
701 GTCGTGTAAGTGGCTCCGCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTTGCCGTGAACGTTTCCCTATCAGTGATAGAGATCTCCC
801 TATCAGTGATAGAGATCTTCGCAACGGGTTTCCGCCAGAACACAGCTGAAGCTTACCCGGTACCATGGGAATTCAGCTTGGATCCAGATCTGGTAC
901 CTCTAGACTCGAGATGCATGATATCGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGC
1001 TTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACAACAACAAATTCATTCTTTTATGTTTCAGG
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1201 CAAGCCTCTACTTGAATCCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTGCAGCCTCACCTCTTTCA
1301 TGGAGTTTAAGATATAGTGATTTTCCCAAGTTTGAAGTACTCTTTCATTTCTTTATGTTTTAAATGCAGTACCTCCACATTCCCTTTTATGAAAA
1401 TATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTA
1501 GTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCGAATTCTCGACTCATTCTTTGCCCTCGGACGA
1601 GTGCTGGGGCGTCGGTTTTCACTATCGGCGAGTACTTCTACACAGCCATCGGTCCAGACGGCCGCTTCTGCGGGCGATTGTGTACGCCGACAGTCC
334 T S P R R N G S D A L V E V C G D T W V A A S R R A I Q T R G V T G
1701 CGGCTCCGGATCGGACGATTGCGTCGCATCGACCTGCGCCAAAGTGCATCATCGAAATTGCCGTCAACCAAGCTCTGATAGAGTTGGTCAAGACCAAT
301 A G S R V I A D C R G Q A W A A D D F N G D V L S Q Y L Q D L G I
1801 CGGAGCATATACGCCGGAGCCGCGGATCTCGAAAGTCCGGATCGCTCCGCTCGAAGTAGCGCTGCTGCTCATAACAAGCAACCAGGCCCTC
268 R L M Y A R L R P S G A L E P H R R E F Y R T Q Q E M C A L W P R
1901 CAGAAGAAGATGTTGGCGACCTCGTATTGGGAATCCCCGAACATCGCCTCGCTCCAGTCAATGACCGCTGTTATGCGGCCATTGTCGTCAGGACATTGT
234 W F F I N A V E Y Q S D G F M A E S W D I V A T I R G N D T L V N N
2001 TGGAGCCGAAATCCGCGTGCACGAGTCCGGACTTCCGGGCGAGTCCGCGCCAAAGCATCAGTCTATCGAGAGCTGCGCGACGGACGCACTGACGNT
201 S G F D A H V L H R V E P C D E A W L M L E D L A Q A V S A S V T
2101 GTCGTCCATCACAGTTTCCAGTGATACACATGGGGATCAGCAATCGCGCATATGAAATCACGCCATGTAGTGTATTGACCGATTCTTGGGTCCGAAT
168 D D M V T Q W H Y V H P D A I A C I F D R W T T Y Q G I G Q P G F
2201 GGGCCGAACCCGCTCGTCTGGCTAAGATCGGCCGAGCGATCGCATCCATGAGCTCCGCGACGGGTTGCAGAACAGCGGGCAGTTCCGTTTTAGGCAGGT
134 P G F G S T Q S L D A A A I A D M L E A V P Q L V A P L E T E P L D
2301 CTTGCAACGTGACACCCTGTGCACGGCGGGAGATGCAATAGGTGAGGCTCTCGCTGAATCCCAATGTCAAGCACTTCCGGAATCGGGAGCGCGCCGA
101 Q L T V G Q A R R S I C Y T L S E S F E G I D L V E P I P L A A S
2401 TGCAAAGTGCCGATAAACATAACGATCTTTGTAGAAACCATCGGCGCAGCTATTTACCCGAGGACATATCCAGGCCCTCTACATCGAAGCTGAAAGCA
68 A F H R Y V Y R D K Y F G D A C S N V R L V Y G R G G V D F S F A
2501 CGAGATTTCTCGCCCTCCGAGAGCTGCATCAGGTCCGAGACGCTGTCGAACTTTTCGATCAGAAACTTCCGCGACAGACGTCGCGGTGAGTTGAGGCTTTT
34 R S E E G E S L Q M L D S V S D F K E I L F K A V S T A T L E P K K
2601 TCATGATGGCCCTCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAATCCGGTTCCTTGAATTAGCGGTGTTTTT
1 M
2701 ACAACACCTAAAAAGGGTTTAAAGATACCTTTGAACCGCTAAGAAGCCCGAGAATTAGTCCGCTCAAACTCAAGGGGACAAATCCAAAAATGACT
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4201 CCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAG
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4601 TGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTC
4701 GGAAAAAGAGTTGGTAGCTCTTGATCCGCAAAACAAACCACCCTGGTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGAT
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