

Product usage

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

Important Limited Use information for pTiGer4-Lucia

The purchase of the pTiGer4-Lucia 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.

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



pTiGer4-Lucia

A multigenic plasmid for inducible Lucia luciferase expression, selectable with Puromycin

Catalog code: ptg4-lc

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

For research use only

Version 24A16-NJ

PRODUCT INFORMATION

Contents:

- 20 µg of pTiGer4-Lucia provided as lyophilized DNA
- 1 ml of Puromycin (10 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 Puromycin 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 pTiGer4-Lucia plasmid encodes the Lucia luciferase and the Puromycin 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 Lucia expression 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 Lucia-encoding gene is transcribed.

pTiGer-SEAP and pTiGer-eGFP 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-1 α (EF-1 α)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.
- **Lucia** is a secreted coelenterazine luciferase encoded by a synthetic gene developed by InvivoGen. It generates 1000-fold higher bioluminescent signal compared to the commonly used Firefly and Renilla luciferases. Lucia luciferase activity can be evaluated using QUANTI-Luc™ 4 Lucia/Gaussia, an assay reagent containing all the components required to quantitatively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases.

- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.

Puromycin 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 *pac* gene in *E. coli*.
- **pac:** The resistance to Puromycin is conferred by the *pac* gene from *Streptomyces* which encodes a N-acetyl-transferase. The *pac* 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-1 α enhances *pac*-encoding mRNA stability and protein translation.
- **hβGlo pAn:** The human β -Globin pAn is a strong polyadenylation signal placed downstream of *pac*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 1 α 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 mRNA 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 DH5 α .

Puromycin usage

Puromycin can be used at 100-125 µg/ml in *E. coli* in liquid or solid media and at 1-10 µg/ml to select Puromycin-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
Puromycin	Selection antibiotic	ant-pr1
QUANTI-Luc™ 4 Lucia/Gaussia	Luciferase Detection	rep-qlc4lg1

TECHNICAL SUPPORT

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

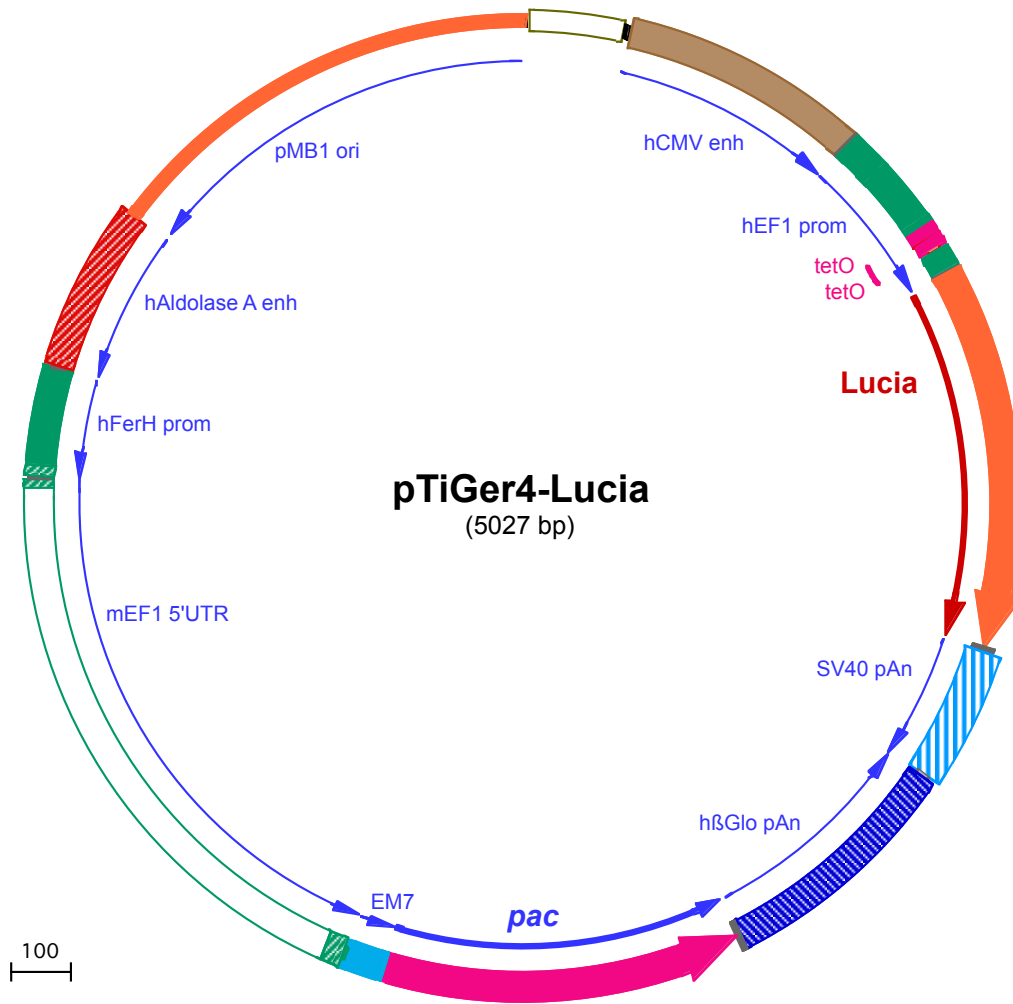
InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Asia: +852 3622-3480

E-mail: info@invivogen.com

 **InvivoGen**
www.invivogen.com



1 CTCGAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACG
101 AAACAAAACAAACTAGCAAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGACCTGCAGGCGTTACATAACTTACGGTA
201 AATGGCCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTC
301 AATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC
401 CGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTTGG
501 CAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTTACTAGTCAGTGGCC
601 AGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCGGCAATTGATCCGGTGCCTAGAGAAGTGGCGCGGGTAACTGGGAAAGTGAT
701 GTCGTGTAAGTGGCTCCGCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTTGCCGTGAACGTTCCCTATCAGTGATAGAGATCTCCC
801 TATCAGTGATAGAGATCTTTCGCAACGGGTTGCCGCCAGAACACAGCTGAAGCTTACCCGGTACCATTGAAATCAAGGTGCTGTTTGCCTCATCTGT
120 I A V A E A K P T E I N E D L N I A A V A S N F A T T D L E T D L
1001 TCACCAACTGGGAGACCATGAATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGTGCCTGGCAAAAACCTCCCCCAGA
45 F T N W E T M N V I S T D T E Q V N T D A D R G K L P G K K L P P D
1101 TGTCTGAGGGAGCTGGAGGCCAATGCCAGAAGGGCTGGTTGCACAAGAGGCTGCCTCATTTGCCTCTCCACATTAAGTGCACCCCTAAGATGAAGAAA
78 V L R E L E A N A R R A G C T R G C L I C L S H I K C T P K M K K
1201 TTTATCCCTGGCAGGTGCCACACTTATGAAGGTGAAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCAGAGATTCTGGCTTCA
112 F I P G R C H T Y E G E K E S A Q G G I G E A I V D I P E I P G F
1301 AGGATAAGGAGCCACTGGACCAGTTTATTGCTCAAGTGGACCTCTGTCTGATTGCACCACTGGCTGTCTGAAGGGCCTGCCAATGTCCAGTGTCTGA
145 K D K E P L D Q F I A Q V D L C A D C T T G C L K G L A N V Q C S D
1401 CCTCTGAAGAAGTGGCTTCCCAGAGGTGTACCCTTTTCCAGCAAGATTGAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGACAGATGAGCT
178 L L K K W L P Q R C T T F A S K I Q G R V D K I K G L A G D R •
1501 AGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTG
1601 CTTTATTTGTAACCATTATAAGCTGCAATAAACAGTTAACAACAACAATTGCATTATTTTATGTTTCAGGTTCCAGGGGAGGTGTGGGAGGTTTTTTA
1701 AAGCAAGTAAACCTCTACAATGTGGTATGGAATTCTAAATACAGCATAGCAAACTTTAACTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAG
1801 GGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTCATGGAGTTAAGATATAGTGATTTTCCC
1901 AAGGTTTGAAGTACTCTTCAATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCTTTTATGTAATAATCAGAAATAATTAATACATCATT
2001 GCAATGAAAATAAATGTTTTTATTAGGCAGAAATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGACTTAGGGAACAAGGAACC
2101 TTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTCAGGTTAAAGCTCCAGGCTTCTTGTGCATGCACCAAGTTCTTGGGCTTCTGGAACCTCA
200 A G P K R T M C W T R P G E P V E
2201 ACATCAGCTGTACAGTGAATCCAGTCTTTTCAAAAAGGCAGGTTTCTGGGAGCAGAAGTTCCAGAAAGGCAGGAACCTCCAGCCCTTTCAGCAGCTT
181 V D A T V T F G L R E Y F P L N R P A S T E L F A P V G A R E A A E
2301 CAACTCCAGGCAGAAACAAGCAGATCCAGACCCTTTCCCTGGTGGTCAGGGCTCACTCCAACAGTTGCCAGAAACCAAGCTGGCTCTTTTGGCCTGTG
148 V G P L V V A S G L G K G Q H D P S V G V T A L F W A P E K P R H
2401 TGGTGCCAGCAGACCTTCCATTTGTTGTGTGCTGCCAGCCTGCTCCAGAGAGCTCAGCCATTCTTGGTCCAATTTCCAGAAAACAGCACCAGCTTCA
115 P A L L G E M Q Q Q A A L R S G S L E A M R P G I E A F V A G A E
2501 ACAGACTCAGGTGTTGTCCAACCTGCAACAGCAGCTCCATCATCTGCAACCCAACTTTTCCAATGTCCAGTCCCCTCTGGTGGGAAGAGTTCTTGCA
81 V S E P T T W V A V A A G D D A V W V K G I D L G V R T L F L E Q L
2601 GTTCTGTACCCTCTCAATGTGCTGTGAGGTTCAACTGTGTGCTTGTGAGGGTGTGCTGCAAAAAGCAGCAGCCAGTGTCTCACAGCTCTTGGAAC
48 E T V R E I H R D P D V T H R T A P Y D A F A A A L T R V A R P V
2701 ATCATCTCTGGTTGCCAGCCTCACTGTGGGTTTGTACTCAGTCATGGTGGCCCTCTATAGTGAGTCGATTATATACTATGCCGATATACTATGCCGATGA
15 D D R T A L R V T P K Y E T M
2801 TTAATTGCAATCCGGTTGCTTTGAATTAGCGGTGGTTTTTCAACACCTAAAAAGGGTTTAAAAGATACCTTTGAACCGCTAAGAAGCCCGAGAATTA
2901 GCTCCGCTCAAACTCAAGGGGACAAATCCAAAAATGACTTCCAGCGCCAGGCTGGCCTGACTAGTCTCCACCCACCAATGTGAACAACTCCAACGC
3001 CATTACATCCCCTCCCCCGCCGACTAGCCGTGCTCAAAAGCCGAGGTGACTATTGCGGCCGATAGGACCACGGGGTACAGGAAGCAGCAGCCGGT
3101 GAGGGACCAGGCCCTTCTCTTTGTGTGGTGACTCACCCGCCGTCACCCGGGTGCCCGTCTCCATTTTGTGCTCTTGAACAGGGCCCGGGAG
3201 CGGCCATCTTCCACGCACGCAACTGGTGGCGGACGGGATGGCCTCACCTAGTTAGGGAGGCAGGGCAACGCGCGCCGCAAGCCAGATCGTGCCGGG

3301 TGCTGGGGCCACATGGCCTCGGCACGCTAACCCAGCCTGGTTGCTTCGGGAAAAACCCAGGCCTGCCCATCCAGGTGGCGTCGGACATGTGCTCCG
3401 AAGGCGGGCGGGCCCCAGCCGCACTCTGTCCCTCCATTCTCCCAACCATGACCTCTCCGGGCTCCGGGCGAGCAAGCCCCGACCCCTCCCTTTGTT
3501 AGCCCCATTGCTGAACGGCAATCGAAGGCAGCAGGGCAACAACAACAAAAAAAAAAGACCAGAGTGCGGCCGGAGTAGCACGCGGCGGGCGGGGA
3601 CACCACGCTAGGCCTCAAGCCGACACGAGGCGAGGCTACGGGGTTGCCGCTAGGCCTCGACTCTGCCTCCCGCGCCGCCGCAACTCGAAGCGGGAAT
3701 GCTCGCAGCTAATCCCCGCCGACGACAGCGGGCCCGCCGCTCGGAGCAGGACCTCCAGCTCGGCGGCCGGAAGCCACACCCGCCCTCACCTGC
3801 GTTCTGACGGCAAGCTTCGGCGAAGAACGTCTGGCCCTGCGGGTCGCTTGTGGTCTTTATAGCCGCTCGGCGTCAGGCCGCCCGGCAATCAGCGC
3901 CGCCCGCCCGAGCCCGCTCTTCGGTGGGCGGGACCCCGCCCTGCTGTGGGGAGGGCGGCCGCTGGAGGCCCTCGCGCTCTGGCGGAACTA
4001 GTCGACGGACTGGGTACGGGCCCCCGAGAGGCGCAGCCAGAGGCCGCTAGGAAGGGGGCGCCGAGAACACGATCCCTCCCCACCCCTC
4101 GGACGTGACTCGGACCACATCCCGGGTTCGCTAGGGCCCTCCCTTCTGCTCCTTTCCCGAGCCTGGCGGCTCTGGGGCGCCGTGACTCAGCCAGAAT
4201 GTTGGCAATGGGGAGGGCGGAACGGGGAAGTGGAGGACCGGATGGAAAAGTCGAAACGAAGGAAGCTGAGTTTCGCTGCAGGTTAATTAAGAACAT
4301 GTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGA
4401 CGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCTGGAAGCTCCCTCGTGCGCTCTCTGTTCCGACCCTGCCG
4501 TTACCGGATACCTGTCCGCTTTCTCCCTTCGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAA
4601 GCTGGGCTGTGTGCACGAACCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGTAAGACACGACTTATGCCA
4701 CTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAA
4801 CAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCGTGGTAGCGGTGGTTT
4901 TTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAACCA
5001 CGTTAAGGGATTTTGGTCATGGCTAGT