

Product usage

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

Important Limited Use information for pTiGer2-Lucia

The purchase of the pTiGer2-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.

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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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pTiGer2-Lucia

A multigenic plasmid for inducible Lucia luciferase expression, selectable with Zeocin®

Catalog code: ptg2-lc

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

For research use only

Version 24A16-NJ

PRODUCT INFORMATION

Contents:

- 20 µg of pTiGer2-Lucia 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-Lucia plasmid encodes the Lucia luciferase 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 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.

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-1 α enhances *Sh ble*-encoding mRNA stability and protein translation.
- **h β Glo 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 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 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 DH5 α .

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
QUANTI-Luc™ 4 Lucia/Gaussia	Luciferase Detection	rep-qlc4lg1

TECHNICAL SUPPORT

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

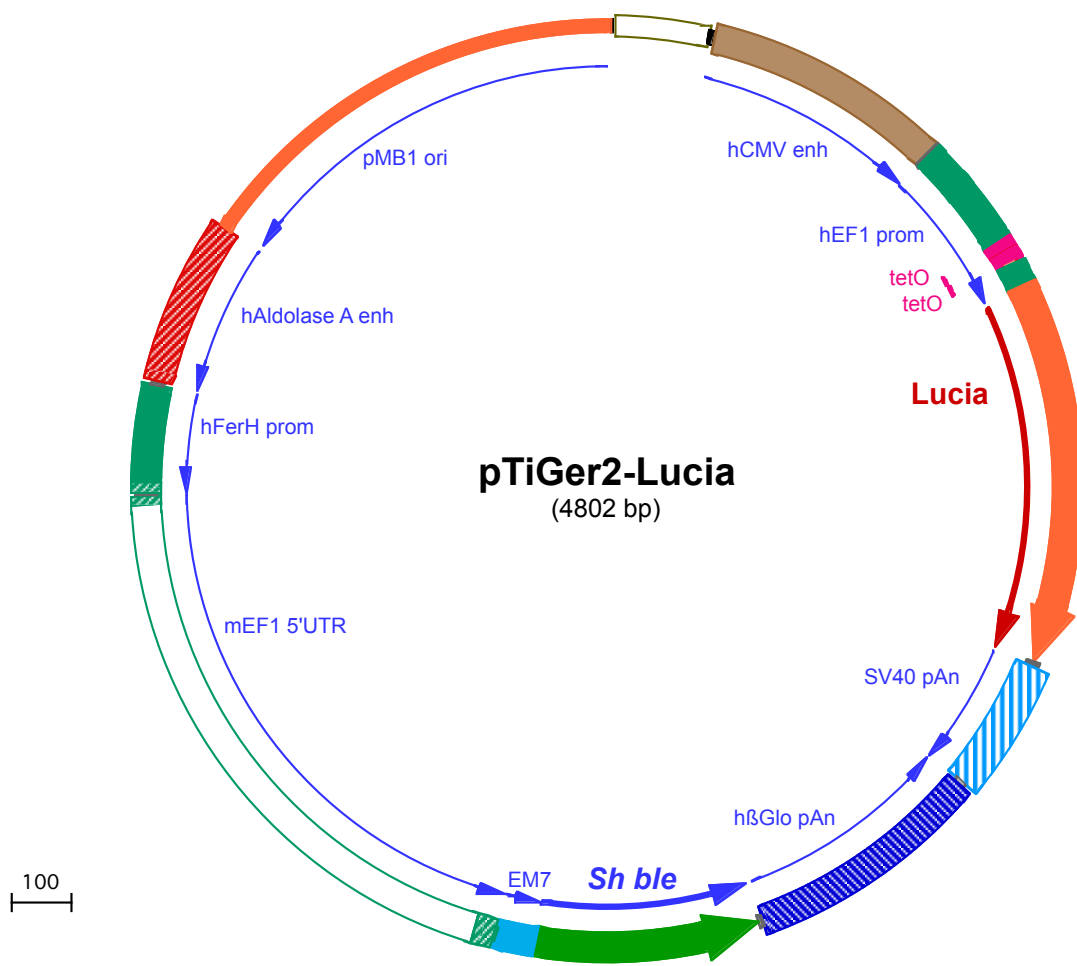
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1 CTCGAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAAACAAACG
101 AAACAAAACAACTAGCAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGACCTGCAGGCGTTACATAACTTACGGTA
201 AATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTC
301 AATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC
401 CGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTTGG
501 CAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTTACTAGTCAGTGGCC
601 AGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTCGGCAATTGATCCGGTGCCTAGAGAAGTGGCGCGGGTAACTGGGAAAGTGAT
701 GTCGTGACTGGCTCCGCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTTGCCGTGAACGTTTCCCTATCAGTGATAGAGATCTCCC
801 TATCAGTGATAGAGATCTTTCGAACGGGTTTCCGCCAGAACACAGCTGAAGCTTCA CCGGTCACCATGGAATCAAGGTGCTGTTTGCCTCATCTGT
12 M E I K V L F A L I C
901 ATTGCTGTTGCTGAGGCAAAACCCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACCTTGGCCACACAGATCTTGAGACTGACCTGT
12 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 TGTCTGAGGGAGCTGGAGGCCAATGCCAGAAGGGCTGGTTGCACAAGAGGCTGCCTCATTGCTCTCCACATTAAGTGCACCCCTAAGATGAAGAAA
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 TTTATCCCTGGCAGGTGCCACACTTATGAAGGTGAAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCAGAGATCTGGCTTCA
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 AGGATAAGGAGCCACTGGACAGTTTATTGCTCAAGTGGACCTCTGTCTGATTGCACCACTGGCTGTCTGAAGGGCTTCCCAATGTCCAGTGTCTGA
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 CCTCCTGAAGAAGTGGCTTCCCAGAGGTGTACCCTTTTCCAGCAAGATTCAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGACAGATGAGCT
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 AGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTG
1601 CTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTATTTTATGTTTCAGGTTCCAGGGGAGGTGTGGGAGTTTTTTA
1701 AAGCAAGTAAACCTCTACAATGTGGTATGGAATTCTAAATACAGCATAGCAAACCTTAACTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAG
1801 GGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTCATGGAGTTAAGATATAGTGATTTTCCC
1901 AAGGTTTGAAGTACTCTTCAATTTCTTTATGTTTTAAATGCACTGACCTCCACATCCCTTTTATGTAATAATTCAGAAATAATTTAAATACATCATT
2001 GCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAGGAACC
2101 TTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCCTTGCACAAAAGTGCACGCAAGTGCAGGCGGCTCGCGCAGG
125 D Q E E A V F H V C N G A P D R L
2201 GCGAACTCCCGCCCCACGGTCTCGCCGATCTCGGTATGGCCGGCCGGAGGCGTCCCGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACA
106 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 A Y L
2301 GCTCGTCCAGGCCGCGCACCCACCCAGGCCAGGGTGTGTCGGCACCACTGGTCTGGACCGGCTGATGAACAGGGTACGTCGTCCCGGACCA
73 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 R V V
2401 ACCGGCAAGTGTCTCCACGAAGTCCCGGAGAACCAGCCGGTCCGTCAGAACTCGACCGTCCGGGACGTCGCGCGGTTGAGCACCGGAACG
40 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 V P V
2501 GCACTGGTCAACTTGGCCATGATGGCCCTCTATAGTGTGATGCTATTACTATGCGGATATACTATGCGGATGATTAATTGTCAATCCGGTTGCTTTGA
6 A S T L K A M
2601 ATTAGCGTGGTTTTCACAAACCTAAAAAGGGTTAAAAGATACCTTTGAACCGCTAAGAAGCCGAGAAATTAGCTCCGCTCAAACTCAAGGGGACA
2701 AATTCAAAAATGACTTCCAGCGCCAGGCTGGCCTGACTAGTCTCCACCCACCAATGTGAACAAAATCCAACGCCATTACATCCCCTCCCCCGCCGG
2801 ACTAGCCGTGCTCAAAAGCCGAGGTGACTATTGCGCCGATAGGACCACGGGTACAGGAAGCAGCAGCCGGTGGAGGACCAGGCCCTTCTCTTTGT
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3301 AAGGCAGCAGGGCAACAACAACAAAAAAGACCAGAGTGCGGCCGGAGTAGCACGCGGGCGGGCGGGACACCACGCTAGGCCTCAAGCCGGAC
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3701 GGTGGGCGGGACCCGCCCTGCTGTGGGGAGGGCGGCCGCTGGAGGCCCTCGCGGCTCTGGCGGAACTAGT**CGACGGACTGGGCTACGGCCGC**
3801 **CCCCGAGAGGCGCAGCCAGAGGCCGCTAGGAAGGGCGGGCGCCGAGAACACGATCCCTCCCCACCCCTCGGACGTGACTCGGACCACATCCCGC**
3901 **GGTTCGCTAGGGCCCTCCTTCTGCTCCTTCCCCAGCCTGGCGGCTCTGGGGCGCGTGACTCAGCCAGAATGTTGGCAATGGGGAGGGCGGAACG**
4001 **GGGAAGTGAGGACGCGGATGGAAAAGTCGAAACGAAGGAAGCTGAGTTTCGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGAAAAGGCC**
4101 **AGGAACCGTAAAAAGCCGCTTGTGGCGTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACC**
4201 **CGACAGGACTATAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTCT**
4301 **CCCTTCGGGAAGCGTGGCGTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGTGCACGAACCCCC**
4401 **G TTCAGCCCGACCCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGA**
4501 **TTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCT**
4601 **GAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACG**
4701 **CGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTA**
4801 **GT**
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