

# Product usage

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

## Important Limited Use information for pTiGer3-Lucia

The purchase of the pTiGer3-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](mailto: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](mailto:info@invivogen.com)



# pTiGer3-Lucia

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

Catalog code: ptg3-lc

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

For research use only

Version 24A16-NJ

## PRODUCT INFORMATION

### Contents:

- 20 µg of pTiGer3-Lucia 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 tetracycline-inducible reporter gene. The pTiGer3-Lucia plasmid encodes the Lucia luciferase and the Hygromycin 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) protein<sup>1</sup>, 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 $\alpha$  (EF-1 $\alpha$ )<sup>2</sup>.
- **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)<sup>1</sup>.
- **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 mRNA<sup>3</sup>.

### 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*.
- **mEF15'UTR:** The 5'UTR (untranslated region) of the murine EF-1 $\alpha$  enhances *hph*-encoding mRNA stability and protein translation.
- **h $\beta$ Glo pAn:** The human  $\beta$ -Globin pAn is a strong polyadenylation signal placed downstream of *hph*<sup>4</sup>.

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 $\alpha$  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  $\beta$ -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 $\alpha$ .

### 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/reptor-cells>.

## RELATED PRODUCTS

| Product                     | Description          | Cat. Code   |
|-----------------------------|----------------------|-------------|
| Hygromycin                  | Selection antibiotic | ant-hg-1    |
| QUANTI-Luc™ 4 Lucia/Gaussia | Luciferase Detection | rep-qlc4lg1 |

## TECHNICAL SUPPORT

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

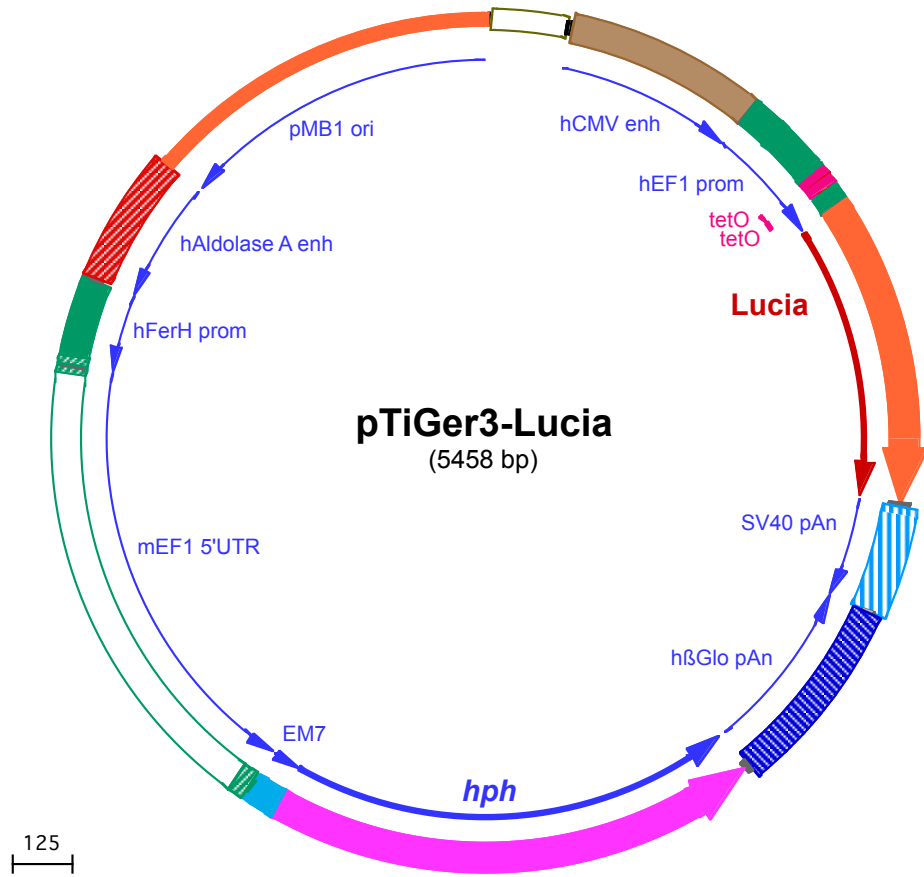
InvivoGen USA (International): +1 (858) 457-5873

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

InvivoGen Asia: +852 3622-3480

E-mail: [info@invivogen.com](mailto:info@invivogen.com)

 **InvivoGen**  
www.invivogen.com



1 CTCGAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAAACAAACG  
101 AAACAAAACAACTAGCAAAATAGGCTGTCCCAAGTCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGACCTGCAGGCGTTACATAACTTACGGTA  
201 AATGGCCCCGCTGGTGACCGCCCAACGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCATTGACGTC  
301 AATGGGTGGAGTATTTACGGTAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCC  
401 CGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGATGATGCGGTTTTGG  
501 CAGTACATCAATGGGCGTGGATAGCGGTTTACTCACGGGGATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGTTTACTAGTACAGTGGCC  
601 AGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAGGGTTCGGCAATTGATCCGGTGCCTAGAGAAGTGGCGCGGGTAACTGGGAAAGTGAT  
701 GTCGTGACTGGCTCCGCCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTTGCCGTGAACGTTTCCCTATCAGTGATAGAGATCTCCC  
801 TATCAGTGATAGAGATCTTTCGAACGGGTTTCCGCCAGAACACAGCTGAAGCTTACCCGGTACCATTGAAATCAAGGTGCTGTTTGCCTCATCTGT  
12 M E I K V L F A L I C  
901 ATTGCTGTTGCTGAGGCAAAACCCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACCTTGGCCACCACAGATCTTGAGACTGACCTGT  
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 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 AGGATAAGGAGCCACTGGACAGTTTATTGCTCAAGTGGACCTCTGTCTGATTGCACCACTGGCTGTCTGAAGGGCCTGCCAATGCCAGTCTGTA  
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 CCTCTGAAGAAGTGGCTTCCAGAGGTGTACCCTTTTCCAGCAAGATTGAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGACAGATGAGCT  
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 AAGCAAGTAAACCTCTACAATGTGGTATGGAATTCTAAATACAGCATAGCAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAG  
1801 GGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTCATGGAGTTAAGATATAGTGATTTTCCC  
1901 AAGGTTTGAACACTAGCTCTTCAATTTCTTTATGTTTTAAATGCACTGACCTCCACATCCCTTTTATGTAATAATCAGAAATAATTAATACATCATT  
2001 GCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAGGAACC  
2101 TTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCGAATTCTCGACTCATTCTTTGCCCTCGGACGAGTGTGGGGCGTGGTTTTCCACTATCGG  
342 E K A R P R T S P R R N G S D A  
2201 CGAGTACTTCTACACAGCCATCGGTCCAGACGGCCGCGCTTCTGCGGGCGATTGTGTACGCCGACAGTCCCGGCTCCGGATCGGACGATTGCGTCGCA  
325 L V E V C G D T W V A A S R R A I Q T R G V T G A G S R V I A D C  
2301 TCGACCTGCGCCAAGTGCATCATCGAAATTGCCGTCAACCAAGCTCTGATAGAGTTGGTCAAGACCAATGCGGAGCATATACGCCGGAGCCGCGGC  
292 R G Q A W A A D D F N G D V L S Q Y L Q D L G I R L M Y A R L R P  
2401 GATCTGCAAGCTCCGGATCCCTCCGCTCGAAGTAGCGCGTCTGCTGCTCATAAGCAACCAGCGCTCCAGAAGAAGATGTTGGCAGCTCGTATT  
258 S G A L E P H R R E F Y R T Q Q E M C A L W P R W F F I N A V E Y Q  
2501 GGGAAATCCCCGAACATCGCTCGCTCCAGTCAATGACCGCTGTTATGCGGCCATTGTCCTGAGGACATTGTTGGAGCCGAAATCCGCGTGCACGAGGTG  
225 S D G F M A E S W D I V A T I R G N D T L V N N S G F D A H V L H  
2601 CCGGACTTCGGGGCAGTCTCGGCCAAAGCATCAGCTCATCGAGAGCTGCGCGACGGACGCACTGACGGTGTCTGCATCACAGTTTGCCAGTGATAC  
192 R V E P C D E A W L M L E D L A Q A V S A S V T D D M V T Q W H Y  
2701 ACATGGGGATCAGCAATCGGCATATGAAATCACGCCATGTAGTGTATTGACCGATTCTTGGCGTCCGAATGGGCGAACCCTCGTCTGGCTAAGAT  
158 V H P D A I A C I F D R W T T Y Q G I G Q P G F G S T Q P G S L D  
2801 CGGCCGACGAGTTCGATCAGCTCCGCGACGGGTTGCAGAACAGCGGGGAGTTCGGTTTTAGGAGGCTTGAACGTCGACACCTGTGACCGGGC  
125 A A A I A D M L E A V P Q L V A P L E T E P L D Q L T V G Q A R R  
2901 GGAGATGCAATAGGTGAGGCTCTCGTGAATTTCCCAATGTCAAGCACTTCCGGAATCGGGAGCGGGCCGATGCAAAGTCCGATAAACATAACGATCT  
92 S I C Y T L S E S F E G I D L V E P I P L A A S A F H R Y V Y R D  
3001 TTGTAGAAACATCGGCGAGCTATTTACCCGACGACATATCCACGCCCTCTACATCGAAGCTGAAAGCAGGAGATTCTTCCGCTCCGAGAGCTGCA  
58 K Y F G D A C S N V R L V Y G R G G V D F S F A R S E E G E S L Q M  
3101 TCAGGTCCGAGACGCTGCAACTTTTCGATCAGAAACTTCGGACAGACGTCGCGGTGAGTTTCCAGGCTTTTCATGATGGCCTCTATAGTGAGTCTG  
25 L D S V S D F K E I L F K A V S T A T A L E P K K M  
3201 ATTATACTATGCCGATATACTATGCCGATGATTAATTGCAATCCGGTTGCTTTGAATTAGCGGTGGTTTTTCAACACCTAAAAAGGGTTTAAAGAT

3301 ACCTTTGAACCGCTAAGAAGCCCGAGAATTAGCTCCGCTCAAACTCAAGGGGACAAATTCCAAAATGACTCCAGCGCCAGGCTGGCCTGACTAGTCT  
3401 CCACCCACCAAATGTGAACAACTCCAACGCCATTACATCCCCTCCCCCGCCGACTAGCCGTGCTCAAAAGCCGAGGTGACTATTGCGGCCGATAG  
3501 GACCACGGGGTACAGGAAGCAGCAGCCGGTGAGGGACCAGGCCCTCTTCTTTGTGTGGTGACTCACCCGCCCGCTCCACCGGGCTGCCGCTCTCC  
3601 ATTTTGAAGCTCTTGCAACAGGGCCCGGAGCGGCATCTTTCCACGCACGCAACTGGTGCCGGACGGGATGGCCTACCCTAGTTAGGGAGGCAGGGCA  
3701 ACGCGGCGCCGCAAGCCAGATCGTGCCGGTGTGGGGCCACATGGCCTCGGCACGCTAACCCAGCCTGGTTGCTTCGGGAAAAACCCAGGCCTCGC  
3801 CCCATCCAGGTGGCGTCGGACATGTGCTCCGAAGCGGGCGGGCCCCAGCCGCACTCCTGTCCCTCATTCTCCCAACCATGACCTCTCCGGGCTCC  
3901 GGGCGAGCAAGCCCCGACCCTCCCTTTGTAGCCCTATTGCTGAACGGCAATCGAAGGCAGCAGGGCAACAACAACAAAAAAAAAAGACCAGAGTG  
4001 CGGCCGGAGTAGCAGCGGGCGGGCGGACACCACGCTAGGCCTCAAGCCGGACACGAGGCAGGCTACGGGGTTGCCGTAGGCCTCGACTCTGCC  
4101 TCCCGCGCCGCCGCAACTCGAAGCGGGAATGCTCGCAGCTAATCCCCGCCGACAGCGGGGCCCGCCGCTCGGAGCAGGACCTCCAGCTCGGCGG  
4201 CCCGCGAAGCCACACCCGCCCTCACCTGCGTTCTGACGGCAAGCTTCGGCGAAGAAGCTTGCCCTGCGGGTTCGCTTGTGGTCTCTTATAGCCGCT  
4301 CGGCGTCAGGCCCGCCCGCCAATCAGCGCCCGCCCGAGCCGCTCTTCGGTGGCGGGACCCCGCCCTGCTGTGGGGAGGGCGGGCCG  
4401 CTGGAGGCCCTCGCGCTCTGGCGGAAGTACTAGTCGACGGACTGGGCTACGGGCCGCCCGAGAGGCGCAGCCAGAGGCCGCTAGGAAGGGCGGGC  
4501 GCCGAGAACAGTACCTCCCAACCCCTCGGACGTGACTCGGACCACATCCCGGGTTCGCTAGGGCCCTCCCTTCTGCTCTTTCCCAAGCCTGGC  
4601 GCGCTCTGGGGCGCCGTGACTCAGCCAGAATGTTGGCAATGGGGAGGGCGGAACGGGAAGTGGAGGACGCGGATGGAAAAGTCGGAACGAAGGAAGC  
4701 TGAGTTTCGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGCCAGCAAAAGCCAGGAACCGTAAAAAGCCGCTTGTGGCGTTTTTCCATAGGCT  
4801 CCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAAGGTTGGCAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCC  
4901 CTCGTGCGCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTCTCCCTTCGGGAAGCGTGGCGTTTCTCATAGCTCACGCTGTAGGT  
5001 ATCTCAGTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGA  
5101 GTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAG  
5201 TGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCG  
5301 GCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTAC  
5401 GGGGTCTGACGCTCAGTGAACGAAACTCACGTTAAGGGATTTTGGTCATGGCTAGT