

STOP

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

Important Limited Use License information for pNiFty3-T-Lucia

The purchase of the pNiFty3-T-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 InvivoGen, 10515 Vista Sorrento Parkway San Diego, CA 92121 USA. Tel: 858-457-5873 Fax: 858-457-5843.

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 Hong Kong : +852 3-622-34-80
E-mail: info@invivogen.com



pNiFty3-T-Lucia

An NFAT-inducible secreted luciferase reporter plasmid selectable with Zeocin™

Catalog code: pnf3-lc5

For research use only

Version 20L03-MM

PRODUCT INFORMATION

Content:

- 20 µg of pNiFty3-T-Lucia provided as lyophilized DNA
- 1 ml of Zeocin™ (100 mg/ml)

Storage and stability:

- Products are shipped at room temperature.
- Store lyophilized DNA at -20 °C.
- Store Zeocin™ at 4 °C or at -20 °C. The expiry date is specified on the product label.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.

GENERAL PRODUCT USE

Pattern recognition receptor (PRR) activation triggers a complex signaling cascade that leads to the activation of different transcription factors, each playing an important role in the subsequent immune response. To monitor the induction of PRR signaling in response to ligand stimulation in a simple and efficient manner, InvivoGen has designed pNiFty, a family of reporter plasmids expressing a reporter gene under the control of a minimal promoter inducible by these different transcription factors, either individually or in combination. Most pNiFty plasmids are selectable with Zeocin™ in both *E. coli* and mammalian cells, and can be used to generate stable clones. pNiFty plasmids are composed of three key elements: a proximal promoter, repeated transcription factor binding sites (TFBS) and a reporter gene. The proximal promoters are shorter than 500 bp and contain transcription factor binding sites. Upon stimulation in 293 cells, their expression level remains undetectable. With the addition of repeated TFBS, the proximal promoters become inducible by the appropriate stimulus and drive the expression of the reporter gene. by the appropriate stimulus and drive the expression of the reporter gene.

PLASMID FEATURES

- **NFAT binding site:** Nuclear factor of activated T-cell (NFAT) is a family of transcription factors expressed in T cells, but also in other classes of immune and non-immune cells¹. NFAT is activated by stimulation of receptors coupled to calcium mobilization, such as the PRRs Dectin-1 and Mincle^{2,3}. Calcium mobilization induces the calmodulin-dependent phosphatase calcineurin leading to NFAT activation. NFAT binds to a 9 bp element, with the consensus sequence (A/T)GGAAA(A/N)(A/T/C)N.
- **IFN-β promoter:** the mouse IFN-β minimal promoter comprises several positive regulatory domains that bind different cooperating transcription factors such as NF-κB, IRF3 and IRF7².
- **Lucia luciferase** is a synthetic CpG-free gene encoding a secreted coelenterazine-utilizing luciferase. ORF size (from ATG to stop codon): 634 bp Lucia luciferase activity can be evaluated using QUANTI-Luc™ (cat. code: rep-qlc1), 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.

- **Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.
- **EF1/HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter⁵ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁶. The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Zeo:** Resistance to the antibiotic Zeocin™ is conferred by the *Sh ble* gene from *Streptoaloteichus hindustanus*. The *Sh ble* gene is driven by the EF1-HTLV promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.
- **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁷.

1. Rao A. et al., 1997. Transcription factors of the NFAT family: regulation and function. Annu Rev Immunol. 15:707-47. 2. Goodridge HS. et al., 2007. Dectin-1 stimulation by *Candida albicans* yeast or zymosan triggers NFAT activation in macrophages and dendritic cells. J Immunol. 178(5):3107-15. 3. Yamasaki S. et al., 2009. C-type lectin Mincle is an activating receptor for pathogenic fungus, *Malassezia*. PNAS. 106(6):1897-902. 4. Vojdani G. et al., 1988. Structure and characterization of a murine chromosomal fragment containing the interferon beta gene. J Mol Biol. 204(2):221-31. 5. Kim D. et al., 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. Gene 91(2): 217-23. 6. Takebe Y. et al., 1988. SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol. Cell Biol. 1: 466-72. 7. Yu J. & Russell J., 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 containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile H₂O. Store resuspended plasmid at -20 °C.

Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5α.

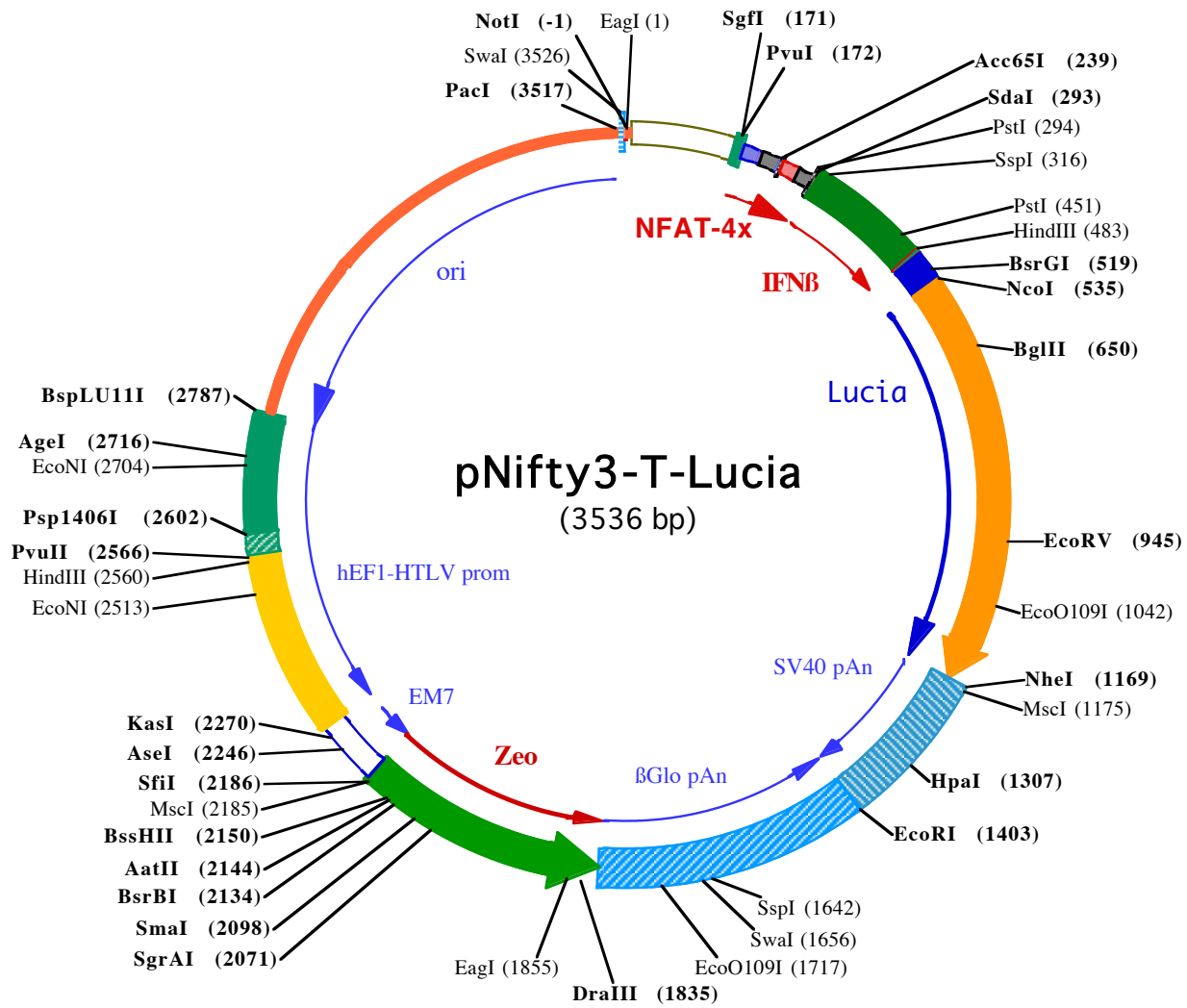
Zeocin™ usage

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

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 Hong Kong : +852 3-622-34-80
E-mail: info@invivogen.com





100
|-----|

EagI (1)
NotI (-1)
1 CCGGCCCAATAAAATATCTTTATTTTATTACATCTGTGTGTTGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACA
PvuII (172)
SgfI (171)
101 AAACAACTAGCAAAATAGGCTGTCCCCAGTGAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCGGAGGAAAACTGTTTCATAC
PstI (294)
SdaI (293)
201 AGAAGGGTGGAGGAAAACTGTTTCATACAGAAGGGGTGTACCCAAAGAGGAAAAATTTGTTTCATACAGAAGGAGGAAAACTGTTTCATACCTGCAG
SspI (316)
301 GagcttgaataaaatgaatattagaagctgtagaataagagaaaatgacagaggaAAACTGAAAGGgAGAACTGAAAGTgGgaaattcctctgaggcag
PstI (451)
HindIII (483)
401 aaaggaccatccctTATAAatgacacaggccatgaaggaagatcattctcactgcagcctttgacagcctttgcctcatcttgAAGCTTCTGCCTTCT
BsrGI (519) NcoI (535)
499 CCCTCTGTGAGTTTGGTTGGTGTACAGTAGCTTCCACCATGGAAATCAAGGTGCTGTTTGCCTCATCTGTATTGCTGTTGCTGAGGCAAAACCCACTG
BglII (650)
599 AAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACCTTTGCCACCACAGATCTTGAGACTGACCTGTTCCACCAACTGGGAGACCATGAATGTGAT
21 I u l l eAsnG l uAspLeuAsn l eAl aAl aValAl aSerAsnPheAl aThr ThrAspLeuG l uThrAspLeuPheThrAsnTrpG l uThr MetAsnVal l l
699 TAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAACTCCCCCAGATGTCCTGAGGAGCTGGAGGCCAATGCC
54 eSer ThrAspThr Gl uGl nValAsnThrAspAl aAspArgGl yLysLeuP roGl yLysLysLeuP roP roAspVal l LeuArgGl uLeuGl uAl aAsnAl a
799 AGAAGGGCTGTTGCACAAGAGGCTGCCTCATTGGCTCTCCACATTAAGTGCACCCTAAGATGAAGAAATTTATCCCTGGCAGGTGCCACACTTATG
88 ArgArgAl aGl yCysThr ArgGl yCysLeu l eCysLeuSer Hi s l l eLysCysThr ProLysMetLysLysPhe l l eProGl yArgCysHi sThr TyrG
EcoRV (945)
899 AAGGTGAAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCAGAGATTCCTGGCTTCAAGGATAAGGAGCCACTGGACCAGTTTAT
121 I uGl yGl uLysGl uSer Al aGl nGl yGl y l l eGl yGl uAl a l l eValAsp l l eP roGl u l l eP roGl yPheLysAspLysGl uP roLeuAspGl nPhe l l
EcoO109I (1042)
999 TGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAAATGCCAGTCTGACTCTGAAGAAAGTGGCTTCCAGAGG
154 eAl aGl nVal AspLeuCysAl aAspCysThr Thr Gl yCysLeuLysGl yLeuAl aAsnVal l Gl nCysSerAspLeuLeuLysLysTrpLeuProGl nArg
MscI (1175)
NheI (1169)
1099 TGTACCCTTTTGCAGCAAGATTCAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGGACAGATGATAGCTAGCTGGCCAGACATGATAAGATACAT
188 CysThr ThrPheAl aSer Lys l l eGl nGl yArgVal AspLys l l eLysGl yLeuAl aGl yAspArg•••
1199 TGATGAGTTTGGACAAACCACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGC
HpaI (1307)
1299 AATAACAAGTTAAACAACAATTCATTCATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTG
EcoRI (1403)
1399 GTATGGAATCTAAATACAGCATAGCAAACTTTAACTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGG
1499 GCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAAGATATAGTGTATTTTCCCAAGGTTTGAAGTCTTTCATTCT
SspI (1642) SmaI (1656)
1599 TTATGTTTTAAATGCACTGACCTCCACATTCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTA
EcoO109I (1717)
1699 GGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTTGGACAGCAAGAAA
DraIII (1835) EagI (1855)
1799 CCGAGCTTCTAGCTTATCCTCAGTCTGCTCTGCCACAAAGTGCACGAGTTCGCCGGCGGTCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTC
127•••Gl y•••AspGl nGl uGl uAl aVal l PheHi sVal CysAsnGl yAl aP roAspArgLeuAl aPheGl uArgGl yTrpP roGl nGl u
1899 GCCGATCTCGGTCATGGCCGGCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCTACAGCTCTCCAGGCCGCGCACCCACACC
98 Gl y l l eGl uThr MetAl aP roGl ySer Al aAspArgPheAsnThr Ser Val l Gl uSer TrpGl uAl aTyrLeuGl uAspLeuGl yArgVal l TrpVal l T
SgrAI (2071)
1999 CAGGCCAGGTGTTGTCCGGCACCCTGGTCTGGACCGGCTGATGAACAGGGTACGCTGCTCCGGACCACCCGGCGAAGTCTCTCCACGAAGT
64 rP al aLeuThrAsnAspP roVal l Gl nAspGl nVal Al aSer l l ePheLeuThr Val AspAspArgVal l Gl yAl aPheAspAspGl uVal l PheAs
AatII (2144) SfiI (2186)
SmaI (2098) BsrBI (2134) BssHIII (2150) MscI (2185)
2099 CCCGGGAGAACCCGAGCCGGTCCGAGAACTCGACCGTCCGGCAGCTCGCGCGGTGAGCACCAGGACCGCACTGGTCAACTGGCCATGATGGC
31 pArgSer PheGl yLeuArgAspThr TrpPheGl uVal Al aGl yAl aVal AspArgAl aThr LeuVal l P roVal Al aSer Thr LeuLysAl aMet ←
AseI (2246) KasI (2270)
2199 CCTCTATAGTGAGTCGATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTTGTAGGCCCGGTACAGCTTGATCTGTAAC
2299 GGCGCAGAACAGAAAACGAAACAAAGCCTAGAGTTGAGCAAGCAGGGTTCAGGCAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCCAAGGGAGCGC
2399 CGGACAAAGGCCCGTCTCGACCTGAGCTTTAACTTACCTAGACGGCGGACGCAGTTCAGGAGGCACCACAGGCGGGAGGCGGACGAGGACTCAAC
PvuII (2566)
EcoNI (2513) HindIII (2560)
2499 CGGCGTGGATGGCGGCTCAGGTAGGGCGGCGGCGCTGAAGGAGAGATGCGAGCCCTCGAAGCTTCAGCTGTGTTCTGGCGGCAAAACCGTTGCGAA
Psp1406I (2602)
2599 AAAGAACGTTACGGCGACTACTGCACTTATATACGGTCTCCCCACCCTCGGAAAAAGGGAGCCAGTACACGACATCACTTCCAGTTTACCC

EcoNI (2704) AgeI (2716) BspLU11I (2787)

2699 GCGCCACCTTCTCTAGGCACCGTTCAATTGCGACCCCTCCCCCAACTTCTCGGGGACTGTGGGCGATGTGCGCTCTGCCACTGACACATGTGAGCA

2799 AAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAA

2899 GTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCTGCCGCTTACCGG

2999 ATACCTGTCCGCCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGC

3099 TGTGTGCACGAACCCCGTTTCAGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATGCCACTGGCAG

3199 CAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATT

3299 TGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCCTGGTAGCGGTGTTTTTTGTT

3399 TGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAAACGAAAACTCACGTTAAG

Swal (3526)

Pacl (3517)

3499 GGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA