

STOP

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TECHNICAL SUPPORT

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pNiFty3-TAN-Lucia

An inducible reporter plasmid selectable with Zeocin™

Catalog code: pnf3-lc8

For research use only

Version 20L03-MM

PRODUCT INFORMATION

Content:

- 20 µg of pNiFty3-TAN-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.
- **AP-1 binding site:** Activator protein 1 (AP-1) is a transcription factor activated by most PRRs. AP-1 is a heterodimeric complex composed of members of Fos, Jun and, ATF protein families. AP-1 binds to the TPA responsive element (TRE: TGAG/CTCA)⁴. AP-1 activation in TLR signaling is mostly mediated by MAP kinases such as c-Jun N-terminal kinase (JNK), p38 and extracellular signal regulated kinase (ERK).

- **NF-κB binding site:** Nuclear factor (NF)-κB is a “rapid-acting” primary transcription factor activated by a wide variety of PRRs. NF-κB is a protein complex that belongs to the Rel-homology domain-containing protein family. The prototypical NF-κB is composed of the p65(RelA) and p50 subunits⁵. NF-κB binds specific decameric DNA sequences (GGGRNNYYCC, R-purine Y=pyrimidine) and activates genes involved in the regulation of the innate and adaptive immune response.

- **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 IRF7⁶.

- **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 promoter⁷ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁸. 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 *Streptoalloteichus 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 transcription⁹.

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. Hess J. et al., 2004. AP-1 subunits: quarrel and harmony among siblings. *J Cell Sci.* 117(Pt 25):5965-73.
5. Kawai T. & Akira S., 2007. Signaling to NF-kappaB by Toll-like receptors. *Trends Mol Med.* 13(11):460-9.
6. Vodjdani G. et al., 1988. Structure and characterization of a murine chromosomal fragment containing the interferon beta gene. *J Mol Biol.* 204(2):221-31.
7. Kim D. et al., 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. *Gene* 91 (2): 217-223.
8. 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: 466-472.
9. 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.

TECHNICAL SUPPORT

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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.

RELATED PRODUCTS

Product	Catalog Code
ChemiComp GT116	gt116-11
QUANTI-Luc™	rep-qlc1
Zeocin™	ant-zn-1

TECHNICAL SUPPORT

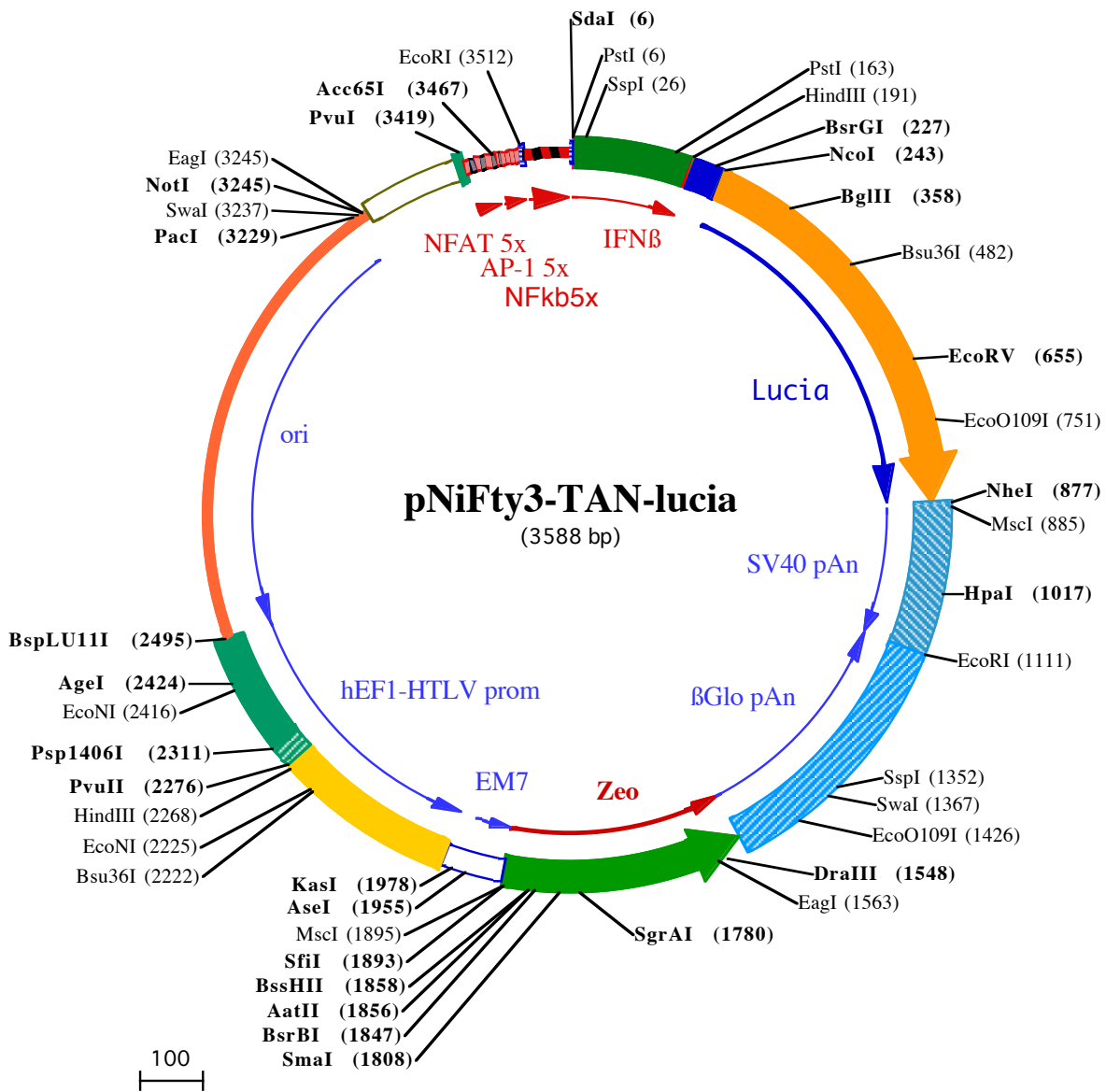
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PstI (6)
SdaI (6)
 1 CCTGCAGGagcttgaataaaatgaatattagaagctggtagaataagagaaatgacagaggaAAACTGAAAGGgAGAACTGAAAGTggaattcctct

PstI (163) HindIII (191)
 101 gaggcagaaaggaccatccct**TATAAA**tagcacagggccatgaaggaagatcattctcactgcagcctttgacagcctttgctcatcttg**AGCTTCT**

BsrGI (227) **NcoI (243)**
 199 GCCTTCTCCCTCCTGTGAGTTGGTGTACAGTAGCTCCAC**CATGGAAATCAAGGTGCTGTTGCCCTCATCTGTATTGCTGTTGCTGAGGCAAAA**
 1▶MetGI u l l eLysVal l e uPheAl aLeu l eCys l eAl aVal l Al aGl uAl aLys

BglIII (358)
 299 C**CCACTGAAATCAATGAAGACCTCAATATAGCTGTGGCTCCA**ACTTTGCCACCACAGATCTTGAGACTGACCTGTT**CACTGGGAGACCATGA**
 19▶ProThr Gl u l l eAsnGl uAspLeuAsn l eAl aAl aVal l Al aSerAsnPheAl aThr ThrAspLeuGl uThrAspLeuPheThrAsnTrpGl uThr Me tA

Bsu36I (482)
 399 ATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAACTCCCCAGATGTCTGAGGGAGCTGGAGGC
 52▶snVal l l eSer ThrAspThr Gl uGl nVal l AsnThrAspAl aAspArgGl yLysLeuP roGl yLysLysLeuP roP roAspVal l e uArgGl uLeuGl uAl

499 CAATGCCAGAAGGGCTGGTGCACAAGAGGCTGCCTCATTGGCTCTCCACATTAAGTGCACCCCTAAGATGAAGAAATTTATCCCTGGCAGGTGCCAC
 85▶aAsnAl aArgArgAl aGl yCysThrArgGl yCysLeu l eCysLeuSer Hi s l l eLysCysThr P roLysMe tLysLysPhe l l eP roGl yAr gCysHi s

EcoRV (655)
 599 ACTTATGAAGGTGAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGCAATTGTTGATATCCAGAGATTCTGGCTCAAGGATAAGGAGCCACTGGACC
 119▶Thr TyrGl uGl yGl uLysGl uSerAl aGl nGl yGl y l l eGl yGl uAl a l l eValAsp l l eP roGl u l l eP roGl yPheLysAspLysGl uP roLeuAspG

EcoO109I (751)
 699 AGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCTTGCCAATGTCCAGTGCTCTGACCTCTGAAGAAGTGGCTTCC
 152▶ l nPhe l l eAl aGl nVal l AspLeuCysAl aAspCysThr Thr Gl yCysLeuLysGl yLeuAl aAsnVal l Gl nCysSerAspLeuLeuLysLysTrpLeuP r

MscI (885)
 799 CCAGAGGTGTACCACTTTTCCAGCAAGATTCAAGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGGACAGATGATAGCTAGTGGCCAGACATGATAA
 185▶oGl nArgCysThr Thr PheAl aSer Lys l l eGl nGl yAr gVal l AspLys l l eLysGl yLeuAl aGl yAspArg•••

NheI (877)
 899 GATACATTGATGAGTTTGGACAAACCACAACACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTAT

HpaI (1017)
 999 AAGCTGCAATAACAAGTTAAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTAC

EcoRI (1111)
 1099 AAATGTGGTATGGAATTTCAAATACAGCATAGCAAACTTTAACCTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGC

1199 ATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTCTTTCATGGAGTTAAGATATAGTGATTTTCCCAAGTTTGAAGTAGCTCTT

SspI (1352) **Swal (1367)**
 1299 CATTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTATAGTAAAAATTCAGAAATAATTTAAATACATCATTGCAATGAAATAAATGTTT

EcoO109I (1426)
 1399 TTTATTAGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAG

DraIII (1548) **EagI (1563)**
 1499 CAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGTTCGCGAGGGCGAACTCCCGCCCCCAGC
 127▶•••Gl y•••AspGl nGl uGl uAl aVal l PheHi sVal l CysAsnGl yAl aP roAspArgLeuAl aPheGl uArgGl yTrpP r

1599 GCTGCTCGCCGATCTCGGTATGGCCGGCCGGAGGCGTCCCGAAGTTCGTGGACACGACCTCCGACCCTCGCGGTACAGCTCGTCCAGGCCGCGCAC
 100▶oGl nGl uGl y l l eGl uThr Me tAl aP roGl ySerAl aAspArgPheAsnThr Ser Val l Val l Gl uSer TrpGl uAl aTyrLeuGl uAspLeuGl yAr gVal l

SgrAI (1780)
 1699 CCACACCCAGGCCAGGGTGTGTCCGGCACCACTGGTCTGGACCGCTGATGAACAGGGTACGTCGTCGCCGACCACACCGGCCAAAGTCTGCTCCTC
 67▶TrpVal l TrpAl aLeuThrAsnAspP roVal l Val l Gl nAspGl nVal l Al aSer l l ePheLeuThr Val l AspAspArgVal l Val l Gl yAl aPheAspAspGl uV

AatII (1856) **SfiI (1893)**
 1799 ACGAAGTCCCGGAGAACCCGAGCCGGTCCGAGAACTCGACCGTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTGGCCA
 33▶al PheAspArgSer PheGl yLeuArgAspThr TrpPheGl uVal l Al aGl yAl aVal l AspArgAl aThr LeuVal l P roVal l Al aSer Thr LeuLysAl aMe

BsrBI (1847) **BssHII (1858)**
 1899 TGATGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTTGTAGGCGCCGTCACAGCTTGAT
 0▶t

AseI (1955) **KasI (1978)**
 1999 CTGTAACGGCGCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAAGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCCAAG

2099 GGAGCGCCGGACAAAGGCCCGGTCTCGACCTGAGCTTTAACTTACCTAGACGGCGGACGCAGTTCAAGAGGCACCACAGGGGAGGGCGGAGAACCGG

EcoNI (2225) **PvuII (2276)**
Bsu36I (2222) **HindIII (2268)**
 2199 ACTCAACCGCGTGGATGGCGGCCCTCAGGTAGGGCGGGCGCGTGAAGGAGAGATGCGAGCCCTCGAAGCTTCACTGTGTTCTGGCGCAAACCCG

Psp1406I (2311)
 2299 TTGCGAAAAAGAAGTTCACGGCGACTACTGCACTTATATACGGTCTCCCCACCCTCGGGAAAAAGGCGGAGCCAGTACACGACATCACTTTCCAGT

EcoNI (2416) **AgeI (2424)** **BspLUII[™]46; 7+**
 2399 TTACCCCGCCACCTTCTAGGCACCGTTCAATTGCCACCCCTCCCCCAACTTCTCGGGACTGTGGCGATGTGCGCTCTGCCACTGACACAT

2499 GTGAGCAAAGGCCAGCAAAGGCCAGAACCGTAAAAAGCCGCGTGTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGA

2599 CGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGGGTTTCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCTGCCGC
2699 TTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAA
2799 GCTGGGCTGTGTGCACGAACCCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCA
2899 CTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAA
2999 CAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTT
3099 TTTTGTTCGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACTCA

3199 **PacI (3229)** **Swal (3237)** **EagI (3245)** **NotI (3245)**
CGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTG
3299 TGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTT
3399 **PvuI (3419)** **Acc65I (3467)**
CTCTATCGAAGGATCTGCGATCGCTGGAAGATTGGAAAGACTGGAAGATAGGAAACACTGGAAGAGGTACCTGAGTCACTGACTCAGTGAGTCACTG
3499 **EcoRI (3512)**
ACTCAGTGAGTAAGAATCTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACT