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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 cells[1]. 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 subunits[5]. NF-κB binds specific decameric DNA sequences (GGGRNNYYCC, R-purine Y=pyrimidine) and activates genes involved in the regulation of the innate and adaptative 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 IRF7[6].

• **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 quantitively 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 promoter[7] and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat[8]. 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 transcription[9].

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

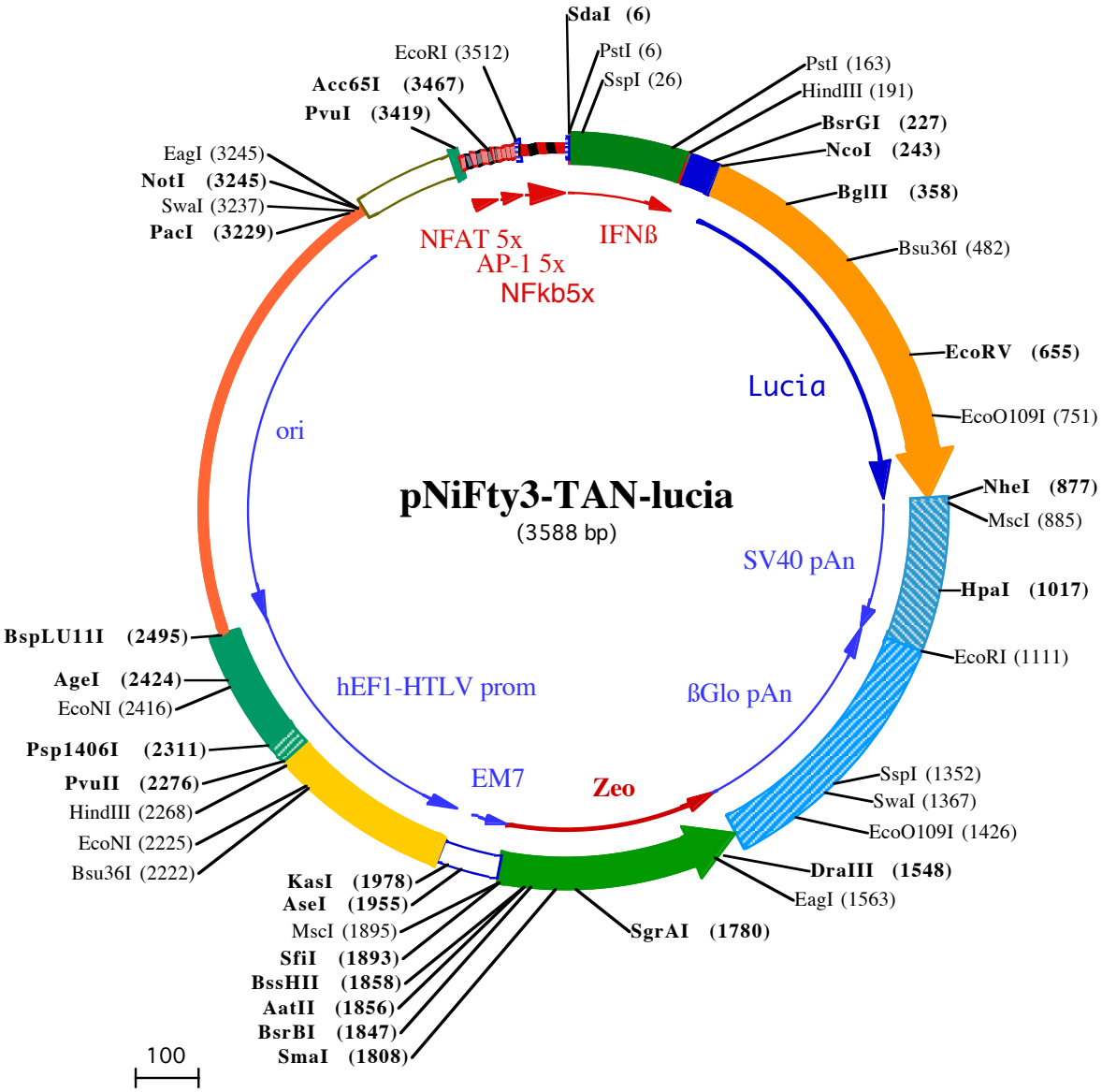
Plasmid resuspension
Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile H_2O. Store resuspended plasmid at -20 °C.

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


pNiFty3-TAN-lucia
(3588 bp)
SdaI (6)
PstI (6)
SspI (26)
PstI (163)
HindIII (191)
BsrGI (227)
NcoI (243)
BglII (358)
Bsu36I (482)
EcoRV (655)
EcoO109I (751)
NheI (877)
MscI (885)
HpaI (1017)
EcoRI (1111)
SspI (1352)
SwaI (1367)
EcoO109I (1426)
DraIII (1548)
EagI (1563)
SgrAI (1780)
KasI (1978)
AseI (1955)
MscI (1895)
SfiI (1893)
BssHII (1858)
AatII (1856)
BsrBI (1847)
SmaI (1808)
BspLU11I (2495)
AgeI (2424)
EcoNI (2416)
Psp1406I (2311)
PvuII (2276)
HindIII (2268)
EcoNI (2225)
Bsu36I (2222)
EcoRI (3512)
Acc65I (3467)
PvuI (3419)
EagI (3245)
NotI (3245)
SwaI (3237)
PacI (3229)
NFAT 5x
AP-1 5x
NFkb5x
IFNß
Lucia
ori
SV40 pAn
hEF1-HTLV prom
ßGlo pAn
EM7
Zeo
100


PstI (6)
SdaI (6)
SspI (26)
PstI (163)
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BspLU11I (2495)


2599 CGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGC
2699 TTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAA
2799 GCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCA
2899 CTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAA
2999 CAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTT
3099 TTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCA
SwaI (3237)
EagI (3245)
PacI (3229)
NotI (3245)
3199 CGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTG
3299 TGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTT
PvuI (3419)
Acc65I (3467)
3399 CTCTATCGAAGGATCTGCGATCGCTGGAAAGATTGGAAAGACTGGAAAGATAGGAAACACTGGAAAGAGGTACCTGAGTCACTGACTCAGTGAGTCACTG
EcoRI (3512)
3499 ACTCAGTGAGTAAGAATTCTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACT