

STOP

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TECHNICAL SUPPORT

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pNiFty3-AN-Lucia

An inducible reporter plasmid selectable with Zeocin™

Catalog code: pnf3-lc6

For research use only

Version 20L03-MM

PRODUCT INFORMATION

Content:

- 20 µg of pNiFty3-AN-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

- **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)¹. 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 subunits². 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 IRF7³.

- **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 promoter⁴ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁵. 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 transcription⁶.

1. Hess J. *et al.*, 2004. AP-1 subunits: quarrel and harmony among siblings. *J Cell Sci.* 117(25):5965-73. 2. Kawai T. & Akira S., 2007. Signaling to NF-kappaB by Toll-like receptors. *Trends Mol Med.* 13(11):460-9. 3. 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. 4. 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. 5. 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. 6. 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

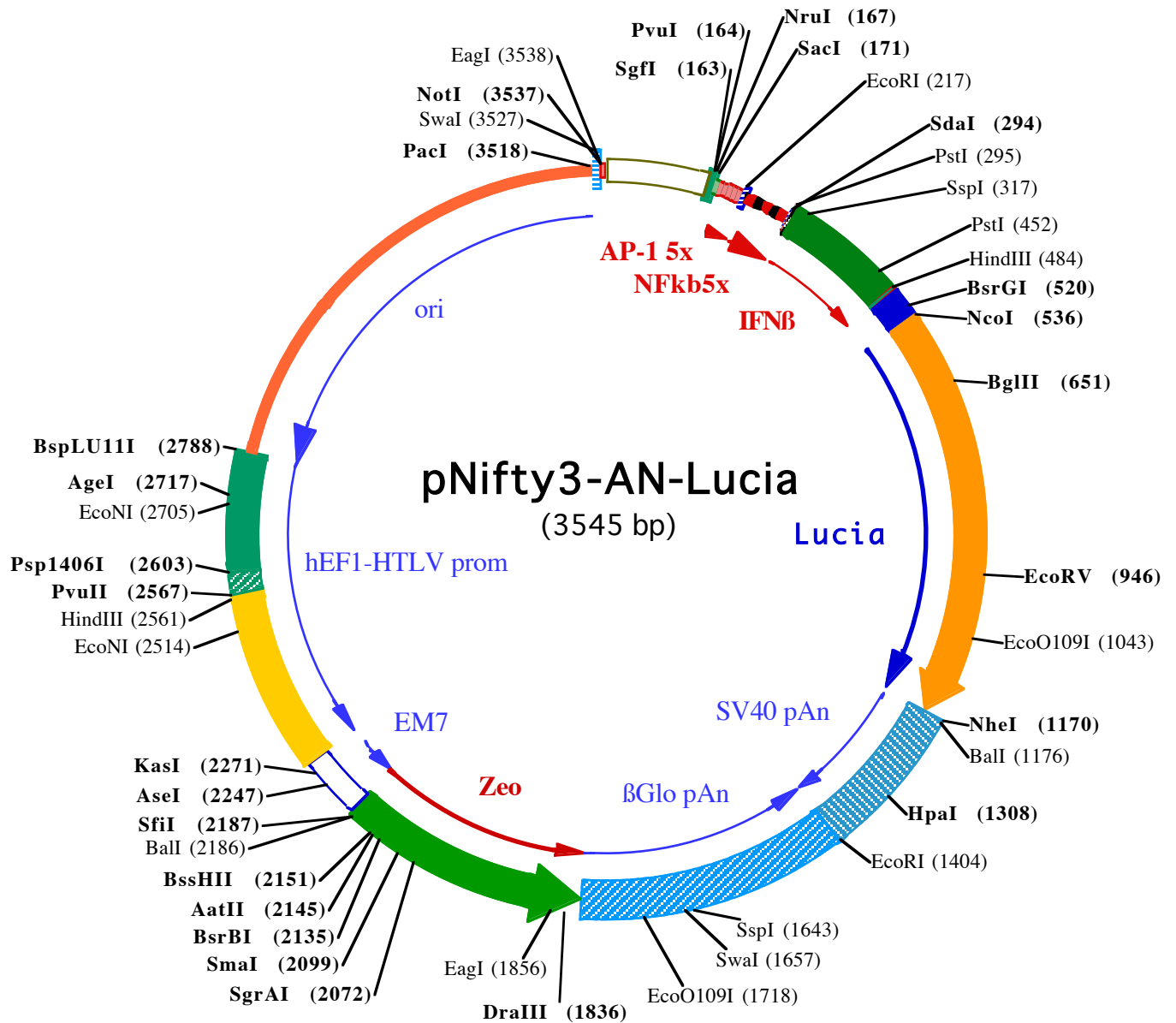
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1 AATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAAAC

SacI (171)
NruI (167)
PvuII (164)
SgfI (163)

101 TAGCAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCGAGCTCTGAGTCACTGACTCAGTGAGTCA

PstI (295)
SdaI (294)

201 CTGACTCAGTGAGTAAGGAATTCCTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTGGGGACTTTCCACTCTGCA

EcoRI (217)
SspI (317)

301 GGagcttgaataaaatgaatattagaagctgtagaataagaaaaatgacagaggaAAACTGAAAGGgAGAACTGAAAGTggaattcctctgaggca

PstI (452)
HindIII (484)

401 gaaaggaccatccctTATAAAtagcacaggccatgaaggaagatcattctcactgcagcctttgacagcctttgcctcatcttgAAGCTCTGCCTTC

BsrGI (520)
NcoI (536)

499 TCCCTCCTGTGAGTTGGTGGTGTACAGTAGCTTCCACCATGGAATCAAGGTGCTGTTGGCCCTCATCTGTATTGCTGTTGCTGAGGCCAAAACCCACT

BglIII (651)

599 GAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACCTTTGCCACCACAGATCTTGAGACTGACCTGTTACCAACTGGGAGACCATGAATGTGA

21▶ Gl u l eAsnGl uAspLeuAsn l eAl aAl aValAl aSerAsnPheAl aThr ThrAspLeuGl uThrAspLeuPheThrAsnTrpGl uThr MetAsnVal l

699 TTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCTGGCAAAAACTCCCCAGATGCTCTGAGGGAGCTGGAGGCCAATGC

54▶ l eSer ThrAspThr Gl uGl nValAsnThrAspAl aAspArgGl yLysLeuProGl yLysLysLeuP roProAspVal l euArgGl uLeuGl uAl aAsnAl

799 CAGAAGGGCTGGTTGCACAAGAGGCTGCCTCATTGCTCTCCACATTAAGTGCACCCCTAAGTGAAGAAATTTATCCCTGGCAGGTGCCACACTTAT

87▶ aArgArgAl aGl yCysThrArgGl yCysLeu l eCysLeuSer Hi s l l eLysCysThr P roLysMetLysLysPhe l l eP roGl yArgCysHi sThr Tyr

EcoRV (946)

899 GAAGGTGAAAAGGAGTCTGCTCAGGGAGGATTGGAGAGGCAATTTGATATCCCAGAGATTCTGGCTTCAAGGATAAGGAGCCACTGGACCAGTTTA

121▶ Gl 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

EcoO109I (1043)

999 TTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAATGTCCAAGTCTGCTGACCTCTGAAGAAGTGGCTTCCCAGAG

154▶ l eAl aGl nVal AspLeuCysAl aAspCysThr Thr Gl yCysLeuLysGl yLeuAl aAsnVal Gl nCysSerAspLeuLeuLysLysTrpLeuP roGl nAr

Ball (1176)
NheI (1170)

1099 GTGTACCACCTTTTCCAGCAAGATTCAAGGTAGGGTGACAAAATCAAGGGTCTGGCTGGGACAGATGATAGCTAGCTGGCCAGACATGATAAGATACA

187▶ gCysThr Thr PheAl aSer Lys l l eGl nGl yArgVal AspLys l l eLysGl yLeuAl aGl yAspArg●●●

1199 TTGATGAGTTGGACAAACCACAACCTAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTG

HpaI (1308)

1299 CAATAAACAAGTTAAACAACAACAAATTGCATTCAATTTATGTTTCAGGTTCAAGGGAGGTGTGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGT

EcoRI (1404)

1399 GGTATGGAATCTAAAATACAGCATAGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGG

1499 GGCTGTTGCCAATGTGCATTAGCTGTTGCAGCCTCACCTTCTTCATGGAGTTAAGATATAGTGTATTTTCCAAGGTTTGAAGTCTCTTCATTTT

SspI (1643)
SwaI (1657)

1599 TTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTTAGTAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAATGTTTTTTATT

EcoO109I (1718)

1699 AGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGACTTAGGGAACAAGGAACCTTTAATAGAAATTTGGACAGCAAGAA

DraIII (1836)
EagI (1856)

1799 AGCGAGCTTCTAGCTTATCCTCAGTCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGTTCGCGCAGGGCGAACTCCGCCCCACGGCTGCT

1274●●●Gl y●●●AspGl nGl uGl uAl aVal l PheHi sVal CysAsnGl yAl aP roAspArgLeuAl aPheGl uArgGl yTrpP roGl nGl

1899 CGCCGATCTCGGTATGCGCCGCGCCGAGGCGTCCCGAAGTTCCTGGACACGACCTCCGACCCTCGGCGTACAGCTCGTCCAGGCGCGCACCCACAC

984▶ uGl y l l eGl uThr Me tAl aP roGl ySer Al aAspArgPheAsnThr Ser Val l Val Gl uSer TrpGl uAl aTyrLeuGl uAspLeuGl yArgVal l TrpVal l

SgrAI (2072)

1999 CCAGGCCAGGGTGTTCGGCACCACCTGGTCTGGACCGCTGATGAACAGGGTTCACGTCGTCGGACCACACCGGCGAAGTCTGCTCCACGAAG

654▶ TrpAl aLeuThrAsnAspP roVal l Val Gl nAspGl nValAl aSer l l ePheLeuThr Val AspAspArgVal l Val Gl yAl aPheAspAspGl uVal l PheA

SmaI (2099)
AatIII (2145)
BsrBI (2135)
BssHIII (2151)
SfiI (2187)
Ball (2186)

2099 TCCCGGAGAACCCGAGCCGGTCCGATCCAGAACTCGACCGCTCCGGCGACGTCGCGCGGGTGGAGCACCAGGCAAGGCACTGGTCACTTGGCCATGATGG

314▶ spArgSer PheGl yLeuArgAspThr TrpPheGl uValAl aGl yAl aVal AspArgAl aThr LeuVal l P roValAl aSer Thr LeuLysAl aMe t

AseI (2247)
KasI (2271)

2199 CCCTCCTATAGTGAGTCGATTATACTATGCCGATATACTATGCCGATGATTAATGTCAACTACTGTTTGTAGGCGCCGGTCACAGCTTGATCTGTAA

2299 CGGCGCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCCAAGGGAGCG

2399 CCGGACAAAGGCCCGGTCTCGACCTGAGCTTTAACTTACCTAGACGGCGACGCAGTTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAA

PvuII (2567)
HindIII (2561)

2499 CCGGCGTGGATGGCGGCTCAGGTAGGGCGGGCGCGCTGAAGGAGAGATGCGAGCCCTCGAAGCTTCAAGCTGTGTTCTGGCGCAAACCGTTGCGA

Psp1406I (2603)

2599 AAAAGAACGTTACGCGGACTACTGCATTATATACGGTCTCCCCACCCTCGGAAAAAGCGGAGCCAGTACACGACATCACTTTCCAGTTTACCC

EcoNI (2705) AgeI (2717) BspLU11I (2788)

2699 CGCGCCACCTTCTCTAGGCACCGTTCAATTGCCGACCCCTCCCCCAACTTCTCGGGGACTGTGGGCGATGTGCGCTCTGCCACTGACACATGTGAGC

2799 AAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCA

2899 AGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCTGCCGCTTACCG

2999 GATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGG

3099 CTGTGTGCACGAACCCCGTTAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCA

3199 GCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTAT

3299 TTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTTGT

3399 TTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAA

Swal (3527) EagI (3538)

PacI (3518) NotI (3537)

3499 GGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGC