

# STOP

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### 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](mailto:info@invivogen.com)



# pNiFty3-A-Lucia

An AP-1-inducible secreted luciferase reporter plasmid selectable with Zeocin™

Catalog code: pnf3-lc3

For research use only

Version 20L03-MM

## PRODUCT INFORMATION

### Content:

- 20 µg of pNiFty3-A-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.

## 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)<sup>1</sup>. 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).
- **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<sup>2</sup>.
- **Lucia luciferase** is a synthetic CpG-free gene that codes for 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 promoter<sup>3</sup> 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<sup>4</sup>. 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<sup>5</sup>.

1. Hess J. *et al.*, 2004. AP-1 subunits: quarrel and harmony among siblings. *J Cell Sci.* 117(25):5965-73. 2. 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. 3. 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. 4. 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. 5. 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 H<sub>2</sub>O. 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 DH5a.

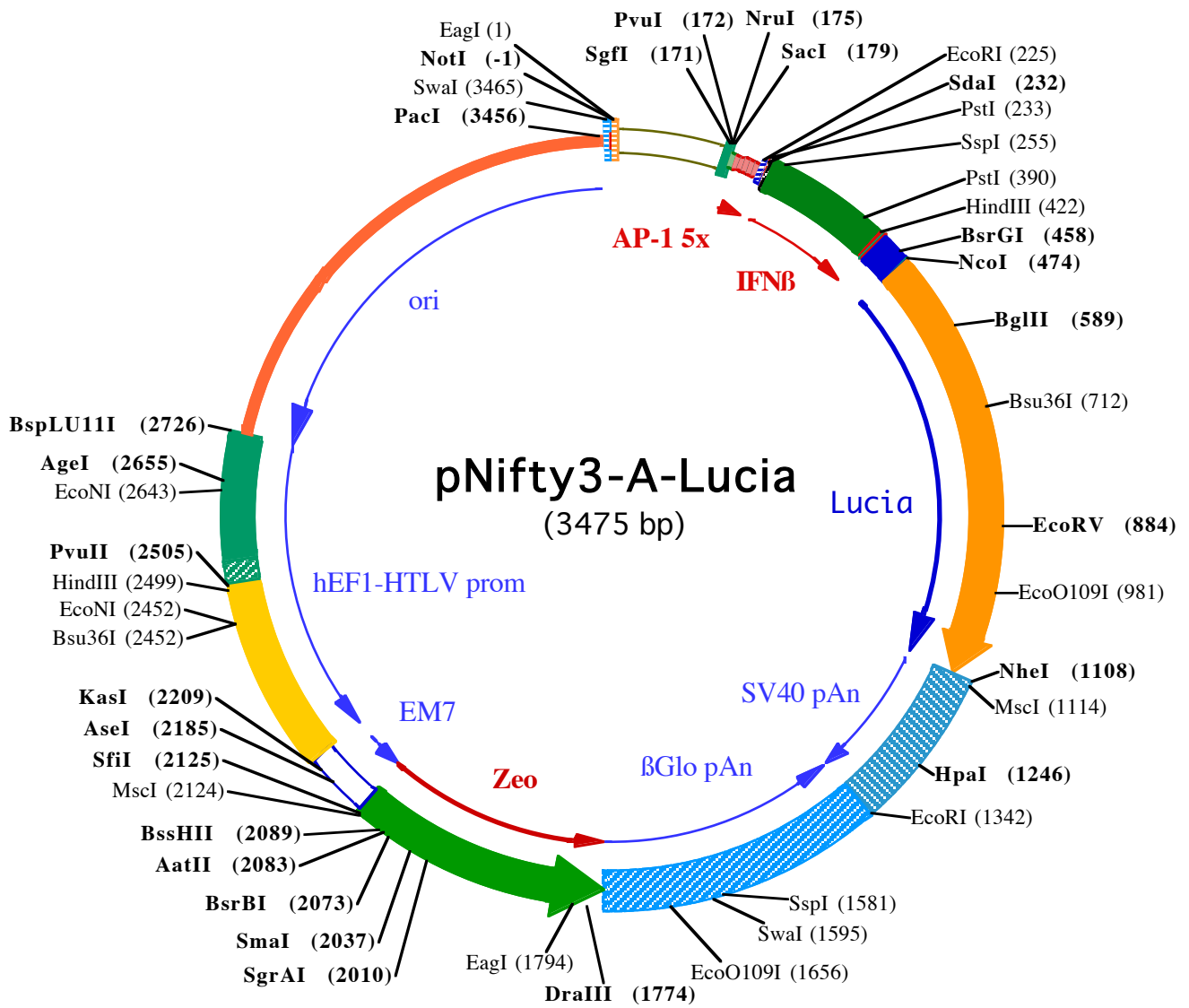
### Zeocin™ usage

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  
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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 GCGGCCGCAATAAAATATCTTTATTTTATTACATCTGTGTGTTGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACA  
SacI (179)  
NruI (175)  
PvuI (172)  
SgfI (171)  
101 AAACAACTAGCAAATAGGCTGTCCCGAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCGCGATCGGAGCTCTGAGTCACTGACTCA  
SdaI (232)  
EcoRI (225) PstI (233) SspI (255)  
201 GTGAGTCACTGACTCAGTGAGTAAAGGAATTCTCTGCAGGagcttgaataaaatgaatattagaagctgtagaataagagaaaatgacagaggaAAACT  
PstI (390)  
301 GAAAGGgAGAACTGAAAGTggaattcctctgaggcagaaggaccatccctTATAAAtagcacaggccatgaaggaagatcattctcactgcagcc  
HindIII (422) BsrGI (458) NcoI (474)  
399 tttgacagcctttgctcatcttgAAGCTTCTGCCTTCTCCCTCTGTGAGTTTGGTTGGTGTACAGTAGCTTCCACCATGGAAATCAAGGTGCTGTTTG  
1 MetGI uI l eLysVal l euPheA  
BglIII (589)  
499 CCCTCATCTGTATTGCTGTTGCTGAGGCAAACCCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCTCCAACCTTGGCCACCAGATCTTGA  
8 l aLeu l l eCys l l eAl aVal l Al aGl uAl aLysProThr Gl u l l eAsnGl uAspLeuAsn l l eAl aAl aVal l Al aSerAsnPheAl aThr ThrAspLeuGl  
599 GACTGACCTGTTCAACAAGTGGGAGACCATGAATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAA  
4 l uThr AspLeuPheThrAsnTrpGl uThr MetAsnVal l l eSer ThrAspThr Gl uGl nVal l AsnThrAspAl aAspArgGl yLysLeuProGl yLysLys  
Bsu36I (712)  
699 CCCCCCAGATGCTCTGAGGGAGCTGGAGCCAATGCCAGAAGGGCTGTTGCACAAGAGGCTGCCTCATTGGCTCTCCACATTAAGTGCACCCCTA  
7 l uP roP roAspVal l euArgGl uLeuGl uAl aAsnAl aArgArgAl aGl yCysThr ArgGl yCysLeu l l eCysLeuSer Hi s l l eLysCysThr P roL  
EcoRV (884)  
799 AGATGAAGAAATTTATCCCTGGCAGGTGCCACACTTATGAAGGTGAAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCAGAGAT  
10 l ysMetLysLysPhe l l eP roGl yA rgCysHi sThr TyrGl uGl yGl uLysGl uSerAl aGl nGl yGl y l l eGl yGl uAl a l l eVal l Asp l l eP roGl u l l  
EcoO109I (981)  
899 TCCTGGCTTCAAGGATAAGGAGCCACTGGACCAGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCTTGGCAATGTC  
14 l eP roGl yPheLysAspLysGl uP roLeuAspGl nPhe l l eAl aGl nVal l AspLeuCysAl aAspCysThr Thr Gl yCysLeuLysGl yLeuAl aAsnVal  
999 CAGTGCTCTGACCTCTGAAGAAGTGGCTTCCCCAGAGGTGTACCACTTTTCCAGCAAGATTACAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGG  
17 l Gl nCysSerAspLeuLeuLysLysTrpLeuP roGl nArgCysThr Thr PheAl aSer Lys l l eGl nGl yA rgVal l AspLys l l eLysGl yLeuAl aGl yA  
MscI (1114)  
NheI (1108)  
1099 ACAGATGATAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAAT  
20 l spArg●●●  
HpaI (1246)  
1199 TGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAGTTAAACAACAACAAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGGTG  
EcoRI (1342)  
1299 GGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAAATCTAAAATACAGCATAGCAAACTTTAACCTCCAAATCAAGCCTCTACTTGA  
1399 ATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTAAGATAT  
SspI (1581) SwaI (1595)  
1499 AGTGTATTTTCCAAGGTTTGAAGTACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTATGTAATAATTCAGAAATAATT  
EcoO109I (1656)  
1599 TAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTGTGGACTTAGG  
DraIII (1774) EagI (1794)  
1699 GAACAAAGGAACCTTTAATAGAAATGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGC  
127 l ●●●Gl y●●●AspGl nGl uGl uAl aVal l PheHi sVal l CysAsnGl yAl a  
1799 CGGGTCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGGTATGGCCGCGCCGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGAC  
11 l P roAspArgLeuAl aPheGl uArgGl yTrpP roGl nGl uGl y l l eGl uThr MetAl aP roGl ySer Al aAspArgPheAsnThr Ser Val l Gl uSer T  
1899 CACTCGGCGTACAGCTCTGCCAGGCGCGCACCCACCCAGGCCAGGGTGTGTCCGGCACCTGTCTGACCCGCGCTGATGAACAGGGTCAAGT  
77 l rP gl uAl aTyrLeuGl uAspLeuGl yArgVal l TrpVal l TrpAl aLeuThrAsnAspP roVal l Val l Gl nAspGl nVal l Al aSer l l ePheLeuThr Val l As  
AatII (2083)  
SgrAI (2010) SmaI (2037) BsrBI (2073) BssHII (2089)  
1999 CGTCCCGGACACACCGGCGAAGTCTCTCCACGAAGTCCCGGAGAACCCGAGCCGGTCCGAGTCCAGAACTCGACCCGCTCCGGCGACGTCGCGCGGGT  
44 l pAspArgVal l Val l Gl yAl aPheAspAspGl uVal l PheAspArgSer PheGl yLeuArgAspThr TrpPheGl uVal l Al aGl yAl aVal l AspArgAl aThr  
SfiI (2125) MscI (2124) AseI (2185)  
2099 GAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCCCTCTATAGTGTGATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAC  
11 l LeuVal l P roVal l Al aSer Thr LeuLysAl aMet  
KasI (2209)  
2199 TACTGTTTGTAGGCGCGGTACAGCTTGATCTGTAAACGGCGCAGAACGAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGT  
2299 GGAGAGCCGGCTGAGTCTAGGTAGGCTCCAAGGGAGCGCCGACAAAGGCCCGTCTCGACCTGAGCTTTAAACTTACTAGACGGCGGACGAGTTTCAG  
EcoNI (2452) Bsu36I (2452)  
2399 GAGGCACCACAGGCGGGAGCGGCCAGAACGCGACTCAACCGCGTGGATGGCGGCCCTCAGGTAGGGCGGGCGCGGTGAAGGAGAGATGCGAGCCCTC  
PvuII (2505) HindIII (2499)  
2499 GAAGCTTCAGCTGTGTTCTGGCGCAAACCGTTGCGAAAAGAAGTTCACGGCGACTACTGCCTTATATACGGTTCTCCCCACCTCGGGAAAAAG

EcoNI (2643) AgeI (2655)

2599 GCGGAGCCAGTACACGACATCACTTTCCAGTTTACCCCGCGCCACCTTCTAGGCACCGGTTCAATTGCCGACCCCTCCCCCAACTTCTCGGGGACT

BspLU11I (2726)

2699 GTGGGGGATGTGCGCTCTGCCCACTGACACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGC

2799 TCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTC

2899 CCTCGTGCCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCCTTCTCCCTTCGGGAAGCGTGGCGCTTTCATAGCTCACGCTGTAGG

2999 TATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTG

3099 AGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAA

3199 GTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCC

3299 GGCAAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTA

SwaI (3465)

PacI (3456)

3399 CGGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAA**CATTTAAATCA**