

STOP

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TECHNICAL SUPPORT

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pNiFty3-I-Lucia

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

Catalog code: pnf3-lc4

For research use only

Version 20L03-MM

PRODUCT INFORMATION

Content:

- 20 µg of pNiFty3-I-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

- **ISRE binding site:** PRRs involved in the antiviral response induce the activation of interferon regulatory factors (IRFs) and the production of type I interferons (IFNs). IFNs trigger the formation of the ISGF3 complex which contains signal transducer and activator of transcription (STAT) 1, STAT2 and IRF9. ISGF3 and IRFs bind to specific nucleotide sequences called interferon-stimulated response elements (ISREs; AGTTTCNNTTCC) in the promoter of IFN-stimulated genes (ISGs) leading to their activation¹.
- **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 promoter³ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat⁴. 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 transcription⁵.

1. **Wesoly J. et al., 2007.** STAT activation and differential complex formation dictate selectivity of interferon responses. *Acta Biochim Pol.* 54(1):27-38. 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): 217-23. 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: 466-72. 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 H₂O. Store resuspended plasmid at -20 °C.

Plasmid amplification and cloning

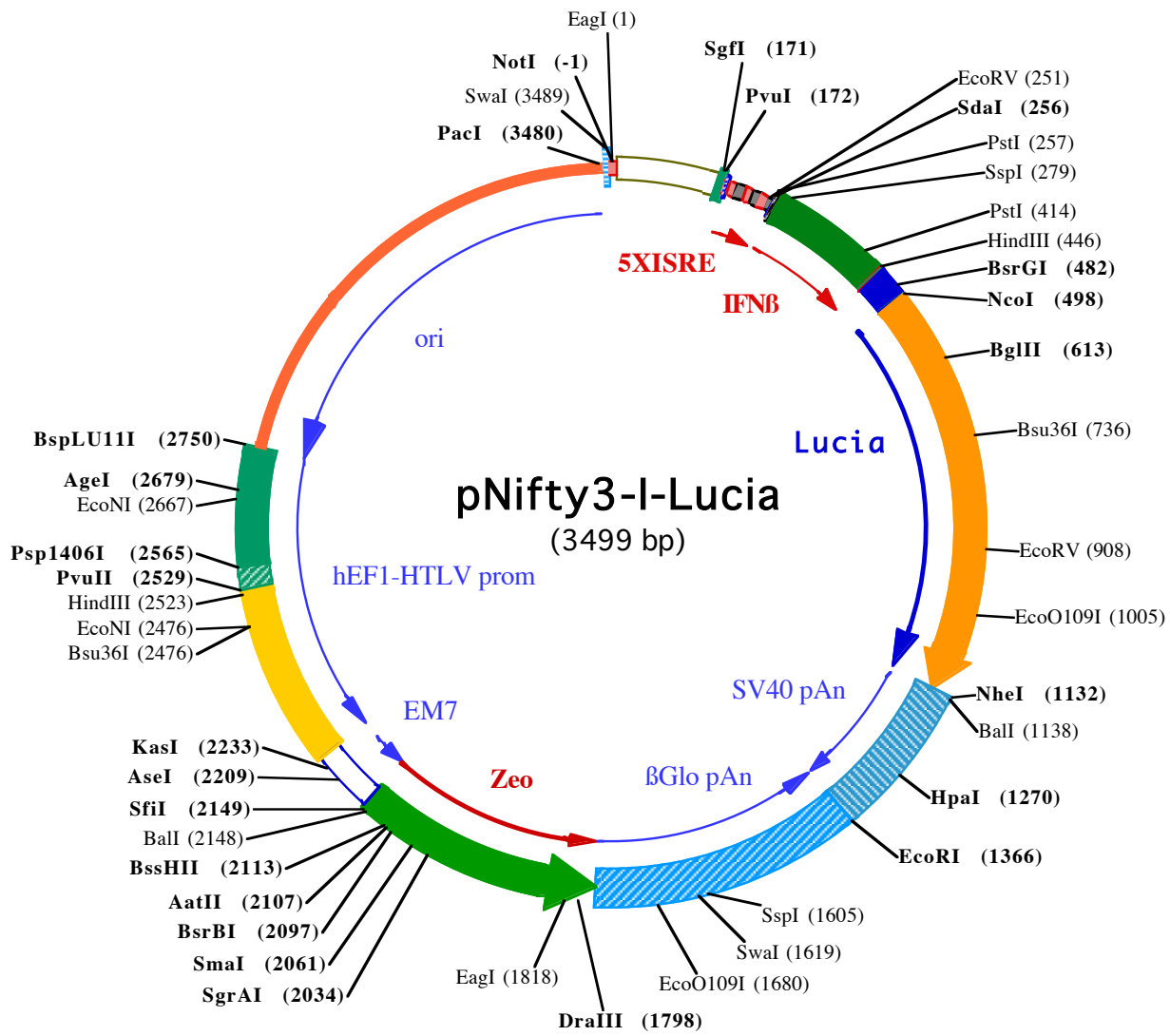
Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5α.

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

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100

EagI (1)
NotI (-1)
1 GCGGCCGCAATAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTGTGTAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACA

PvuII (172)
SgfI (171)
106 AACTAGCAAATAGGCTGCCCCAGTGCAGTGCAGGTGCCAGAACATTTCTATCGAAGGATCTGCGATCGCTGAATTAGTTTCACCTTCCAGTTTCAGTTTC

PstI (257)
SdaI (256)
EcoRV (251) SspI (279)
211 CAGTTTCATTTCCAGTTTCATTTCCAGTTTCATTTCTGATATCCTGCAGGagcttgaataaaatgaatattagaagctgttagaataagagaaaatgacag

PstI (414)
316 aggaAAACTGAAAGGgAGAAGTGAAGTggaaattcctctgaggcagaaggaccatccctTATAAAtagcacaggccatgaaggaagatcattctcactgc

HindIII (446) BsrGI (482) NcoI (498)
419 agcctttgacagcctttgctcattcttAGCTTCTGCTTCTCCCTCTGTGAGTTTGGTGGTGTACAGTAGCTTCCACCATGGAAATCAAGGTGCTGTTTGC

BglIII (613)
524 CCTCATCTGTATTGCTGTTGCTGAGGCAAAACCCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACCTTGGCCACCAGATCTTGAGACTGA

81aLeuI l eCysI l eAl aVaI Al aGl uAl aLysProThrGl uI l eAsnGl uAspLeuAsnI l eAl aAl aVaI Al aSerAsnPheAl aThr ThrAspLeuGl uThrAs
629 CTTGTTCAACCACTGGGAGACCATGAATGTGATTAGCACTGACACAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAACCTCCCCCAGA

43pLeuPheThrAsnTrpGl uThr Me tAsnVal I l eSer ThrAspThr Gl uGl nVal lAsnThrAspAl aAspArgGl yLysLeuP roGl yLysLysLeuP roP roAs
Bsu36I (736)
734 TGTCTGAGGGAGCTGGAGGCCAATGCCAGAAAGGCTGGTGCACAAGAGGCTGCCTCATTGCTCTCCACATTAAGTGACCCCTAAGATGAAGAAATTTAT

78pVal lLeuArgGl uLeuGl uAl aAsnAl aArgArgAl aGl yCysThr ArgGl yCysLeuI l eCysLeuSer Hi sI l eLysCysThr ProLysMetLysLysPheI l
EcoRV (908)
839 CCCTGGCAGGTGCCACACTTATGAAGGTGAAAAGGAGTCTGCTCAGGAGGGATTGGAGAGCAATTTGTGATATCCAGAGATTCTGGCTTCAAGGATAAGGA

113pEProGl yArgCysHi sThr TyrGl uGl yGl uLysGl uSer Al aGl nGl yGl yI l eGl yGl uAl aI l eVal lAspI l eP roGl uI l eP roGl yPheLysAspLysGl
EcoO109I (1005)
944 GCCACTGGACAGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCTTGGCAATGTCCAGTGTCTGACCTCTGAAGAAGTG

148pUProLeuAspGl nPheI l eAl aGl nVal lAspLeuCysAl aAspCysThr Thr Gl yCysLeuLysGl yLeuAl aAsnVal lGl nCysSer AspLeuLeuLysLysTr
Ball (1138)
1049 GTTCCCCAGAGGTGTACCCTTTTGGCAGCAAGATTCAAGGGTCTGGCTGGGGACAGATGATAGCTAGCTGGCCAGACATGATA

183pLeuP roGl nArgCysThr Thr PheAl aSerLysI l eGl nGl yArgVal lAspLysI l eLysGl yLeuAl aGl yAspArg•••
1154 AGATACATTGATGAGTTTGGACAAACCACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGC

HpaI (1270)
1259 TGCAATAAACAAAGTTAACAAACAATTCATTCTTTTATGTTTCAGGTTTCAGGGGAGGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGT

EcoRI (1366)
1364 ATGGAATTCATAAATACAGCATAGCAAACTTTAACCTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGCTGTTG

1469 CCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTCATGGAGTTTAAAGATATAGTGATTTTTCCCAAGGTTTGAAC TAGCTCTTCATTTCTTTATGTTTTAAA

SspI (1605) SwaI (1619)
1574 TGCCTGACCTCCACATTCCCTTTTTAGTAAAATATTCAGAAAATAATTTAAATACATCATTGCAATGAAAATAATGTTTTTATTAGGCAGAATCCAGATGCT

EcoO109I (1680)
1679 CAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTAAATAGAAATTTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCA

1274•••Gl y•••
1784 GTCCTGCTCCTCTGCCACAAAGTGCACGAGTTGCCGGCCGGTGCAGGCGAATCCCGCCCCACGGCTGCTGCGCATCTCGGTATGCGCCGGCCCGGA

124AspGl nGl uGl uAl aVal lPheHi sVal lCysAsnGl yAl aP roAspArgLeuAl aPheGl uArgGl yTrpP roGl nGl uGl yI l eGl uThr Me tAl aP roGl ySer
1889 GCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCTACAGCTGCTCCAGGCGCGCACCCACCCAGCCAGGGTGTTCGGCCACCCTGGTC

89Al aAspArgPheAsnThr Ser Val lVal lGl uSer TrpGl uAl aTyrLeuGl uAspLeuGl yArgVal lTrpVal lTrpAl aLeuThrAsnAspP roVal lVal lGl nAsp
SgrAI (2034) SmaI (2061) BsrBI 2097
1994 CTGGACCGCTGATGAACAGGGTACGCTGCTCCCGACACCGCGAAGTCTCCTCCACGAAGTCCCGGGAGAACCAGCGGCTCGGTCCAGAAGTCCGAC

54Gl nVal lAl aSerI l ePheLeuThr Val lAspAspArgVal lVal lGl yAl aPheAspAspGl uVal lPheAspArgSer PheGl yLeuArgAspThr TrpPheGl uVal l
BssHII (2113) SfiI (2149)
2099 CGCTCCGGGACGTCGCGCGGCTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCCCTCTATAGTAGTCTATTATACTATGCGGATATACTATG

19Al aGl yAl aVal lAspArgAl aThr LeuVal lP roVal lAl aSer Thr LeuLysAl aMet
AatII (2107) Ball (2148)
2204 CCGATGATTAATGTCAACTACTGTTTGTAGGCGCCGTACAGCTTGATCTGTAACGGCGCAGAACGAAACAAAGACGTAGAGTTGAGCAAGCAGG

AseI (2209) KasI (2233)
2309 GTCAGGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCAAGGGAGCGCCGACAAAGGCCCGGTCTGACCTGAGCTTTAAACTTACCTAGACGGCGGAC

EcoNI (2476) Bsu36I (2476)
2414 GCAGTTCAGGAGGCCACCAGCGGGAGCGGCAGAACCGGACTCAACCGCGTGGATGCCGGCCTCAGGTAGGGCGGGCGCGTGAAGGAGAGATGCGAGCC

PvuII (2529)
HindIII (2523) Psp1406I (2565)
2519 CTTGAAAGCTTCAAGCTGTGTTCTGGCGGCAAAACCCGTTGCGAAAAGAACGTTCAAGGCGACTACTGCACTTATATACGGTTCTCCCCACCTCGGGAAAAAGG

EcoNI (2667) AgeI (2679)
2624 CGGAGCCAGTACACGACATCACTTTCCAGTTTACCCCGGCCACTTCTTAGGCACCGGTTCAATTGCCGACCCTCCCCCAACTTCTCGGGACTGTGGCC

BspLU11I (2750)
2729 GATGTGCGCTCTGCCACTGACACATGTGAGCAAAAGGCCAGAAAAGGCCAGAACCGTAAAAAGCCGCTTCTGCTGGCGTTTTTCCATAGGCTCCGCCCTCT

2834 GACGAGCATCAAAAAATCGACGCTCAAGTCAAGGTGGCGAAAACCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTGTCGCTCTCCT

2939 GTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCCGGAAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTC

3044 GTTCGCTCAAGCTGGGCTGTGTGCACGAACCCCGTTACGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCAACCCGGTAAGACACGACTTA

3149 TCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGA

3254 ACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTT

3359 GTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGG

Swal (3489)

PacI (3480)

3464 ATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA