

STOP

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TECHNICAL SUPPORT

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pNiFty3-Lucia

Secreted luciferase reporter plasmid selectable with Zeocin™

Catalog code: pnf3-lc1

For research use only

Version 20L03-MM

PRODUCT INFORMATION

Content:

- 20 µg of pNiFty3-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.

PLASMID FEATURES

- **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 IRF7¹.
- **Lucia luciferase** is a synthetic CpG-free gene that codes for a secreted coelenterazine-utilizing luciferase.
ORF size (from the ATG to the stop codon): 634 bp
Lucia luciferase activity can be evaluated using QUANTI-Luc™ (catalog 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 promoter² and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat³. 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 transcription⁴.

References

1. **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. 2. **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. 3. **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. 4. **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

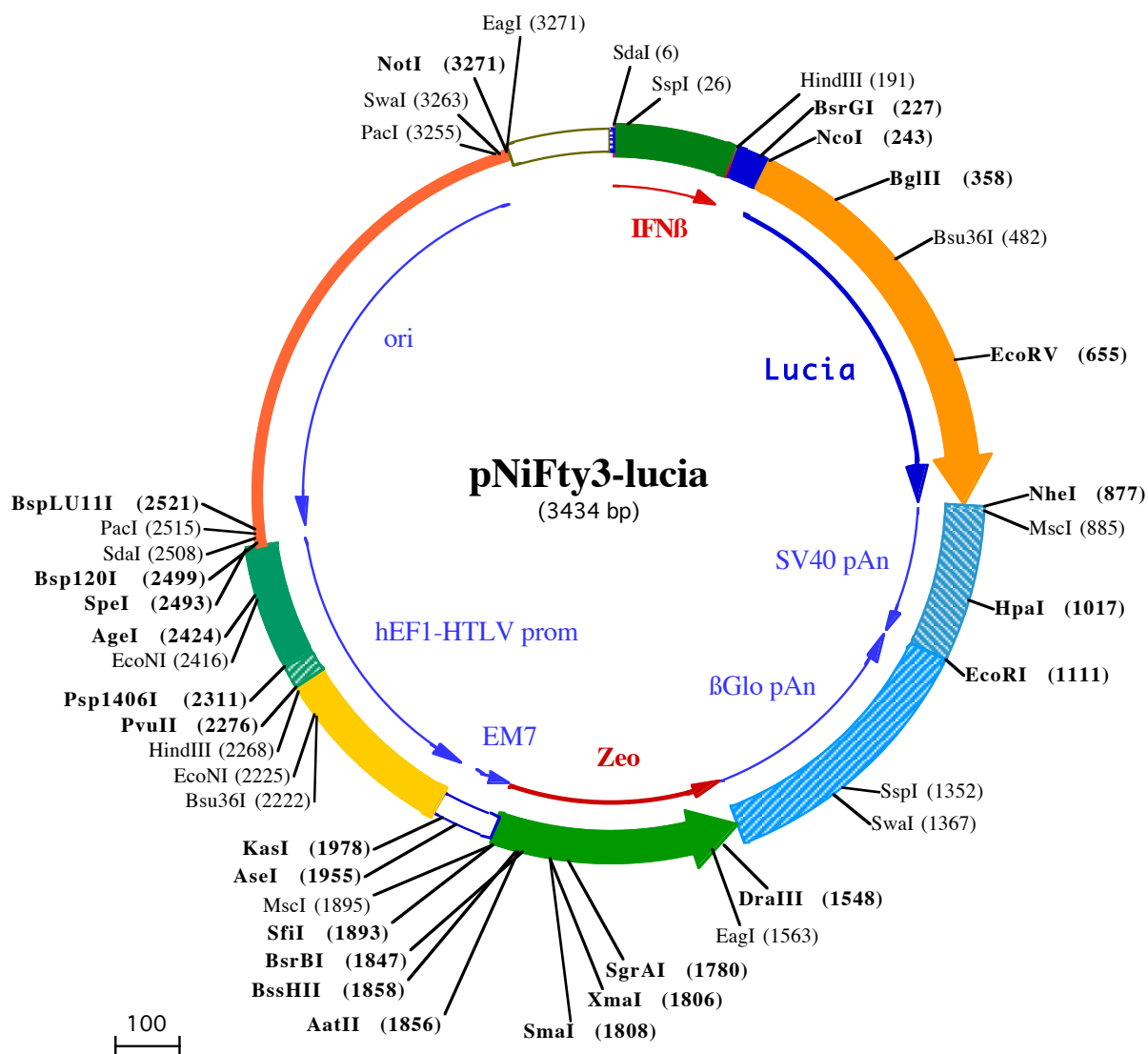
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SdaI (6) SspI (26)
1 CCTGCAGGagccttgaataaaatgaatatttagaagctgttagaataagagaaaatgacagaggaAAACTGAAAGGgAGAAGTAAAAGTggaattcctct
HindIII (191)
101 gaggcagaaaggaccatccctTATAAAtagcacaggccatgaaggaagatcattctcactgcagcctttgacagcctttgctcatcttgAGCTTCT
BsrGI (227) NcoI (243)
199 GCCTTCTCCCTCCTGTGAGTTGGTTGGTGTACAGTAGCTCCACCATGGAAATCAAGGTGCTGTTTGCCTCATCTGTATTGCTGTTGCTGAGGCCAAA
1▶MetGluLeuLysValLeuPheAlaLeuLeuCysIleAlaValAlaGluAlaLys
BglIII (358)
299 CCCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACTTGGCCACCACAGATCTTGAGACTGACCTGTTCACTGGGAGACCTGAA
19▶ProThrGluLeuAsnGluAspLeuAsnIleAlaAlaValAlaSerAsnPheAlaThrThrAspLeuGluThrAspLeuPheThrAsnTrpGluThrMetA
Bsu36I (482)
399 ATGTGATTAGCACTGACACAGAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCTGGCAAAAACTCCCCCAGATGCTCTGAGGGAGCTGGAGGC
52▶snValIleSerThrAspThrGluGluValAsnThrAspAlaAspArgGluLysLeuProGluLysLysLeuProProAspValLeuArgGluLeuGluAla
499 CAATGCCAGAAAGGCTGGTTGCACAGAGGCTGCCTCATTGGCTCTCCACATTAAGTGCACCCCTAAGATGAAGAAATTTATCCCTGGCAGGTGCCAC
85▶aAsnAlaArgArgAlaGluCysThrArgGluCysLeuLeuCysLeuSerHisIleLysCysThrProLysMetLysLysPheIleProGluYArgCysHis
EcoRV (655)
599 ACTTATGAAGGTGAAAAGGAGTCTGCTCAGGGAGGGATTGGAGAGGCAATTGTTGATATCCAGAGATTCTGGCTTCAAGGATAAGGAGCCACTGGACC
119▶ThrTyrGluGluLysGluSerAlaGluNluGluYleGluYluAlaIleValAspIleProGluIleProGluPheLysAspLysGluProLeuAspG
699 AGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCTTGCCAATGTCCAGTGCTCTGACCTCTGAAGAAGTGGCTTCC
152▶IlePheIleAlaGluValAspLeuCysAlaAspCysThrThrGluCysLeuLysGluLeuAlaAsnValGluNcysSerAspLeuLysLysTrpLeuP
MscI (885)
799 CCAGAGGTGTACCACTTTTGCAGCAAGATTCAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGACAGATGATAGCTAGCTGGCCAGACATGATAA
185▶oGluNArgCysThrThrPheAlaSerLysIleGluNluYArgValAspLysIleLysGluYLeuAlaGluYAspArg●●●
899 GATACATTGATGAGTTTGGACAAACCACAACACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACATTAT
HpaI (1017)
999 AAGCTGCAATAAACAAAGTTAAACAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTAC
EcoRI (1111)
1099 AAATGTGGTATGGAATCTAAAATACAGCATAGCAAACTTTAACCTCCAATCAAGCCTCTACTGAATCCTTTCTGAGGGATGAATAAGGCATAGGC
1199 ATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTAAGATATAGTGATTTTCCCAAGGTTTGAAGTAGCTCTT
SspI (1352) SwaI (1367)
1299 CATTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTGTAGTAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTT
1399 TTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAG
DraIII (1548) EagI (1563)
1499 CAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGTGCGCGAGGGCGAACTCCCGCCCCACG
1274●●●Gly●●●AspGluNluGluAlaValPheHisValCysAsnGluYAlaProAspArgLeuAlaPheGluArgGluYTrpPro
1599 GCTGCTCGCGCATCTGGTCATGGCCGGCCGGAGGCGTCCCGAAGTCTGTGGACACGACCTCCGACCCTCGGCGTACAGCTCGTCCAGGCCGCGCAC
1000oGluNluGluYleGluThrMetAlaProGluYSerAlaAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGluYArgVal
SgrAI (1780)
1699 CCACACCCAGGCCAGGGTGTGTCCGGCACCACCTGGTCTGGACCGCGCTGATGAACAGGGTCACGTCGTCGCCGACCACACCGCGGAAGTCGTCTCC
674TrpValTrpAlaLeuThrAsnAspProValValGluNAspGluValAlaSerIlePheLeuThrValAspAspArgValValGluYAlaPheAspAspGluUV
XmaI (1806) AatII (1856) SfiI (1893)
1799 ACGAAGTCCCGGGAGAACCCGAGCCGGTCCGAGAACTCGACCGCTCCGGCGACGTCGCGCGGTTGAGCACCGGAACCGCACTGGTCAACTTGGCCA
334AlPheAspArgSerPheGluYLeuArgAspThrTrpPheGluValAlaGluYAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMe
SmaI (1808) BsrBI (1847) BssHII (1858) MscI (1895)
1899 TGATGGCCCTCTATAGTGAGTCGATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTTGTAGGCGCGGTCACAGCTTGAT
04t
1999 CTGTAACGGCGCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAAGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCCAAG
2099 GGAGCGCCGGACAAAGGCCCGTCTCGACCTGAGCTTTAACTTACCTAGACGGCGACGAGTTCAAGAGGACACCAGGCGGGAGCGGCGGAGAACCGG
EcoNI (2225) PvuII (2276)
2199 ACTCAACCGCGTGATGGCGCCCTCAGGTAGGGCGGCGCGGTGAAGGAGAGATGCGAGCCCTCGAAGCTTCAGCTGTGTTCTGGCGCAAACCCG
Bsu36I (2222) HindIII (2268)
2299 TTGCGAAAAAGAACGTTACGGCGACTACTGCACTTATATACGGTTCTCCCCACCTCGGGAAAAAGCGGAGCCAGTACACGACATCACTTTCCAGT
Psp1406I (2311)
2399 TTACCCCGCCACCTTCTAGGACCGGTTCAATTGCCGACCCCTCCCCCACTTCTCGGGACTGTGGCGATGTGGCTCTGCCACTGACTAGT
EcoNI (2416) AgeI (2424) SpeI (2493)
2499 GGGCCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCC
SdaI (2508) PaeI (2515)
2499 GGGCCCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCC
Bsp120I (2499) BspLU11I (2521)
2599 CCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCTGGAAAGCTCCCTCGT

2699 GCGCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTTCATAGCTCACGCTGTAGGTATCTC
2799 AGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCA
2899 ACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGGGTGCTACAGAGTTCTTGAAGTGGTG
2999 GCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAA
3099 CAAACCACCGCTGGTAGCGGTGGTTTTTTTGGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGT

EagI (3271)
PacI (3255) SmaI (3263) **NotI (3271)**
3199 CTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCCAATAAAATATCTTTATTTCA
3299 TTACATCTGTGTGGTTGGTTTTTTTGGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCA
3399 GTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA