STOP

Before using this product, please read the Limited Use License statement below:

Important Limited Use License information for pNiFty3-I-Lucia

The purchase of the pNiFty3-I-Lucia vector conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes.

Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic, or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research.

If the purchaser is unwilling to accept the limitations of this limited use statement, InvivoGen is willing to accept return of the product with a full refund. The product must be returned in resaleable condition. For information on purchasing a license to this product for purposes other than research, contact InvivoGen, 10515 Vista Sorrento Parkway San Diego, CA 92121 USA. Tel: 858-457-5873 Fax: 858-457-5843.

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; AGTTTCNNTTTCC) in the promoter of IFN-stimulated genes (ISGs) leading to their activation[1].

• **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[2].

• **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[3] 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[4]. 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[5].

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 H_2O. Store resuspended plasmid at -20 °C.

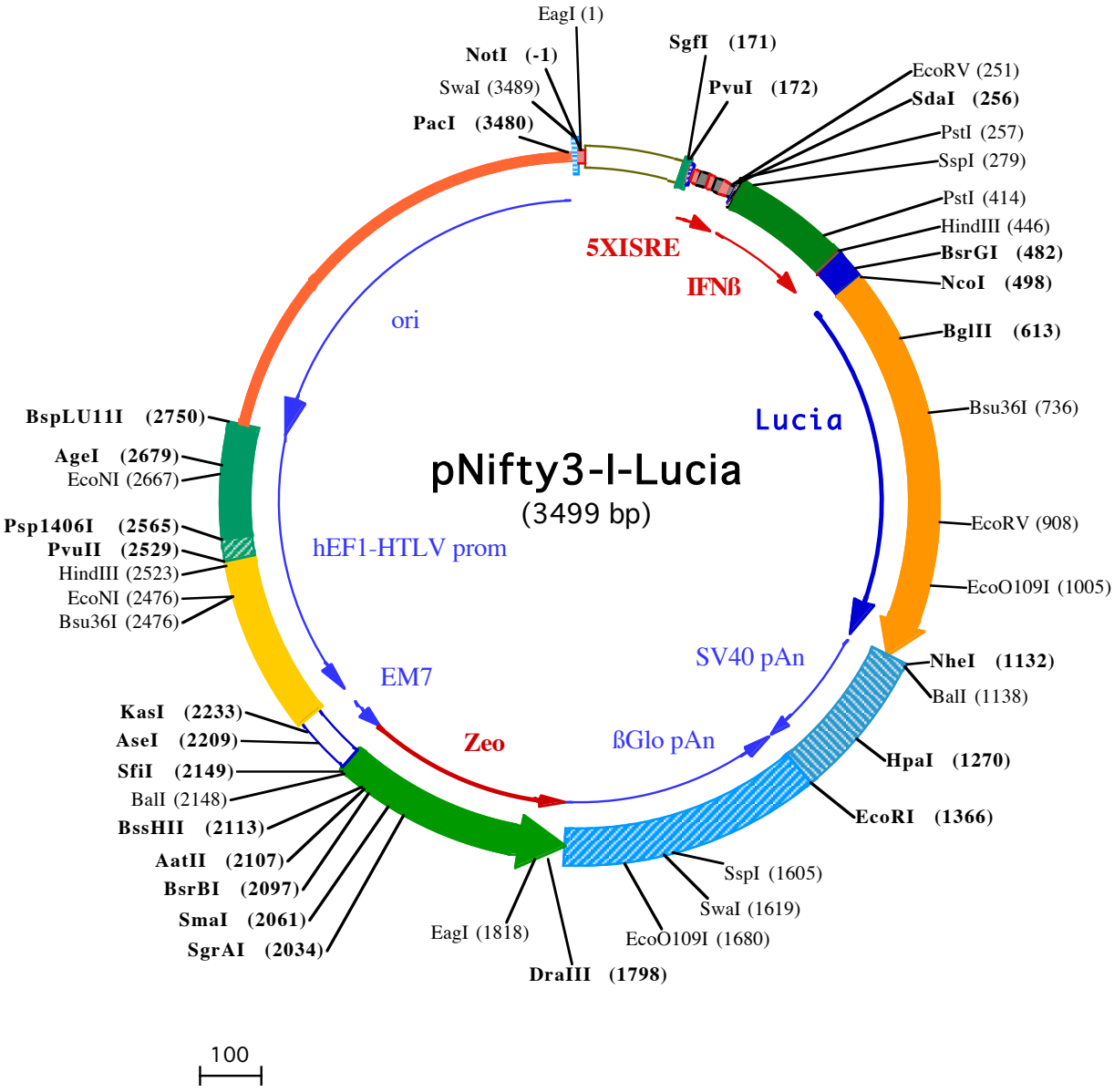
Plasmid amplification and cloning

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.

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


pNifty3-I-Lucia
(3499 bp)
5XISRE
IFNß
Lucia
ori
hEF1-HTLV prom
EM7
Zeo
SV40 pAn
ßGlo pAn
EagI (1)
NotI (-1)
SwaI (3489)
PacI (3480)
SgfI (171)
PvuI (172)
EcoRV (251)
SdaI (256)
PstI (257)
SspI (279)
PstI (414)
HindIII (446)
BsrGI (482)
NcoI (498)
BglII (613)
Bsu36I (736)
EcoRV (908)
EcoO109I (1005)
NheI (1132)
BalI (1138)
HpaI (1270)
EcoRI (1366)
SspI (1605)
SwaI (1619)
EcoO109I (1680)
DraIII (1798)
EagI (1818)
SgrAI (2034)
SmaI (2061)
BsrBI (2097)
AatII (2107)
BssHII (2113)
BalI (2148)
SfiI (2149)
AseI (2209)
KasI (2233)
Bsu36I (2476)
EcoNI (2476)
HindIII (2523)
PvuII (2529)
Psp1406I (2565)
EcoNI (2667)
AgeI (2679)
BspLU11I (2750)
100


EagI (1)
NotI (-1)
PvuI (172)
SgfI (171)
PstI (257)
SdaI (256)
EcoRV (251)
SspI (279)
PstI (414)
HindIII (446)
BsrGI (482)
NcoI (498)
BglII (613)
Bsu36I (736)
EcoRV (908)
EcoO109I (1005)
BalI (1138)
NheI (1132)
HpaI (1270)
EcoRI (1366)
SspI (1605)
SwaI (1619)
EcoO109I (1680)
DraIII (1798)
EagI (1818)
SgrAI (2034)
SmaI (2061)
BsrBI 2097
BssHII (2113)
SfiI (2149)
AatII (2107)
BalI (2148)
AseI (2209)
KasI (2233)
EcoNI (2476)
Bsu36I (2476)
PvuII (2529)
HindIII (2523)
Psp1406I (2565)
EcoNI (2667)
AgeI (2679)
BspLU11I (2750)

3254 ACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTT

3359 GTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGG

SwaI (3489)

PacI (3480)

3464 ATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA