

Product usage

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

Important Limited Use information for pNiFty3-I-Lucia-Zeo

The purchase of the pNiFty3-I-Lucia-Zeo 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 us at outlicensing@invivogen.com.

TECHNICAL SUPPORT

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pNiFty3-I-Lucia-Zeo

IRF-inducible reporter plasmid selectable with Zeocin®

Catalog code: pnf3-lc4

<https://www.invivogen.com/pnifty3-family>

For research use only

Version 23H08-NJ

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized pNiFty3-I-Lucia-Zeo (plasmid DNA)
- 1 ml of Zeocin® (100 mg/ml)

Storage and Stability

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C.
- Resuspended DNA is stable for 1 year at -20°C.
- Store Zeocin® at 4°C or -20°C. The expiry date is specified on the product label.

Quality control

- Plasmid construct is confirmed by restriction analysis and full-length open reading frame (ORF) sequencing.
- After purification by ion exchange chromatography, predominant supercoiled conformation is verified by electrophoresis.

PLASMID FEATURES

- **ISRE-5x mIFN-β** is an engineered murine interferon beta (mIFN-β) promoter comprising different positive regulatory domains that bind transcription factors such as NF-κB, IRF3 and IRF71. This minimal promoter is IRF-specific due to the addition of several interferon-stimulated response elements (ISRE) repeated transcription factor binding sites (TFBS) (AGTTTCNNTTTC)². This feature also enhances the IRF-mediated transcription of the *Lucia* reporter gene.
- **Lucia** is a secreted coelenterazine luciferase encoded by a synthetic gene developed by InvivoGen. It generates 1000-fold higher bioluminescent signal compared to the commonly used Firefly and Renilla luciferases. Lucia luciferase activity can be evaluated using QUANTI-Luc™ 4 Lucia/Gaussia (cat. code: rep-qlc4lg1), an assay reagent containing all the components required to quantitatively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases.
- **SV40 pAn** is the Simian Virus 40 late polyadenylation (pAn) signal and it enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.
- **Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.
- **EF-1α/HTLV hybrid promoter** 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 DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency.

Zeocin® antibiotic selection cassette

- **EM7** is a bacterial promoter that enables the constitutive expression of the Zeocin® resistance gene (*Sh ble*) 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*.
- **Human β-Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of *bsr*. The use of β-globin pAn minimizes interference and possible recombination events with the SV40 pAn signal⁶.

PRODUCT INFORMATION

InvivoGen has designed pNiFty3, a collection of inducible reporter plasmids, to monitor pattern recognition receptor (PRR) activation and cytokine signaling upon ligand stimulation. The pNiFty3-I-Lucia-Zeo plasmid features an IRF-inducible *Lucia luciferase* reporter gene under the control of an engineered mIFN-β promoter. This promoter comprises several ISRE repeated TFBS to enhance the IRF-specific transcription. The subsequent expression of Lucia upon receptor activation is readily measurable in the cell culture supernatant when using QUANTI-Luc™ 4 Lucia/ Gaussia, a Lucia luciferase detection reagent. The pNiFty3-I-Lucia-Zeo plasmid is selectable with Zeocin® in both *E. coli* and mammalian cells, and can be used to generate stable clones.

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 water.
 - Store the resuspended plasmid at -20°C.
- **Plasmid amplification and cloning**

Plasmid amplification and cloning can be performed in *E. coli* GT115 or other commonly used laboratory *E. coli* strains, such as DH5α.
- **Zeocin® usage**

Zeocin® can be used at 25 µg/ml in *E. coli* in liquid or solid media and at 50-200 µg/ml to select Zeocin®-resistant mammalian cells.

RELATED PRODUCTS

| Product | Description | Cat. Code |
|-----------------------------|--------------------------|-------------|
| Zeocin® | Selection antibiotic | ant-zn-1 |
| ChemiComp GT115 | Competent <i>E. coli</i> | gt115-11 |
| QUANTI-Luc™ 4 Lucia/Gaussia | Luciferase Detection | rep-qlc4lg1 |

1. Vodjdani G. *et al.*, 1988. J Mol Biol. 204(2):221-31. 2. Wesoly J. *et al.*, 2007. Acta Biochim Pol. 54(1):27-38 3. Carswell S. & Alwine J., 1989. Mol Cell Biol. 9(10):4248-58. 4. Kim D. *et al.*, 1990. Gene 91 (2): 217-223. 5. Takebe Y. *et al.*, 1988. Mol. Cell Biol. 1: 466-472. 6. Yu J. & Russell J., 2001. Mol Cell Biol, 21(17):5879-88.

TECHNICAL SUPPORT

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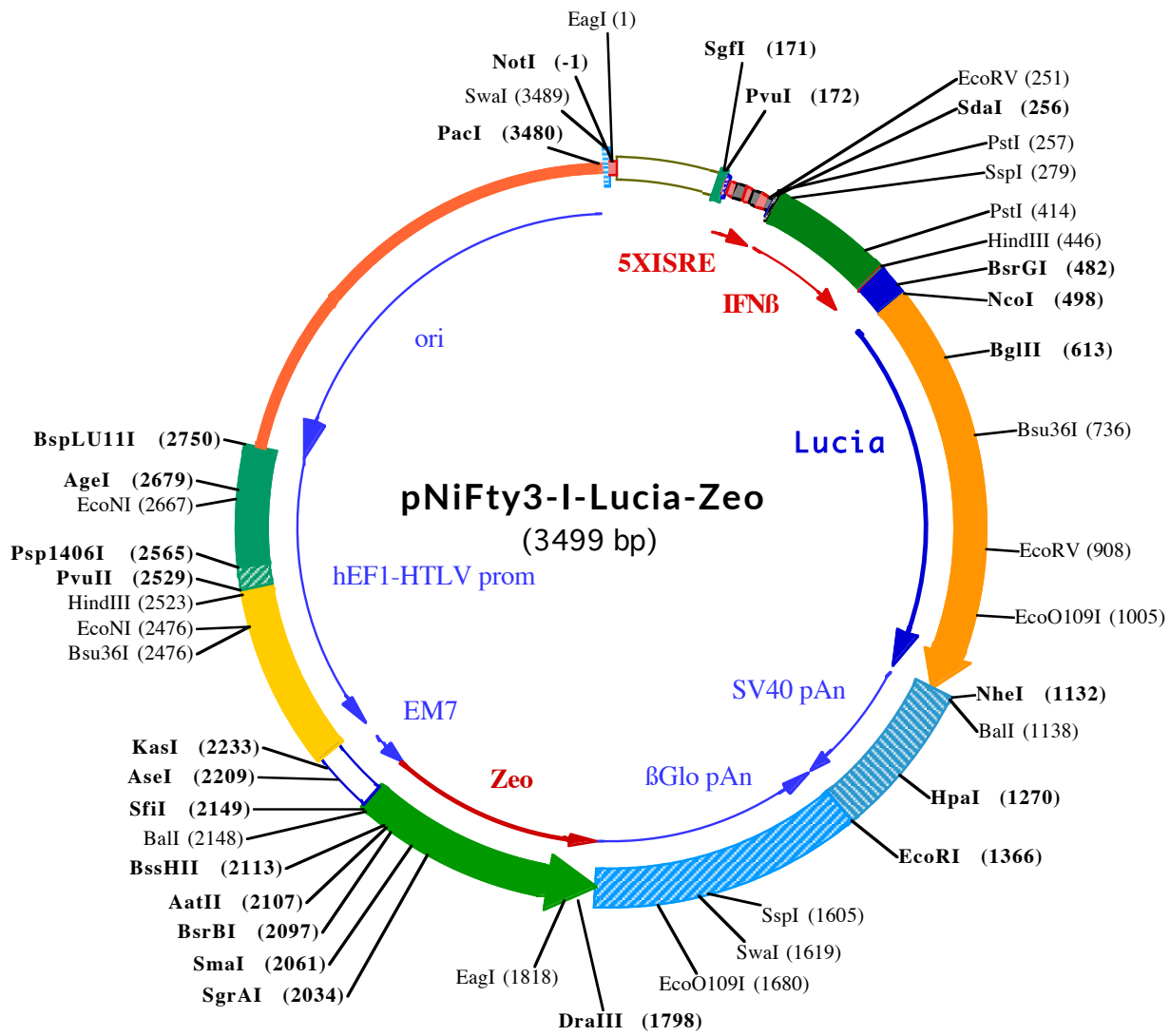
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100

EagI (1)
NotI (-1)
1 GCGGCCGCAATAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTGTGTAATGTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACA

PvuII (172)
SgfI (171)
106 AACTAGCAAATAGGCTGCCCCAGTGCAGGTGCCAGAACATTTCTATCGAAGGATCTGCATCGCTGAATTAGTTTCACTTCCAGTTTCAGTTTC

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

PstI (414)
316 aggaAAACTGAAAGGgAGAAGTGAAGTggaaattcctctgaggcagaaggaccatccctTATAAAtagcacaggccatgaaggaagatcattctcactgc

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

BglIII (613)
524 CCTCATCTGTATTGCTTGTGCTGAGGCAAAACCCACTGAAATCAATGAAGACCTCAATATAGCTGCTGTGGCCTCCAACCTTGGCCACACAGATCTTGAGACTGA

8 aLeuI l eCysI l eAl aVaI aI aGl uAl aLysProThrGl uI l eAsnGl uAspLeuAsnI l eAl aAl aVaI aSerAsnPheAl aThr ThrAspLeuGl uThrAs
629 CCTGTTCCACCAACTGGGAGACCATGAATGTGATTAGCACTGACACAGCAGGTGAACACAGATGCTGACAGGGGCAAGCTGCCTGGCAAAAACCTCCCCCAGA

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

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

113 eP roGl 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 GCCACTGGACAGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCCTGGCCAAATGCCAGTGTCTGACCTCTGAAGAAGTG

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

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

HpaI (1270)
1259 TGCAATAAACAAAGTTAACAAACAATTCATTCTTTTATGTTTCAGGTTTCAGGGGAGGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGT

EcoRI (1366)
1364 ATGGAATTCATAAATACAGCATAGCAAACTTTAACCTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGCTGTTG

1469 CCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTCATGGAGTTTAAAGATATAGTGATTTTTCCCAAGGTTTGAAC TAGCTCTTCATTTCTTTATGTTTTAAA

SspI (1605) SwaI (1619)
1574 TGCCTGACCTCCACATTCCCTTTTTAGTAAAATATTCAGAAAATAATTTAAATACATCATTGCAATGAAAATAATGTTTTTATTAGGCAGAATCCAGATGCT

EcoO109I (1680)
1679 CAAGGCCCTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTAAATAGAAATGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCA

1274•••Gl y•••
1784 GTCCTGCTCCTGCCACAAAGTGCACGAGTTGCCGGCCGGTGCAGGCGAATCCCGCCCCACGGCTGCTGCCGATCTCGGTATGCGCCGGCCCGGA

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

89 Al 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 CTGGACCGCTGATGAACAGGGTACGCTGCTCCGACACACCGGCGAAGTCTCCACGAAGTCCCGGGAGAACCAGCCGCTGCTCCAGAACTCGAC

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

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

AseI (2209) KasI (2233)
2309 GTCAGGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCAAGGGAGCGCCGACAAAGGCCCGGTCTGACCTGAGCTTTAAACTTACCTAGACGGCGGAC

EcoNI (2476) Bsu36I (2476)
2414 GCAGTTCAGGAGGCCACACAGGCGGGAGCGGCAGAACCGACTCAACCGCGTGGATGCCGGCCTCAGGTAGGGCGGGCGCGTGAAGGAGAGATGCGAGCC

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

EcoNI (2667) AgeI (2679)
2624 CGGAGCCAGTACACGACATCACTTTCCAGTTTACCCGCGCCACTTCTTAGGCACCGGTTCAATTGCCGACCCCTCCCCCAACTTCTCGGGACTGTGGCC

BspLU11I (2750)
2729 GATGTGCGCTCTGCCACTGACACATGTGAGCAAAAGGCCAGAAAAGGCCAGAACCGTAAAAAGCCGCTTCTGCTGGCCTTTTCCATAGGCTCCGCCCTCTCT

2834 GACGAGCATCAAAAAATCGACGCTCAAGTCAAGGTGGCGAAAACCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTGCTGCGCTCTCT

2939 GTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCCGGAAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTC

3044 GTTCGCTCAAGCTGGGCTGTGTCACGAACCCCGTTCAGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCAACCCGGTAAGACACGACTTA

3149 TCGCCACTGGCAGCAGCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGA

3254 ACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTT

3359 GTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGG

Swal (3489)

PacI (3480)

3464 ATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA