Product usage

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

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

The purchase of the pNiFty3-I-SEAP-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.

pNiFty3-I-SEAP-Zeo

IRF-inducible reporter plasmid selectable with Zeocin®

Catalog code: pnf3-sp4

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

For research use only

Version 24A17-NJ

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized **pNiFty3-I-SEAP-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 IRF7[1]. This minimal promoter is truly IRF-specific due to the addition of several interferon-stimulated response elements (ISRE) repeated transcription factor binding sites (TFBS) (AGTTTCNNTTTCC)[2]. This feature also enhances the IRF-mediated transcription of the *SEAP* reporter gene.

- **SEAP** is a **s**ecreted form of human **e**mbryonic **a**lkaline **p**hosphatase. It is extremely heat stable and resistant to the inhibitor L-homoarginine. It catalyses the hydrolysis of pNitrophenyl phosphate (pNpp) producing a yellow end product. SEAP levels can be evaluated qualitatively with the naked eye and quantitatively using a spectrophometer in combination with SEAP detection media, such as HEK-Blue™ Detection, or QUANTI-Blue™ Solution, a SEAP detection reagent.

- **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 mRNA[3].

- **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 promoter[4] 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[5]. 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

- **CMV promotor & enhancer** drives the expression of the Zeocin® resistance gene (*Sh ble*) in mammalian cells.
- **EM7** is a bacterial promoter that enables the constitutive expression of the *Sh ble* 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*.
- **Human β-Globin pAn** is a strong polyadenylation (pAn) signal placed downstream of *Sh ble*. The use of β-globin pAn minimizes interference and possible recombination events with the SV40 pAn signal[6].

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-SEAP-Zeo** plasmid features an IRF-inducible *SEAP* 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 SEAP upon receptor activation is readily measurable in the cell culture supernatant when using QUANTI-Blue™ Solution, a SEAP detection reagent. The **pNiFty3-I-SEAP-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 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**

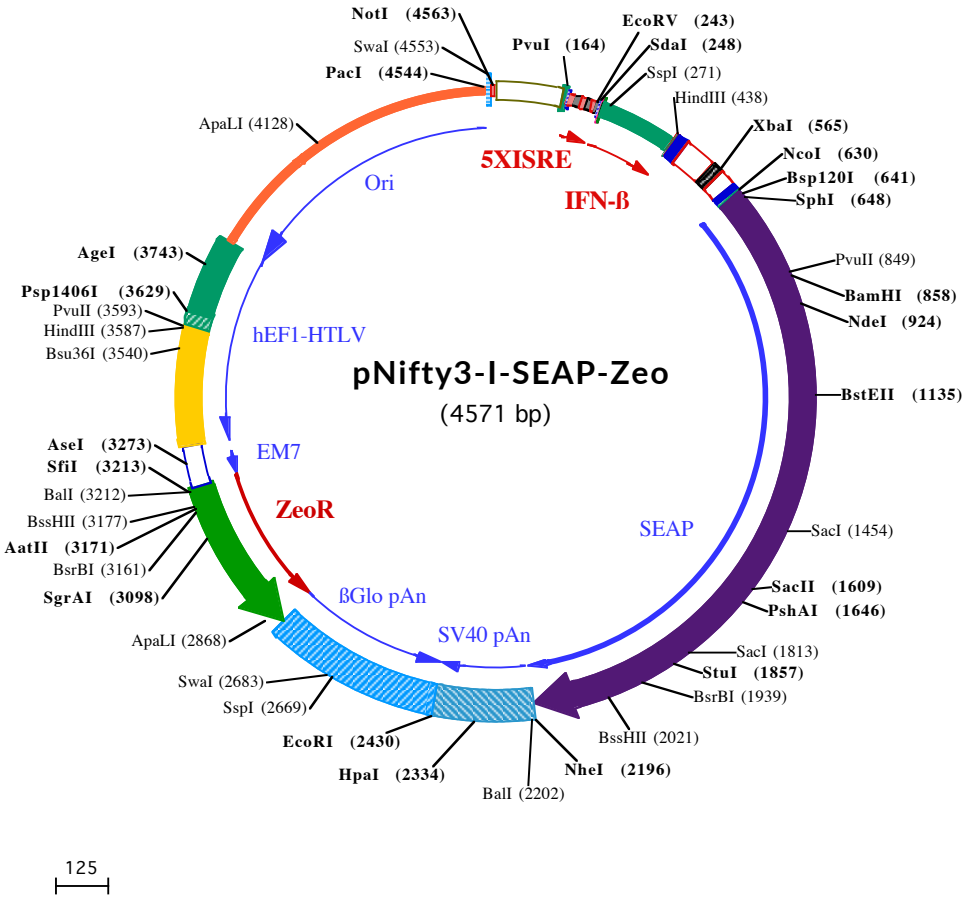
Zeocin® can be used at 25 µg/ml in *E. coli* in liquid or solid media and at 50-200 µg/ml to select Zeocin®-resistant mammalian cells.

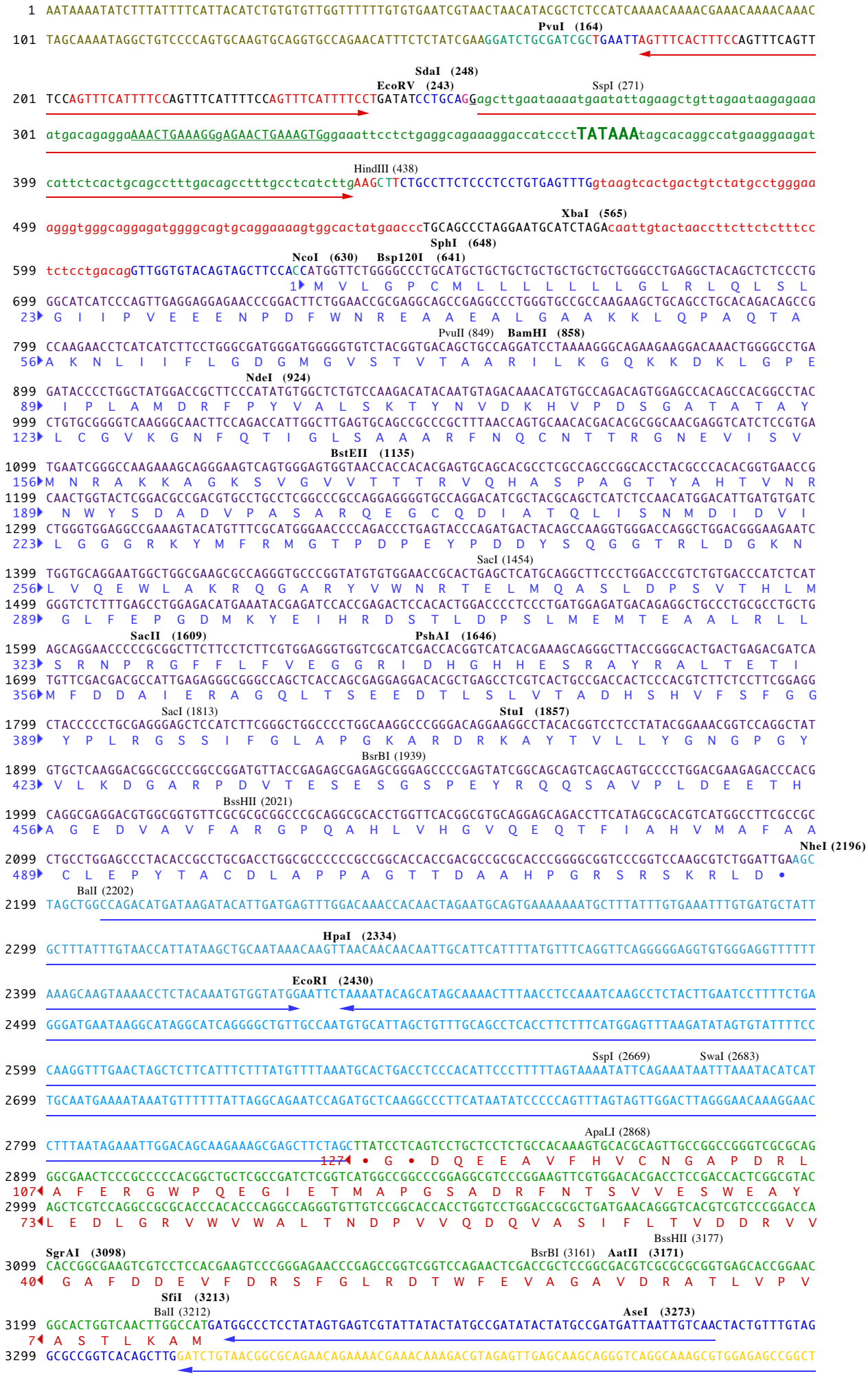
RELATED PRODUCTS

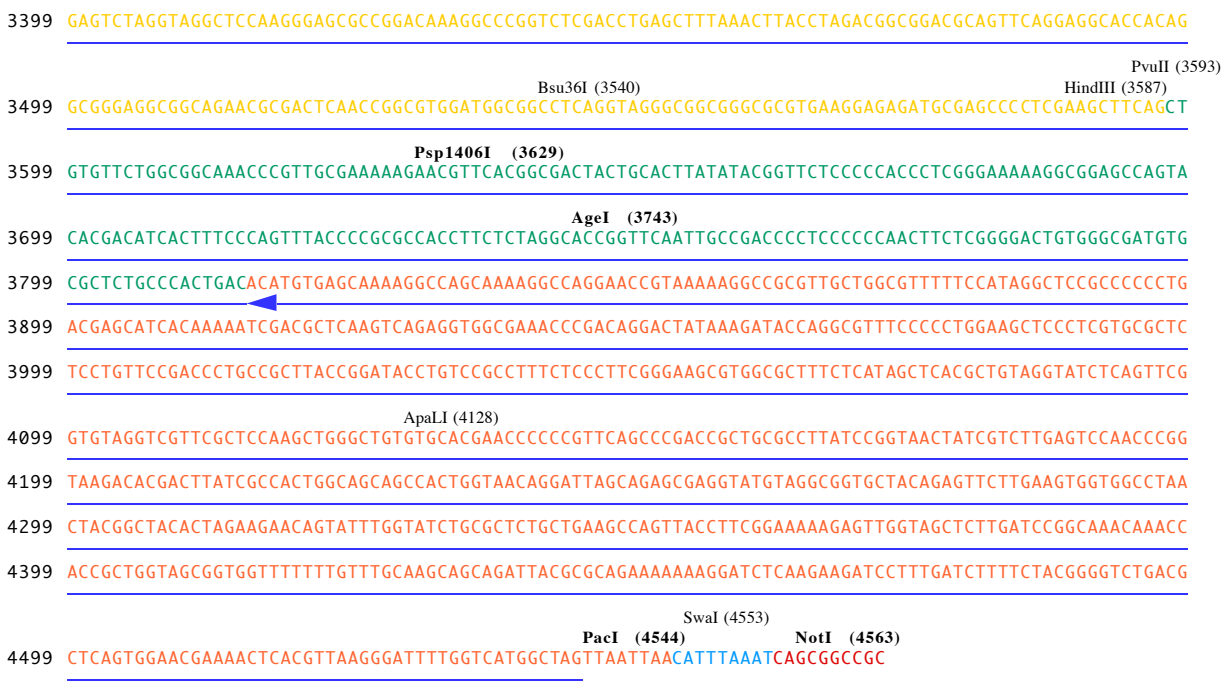
Product	Description	Cat. Code
Zeocin®	Selection antibiotic	ant-zn-1
pNiFty3-I-SEAP-Blasti	Reporter plasmid	pnf3b-sp4
pNiFty3-I-SEAP-Puro	Reporter plasmid	pnf3p-sp4
HEK-Blue™ Detection	SEAP Detection	hb-det2
QUANTI-Blue™ Solution	SEAP Detection	rep-qbs

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
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com


NotI (4563)
SwaI (4553)
PacI (4544)
ApaLI (4128)
PvuI (164)
EcoRV (243)
SdaI (248)
SspI (271)
HindIII (438)
XbaI (565)
NcoI (630)
Bsp120I (641)
SphI (648)
PvuII (849)
BamHI (858)
NdeI (924)
BstEII (1135)
SacI (1454)
SacII (1609)
PshAI (1646)
SacI (1813)
StuI (1857)
BsrBI (1939)
BssHII (2021)
NheI (2196)
BalI (2202)
HpaI (2334)
EcoRI (2430)
SspI (2669)
SwaI (2683)
ApaLI (2868)
SV40 pAn
ßGlo pAn
SEAP
ZeoR
EM7
AseI (3273)
SfiI (3213)
BalI (3212)
BssHII (3177)
AatII (3171)
BsrBI (3161)
SgrAI (3098)
AgeI (3743)
Psp1406I (3629)
PvuII (3593)
HindIII (3587)
Bsu36I (3540)
hEF1-HTLV
Ori
5XISRE
IFN-ß
pNifty3-I-SEAP-Zeo
(4571 bp)
125


PvuI (164)
SdaI (248)
EcoRV (243)
SspI (271)
HindIII (438)
XbaI (565)
SphI (648)
NcoI (630)
Bsp120I (641)
PvuII (849)
BamHI (858)
NdeI (924)
BstEII (1135)
SacI (1454)
SacII (1609)
PshAI (1646)
SacI (1813)
StuI (1857)
BsrBI (1939)
BssHII (2021)
NheI (2196)
BalI (2202)
HpaI (2334)
EcoRI (2430)
SspI (2669)
SwaI (2683)
ApaLI (2868)
BssHII (3177)
SgrAI (3098)
BsrBI (3161)
AatII (3171)
SfiI (3213)
BalI (3212)
AseI (3273)


3399 GAGTCTAGGTAGGCTCCAAGGGAGCGCCGGACAAAGGCCCGGTCTCGACCTGAGCTTTAAACTTACCTAGACGGCGGACGCAGTTCAGGAGGCACCACAG
PvuII (3593)
Bsu36I (3540)
HindIII (3587)
3499 GCGGGAGGCGGCAGAACGCGACTCAACCGGCGTGGATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTGAAGGAGAGATGCGAGCCCCTCGAAGCTTCAGCT
Psp1406I (3629)
3599 GTGTTCTGGCGGCAAACCCGTTGCGAAAAAGAACGTTCACGGCGACTACTGCACTTATATACGGTTCTCCCCCACCCTCGGGAAAAAGGCGGAGCCAGTA
AgeI (3743)
3699 CACGACATCACTTTCCCAGTTTACCCCGCGCCACCTTCTCTAGGCACCGGTTCAATTGCCGACCCCTCCCCCCAACTTCTCGGGGACTGTGGGCGATGTG
3799 CGCTCTGCCCACTGACACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTG
3899 ACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTC
3999 TCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCG
ApaLI (4128)
4099 GTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGG
4199 TAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAA
4299 CTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACC
4399 ACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACG
SwaI (4553)
PacI (4544)
NotI (4563)
4499 CTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGC