Product usage

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

Important Limited Use information for pNiFty2-N-Fluc-Puro

The purchase of the pNiFty2-N-Fluc-Puro 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.

pNiFty2-N-Fluc-Puro

NF-κB-inducible reporter plasmid selectable with Puromycin

Catalog code: pnf2p-fluc

https://www.invivogen.com/pnifty2-family-puro

For research use only

Version 23H16-AK

PRODUCT INFORMATION

Contents

- 20 µg of lyophilized **pNiFty2-N-Fluc-Puro** (plasmid DNA)
- 1 ml of **Puromycin** (10 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 Puromycin 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

- **NF-κB-5x ELAM** is an engineered ELAM (endothelial cell-leukocyte adhesion molecule) promoter combined with five NF-κB repeated transcription factor binding sites (TFBS) (GGGGACTTTCC)[1]. This minimal promoter is truly NF-κB-specific, as it lacks an AP-1/CREB site found in the full-length promoter[1,2]. The addition of the five TFBS enhances the NF-κB-mediated transcription of the *SEAP* reporter gene.

- **Fluc:** The *firefly luciferase (Fluc)* gene encodes for an intracellular luciferase of fireflies and click beetles. This enzyme interacts with D-luciferin as a chemiluminescent substrate to produce light emission peaking at 560 nm. After cell lysis, the reaction can be measured and detected simply, rapidly and with good sensitivity by means of a luminescence-measuring instrument.

- **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.

Puromycin antibiotic selection cassette

- **CMV promotor & enhancer** drives the expression of the Puromycin resistance gene (*Pac*) in mammalian cells.
- **EM7** is a bacterial promoter that enables the constitutive expression of the *Pac* gene in *E. coli*.
- **Puro (resistance to the antibiotic Puromycin)** is conferred by the *Pac* gene from *Streptomyces* which encodes a N-acetyl-transferase. The *Pac* 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 *Pac*. The use of β-globin pAn minimizes interference and possible recombination events with the SV40 pAn signal[6].

PRODUCT INFORMATION

InvivoGen has designed **pNiFty2**, a collection of inducible reporter plasmids, to monitor pattern recognition receptor (PRR) activation and cytokine signaling upon ligand stimulation. The **pNiFty2-N-Fluc-Puro plasmid** features an NF-κB-inducible *Firefly luciferase (Fluc)* reporter gene under the control of an engineered ELAM promoter. This promoter comprises five NF-κB repeated TFBS to enhance the NF-κB-mediated transcription. The subsequent expression of Fluc can be measured and detected simply, rapidly and with good sensitivity by means of a luminescence-measuring instrument. Of note, the Firefly luciferase remains intracellular, and requires cell lysis in order to measure bioluminescence. The **pNiFty2-N-Fluc-Puro plasmid** is selectable with Puromycin in both *E. coli* and mammalian cells, and can be used to generate stable clones.

METHODS

- **Plasmid resuspension**
  - Quickly spin the tube containing 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.

- **Puromycin usage**

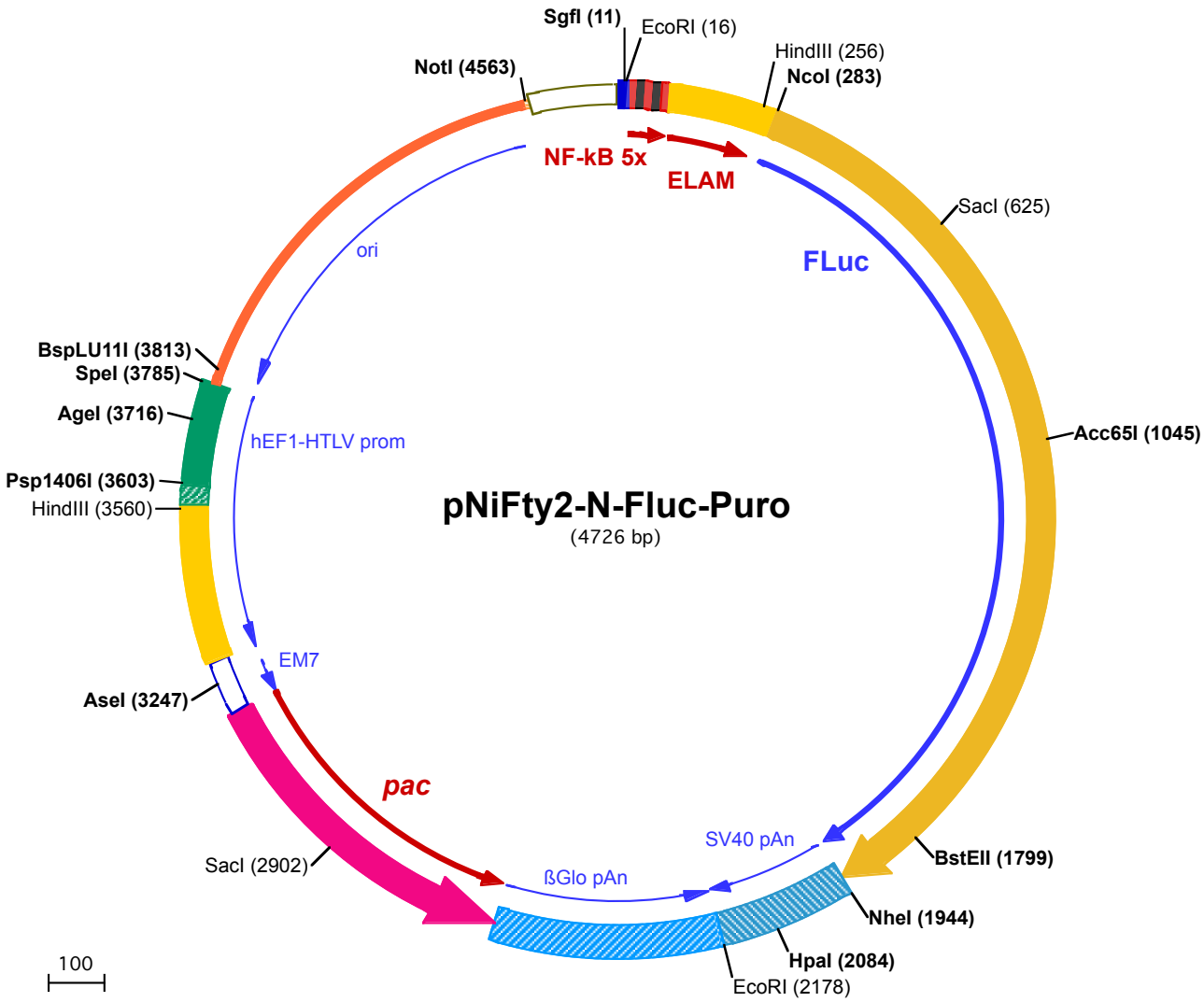
Puromycin can be used at 100-125 µg/ml in *E. coli* in liquid or solid media and at 1-10 µg/ml to select Puromycin-resistant mammalian cells.

RELATED PRODUCTS

Product	Description	Cat. Code
Puromycin	Selection antibiotic	ant-pr-1
pNiFty2-N-Fluc-Blasti	Reporter plasmid	pnf2b-fluc
pNiFty2-N-Fluc-Zeo	Reporter plasmid	pnf2-fluc

1. Schindler U., Baichwal VR., 1994. Mol Cell Biol. 14(9):5820-31. **2. Jensen LE. & Whitehead AS., 2003.** Biotechniques 35:54-58. **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



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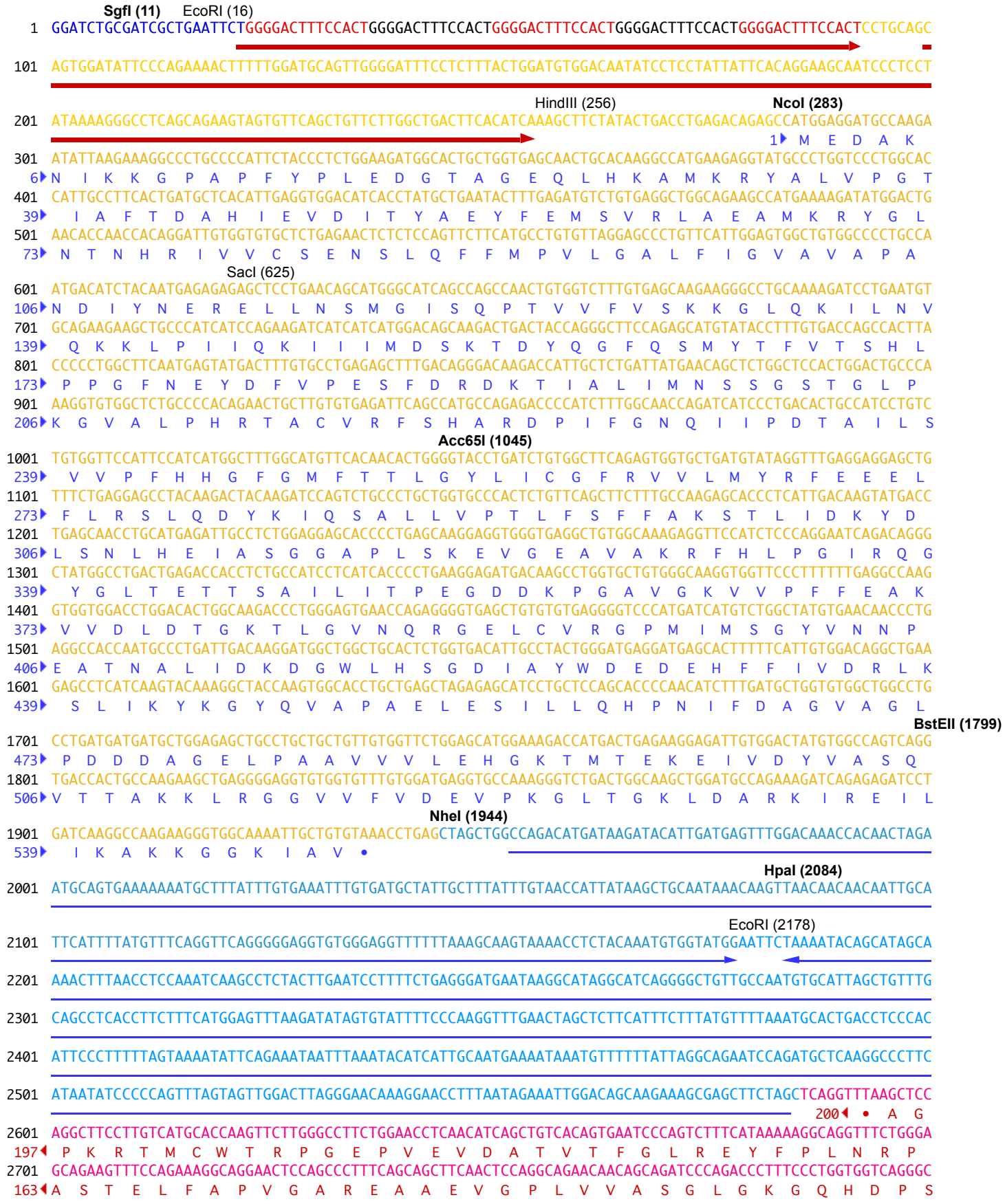
23H21v01b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HindIII (256)
NcoI (283)
SacI (625)
Acc65I (1045)
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HpaI (2084)
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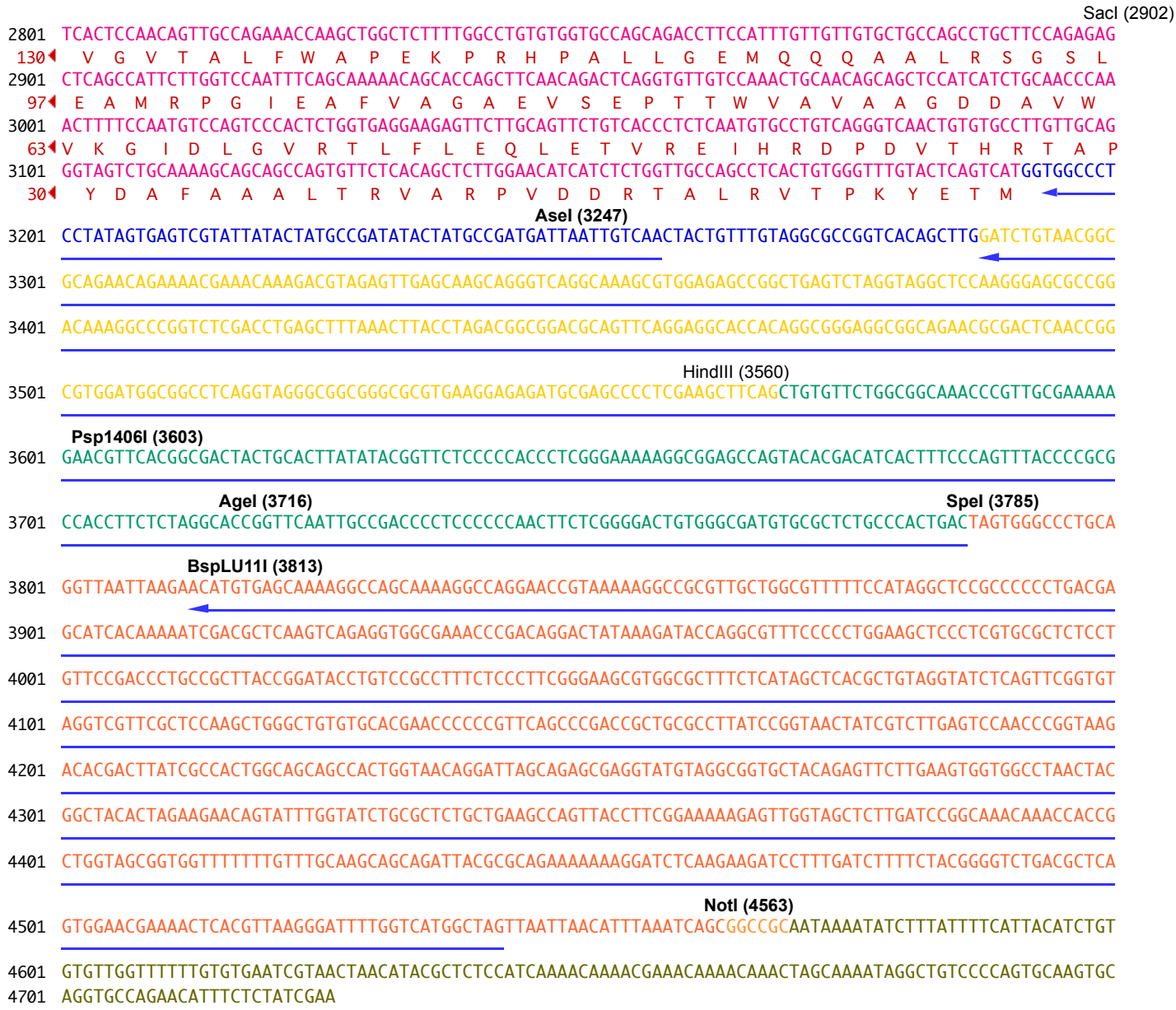
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130 V G V T A L F W A P E K P R H P A L L G E M Q Q Q A A L R S G S L
2901 CTCAGCCATTCTTGGTCCAATTTCAGCAAAAACAGCACCAGCTTCAACAGACTCAGGTGTTGTCCAAACTGCAACAGCAGCTCCATCATCTGCAACCCAA
97 E A M R P G I E A F V A G A E V S E P T T W V A V A A G D D A V W
3001 ACTTTTCCAATGTCCAGTCCCACTCTGGTGAGGAAGAGTTCTTGCAGTTCTGTCACCCTCTCAATGTGCCTGTCAGGGTCAACTGTGTGCCTTGTTGCAG
63 V K G I D L G V R T L F L E Q L E T V R E I H R D P D V T H R T A P
3101 GGTAGTCTGCAAAAGCAGCAGCCAGTGTTCTCACAGCTCTTGGAACATCATCTCTGGTTGCCAGCCTCACTGTGGGTTTGTACTCAGTCATGGTGGCCCT
30 Y D A F A A A L T R V A R P V D D R T A L R V T P K Y E T M
AseI (3247)
3201 CCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAACTACTGTTTGTAGGCGCCGGTCACAGCTTGGATCTGTAACGGC
3301 GCAGAACAGAAAACGAAACAAAGACGTAGAGTTGAGCAAGCAGGGTCAGGCAAAGCGTGGAGAGCCGGCTGAGTCTAGGTAGGCTCCAAGGGAGCGCCGG
3401 ACAAAGGCCCGGTCTCGACCTGAGCTTTAAACTTACCTAGACGGCGGACGCAGTTCAGGAGGCACCACAGGCGGGAGGCGGCAGAACGCGACTCAACCGG
HindIII (3560)
3501 CGTGGATGGCGGCCTCAGGTAGGGCGGCGGGCGCGTGAAGGAGAGATGCGAGCCCCTCGAAGCTTCAGCTGTGTTCTGGCGGCAAACCCGTTGCGAAAAA
Psp1406I (3603)
3601 GAACGTTCACGGCGACTACTGCACTTATATACGGTTCTCCCCCACCCTCGGGAAAAAGGCGGAGCCAGTACACGACATCACTTTCCCAGTTTACCCCGCG
AgeI (3716)
SpeI (3785)
3701 CCACCTTCTCTAGGCACCGGTTCAATTGCCGACCCCTCCCCCCAACTTCTCGGGGACTGTGGGCGATGTGCGCTCTGCCCACTGACTAGTGGGCCCTGCA
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NotI (4563)
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