pVITRO2-neo-GFP/SEAP

An expression plasmid encoding the GFP and SEAP reporter genes

Catalog code: pvitro2-ngfpsp

https://www.invivogen.com/pvitro2-gfpseap

For research use only

Version 19L18-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVITRO2-neo-GFP/SEAP provided as lyophilized DNA

Storage and stability

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C
- Resuspended DNA should be stored at -20°C and is stable for at least 1 year at-20°C. Avoid repeated freeze-thaw cycles.

Quality control

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA was purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE

pVITRO1 and pVITRO2 allow the co-expression of two or more genes from two different transcription units. pVITRO plasmids can be stably transfected in mammalian cells and yield high levels of expression.

pVITRO2-neo-GFP/SEAP contains the reporter genes GFP and SEAP and can be used as a control vector.

pVITRO2-neo-GFP/SEAP also can be used for cloning of open reading frames. Both reporter genes are flanked by unique sites (*Bsp*H I/*Avr* II for GFP and *Nco* I/*Nhe* I for SEAP) that allow for convenient cloning of open reading frames.

PLASMID FEATURES

• **hFerH and hFerL composite promoters:** Ferritin is a 24 subunit protein composed of two subunit types, termed H (heavy) and L (light), which perform complementary functions in the protein. Ferritin is ubiquitously expressed. Its synthesis is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through the interaction between the iron-responsive element (IRE), located in the 5' untranslated region (5'UTR) of the ferritin mRNAs, and the iron regulatory protein[1]. To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.

• **SV40 enhancer** which is comprised of a 72-base-pair repeat allows the enhancement of gene expression in a large host range. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids[2].

• **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (HCMV), located between nucleotides -118 and -524, is composed of unique and repeated sequence motifs. The HCMV enhancer can substitute for the 72-bp repeats of SV40 and is severalfold more active than the SV40 enhancer[3].

• **pMB1 ori:** A minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

• **GFP gene:** This red-shifted variant of the jellyfish GFP gene encodes a green fluorescent protein that absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).

• **FMDV IRES:** The internal ribosome entry site of the Foot and Mouth Disease Virus enables the translation of two open reading frames from one mRNA with high levels of expression[4].

• **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.

• **Neo:** The *neo* gene from Tn5 confers resistance to Kanamycin in *E. coli* and G418 in mammalian cells. In bacteria, *neo* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *neo* is transcribed from the human FerH composite promoter as a polycistronic mRNA and translated via the FMDV IRES.

• **EF1 pAn** is a strong polyadenylation signal. InvivoGen uses a sequence starting after the stop codon of the EF1 cDNA and finishing after a bent structure rich in GT.

• **SEAP** is a secreted form of human embryonic alkaline phosphatase. Unlike endogenous alkaline phosphatases, SEAP 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 expression can be readily quantified by collecting samples of culture medium and measuring the hydrolysis of pNpp with a spectrophometer at 405 nm. SEAP activity that can be readily assessed qualitatively and quantitatively using HEK-Blue™ Detection or QUANTI-Blue™.

• **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA. The efficiency of this signal was first described by Carswell *et al.*[5]

1. Eisenstein RS. and Munro HN. 1990. Translational regulation of ferritin synthesis by iron. Enzyme 44(1-4):42-58. **2. Dean DA. *et al.* 1999.** Sequence requirements for plasmid nuclear import. Exp. Cell. Res. 253:713-22. **3. Boshart M. *et al.* 1985.** A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. Cell 141(2):521-30. **4. Ramesh N *et al.* 1996.** High-titer bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. Nucleic Acids Res. 24(14):2697-700. **5. Carswell S., and Alwine JC. 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol. Cell Biol. 10: 4248-4258.

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 other commonly used laboratory *E. coli* strains, such as DH5α.

Bacterial antibiotic selection

Kanamycin (not provided) is normally used for *E. coli* at a final concentration of 50 µg/ml in liquid or solid media.

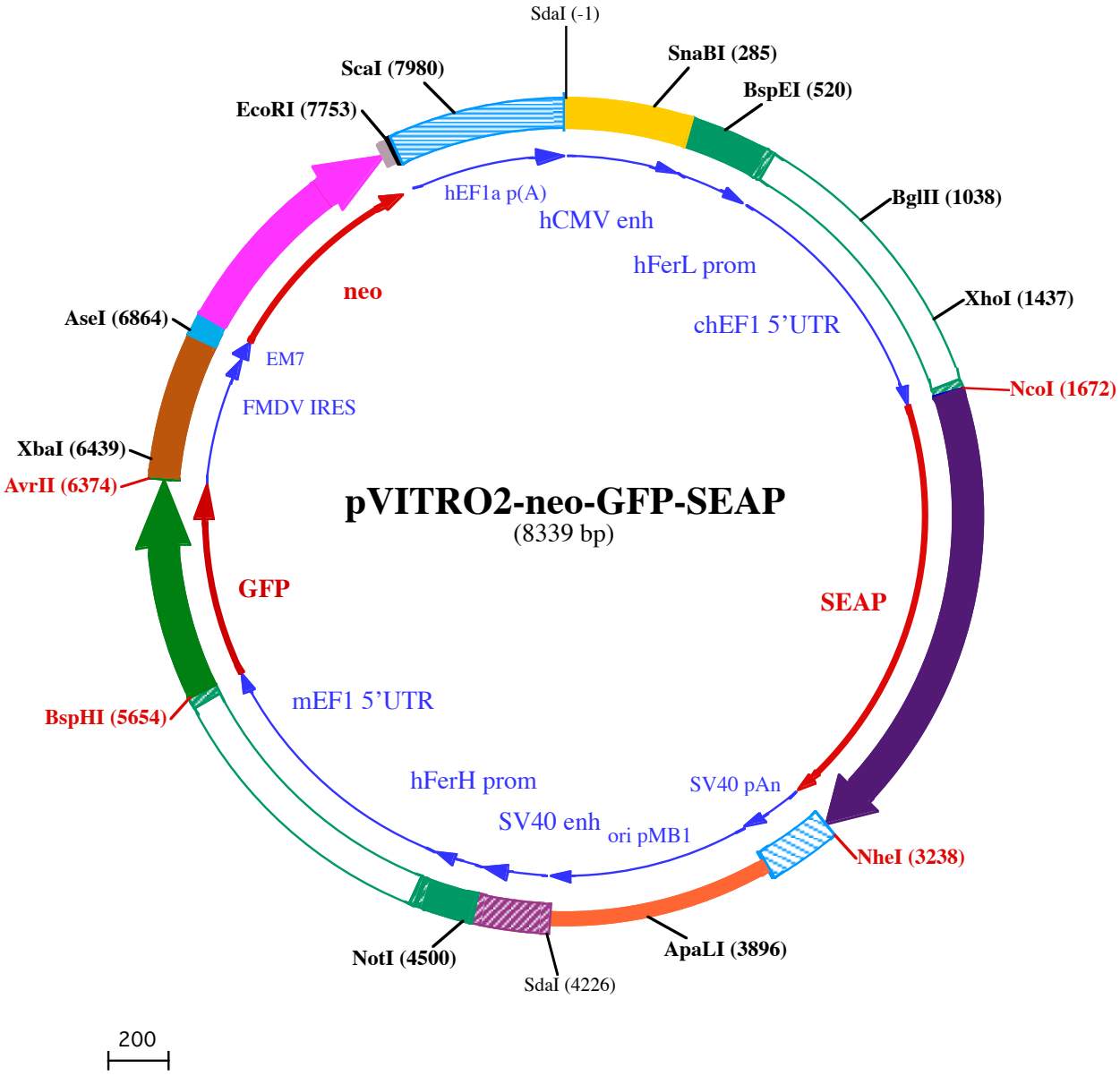
Mammalian antibiotic selection

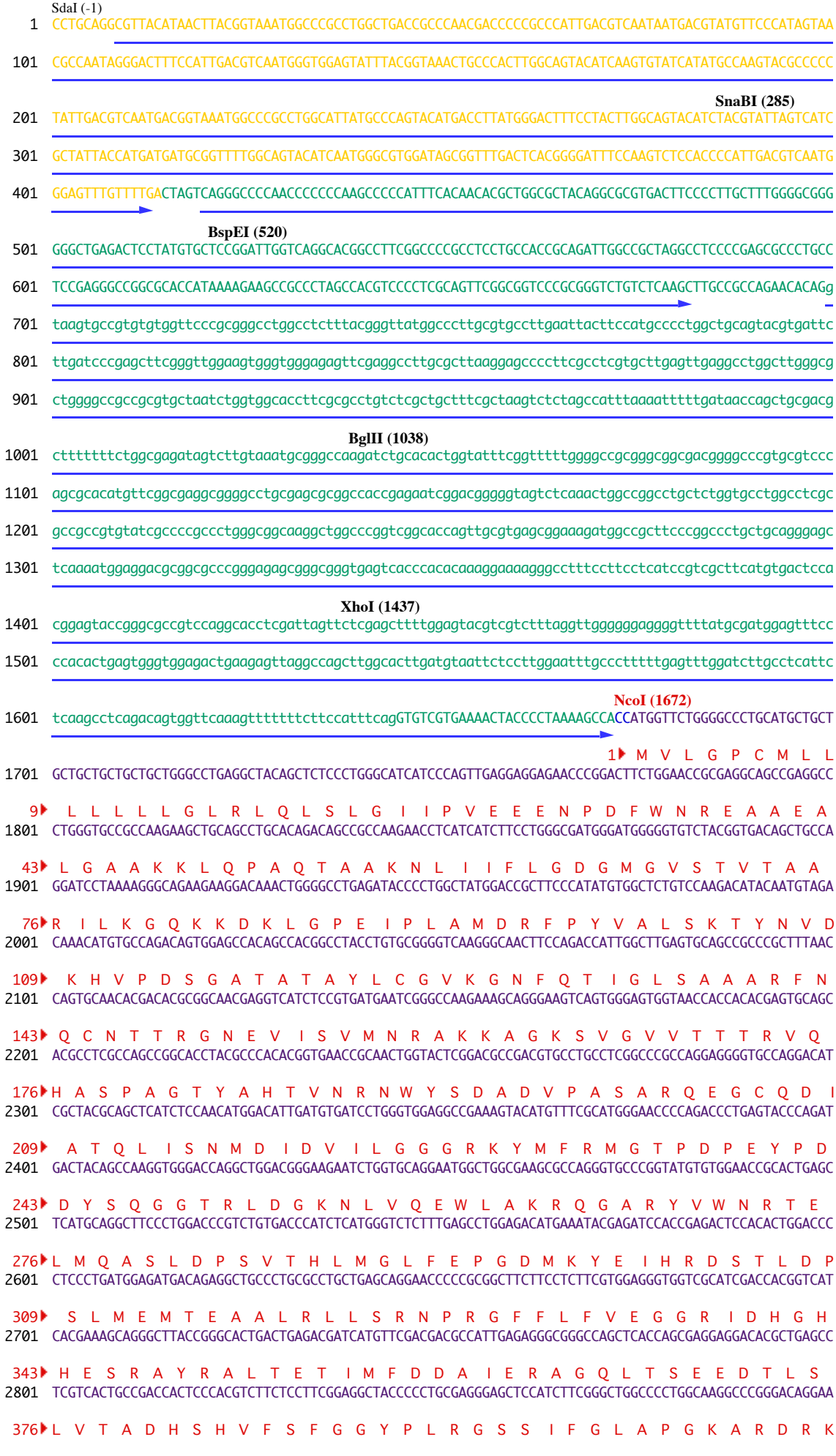
G418 is normally used at a concentration of 400 µg/ml. However, the optimal concentration needs to be determined for your cells.

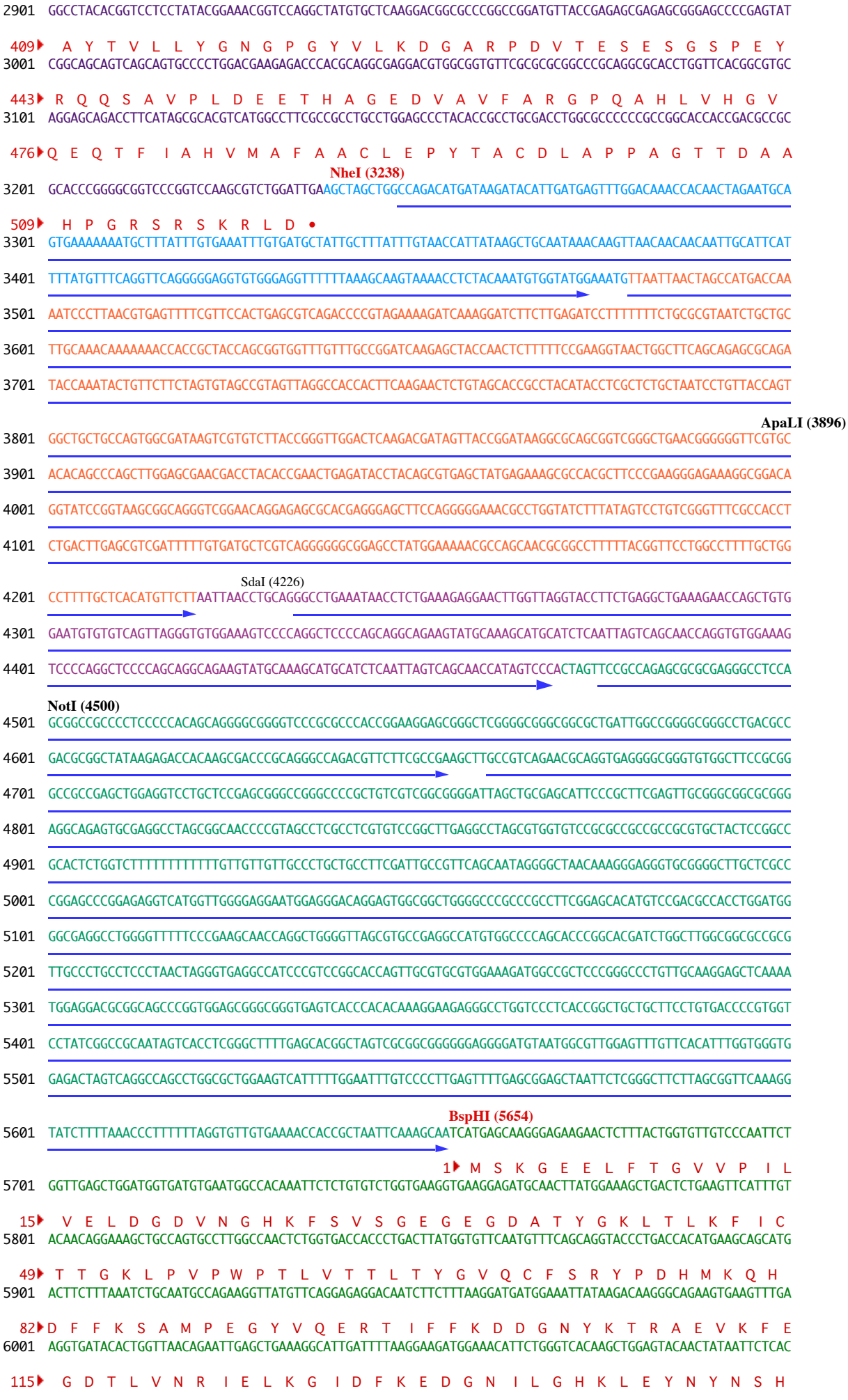
RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116 cells	Competent *E. coli* cells	gt116-11
G418	Selection antibiotic	ant-gn-1

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 3622-3480
E-mail: info@invivogen.com


SdaI (-1)
SnaBI (285)
BspEI (520)
BglII (1038)
XhoI (1437)
NcoI (1672)
NheI (3238)
ApaLI (3896)
SdaI (4226)
NotI (4500)
BspHI (5654)
AvrII (6374)
XbaI (6439)
AseI (6864)
EcoRI (7753)
ScaI (7980)
hEF1a p(A)
hCMV enh
hFerL prom
chEF1 5'UTR
SEAP
SV40 pAn
ori pMB1
SV40 enh
hFerH prom
mEF1 5'UTR
GFP
FMDV IRES
EM7
neo
pVITRO2-neo-GFP-SEAP
(8339 bp)
200


SdaI (-1)
SnaBI (285)
BspEI (520)
BglII (1038)
XhoI (1437)
NcoI (1672)


NheI (3238)
ApaLI (3896)
SdaI (4226)
NotI (4500)
BspHI (5654)

```
6101  AATGTTTACATTATGGCAGATAAGCAGAAGAATGGAATTAAGGTTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAACTGGCAGACCATT

 149  N  V  Y  I  M  A  D  K  Q  K  N  G  I  K  V  N  F  K  I  R  H  N  I  E  D  G  S  V  Q  L  A  D  H
6201  ACCAGCAGAACACCCCTATTGGTGATGGCCCAGTTCTCCTCCCAGATAATCACTATCTCCGCACTCAATCTGCTCTGTCCAAAGACCCTAATGAGAAAAG

 182  Y  Q  Q  N  T  P  I  G  D  G  P  V  L  L  P  D  N  H  Y  L  R  T  Q  S  A  L  S  K  D  P  N  E  K  R
                                                                                    AvrII (6374)
6301  AGACCACATGGTCCTCCTGGAGTTTGTGACAGCAGCAGGAATTACTCTGGGAATGGATGAGCTGTACAAGTAAACCTAGGAGCAGGTTTCCCCAATGACA

 215  D  H  M  V  L  L  E  F  V  T  A  A  G  I  T  L  G  M  D  E  L  Y  K  •
                                XbaI (6439)
6401  CAAAACGTGCAACTTGAAACTCCGCCTGGTCTTTCCAGGTCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTTA

6501  GTAACAGCACTGTTGCTTCGTAGCGGAGCATGACGGCCGTGGGAACTCCTCCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCCACACGGGCCCG

6601  TCATGTGTGCAACCCCAGCACGGCGACTTTACTGCGAAACCCACTTTAAAGTGACATTGAAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCT

6701  TCAGGTACCCCGAGGTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCCTGAATAGGT

                                                                                AseI (6864)
6801  GACCGGAGGTCGGCACCTTTCCTTTGCAATTACTGACCCTATGAATACAACTGACTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAAT

6901  ACGACTCACTATAGGAGGGCCACCATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCAC

                1  M  I  E  Q  D  G  L  H  A  G  S  P  A  A  W  V  E  R  L  F  G  Y  D  W  A
7001  AACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGA

  26  Q  Q  T  I  G  C  S  D  A  A  V  F  R  L  S  A  Q  G  R  P  V  L  F  V  K  T  D  L  S  G  A  L  N  E
7101  ACTGCAAGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTG

  59  L  Q  D  E  A  A  R  L  S  W  L  A  T  T  G  V  P  C  A  A  V  L  D  V  V  T  E  A  G  R  D  W  L
7201  CTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGC

  93  L  L  G  E  V  P  G  Q  D  L  L  S  S  H  L  A  P  A  E  K  V  S  I  M  A  D  A  M  R  R  L  H  T
7301  TTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGA

 126  L  D  P  A  T  C  P  F  D  H  Q  A  K  H  R  I  E  R  A  R  T  R  M  E  A  G  L  V  D  Q  D  D  L  D
7401  CGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGAGCATGCCCGACGGCGAGGATCTCGTCGTGACACATGGCGATGCCTGC

 159  E  E  H  Q  G  L  A  P  A  E  L  F  A  R  L  K  A  S  M  P  D  G  E  D  L  V  V  T  H  G  D  A  C
7501  TTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCC

 193  L  P  N  I  M  V  E  N  G  R  F  S  G  F  I  D  C  G  R  L  G  V  A  D  R  Y  Q  D  I  A  L  A  T
7601  GTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCT

 226  R  D  I  A  E  E  L  G  G  E  W  A  D  R  F  L  V  L  Y  G  I  A  A  P  D  S  Q  R  I  A  F  Y  R  L
                                                EcoRI (7753)
7701  TCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGAATTCGCTAGGATTATCCCTAATACCTGCCACCCCACTCTTAATCA

 259  L  D  E  F  F  •
7801  GTGGTGGAAGAACGGTCTCAGAACTGTTTGTTTCAATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAATGATAACAATGCATCGTAAAACCTTCAG

                                                                                ScaI (7980)
7901  AAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAATCAGTACTTTTTAATGGAAACA

8001  ACTTGACCAAAAATTTGTCACAGAATTTTGAGACCCATTAAAAAAGTTAAATGAGAAACCTGTGTGTTCCTTTGGTCAACACCGAGACATTTAGGTGAAA

8101  GACATCTAATTCTGGTTTTACGAATCTGGAAACTTCTTGAAAATGTAATTCTTGAGTTAACACTTCTGGGTGGAGAATAGGGTTGTTTTCCCCCCACATA

8201  ATTGGAAGGGGAAGGAATATCATTTAAAGCTATGGGAGGGTTGCTTTGATTACAACACTGGAGAGAAATGCAGCATGTTGCTGATTGCCTGTCACTAAAA

8301  CAGGCCAAAAACTGAGTCCTTGGGTTGCATAGAAAGCTG
```