pVITRO2-hygro-GFP/SEAP

A multigenic plasmid for high levels of expression of the GFP and SEAP reporter genes

Catalog code: pvitro2-gfpsp

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

For research use only

Version 20H19-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVITRO2-hygro-GFP/SEAP provided as lyophilized DNA
- 1 ml Hygromycin B Gold at 100 mg/ml

Storage and stability

- Product is shipped at room temperature.
- Upon receipt, store lyophilized DNA at -20°C.
- Resuspended DNA should be stored at -20°C.
- Store Hygromycin B Gold at 4°C or -20°C. The expiry date is specified on the product label.

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

pVITRO is a family of plasmids developed mainly for *in vitro* studies. They allow the ubiquitous and constitutive co-expression of two genes of interest. pVITRO plasmids can be stably transfected in mammalian cells and the genes of interest are expressed at high levels. Each pVITRO plasmid is available with either two multiple cloning sites or two reporter genes.

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

pVITRO2-hygro-GFP/SEAP also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (BspH I/Avr II for GFP and Nco I/Nhe I for SEAP) that allow for convenient cloning of ORF's.

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 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.

Hygromycin B usage:

This antibiotic can be used for *E. coli* at 50-100 µg/ml in liquid or solid media and at 50-500 µg/ml to select Hygromycin-resistant mammalian cells.

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[2]. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells.

• **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*.

• ***hph* gene** confers resistance to Hygromycin B both in *E. coli* and mammalin cells. In bacteria, *hph* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *hph* is transcribed from the CAG 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. & Munro HN. 1990. Translational regulation of ferritin synthesis by iron. Enzyme 44(1-4):42-58. **2. Dean D.A. *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. & Alwine J.C. 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol. Cell Biol. 10:4248-58.

TECHNICAL SUPPORT
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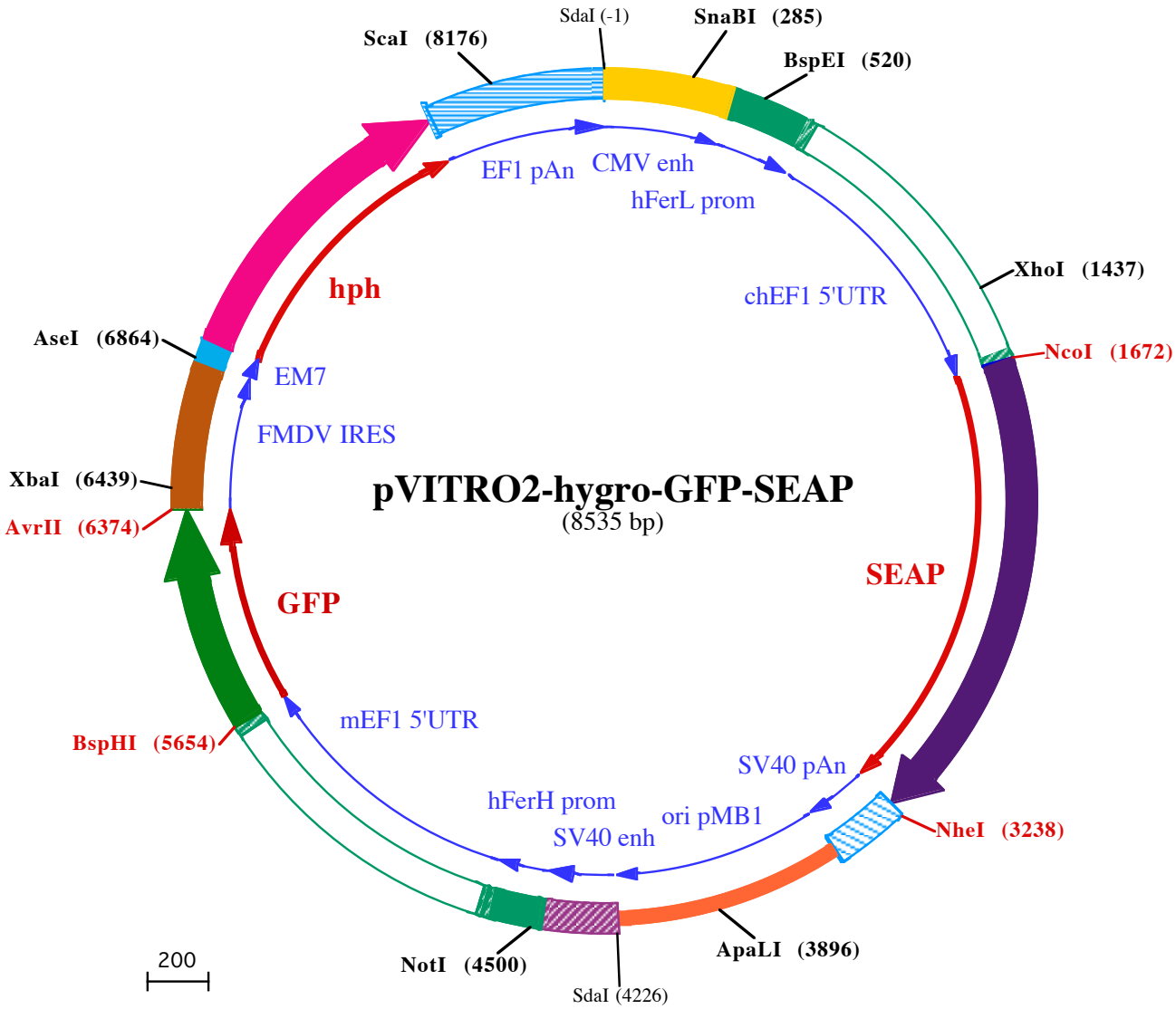
ScaI (8176)
SdaI (-1)
SnaBI (285)
BspEI (520)
XhoI (1437)
NcoI (1672)
NheI (3238)
ApaLI (3896)
SdaI (4226)
NotI (4500)
BspHI (5654)
AvrII (6374)
XbaI (6439)
AseI (6864)
EF1 pAn
CMV enh
hFerL prom
chEF1 5'UTR
hph
EM7
FMDV IRES
pVITRO2-hygro-GFP-SEAP
(8535 bp)
SEAP
GFP
mEF1 5'UTR
SV40 pAn
hFerH prom
ori pMB1
SV40 enh
200

SdaI (-1)

1 CCTGCAGGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAA

101 CGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTA

SnaBI (285)

203 TTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTA

305 TTACCATGATGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGT

406 TTGTTTTGACTAGTCAGGGCCCCAACCCCCCCAAGCCCCCATTTCACAACACGCTGGCGCTACAGGCGCGTGACTTCCCCTTGCTTTGGGGCGGGGGGCTG

BspEI (520)

507 AGACTCCTATGTGCTCCGGATTGGTCAGGCACGGCCTTCGGCCCCGCCTCCTGCCACCGCAGATTGGCCGCTAGGCCTCCCCGAGCGCCCTGCCTCCGAGG

608 GCCGGCGCACCATAAAAGAAGCCGCCCTAGCCACGTCCCCTCGCAGTTCGGCGGTCCCGCGGGTCTGTCTCAAGCTTGCCGCCAGAACACAGgtaagtgcc

709 gtgtgtggttcccgcgggcctggcctctttacgggttatggcccttgcgtgccttgaattacttccatgcccctggctgcagtacgtgattcttgatcccga

811 gcttcgggttggaagtgggtgggagagttcgaggccttgcgcttaaggagccccttcgcctcgtgcttgagttgaggcctggcttgggcgctggggccgccg

913 cgtgctaatctggtggcaccttcgcgcctgtctcgctgctttcgctaagtctctagccatttaaaatttttgataaccagctgcgacgctttttttctggcg

1015 agatagtcttgtaaatgcgggccaagatctgcacactggtatttcggtttttggggccgcgggcggcgacggggcccgtgcgtcccagcgcacatgttcgg

1116 cgaggcggggcctgcgagcgcggccaccgagaatcggacgggggtagtctcaaactggccggcctgctctggtgcctggcctcgcgccgccgtgtatcgccc

1218 cgccctgggcggcaaggctggcccggtcggcaccagttgcgtgagcggaaagatggccgcttcccggccctgctgcagggagctcaaaatggaggacgcggc

1320 gcccgggagagcgggcgggtgagtcacccacacaaaggaaaagggcctttccttcctcatccgtcgcttcatgtgactccacggagtaccgggcgccgtcca

XhoI (1437)

1422 ggcacctcgattagttctcgagcttttggagtacgtcgtctttaggttggggggaggggttttatgcgatggagtttccccacactgagtgggtggagact

1523 gaagagttaggccagcttggcacttgatgtaattctccttggaatttgccctttttgagtttggatcttgcctcattctcaagcctcagacagtggttcaaa

NcoI (1672)

1625 gtttttttcttccatttcagGTGTCGTGAAAACTACCCCTAAAAGCCACCATGGTTCTGGGGCCCTGCATGCTGCTGCTGCTGCTGCTGCTGGGCCTGAGGC

1▸ M V L G P C M L L L L L L L G L R

1727 TACAGCTCTCCCTGGGCATCATCCCAGTTGAGGAGGAGAACCCGGACTTCTGGAACCGCGAGGCAGCCGAGGCCCTGGGTGCCGCCAAGAAGCTGCAGCCTG

18▸ L Q L S L G I I P V E E E N P D F W N R E A A E A L G A A K K L Q P

1829 CACAGACAGCCGCCAAGAACCTCATCATCTTCCTGGGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCCTAAAAGGGCAGAAGAAGGACAAAC

52▸ A Q T A A K N L I I F L G D G M G V S T V T A A R I L K G Q K K D K

1931 TGGGGCCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCA

86▸ L G P E I P L A M D R F P Y V A L S K T Y N V D K H V P D S G A T A

2033 CGGCCTACCTGTGCGGGGTCAAGGGCAACTTCCAGACCATTGGCTTGAGTGCAGCCGCCCGCTTTAACCAGTGCAACACGACACGCGGCAACGAGGTCATCT

120▸ T A Y L C G V K G N F Q T I G L S A A A R F N Q C N T T R G N E V I

2135 CCGTGATGAATCGGGCCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACCACCACACGAGTGCAGCACGCCTCGCCAGCCGGCACCTACGCCCACACGGTGA

154▸ S V M N R A K K A G K S V G V V T T T R V Q H A S P A G T Y A H T V

2237 ACCGCAACTGGTACTCGGACGCCGACGTGCCTGCCTCGGCCCGCCAGGAGGGGTGCCAGGACATCGCTACGCAGCTCATCTCCAACATGGACATTGATGTGA

188▸ N R N W Y S D A D V P A S A R Q E G C Q D I A T Q L I S N M D I D V

2339 TCCTGGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCCAGACCCTGAGTACCCAGATGACTACAGCCAAGGTGGGACCAGGCTGGACGGGAAGAATC

222▸ I L G G G R K Y M F R M G T P D P E Y P D D Y S Q G G T R L D G K N

2441 TGGTGCAGGAATGGCTGGCGAAGCGCCAGGGTGCCCGGTATGTGTGGAACCGCACTGAGCTCATGCAGGCTTCCCTGGACCCGTCTGTGACCCATCTCATGG

256▸ L V Q E W L A K R Q G A R Y V W N R T E L M Q A S L D P S V T H L M

2543 GTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCCTGCTGAGCA

290▸ G L F E P G D M K Y E I H R D S T L D P S L M E M T E A A L R L L S

2645 GGAACCCCCGCGGCTTCTTCCTCTTCGTGGAGGGTGGTCGCATCGACCACGGTCATCACGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTCG

324▸ R N P R G F F L F V E G G R I D H G H H E S R A Y R A L T E T I M F

2747 ACGACGCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGGACACGCTGAGCCTCGTCACTGCCGACCACTCCCACGTCTTCTCCTTCGGAGGCTACCCCC

358▸ D D A I E R A G Q L T S E E D T L S L V T A D H S H V F S F G G Y P

2849 TGCGAGGGAGCTCCATCTTCGGGCTGGCCCCTGGCAAGGCCCGGGACAGGAAGGCCTACACGGTCCTCCTATACGGAAACGGTCCAGGCTATGTGCTCAAGG

392▸ L R G S S I F G L A P G K A R D R K A Y T V L L Y G N G P G Y V L K

2951 ACGGCGCCCGGCCGGATGTTACCGAGAGCGAGAGCGGGAGCCCCGAGTATCGGCAGCAGTCAGCAGTGCCCCTGGACGAAGAGACCCACGCAGGCGAGGACG

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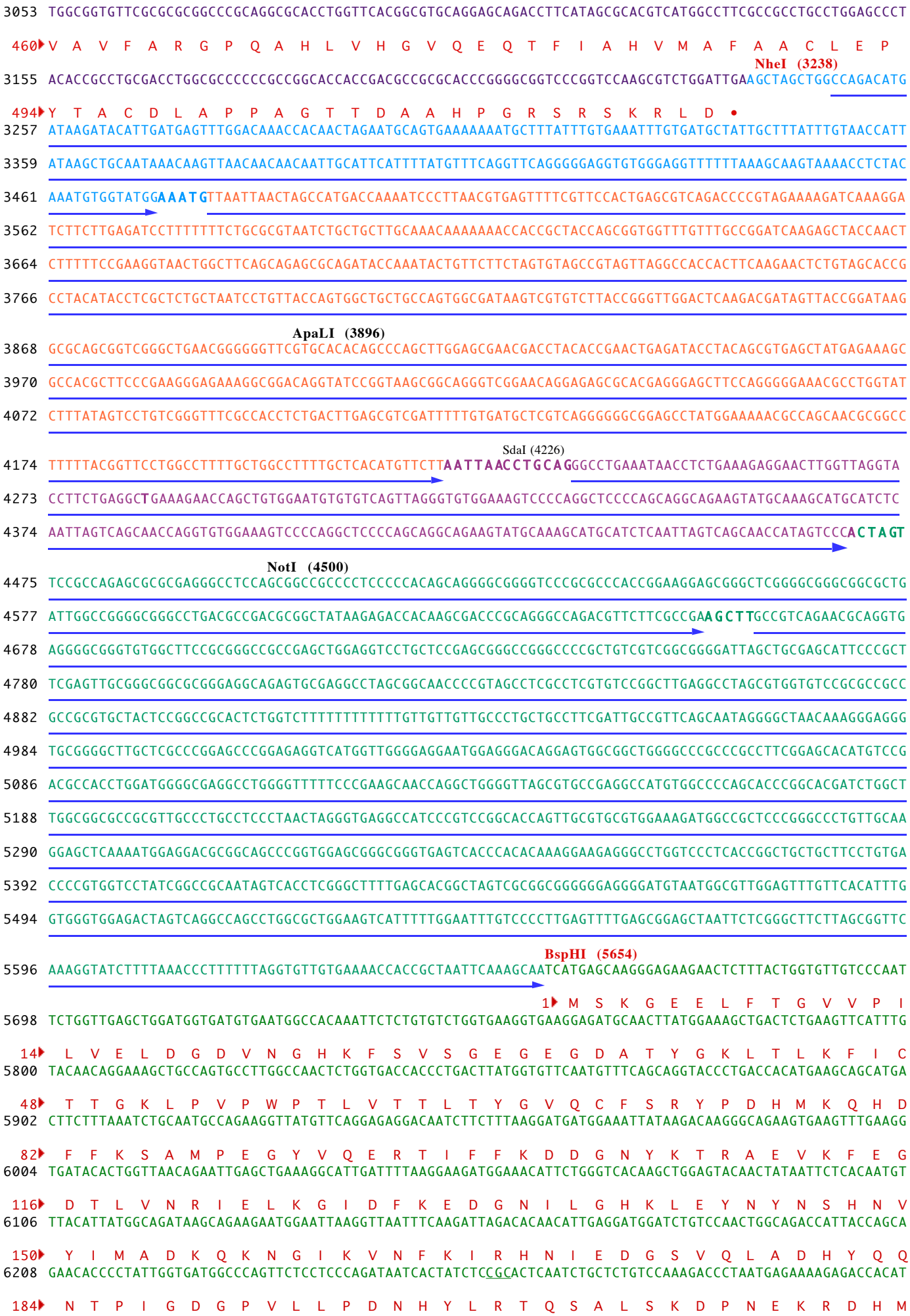
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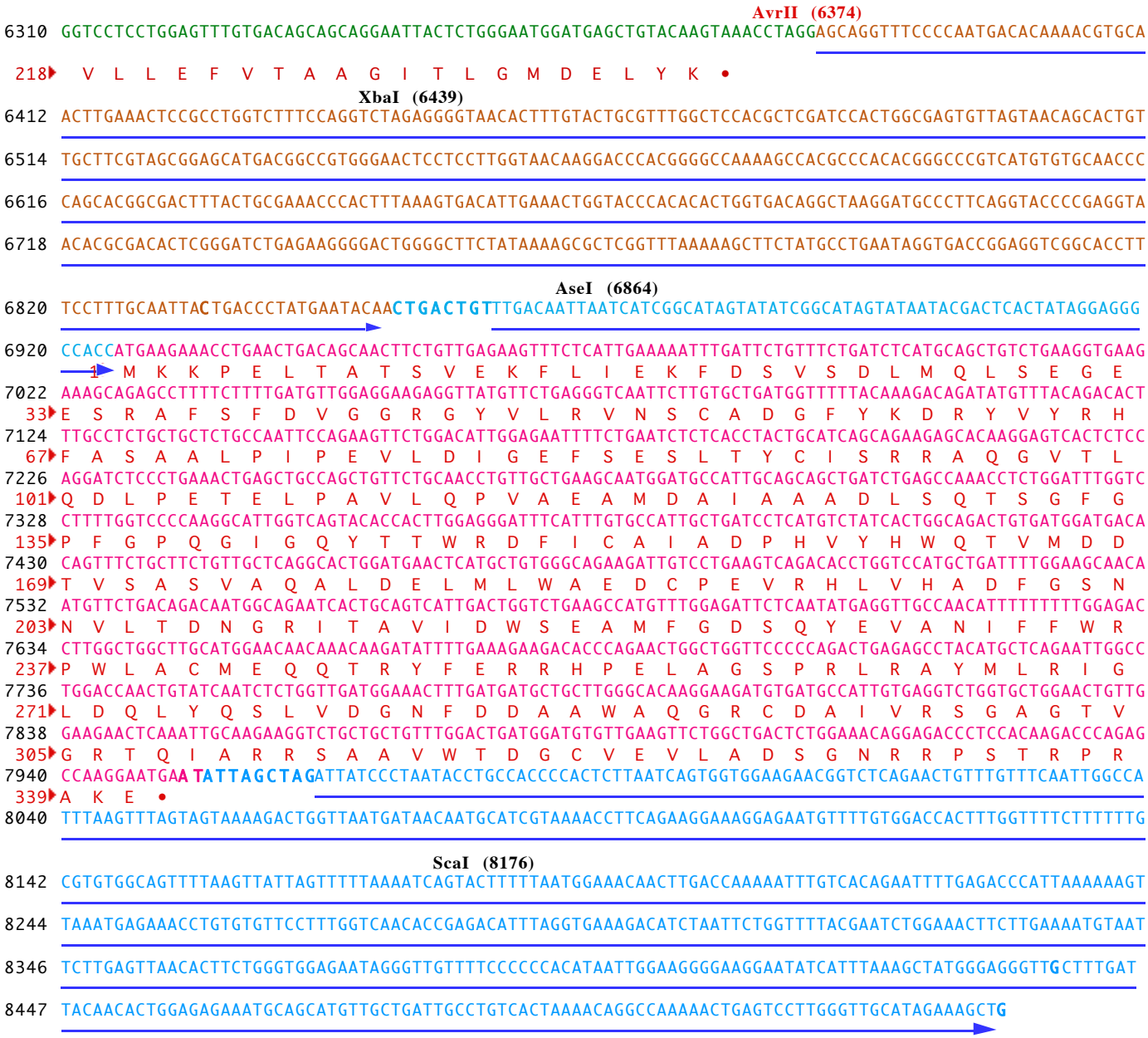
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