STOP

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pVITRO2-blasti-Lucia/SEAP

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

Catalog code: pvitro2-blucsp

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

For research use only

Version 20H17-MM

PRODUCT INFORMATION

Contents:

- 20 µg of pVITRO2-blasti-Lucia/SEAP provided as lyophilized DNA
- 2 x 1 ml blasticidin at 10 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 blasticidin 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 vectors with improved features. pVITRO plasmids allow the co-expression of two or more genes from two different transcription units. pVITRO plasmids can be stably transfected in mammalian cells and are expressed at high levels.

pVITRO2-Lucia/SEAP contains the Lucia luciferase and SEAP reporter genes. pVITRO2-Lucia/SEAP can be used as a control vector. **pVITRO2-Lucia/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 Lucia luciferase and Nco I/Nhe I for SEAP) that allow for convenient cloning of ORFs.

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.

Blasticidin usage

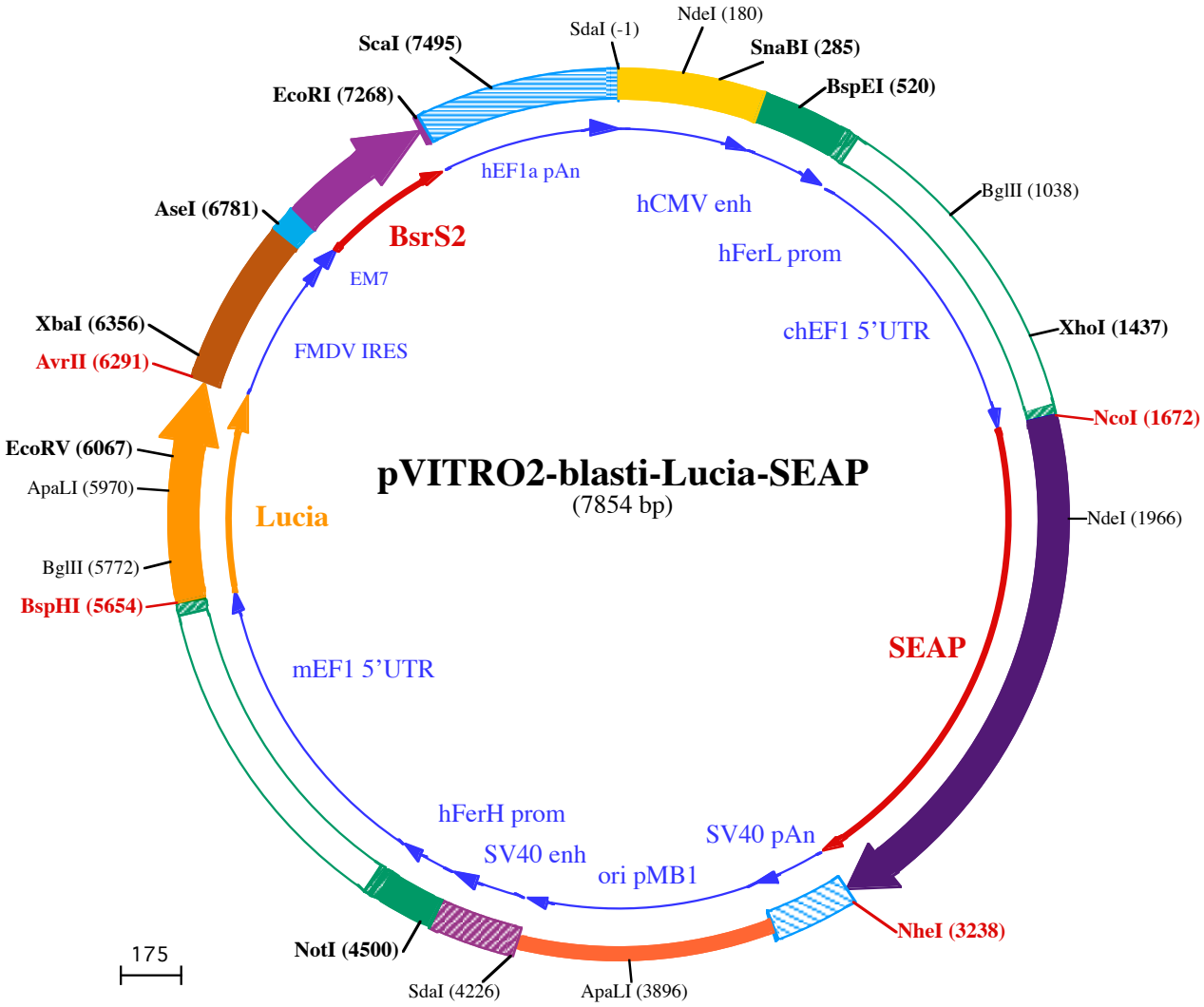
Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

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) is composed of unique and repeated sequence motifs. The HCMV enhancer can substitute for the 72-bp repeats of SV40 and is several-fold more active than the SV40 enhancer[3].
- **pMB1 Ori** is a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **Lucia luciferase** is a synthetic CpG-free gene that codes for a secreted coelenterazine-utilizing luciferase. ORF size (from the ATG to the stop codon): 634 bp. Lucia luciferase activity can be evaluated using QUANTI-Luc™, an assay reagent containing all the components required to quantitively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases.
- **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*.
- **Blasti:** Resistance to blasticidin is conferred by the *bsr* gene from *Bacillus cereus*. In bacteria, *bsr* is expressed from the constitutive *E. coli* EM7 promoter. In mammalian cells, *bsr* is transcribed from the hFerH/mEF1α 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. Kim DW. *et al.*, **1990.** Use of the human elongation factor 1α promoter as a versatile and efficient expression system Gene 91:217-23. **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 41:521-30. **4. Ramesh N. et al., 1996.** High-titer bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. Nucleic Acids Res. 24:2697-700. **5. Carswell S. & Alwine JC. ,1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol. Cell Biol. 9: 4248-58.

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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(7854 bp)
ScaI (7495)
SdaI (-1)
NdeI (180)
SnaBI (285)
BspEI (520)
EcoRI (7268)
BglII (1038)
AseI (6781)
XhoI (1437)
XbaI (6356)
AvrII (6291)
NcoI (1672)
EcoRV (6067)
ApaLI (5970)
NdeI (1966)
BglII (5772)
BspHI (5654)
NheI (3238)
NotI (4500)
SdaI (4226)
ApaLI (3896)
175
hEF1a pAn
hCMV enh
hFerL prom
chEF1 5'UTR
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BsrS2
Lucia
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SV40 enh
ori pMB1
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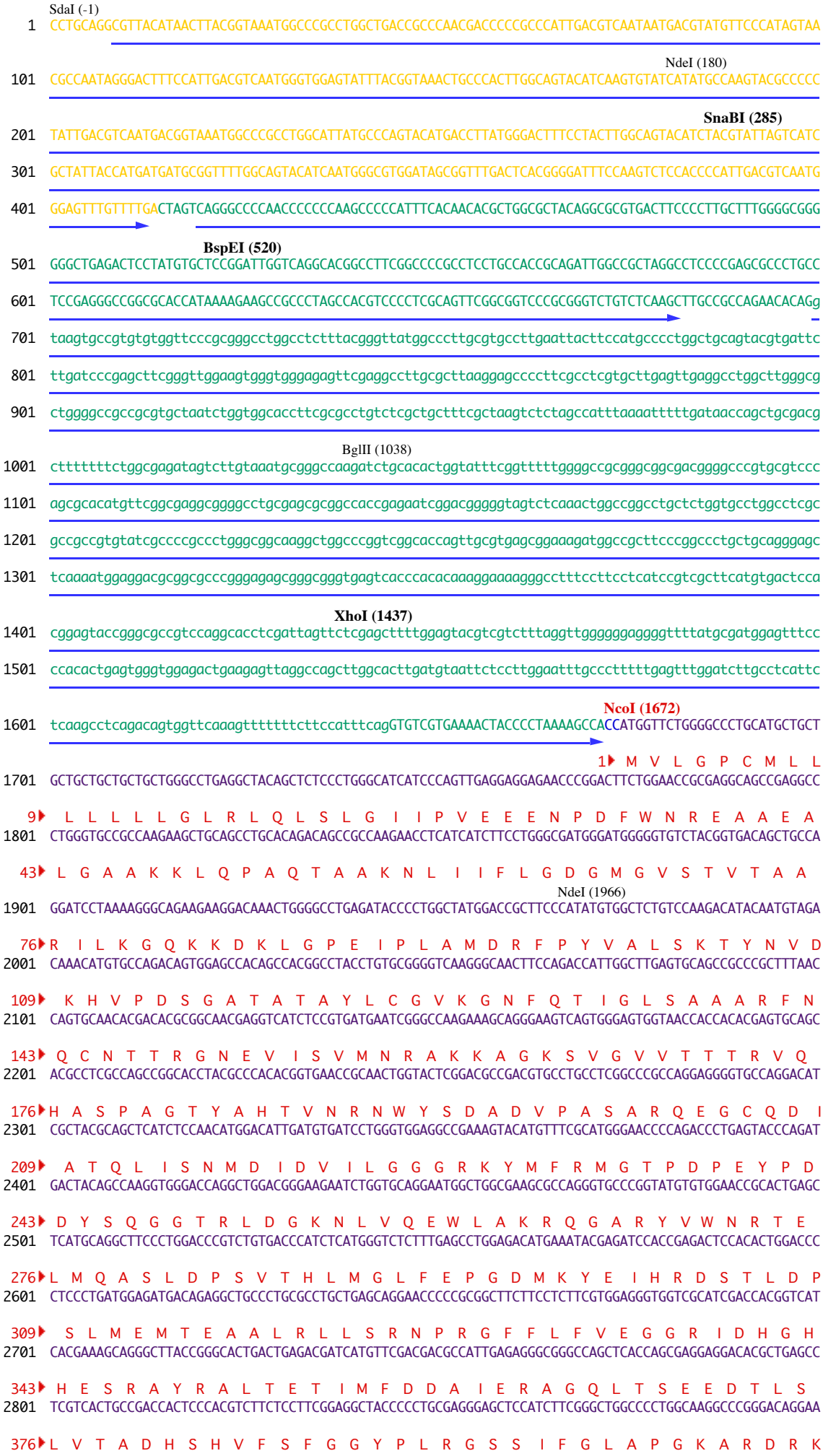
SEAP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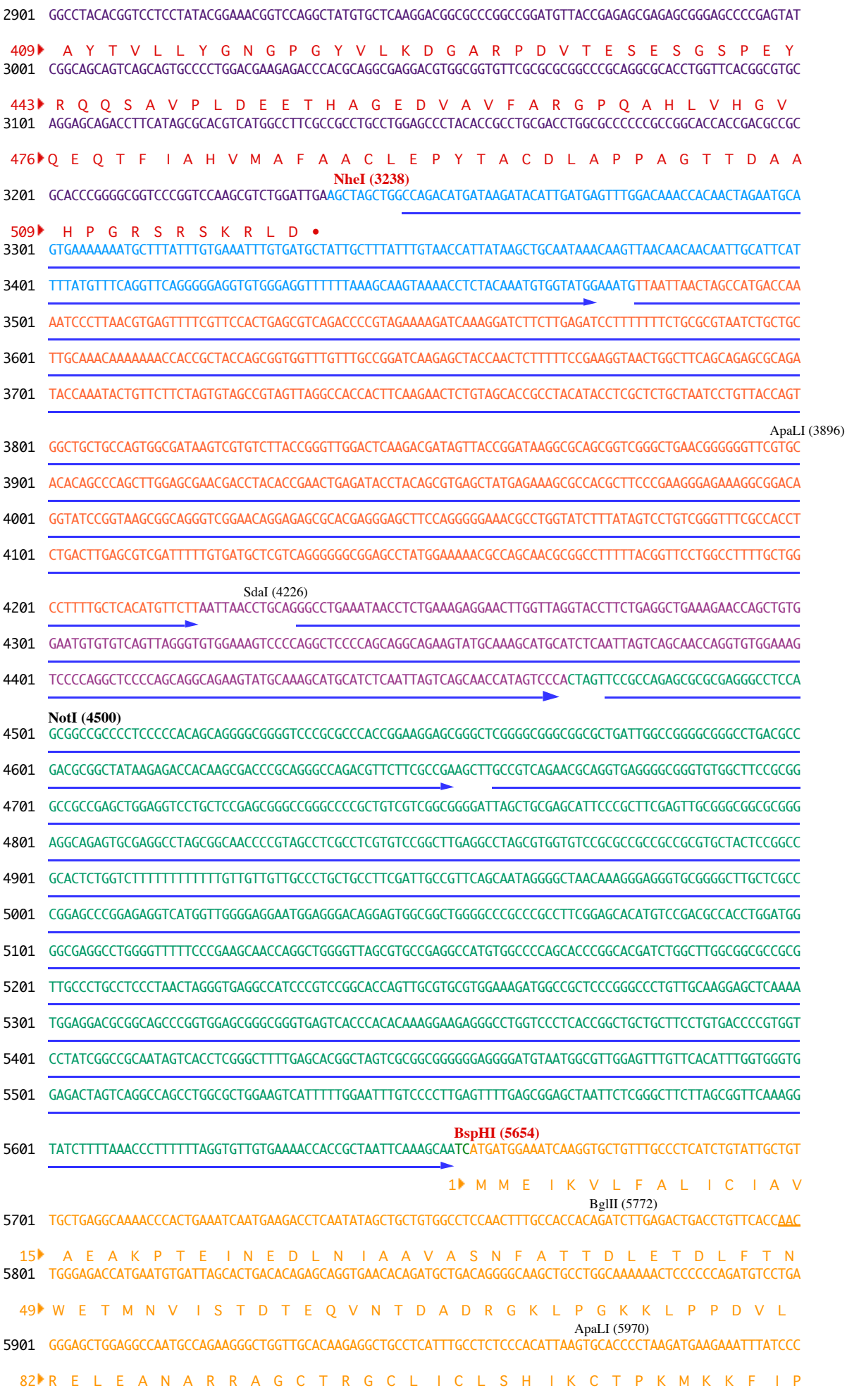
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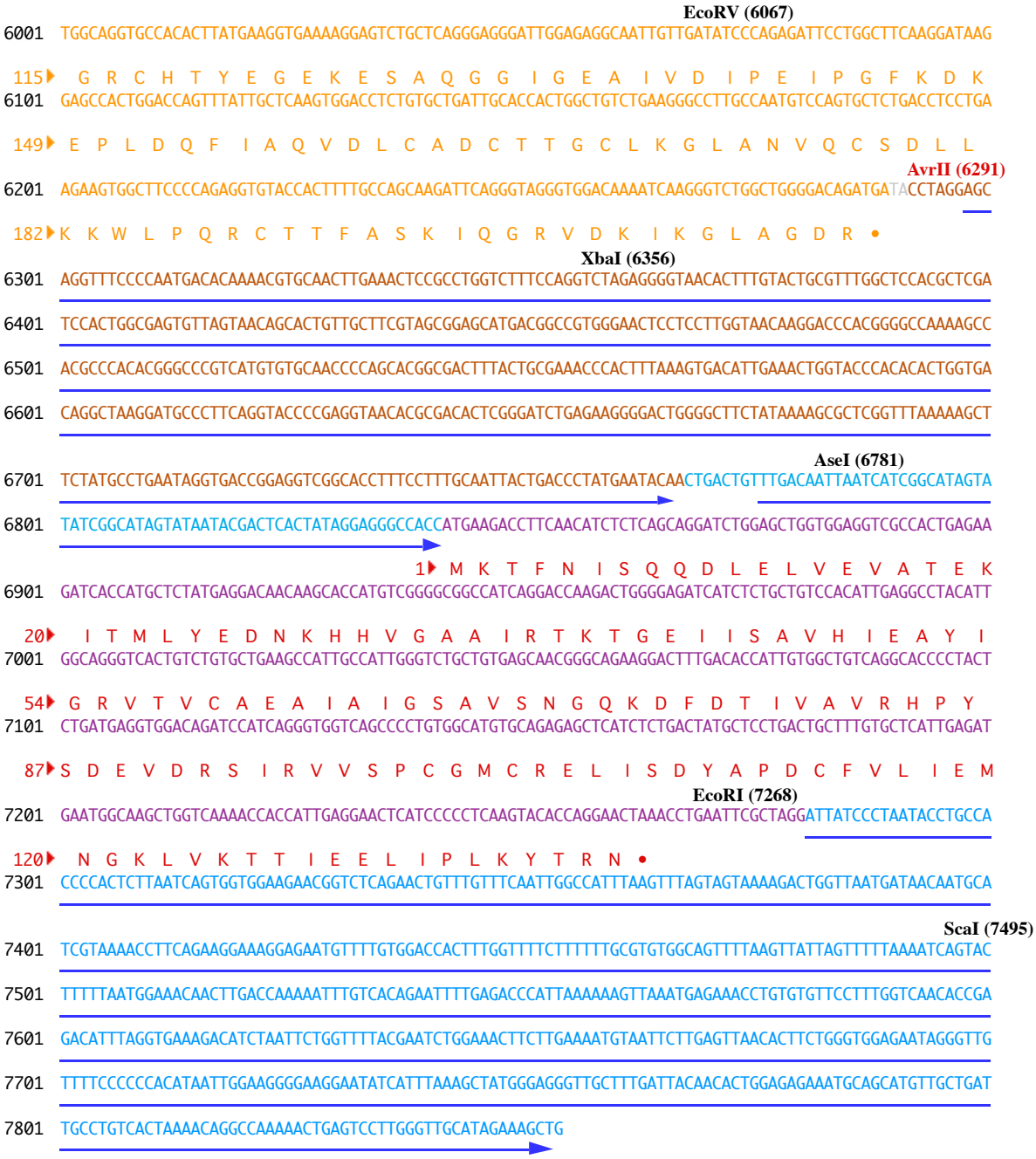
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115 G R C H T Y E G E K E S A Q G G I G E A I V D I P E I P G F K D K
6101 GAGCCACTGGACCAGTTTATTGCTCAAGTGGACCTCTGTGCTGATTGCACCACTGGCTGTCTGAAGGGCCTTGCCAATGTCCAGTGCTCTGACCTCCTGA
149 E P L D Q F I A Q V D L C A D C T T G C L K G L A N V Q C S D L L
AvrII (6291)
6201 AGAAGTGGCTTCCCCAGAGGTGTACCACTTTTGCCAGCAAGATTCAGGGTAGGGTGGACAAAATCAAGGGTCTGGCTGGGGACAGATGATACCTAGGAGC
182 K K W L P Q R C T T F A S K I Q G R V D K I K G L A G D R •
XbaI (6356)
6301 AGGTTTCCCCAATGACACAAAACGTGCAACTTGAAACTCCGCCTGGTCTTTCCAGGTCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGA
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6601 CAGGCTAAGGATGCCCTTCAGGTACCCCGAGGTAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCT
AseI (6781)
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7001 GGCAGGGTCACTGTCTGTGCTGAAGCCATTGCCATTGGGTCTGCTGTGAGCAACGGGCAGAAGGACTTTGACACCATTGTGGCTGTCAGGCACCCCTACT
54 G R V T V C A E A I A I G S A V S N G Q K D F D T I V A V R H P Y
7101 CTGATGAGGTGGACAGATCCATCAGGGTGGTCAGCCCCTGTGGCATGTGCAGAGAGCTCATCTCTGACTATGCTCCTGACTGCTTTGTGCTCATTGAGAT
87 S D E V D R S I R V V S P C G M C R E L I S D Y A P D C F V L I E M
EcoRI (7268)
7201 GAATGGCAAGCTGGTCAAAACCACCATTGAGGAACTCATCCCCCTCAAGTACACCAGGAACTAAACCTGAATTCGCTAGGATTATCCCTAATACCTGCCA
120 N G K L V K T T I E E L I P L K Y T R N •
7301 CCCCACTCTTAATCAGTGGTGGAAGAACGGTCTCAGAACTGTTTGTTTCAATTGGCCATTTAAGTTTAGTAGTAAAAGACTGGTTAATGATAACAATGCA
ScaI (7495)
7401 TCGTAAAACCTTCAGAAGGAAAGGAGAATGTTTTGTGGACCACTTTGGTTTTCTTTTTTGCGTGTGGCAGTTTTAAGTTATTAGTTTTTAAAATCAGTAC
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