pVITRO2-neo-mcs

An innovative multigenic plasmid for high levels of expression

Catalog # pvitro2-nmcs

For research use only

Version # 05E18-MT

PRODUCT INFORMATION

Contents:

- 20 µg of pVITRO2-neo-mcs provided as lyophilized DNA
- 4 pouches of *E. coli* FastMedia™ Kan

Storage and Stability:

- Products are shipped at room temperature. Lyophilized DNA should be resuspended upon receipt and stored at - 20°C (see Methods).
- Lyophilized DNA is stable 12 months at -20°C. Resuspended DNA is stable more than one year at -20°C. Avoid repeated freeze-thaw cycles.
- Store *E. coli* FastMedia™ Kan pouches at room temperature. FastMedia™ is stable 18 months when stored properly.

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-neo-mcs plasmid is selectable in *E. coli* with kanamycin and in mammalian cells with G418. It contains two multiple cloning sites (MCS) for the convenient cloning of two cDNAs.

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 To eliminate the iron regulation of the ferritin promoters, the 5'UTR of FerH and FerL have been replaced by the 5'UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.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[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].

• **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.*[4]

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

• **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[5].

• **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. The neo gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.

• **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.

• **MCS1 and MCS2:** Each multiple cloning site contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning.

MCS1 includes the following restriction sites:

Age I, *Eco* RV, *Bam* HI, *Sal* I and *Avr* II

- *Age* I is compatible with *Bsp* EI and *Sgr* AI.
- *Eco* RV (blunt-end restriction enzyme)
- *Bam* HI is compatible with *Bgl* II, *Bst* YI and *Bcl* I.
- *Sal* I is compatible with *Ava* I *and Xho* I.
- *Avr* II is compatible with *Xba* I, *Spe* I and *Nhe* I.

MCS2 includes the following restriction sites:

Sgr AI, *Bgl* II, *Xho* I *and Nhe* I

- *Sgr* AI is compatible with *Bsp* EI and *Age* I.
- *Bgl* II is compatible with *Bam* HI, *Bst* YI and *Bcl* I.
- *Xho* I is compatible with *Ava* I *and Sal* I.
- *Nhe* I is compatible with *Xba* I, *Spe* I and *Avr* II.

References:

1. Eisenstein RS. and Munro HN., 1990. Translational regulation of ferritin synthesis by iron. Enzyme 44(1-4):42-58
2. Moreau P. *et al.*, 1981. The SV40 72 base repair repeat has a striking effect on gene expression both in SV40 and other chimeric recombinants. Nucleic Acids Res. 9(22):6047-68.
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. 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.
5. 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.

TECHNICAL SUPPORT
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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.

Selection of bacteria with *E. coli* FastMedia™ Kan:

E. coli FastMedia™ Kan is a **fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* FastMedia™ Kan is a TB (liquid) or LB (solid) based medium with kanamycin, and contains stabilizers.

Method:

1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.
2- Add 200 ml of distilled water to the flask
3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave FastMedia™.**
4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
6- Let agar medium cool to 45˚C before pouring plates. Let liquid media cool to 37˚C before seeding bacteria.

Note: *Do not reheat solidified FastMedia™ as the antibiotic will be permanently destroyed by the procedure.*

RELATED PRODUCTS

Product	Quantity	Catalog Code
pVITRO2-neo-GFP/LacZ	20 µg	pvitro2-ngfplacz
G418	1 g	ant-gn-1
	5 mg	ant-gn-5
Fast-Media™ Kan Agar	20 pouches	fas-kn-s
Fast-Media™ Kan TB	20 pouches	fas-kn-l

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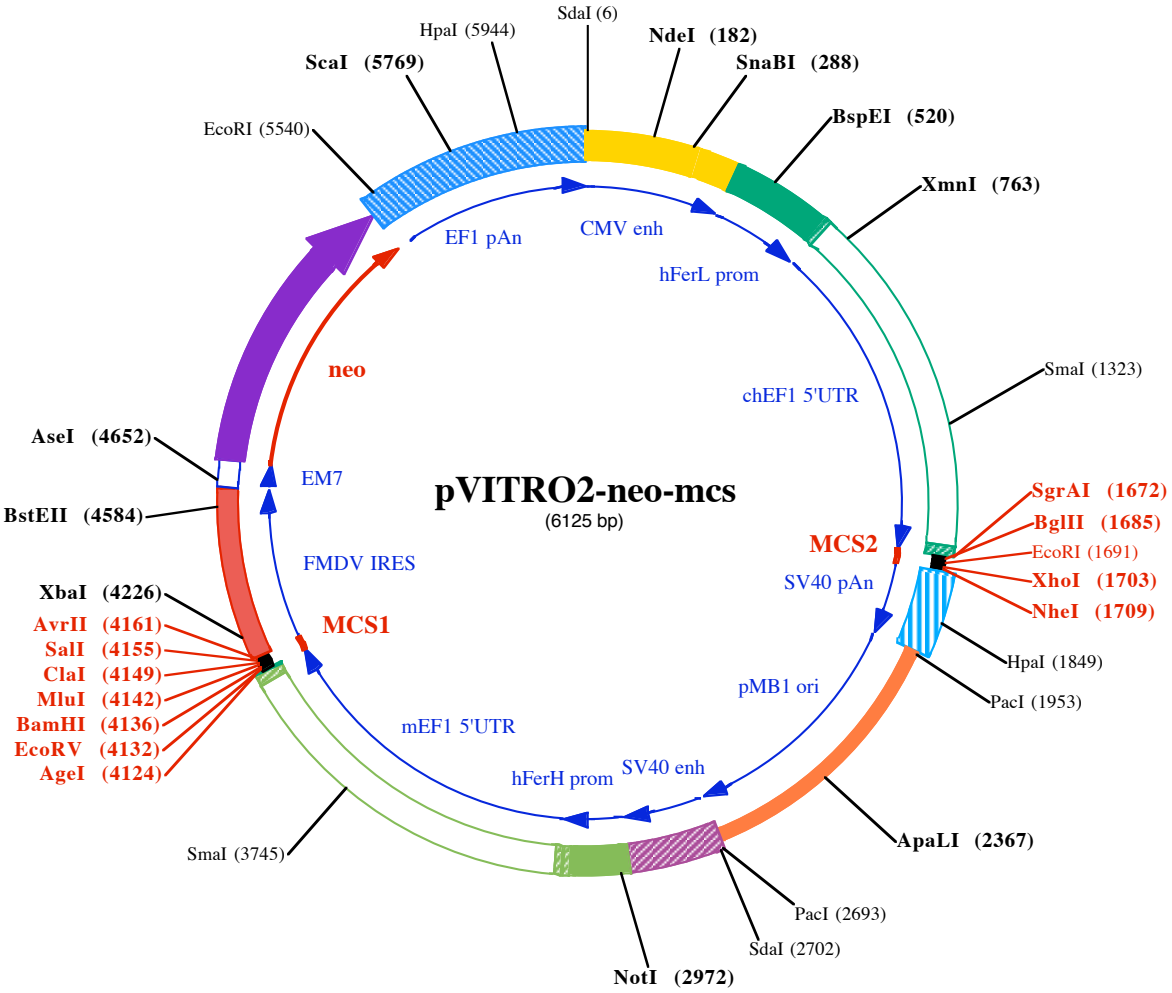
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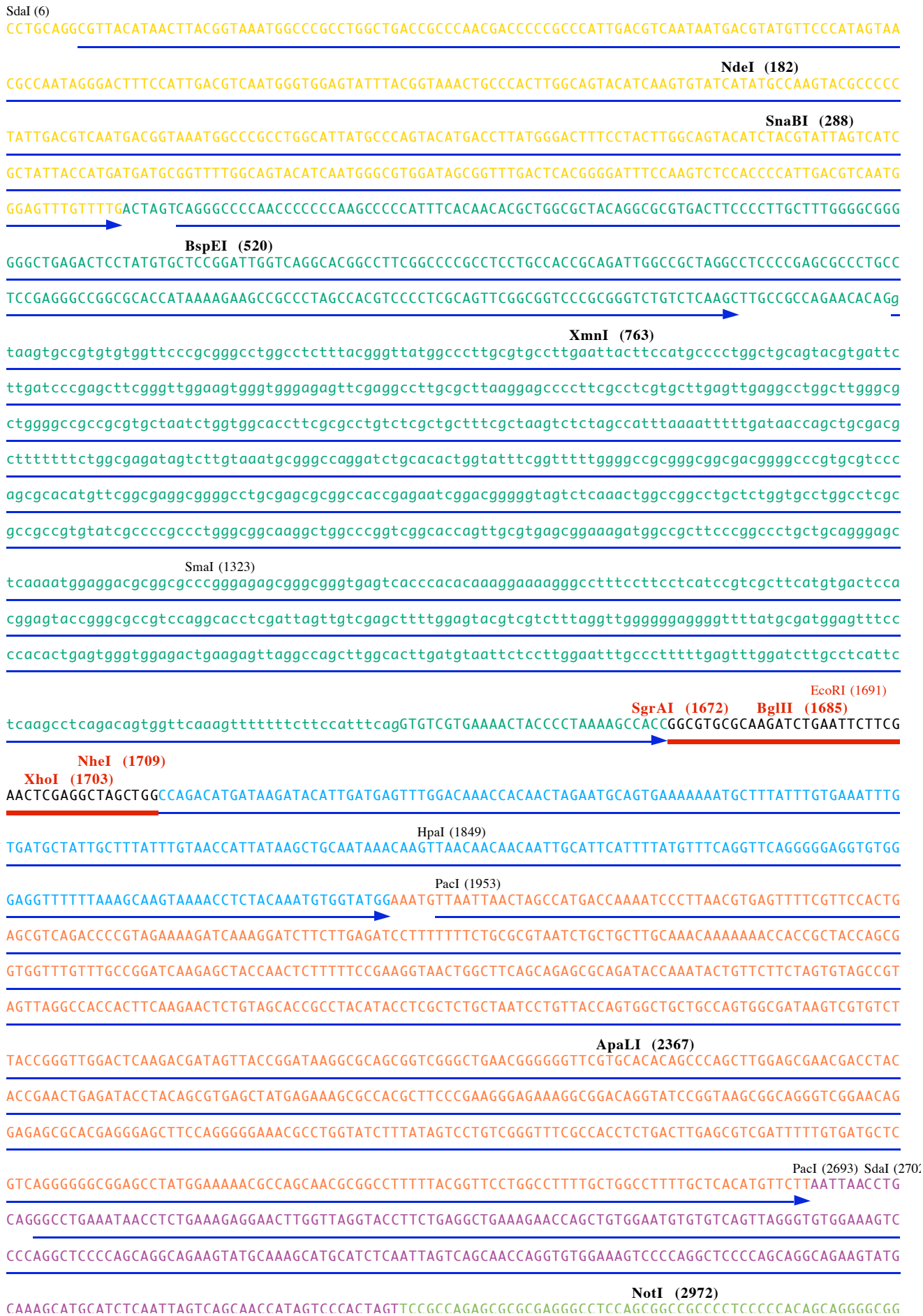
SdaI (6)
NdeI (182)
SnaBI (288)
BspEI (520)
XmnI (763)
SmaI (1323)
SgrAI (1672)
BglII (1685)
EcoRI (1691)
XhoI (1703)
NheI (1709)
HpaI (1849)
PacI (1953)
ApaLI (2367)
PacI (2693)
SdaI (2702)
NotI (2972)
SmaI (3745)
mEF1 5'UTR
hFerH prom
SV40 enh
pMB1 ori
SV40 pAn
MCS2
chEF1 5'UTR
hFerL prom
CMV enh
EF1 pAn
HpaI (5944)
ScaI (5769)
EcoRI (5540)
neo
EM7
FMDV IRES
MCS1
AseI (4652)
BstEII (4584)
XbaI (4226)
AvrII (4161)
SalI (4155)
ClaI (4149)
MluI (4142)
BamHI (4136)
EcoRV (4132)
AgeI (4124)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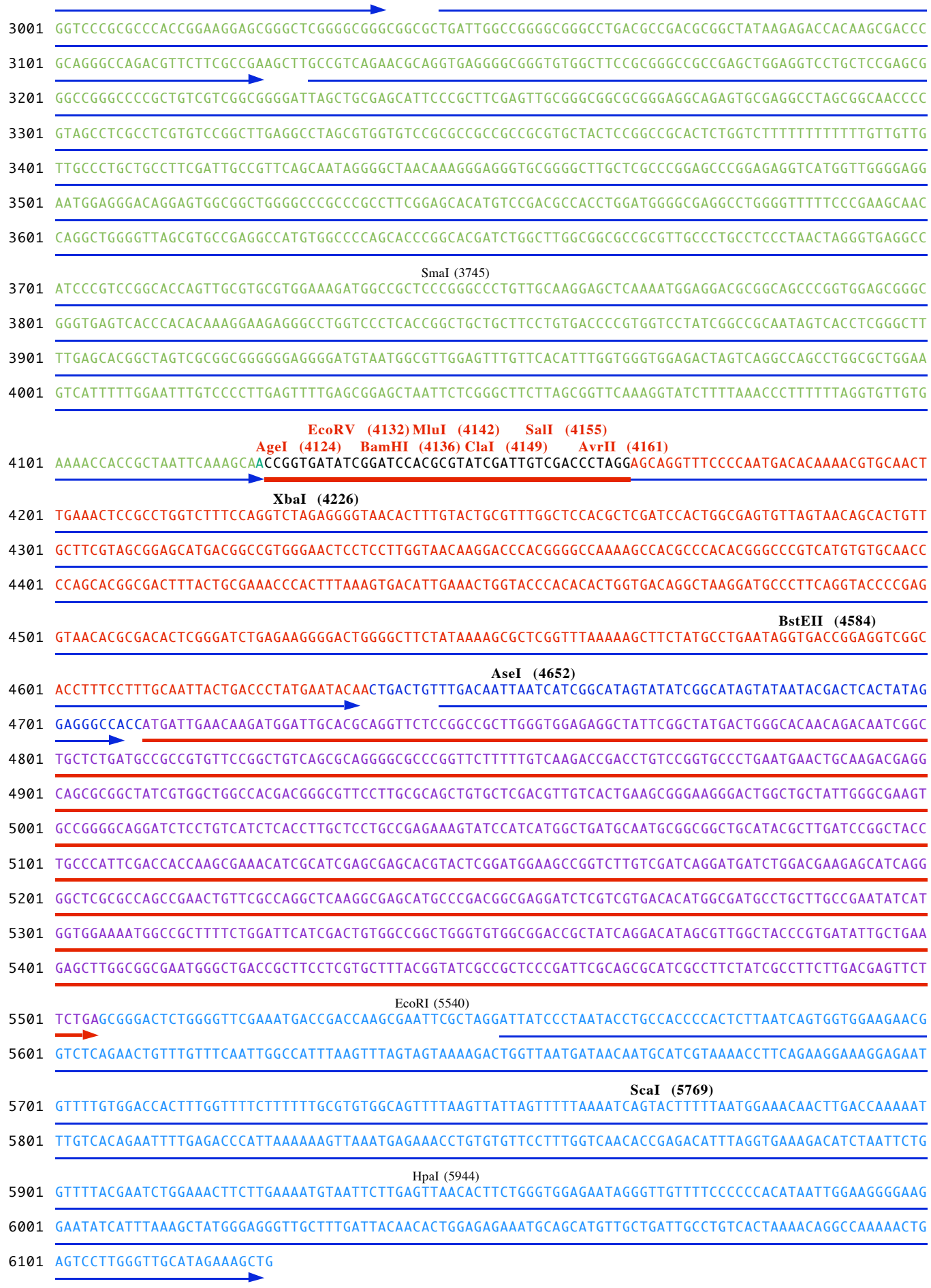
HpaI (1849)
PacI (1953)
ApaLI (2367)
PacI (2693)
SdaI (270
NotI (2972)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AgeI (4124) BamHI (4136) ClaI (4149) AvrII (4161)
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