STOP

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pVIVO1-Lucia/SEAP

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

Catalog code: pvivo1-lucsp

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

For research use only

Version 19J02-MM

PRODUCT INFORMATION

Contents

- 20 µg of pVIVO1-Lucia/SEAP provided as lyophilized DNA
- 1 ml of Hygromycin B Gold (ultrapure Hygromycin B;100 mg/ml)

Storage and Stability

- Product is shipped at room temperature.
- Store lyophilized DNA at -20 °C.
- Resuspended DNA is stable for 12 months when stored at -20 °C. Avoid repeated freeze-thaw cycles.
- Store Hygromycin B Gold at 4 °C or at -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

pVIVO1 is a multigenic vector with two transcription units allowing the combined expression of two genes of interest from a single vector. **pVIVO1-Lucia/SEAP** contains two reporter genes (Lucia luciferase and SEAP) and can be used as a control vector.

pVIVO1-Lucia/SEAP also can be used for cloning of open reading frames (ORF). Both reporter genes are flanked by unique sites (*Bsp*H I/*Avr* II for Lucia luciferase and *Nco* I/*Nhe* I for SEAP) that allow for convenient cloning of ORFs which can be selected from InvivoGen's extensive list of genes.

For more information, visit: https://www.invivogen.com/genes.

PLASMID FEATURES

• **haGRP78 and hGRP94 prom:** The hamster GRP78 and human GRP94 promoters drive weak levels of expression in normal conditions and are induced in stress conditions prevailing inside tumors, such as glucose deprivation and hypoxia[1]. Within the tumor micro-environment, the GRP promoters yield persistent expression whereas the activity of viral promoters declines rapidly[2].

• **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[3].

• **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 severalfold more active than the SV40 enhancer[4].

• **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™ or QUANTI-Luc™, assay reagents containing all the components required to quantitively measure the activity of Lucia luciferase and other coelenterazine-utilizing luciferases.

• **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]

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

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

• **Hygro-ΔCpG** is a new allele of the *hph* gene conferring resistance to hygromycin B. In order to reduce the immunogenicity of this bacterial gene all CpG motifs have been removed by chemically synthesizing the gene. The *Hygro-ΔCpG* gene allows the selection of *E. coli* clones transformed with a pVIVO plasmid.
Note: Stable transfection of mammalian clones cannot be performed due to the absence of a eukaryotic promoter upstream of the Hygro-ΔCpG gene.

• **Term:** The *E. coli rps O* terminator allows efficient transcription termination of the *Hygro-ΔCpG* gene.

• **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™ Solution.

• **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.

REFERENCES

1. Eisenstein RS. & Munro HN. 1990. Translational regulation of ferritin synthesis by iron. Enzyme 44(1-4):42-58. **2. Gazit G. *et al.* 1999.** Use of the glucose starvation-inducible glucose-regulated protein 78 promoter in suicide gene therapy of murine fibrosarcoma. Cancer Res 59: 3100-6 **3. Dean DA. *et al.*, 1999.** Sequence requirements for plasmid nuclear import. Exp. Cell. Res. 253:713-22. **4. Boshart M. *et al.*, 1985.** A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. Cell 41(2):521-30. **5. Carswell S. & Alwine JC. 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol. Cell Biol. 10: 4248-4258.

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 3-622-34-80
E-mail: info@invivogen.com

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

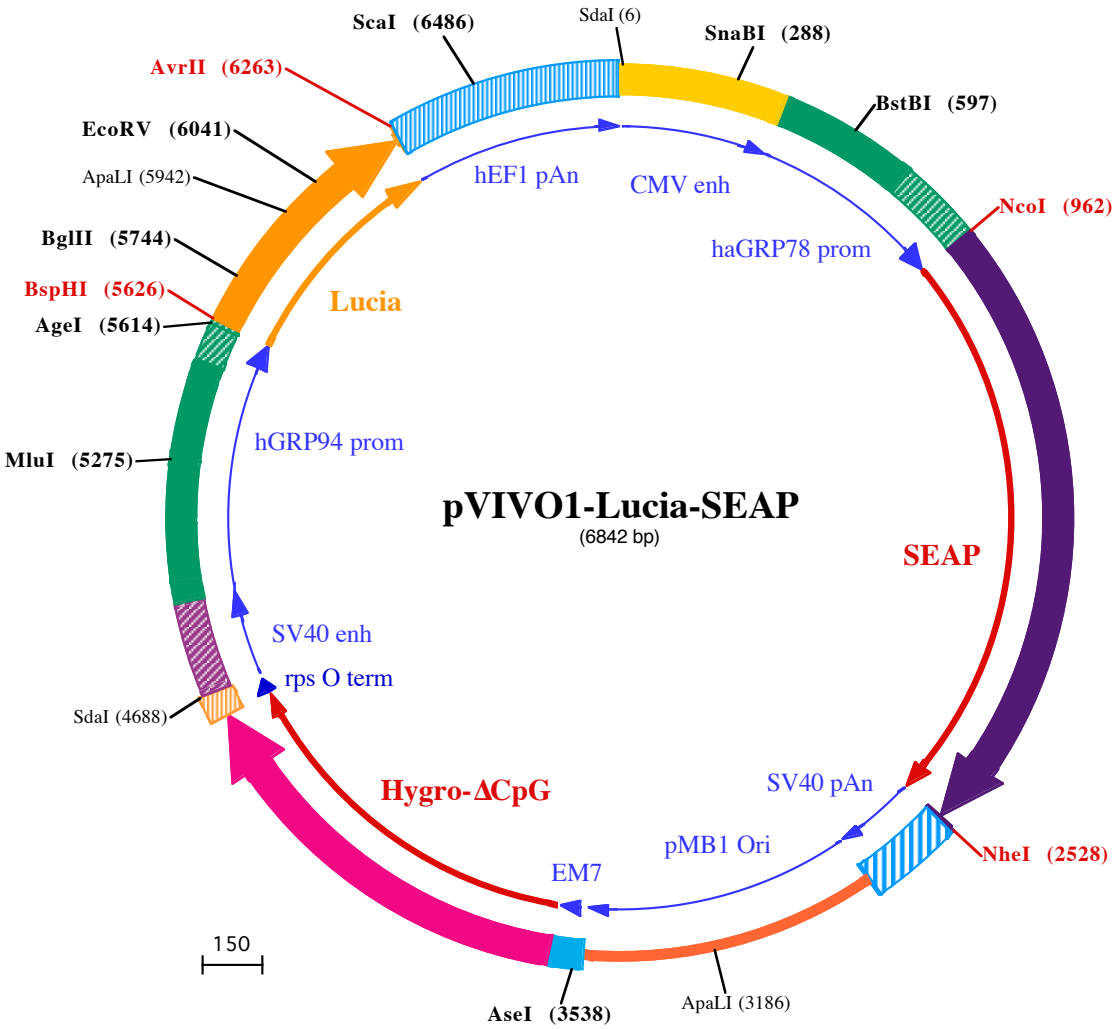
Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.

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.

RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116	Competent *E. coli*	gt116-11
HEK-Blue™ Detection	SEAP detection reagent	hb-det2
Hygromycin B Gold	Selection antibiotic	ant-hg-1
pVIVO1-GFP/LacZ	Dual reporter plasmid	pvivo1-gfplacz
pVIVO1-GFP/SEAP	Dual reporter plasmid	pvivo1-gfpsp
pVIVO1-mcs	Multiple cloning site plasmid	pvivo1-mcs
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
QUANTI-Luc™	Lucia detection reagent	rep-qlc1
QUANTI-Luc™ Gold	Lucia detection reagent	rep-qlcg1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SdaI (6)
SnaBI (288)
AvrII (6263)
BstBI (597)
EcoRV (6041)
ApaLI (5942)
hEF1 pAn
CMV enh
NcoI (962)
BglII (5744)
haGRP78 prom
BspHI (5626)
Lucia
AgeI (5614)
hGRP94 prom
MluI (5275)
pVIVO1-Lucia-SEAP
(6842 bp)
SEAP
SV40 enh
rps O term
SdaI (4688)
Hygro-ΔCpG
SV40 pAn
pMB1 Ori
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EM7
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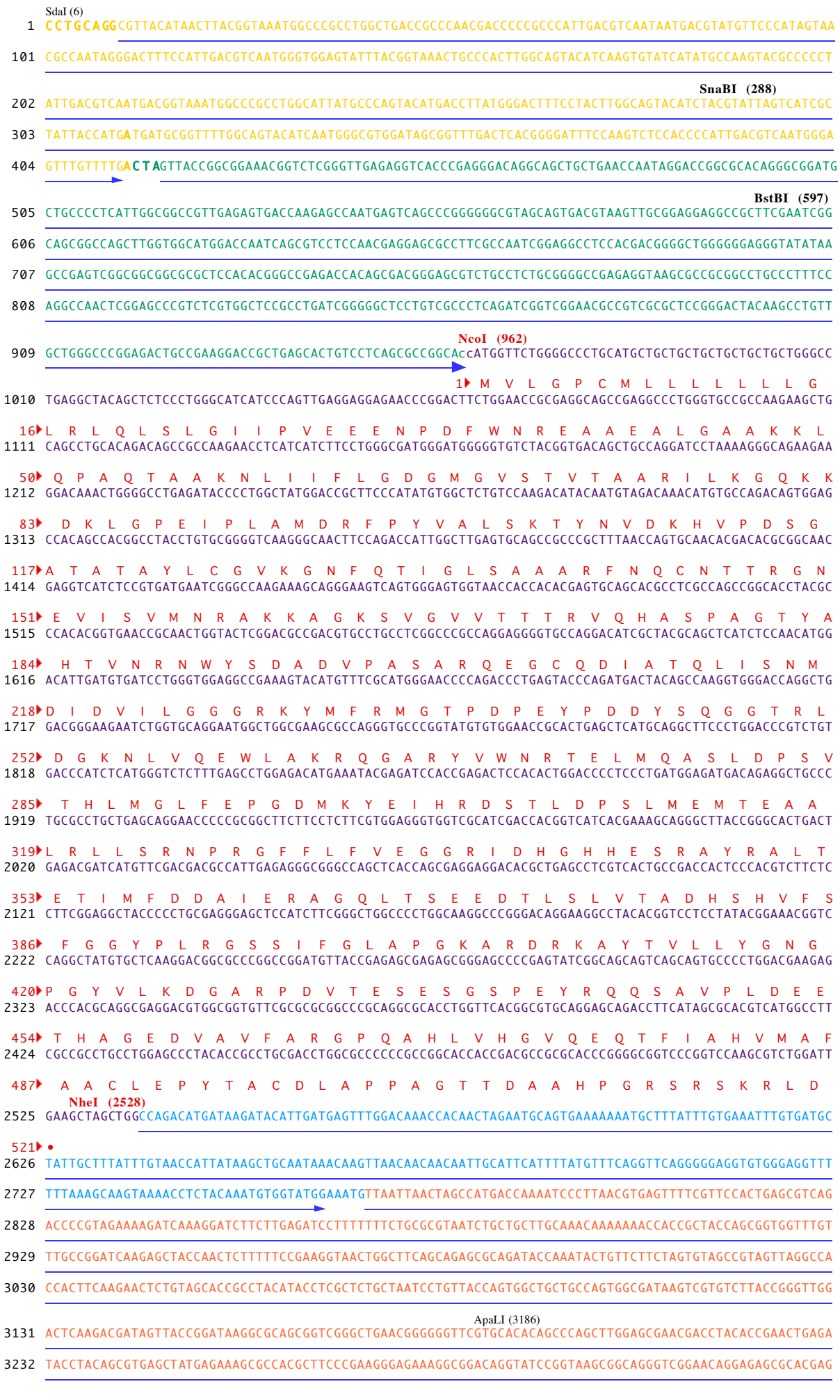
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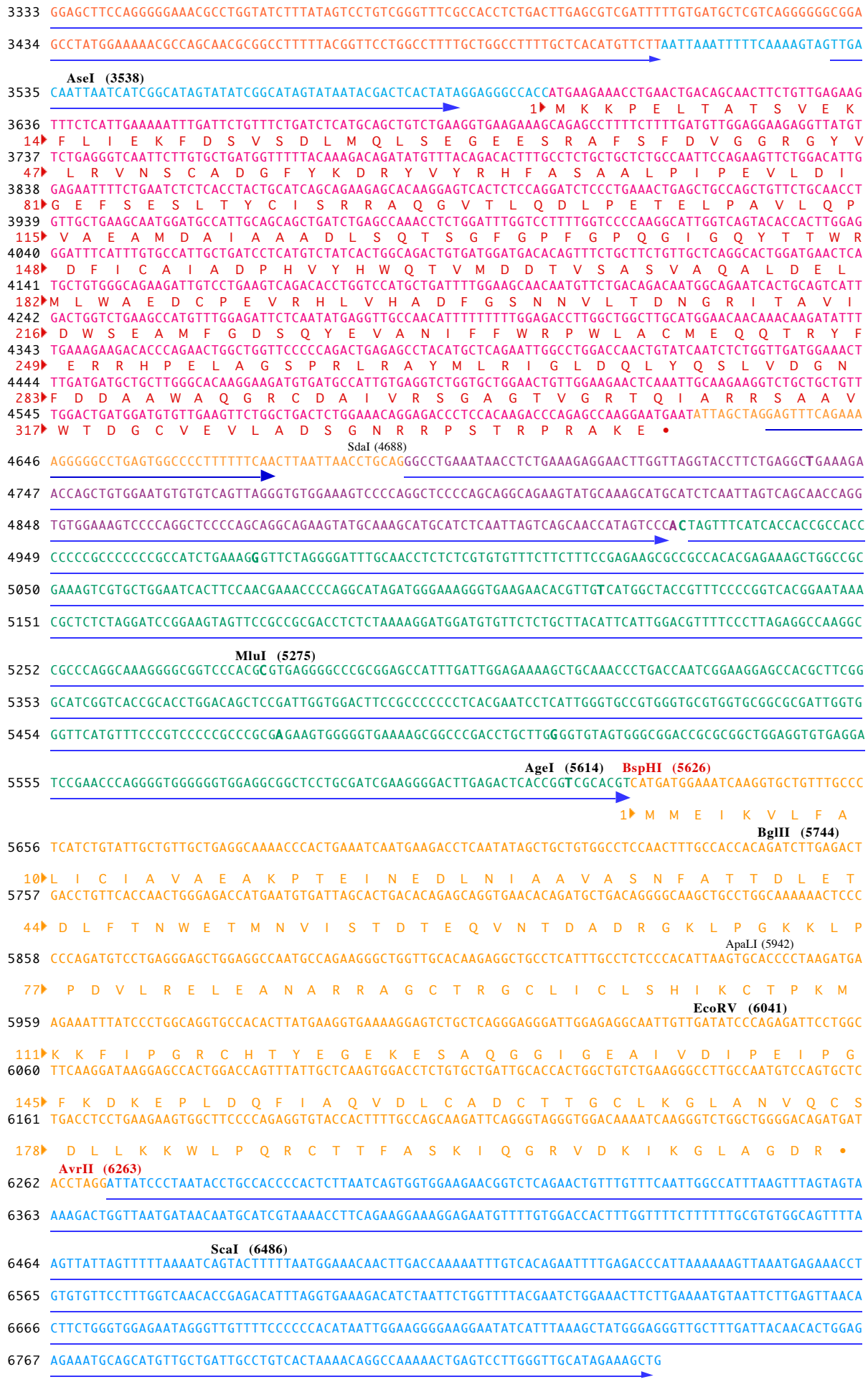
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SdaI (4688)
MluI (5275)
AgeI (5614)
BspHI (5626)
BglII (5744)
ApaLI (5942)
EcoRV (6041)
AvrII (6263)
ScaI (6486)