pMONO-zeo-GFP

A GFP-expression plasmid selectable with Zeocin™

Catalog code: pmonoz-gfp

For research use only

Version 20K26-MM

PRODUCT INFORMATION

Content:

- 20 µg of **pMONO-zeo-gfp** plasmid provided as lyophilized DNA
- 1 ml of Zeocin™ (100 mg/ml)

Storage and Stability:

Product is shipped at room temperature.

Lyophilized DNA should be resuspended upon receipt and stored at - 20°C. 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 Zeocin™ 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

pMONO plasmids are specifically designed for strong and constitutive expression of a gene of interest in a wide variety of cell lines. They allow the selection of stable transfectants and offer a choice of selectable markers. pMONO plasmids contain a unique transcription unit that drives the expression of the gene of interest and the selectable marker through an internal ribosome entry site (IRES). This dual gene expression system ensures that stable clones express the gene of interest.

pMONO-GFP plasmids feature a new allele of the GFP gene called LGFP. They can be used as control vectors or for cloning of an open reading frame, as the LGFP gene is flanked by two unique restriction sites: Bsp HI at the 5' end that encompasses the Start codon, and Avr II at the 3'end.

PLASMID FEATURES

• **SV40/FerH/mEF1a:** pMONO plasmids feature a composite ferritin promoter that confers strong and constitutive expression in a wide range of mammalian cells. The promoter is composed of the ferritin heavy chain (FerH) core promoter[1] fused at its 5' end to the SV40 enhancer, and at its 3' end to the intron-containing 5'UTR of the mouse elongation factor 1 alpha gene. This composite promoter yields similar levels of expression as the CMV promoter in all cell lines tested.

• **LGFP:** 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[2].

• **Zeo:** Resistance to Zeocin™ is conferred by the Sh ble gene from *Streptoalloteichus hindustanus*. In mammalian cells, the *Sh ble* gene is transcribed from the composite ferritin promoter as a polycistronic mRNA and translated through the FMDV IRES. In *E. coli*, *Sh ble* is transcribed from the bacterial EM7 promoter.

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

• **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA[3].

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

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

Zeocin™ usage

This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

References:

1. Eisenstein RS. and Munro HN. 1990. Translational regulation of ferritin synthesis by iron. Enzyme 44(1-4):42-58

2. 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

3. 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
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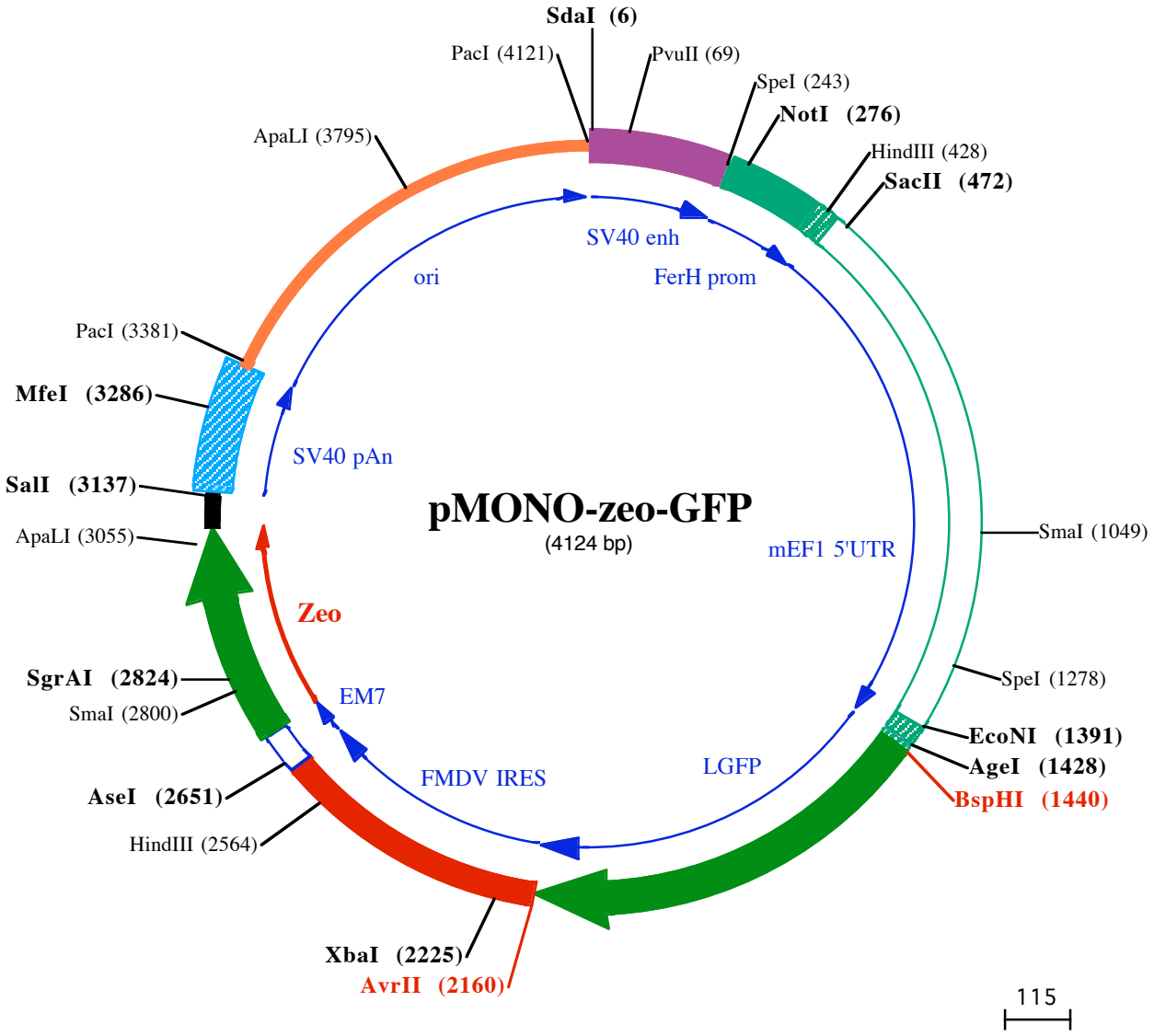
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PvuII (69)
SpeI (243)
NotI (276)
ApaLI (3795)
HindIII (428)
SacII (472)
SV40 enh
ori
FerH prom
PacI (3381)
MfeI (3286)
SV40 pAn
SalI (3137)
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(4124 bp)
ApaLI (3055)
mEF1 5'UTR
SmaI (1049)
Zeo
SgrAI (2824)
EM7
SpeI (1278)
SmaI (2800)
EcoNI (1391)
AgeI (1428)
LGFP
FMDV IRES
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BspHI (1440)
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XbaI (2225)
AvrII (2160)
115

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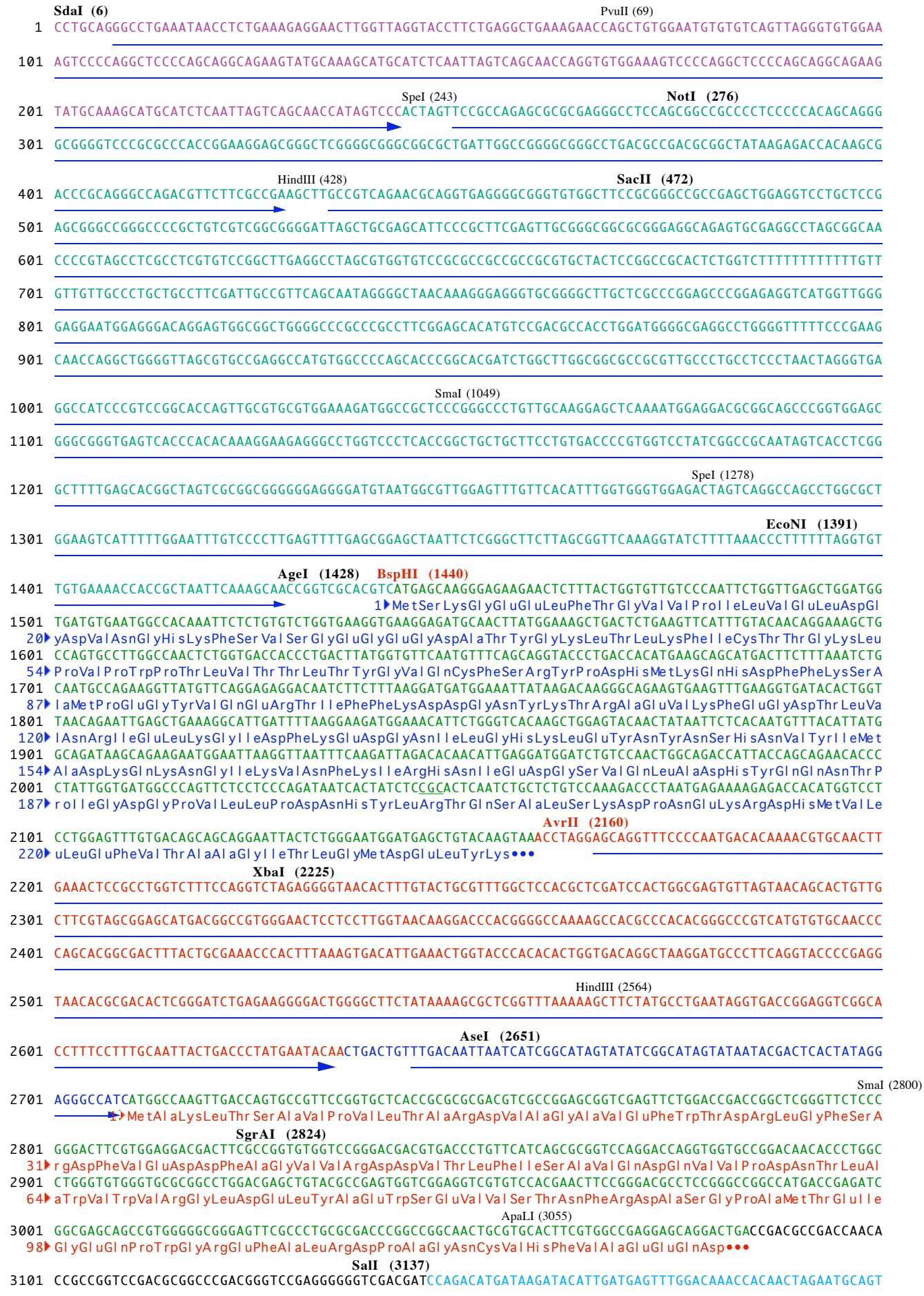
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AvrII (2160)
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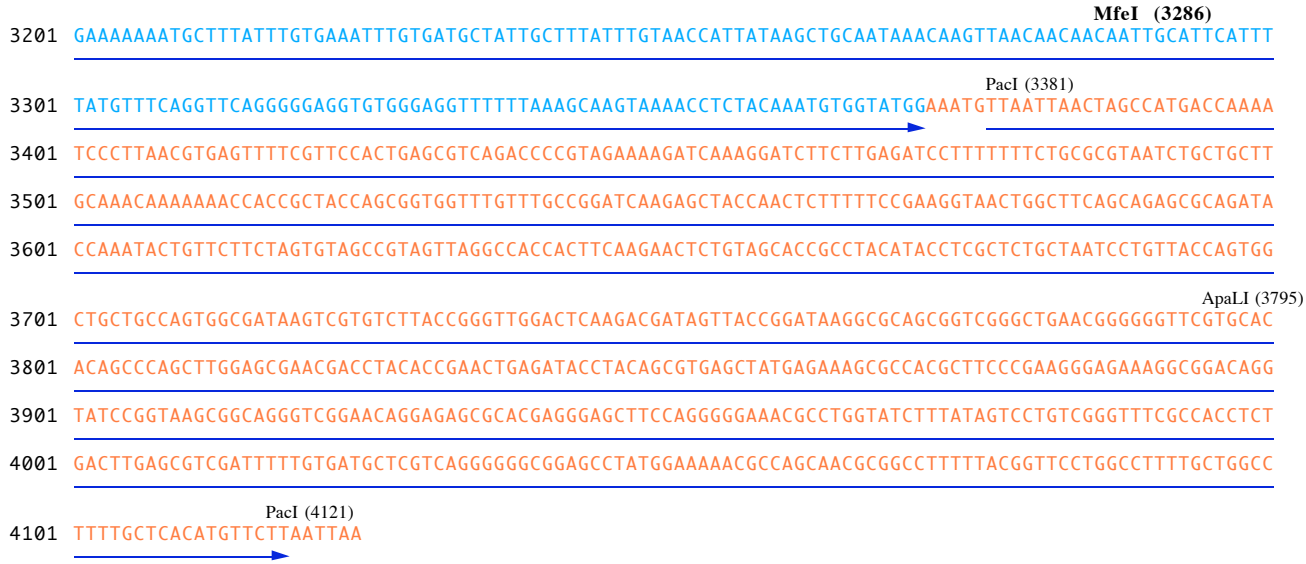
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PacI (3381)
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3501 GCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATA
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ApaLI (3795)
3701 CTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCAC
3801 ACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGG
3901 TATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCT
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