pSELECT-GFPzeo-mcs

Dual expression cassette plasmid for the expression of one gene of interest

Catalog # psetgz-mcs

For research use only

Version 20L01-MM

PRODUCT INFORMATION

Content:

- 20 µg of p**SELECT-GFPzeo-mcs** plasmid provided as lyophilized DNA
- 1 ml of ZeocinÊ (100 mg/ml)

Storage and Stability:

Product is shipped at room temperature. Lyophilized DNA is stable for 1 year when stored at - 20°C. Resuspended DNA is stable for 12 months when stored at -20°C. Avoid repeated freeze-thaw cycles.

Store ZeocinÊ at 4 ÁC or at -20 ÁC. 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

pSELECT 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 variety of selectable markers. pSELECT plasmids contain two expression cassettes: the first drives the expression of the gene of interest and the second drives the expression of a large choice of dominant selectable markers for both *E. coli* and mammalian cells. They are both terminating with a strong polyadenylation signal (polyA) that separates the two expression cassettes thus preventing any transcription interference. The late SV40 polyA terminates the transcription of the gene of interest while the human ß-globin polyA terminates the transcription of the selectable marker.

***Note**: The use of the late SV40 polyA allows you to silence your gene of interest by using the ready-made psiRNA-SV40pA (#psirna3gz21-sv40pa), a plasmid expressing a short hairpin siRNA targeting the late SV40 polyA.*

PLASMID FEATURES

First expression cassette

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter[1] and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat[2]. The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.

• **MCS:** The multiple cloning site contains the following restriction sites:
5' - Sal I, SgrA I, BamH I, Eco47 III, Nco I, Nhe I - 3'
Each restriction site is compatible with many other enzymes, increasing the cloning options.

• **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.

Second expression cassette

• **CMV-HTLV prom:** The strong CMV-HTLV composite promoter combines the human cytomegalovirus immediate-early gene 1 enhancer/promoter isolated from the Towne strain and the 5' untranslated region of the Human T-cell Leukemia Virus. This composite promoter confers high levels of expression in a constitutive manner in a wide range of mammalian cells.

• **EC2K** is a bacterial promoter that enables the constitutive expression of the Zeocin resistance gene in *E. coli*. The EC2K promoter is located within an intron between the GFP and Zeocin resistance genes.

• **GFP::zeo** is a fusion gene that encodes a red-shifted variant of the jellyfish GFP and resistance to Zeocin™ in mammalian cells. This GFP hybrid protein absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm). This fusion gene is very useful to monitor transfection efficiency, standardize gene silencing efficiency, and select clones that stably express an siRNA.

• **ßGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

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 or in 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. Kim, D.W. *et al*. (1990). Gene 2: 217-223.
2. Takebe, Y. *et al*. (1988). Mol. Cell Biol. 1: 466-472.
3. Carswell, S., and Alwine, J.C. (1989). Mol. Cell Biol. 10: 4248-4258.
4. Yu J & Russell JE. (2001). Mol Cell Biol, 21(17):5879-88.

TECHNICAL SUPPORT
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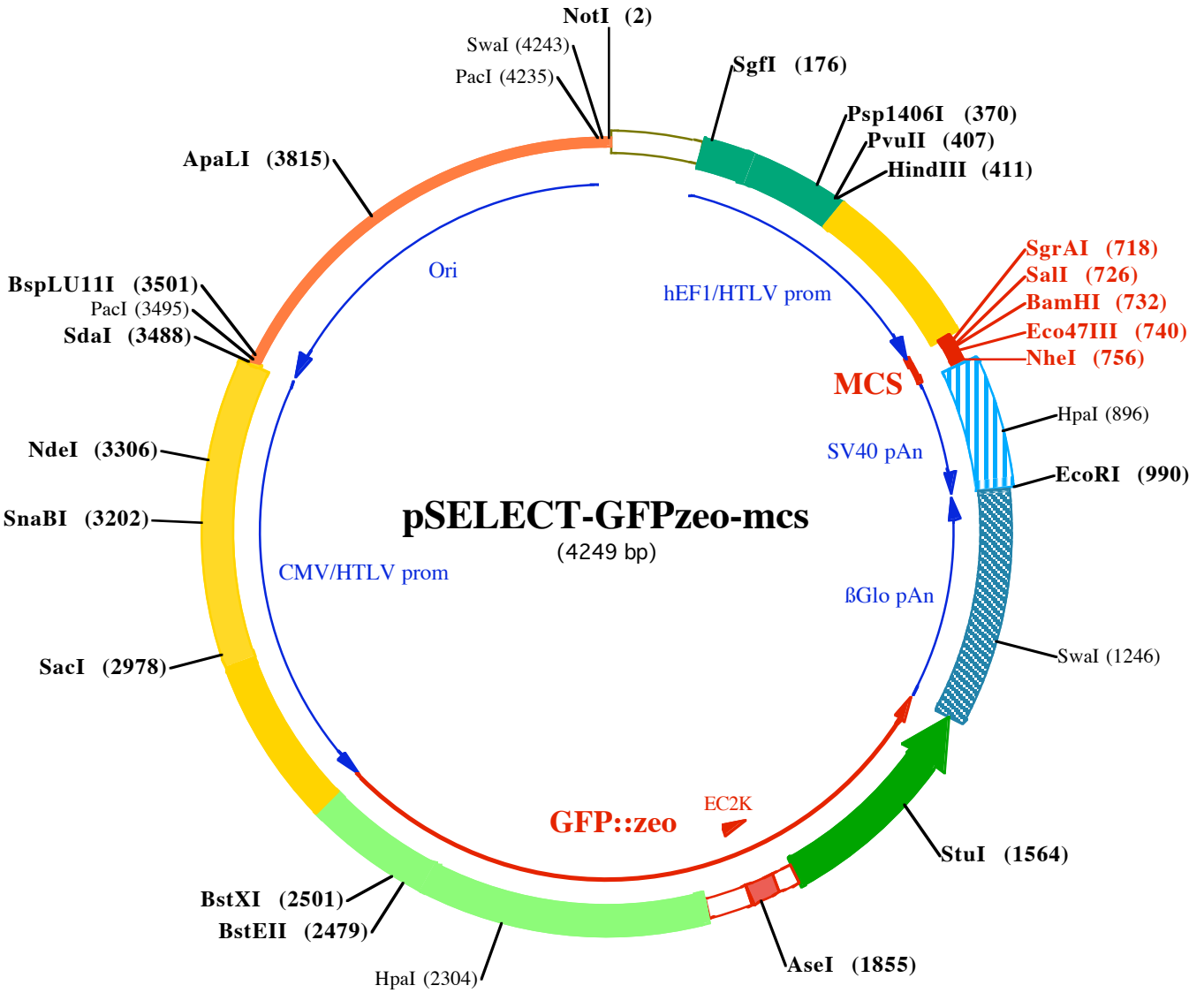
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HindIII (411)
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BamHI (732)
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BspLU11I (3501)
PacI (3495)
SdaI (3488)
Ori
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MCS
HpaI (896)
NdeI (3306)
SV40 pAn
EcoRI (990)
SnaBI (3202)
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(4249 bp)
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ßGlo pAn
SwaI (1246)
SacI (2978)
EC2K
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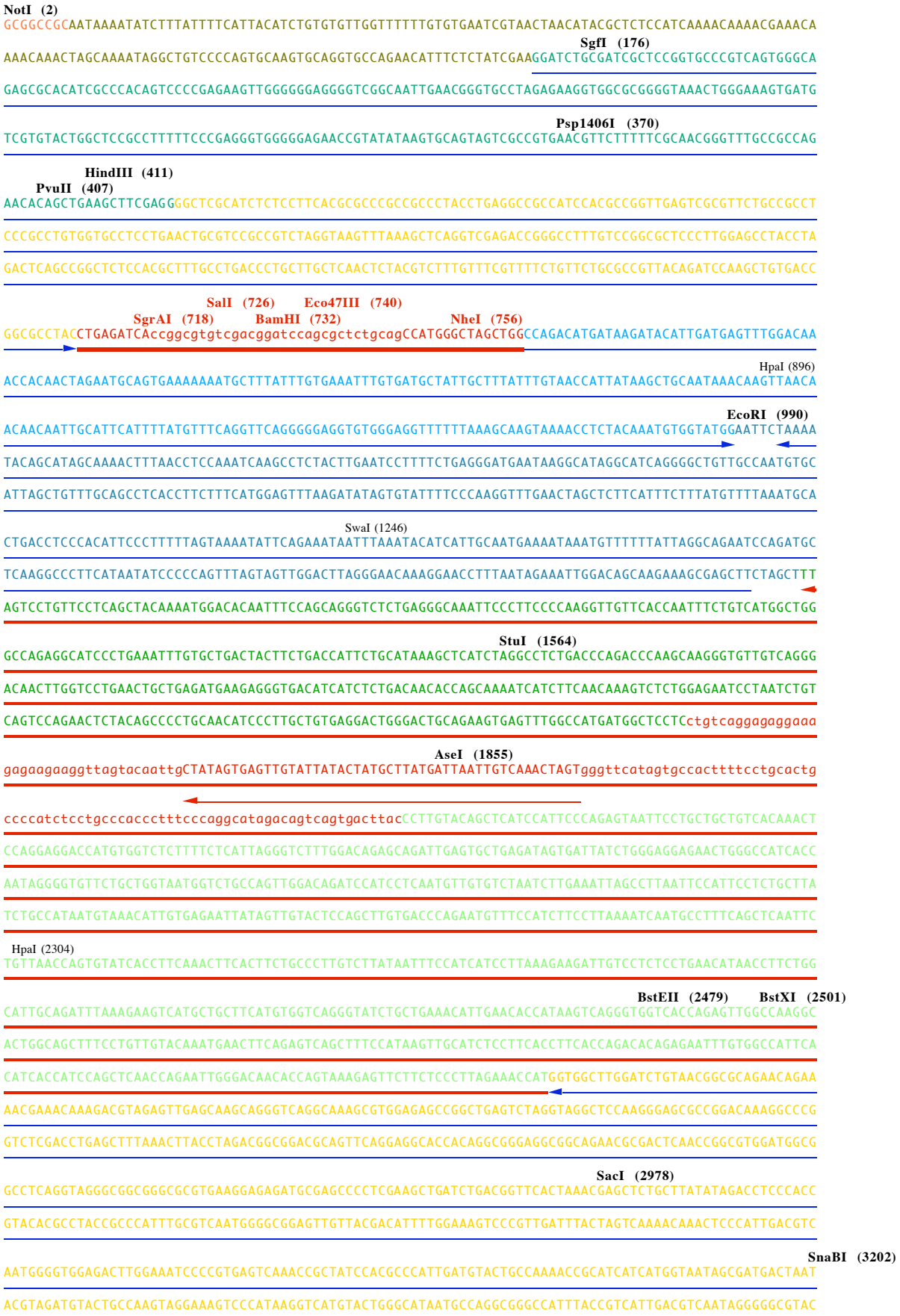
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PacI (4235) SwaI (4243)
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