pSELECT-puro-mcs

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

Catalog code: psetp-mcs

https://www.invivogen.com/pselect-puro

For research use only

Version 19A21-MM

PRODUCT INFORMATION

Contents

- 20 µg of pSELECT-puro-mcs plasmid provided as lyophilized DNA
- 1 ml of Puromycin 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 Puromycin 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

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.
pSELECT-puro-mcs contains a multiple cloning site (MCS) downstream of the composite promoter for convenient cloning of a gene of interest.

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.

Puromycin usage:

This antibiotic can be used for *E. coli* at 100-125 µg/ml in liquid or solid media and at 1-10 µg/ml to select Puromycin-resistant mammalian cells.

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' - SgrA I, Sal 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 enh/prom:** The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli.*
- **Puro:** Resistance to Puromycin is conferred by the *Pac* gene from *Streptomyces* which encodes a N-acetyl-transferase. The *Pac* gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.
- **ßGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

References

1. Kim D.W. *et al.*, 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system.Gene 2: 217-223. **2. Takebe Y. *et al.*, 1988.** SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat.Mol. Cell Biol. 1: 466-472. **3. Carswell S. & Alwine J.C., 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences.Mol. Cell Biol. 10: 4248-4258. **4. Yu J. & Russell J.E., 2001.** Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA.Mol Cell Biol, 21(17):5879-88.

TECHNICAL SUPPORT
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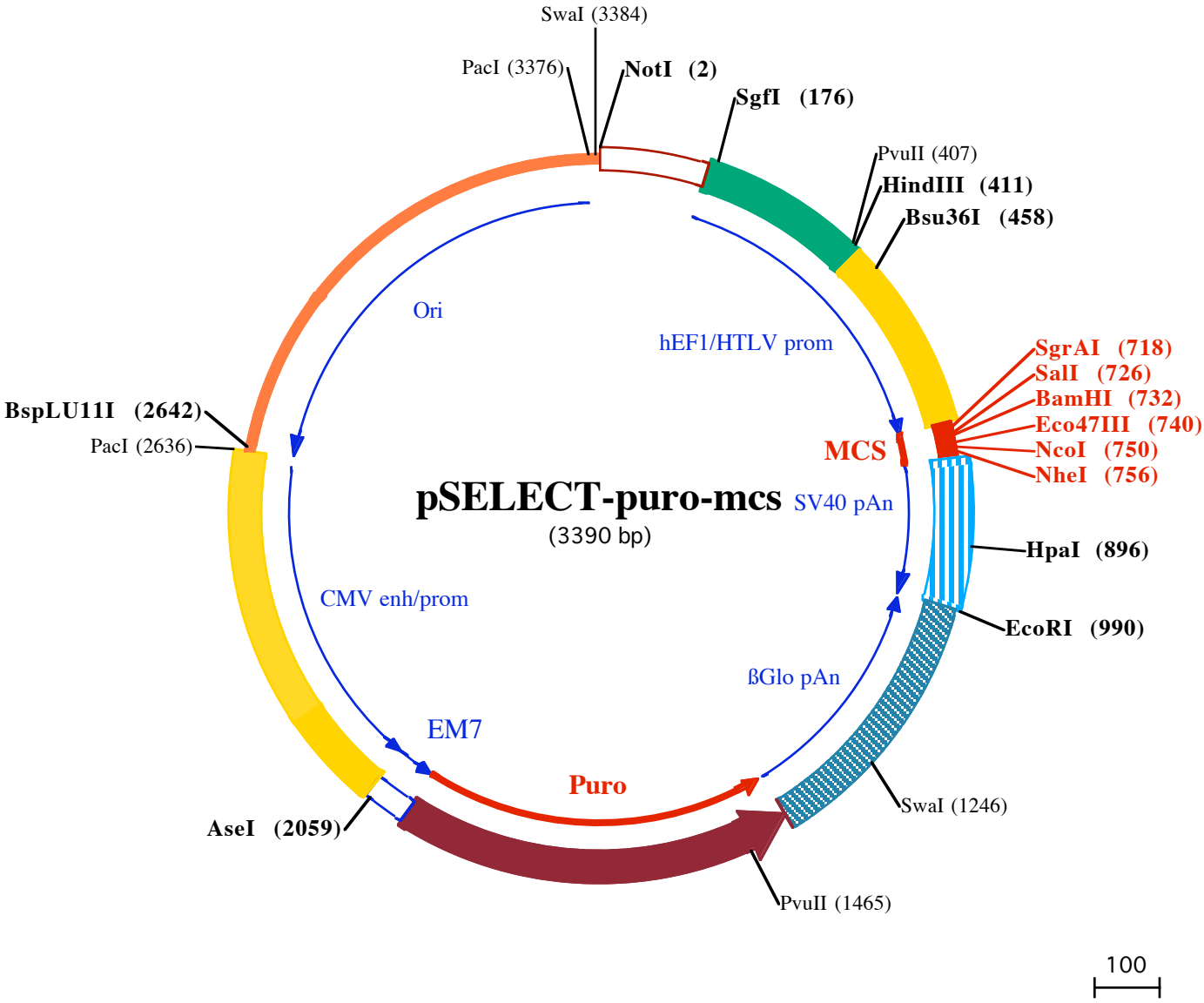
SwaI (3384)
PacI (3376)
NotI (2)
SgfI (176)
PvuII (407)
HindIII (411)
Bsu36I (458)
Ori
hEF1/HTLV prom
SgrAI (718)
SalI (726)
BamHI (732)
Eco47III (740)
NcoI (750)
NheI (756)
BspLU11I (2642)
PacI (2636)
MCS
pSELECT-puro-mcs SV40 pAn
(3390 bp)
HpaI (896)
CMV enh/prom
EcoRI (990)
ßGlo pAn
EM7
Puro
AseI (2059)
SwaI (1246)
PvuII (1465)
100

InvivoGen


NotI (2)
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