pSELECT-puro-mcs

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

Catalog # psetp-mcs

For research use only

Version # 04K22-MT

PRODUCT INFORMATION

Content:

- 20 µg of **pSELECT-puro-mcs** plasmid provided as lyophilized DNA
- 4 pouches of *E. coli* Fast-Media® Puro (2 TB and 2 Agar)

Storage and Stability:

Product is shipped at room temperature.
Lyophilized DNA is stable for 12 months when stored at - 20°C.
Resuspended DNA is stable for 12 months when stored at -20°C. Avoid repeated freeze-thaw cycles.
Store *E. coli* Fast-Media® Puro at room temperature. Fast-Media® pouches are 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

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' - 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*.

• **βGlo 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.

Selection of bacteria with *E. coli* Fast-Media

Fast-Media® is a **fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. Fast-Media® is a TB (liquid) or LB (solid) based medium that already contains the antibiotic.
Fast-Media® Puro can be ordered separately [#fas-pr-l (liquid), #fas-pr-s (solid)].

Method:

1- Pour the contents of a Fast-Media® 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 Fast-Media®.**
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 Fast-Media® as the antibiotic will be permanently destroyed by the procedure.*

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.

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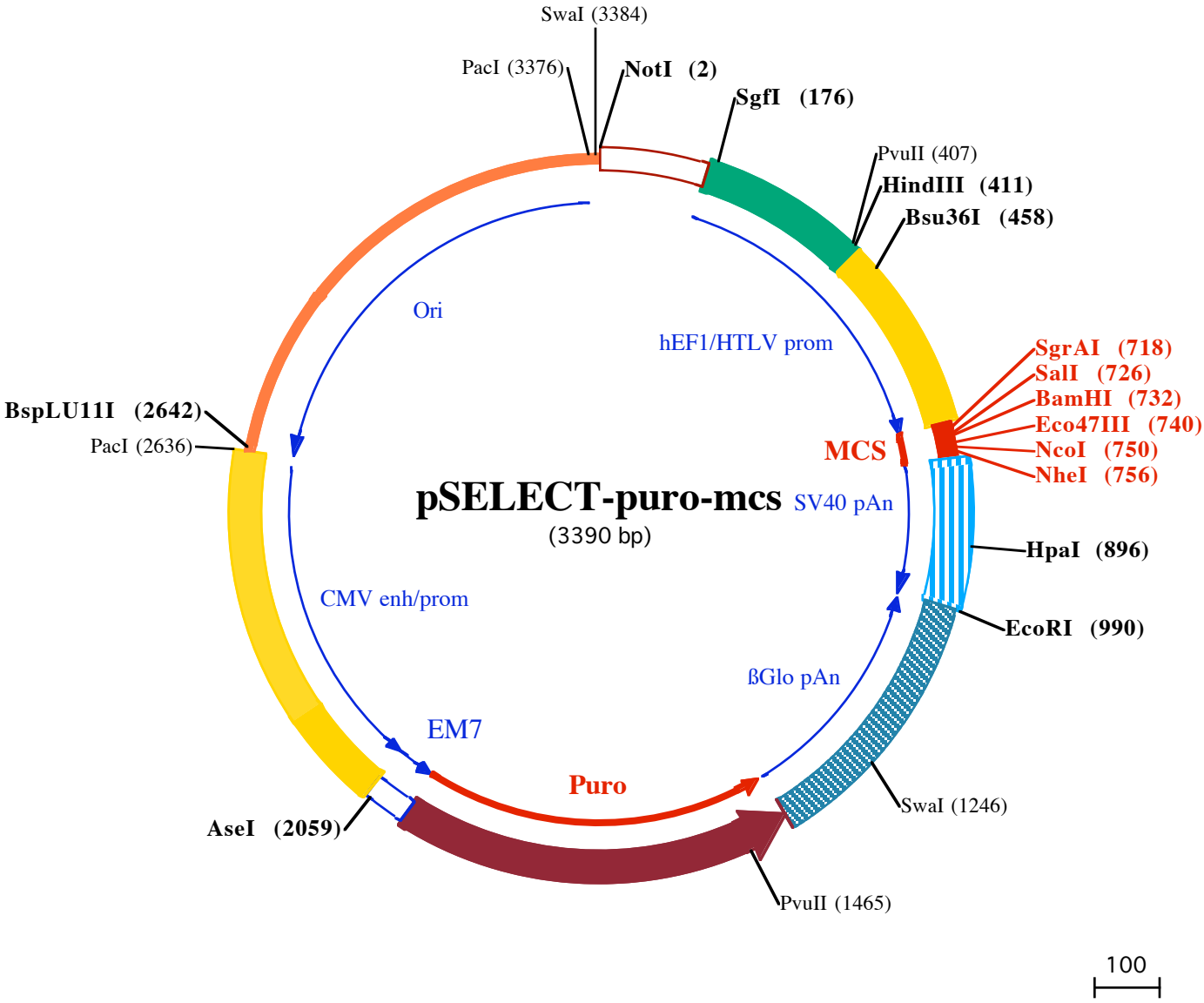
SwaI (3384)
PacI (3376)
NotI (2)
SgfI (176)
PvuII (407)
HindIII (411)
Bsu36I (458)
Ori
hEF1/HTLV prom
SgrAI (718)
SalI (726)
BamHI (732)
Eco47III (740)
NcoI (750)
NheI (756)
BspLU11I (2642)
PacI (2636)
MCS
pSELECT-puro-mcs SV40 pAn
(3390 bp)
HpaI (896)
CMV enh/prom
EcoRI (990)
ßGlo pAn
EM7
Puro
AseI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

InvivoGen


NotI (2)
SgfI (176)
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