

# 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 β-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<sup>1</sup> 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<sup>2</sup>. 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<sup>3</sup>.

• **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<sup>4</sup>.

## 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<sub>2</sub>O. 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.

### TECHNICAL SUPPORT

Toll free (US): 888-457-5873

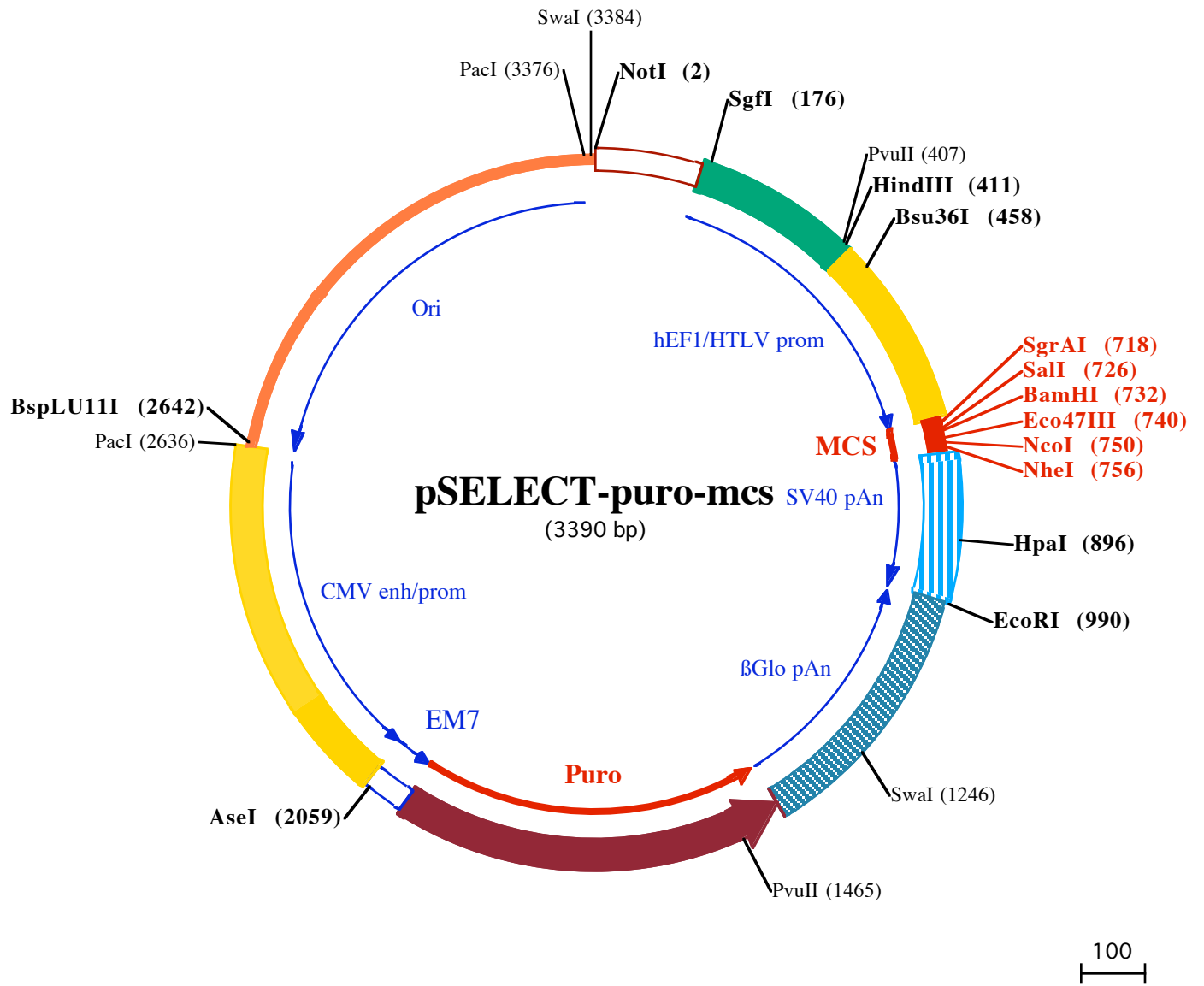
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**NotI (2)**  
1 GCGGCCGAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTGTGTGAATCGTAACATAACATACGCTCTCCATCAAAACAAAACGAAACA

**SgfI (176)**  
101 AAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCA  
201 GAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCTTAGAGAAGTGGCGGGGTAAACTGGGAAAGTGATG  
301 TCGTGTACTGGCTCCGCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAG

**HindIII (411)**  
**PvuII (407)** **Bsu36I (458)**  
401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCTTCACGGCCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCCT  
501 CCCGCTGTGGTGCCTCCTGAAGTGCCTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTA  
601 GACTCAGCCGGCTCTCCACGCTTTCCTGACCTGCTTGTCTCAACTCTACGTCCTTGTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC

**SgrAI (718)** **BamHI (732)** **SalI (726)** **Eco47III (740)** **NheI (756)** **NeoI (750)**  
701 GGCGCTACCTGAGATCACCGCGTGTGACGGATCCAGCGCTCTGCAGCCATGGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAA

**HpaI (896)**  
801 ACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAACAGTTAAAC

**EcoRI (990)**  
901 ACAACAATTGCATTCAATTTATGTTTCAGGTTCCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTTCTAAAA  
1001 TACAGCATAGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGC  
1101 ATTAGCTGTTTGCAGCCTCACCTCTTTTCATGGAGTTAAGATATAGTGATTTTTCCCAAGGTTTGAACAGCTCTTCATTTCTTTATGTTTTAAATGCA

**SwaI (1246)**  
1201 CTGACCTCCACATTCCCTTTTTAGTAAATATTAGAAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTATTAGGCAGAATCCAGATGC  
1301 TCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTCA

**PvuII (1465)**  
1401 GGTTAAGCTCCAGGCTCCTTGTCATGCACCAAGTCTTGGGCCTTCTGGAACCTCAACATCAGCTGTACAGTGAATCCAGTCTTTTCAAAAAAGGC  
200 Met  
1501 AGTTTTCTGGGAGCAGAAGTTCCAGAAAGGCAGGAACCTCCAGCCCTTTCAGCAGCTTCAACTCCAGGCAGAACACAGCAGATCCAGACCCCTTCCCT  
167 Met  
1701 Ser Gl ySer Leu Gl uAl aMet Arg P ro Gl y l l e Gl uAl aPhe Val l a Gl yAl a Gl uVal Gl yPro Leu Val Val Al aSer Gl yLeu Gl yLys Gl yGl  
1801 TCTGCAACCCAACTTTTCCAATGTCCAGTCCACTCTGGTGAGGAAGATCTTGCAGTCTGTCCACCTCTCAATGTGCCTGTCCAGGTCACCTGTGT  
67 Met  
1901 GCCTTGTTCAGGGTAGTCTGCAAAAGCAGCAGCAGCTTCTCACAGCTTTGGAACATCATCTCTGTTGCCAGCCTACTGTGGGTTGTACTCAGT  
34 Met  
2001 CATGGTGGCCCTCTATAGTGAAGTCTATTATACTATGCGGATATACTATGCCGATGATTAATTGTCAAACAGCGTGGATGGCGTCTCCAGCTTATCTG  
1 Met  
2101 ACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCACCCTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAG  
2201 TCCCGTTGATTTACTAGTCAAAACAACTCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGT  
2301 ACTGCCAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAAGTTCATGTACTGGGCATAATGCCA  
2401 GCGGGGCCATTTACCGTCATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACC  
2501 CATTGACGTCAATGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATTATTGACGTCAATGGCGGGGTCGTTGGCGGTGAGCCAGCGGGC

**PaeI (2636)** **BspLU11I (2642)**  
2601 CATTACCCTAAGTTATGTAACCGCTGCAGTTAATTAAGAACCATGTGAGCAAAAAGCCAGCAAAAAGGCCAGGAACCGTAAAAAGCCGCTTGTGGCG  
2701 TTTTTCCATAGGCTCCGCCCTGACGAGCATCAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTC  
2801 CCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCCGGGAAGCGTGGCGCTTCTCATAG  
2901 CTCACGCTGAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCGACCGCTGCGCCTTATCCGGT  
3001 AACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCAGGATGTAGGCGGTGCTA  
3101 CAGAGTCTTGAAAGTGGTGGCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGG  
3201 TAGCTCTTGATCCGGCAACAAACACCGCTGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCT

**PaeI (3376)** **SwaI (3384)**  
3301 TTGATCTTTTCTACGGGCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGATTTTGGTGTGCTAGTTAATTAACATTTAAATCA