

pSELECT-neo-mcs

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

Catalog code: psetn-mcs

<https://www.invivogen.com/pselect-neo>

For research use only

Version 19L13-MM

PRODUCT INFORMATION

Contents

- 20 µg of pSELECT-neo-mcs plasmid provided as lyophilized DNA

Storage and Stability

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C.
- Resuspended DNA should be stored at -20°C and is stable for at least 1 year at -20°C.

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.

PLASMID FEATURES

First expression cassette

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1 α (EF-1 α) core promoter¹ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat². The EF-1 α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1 α 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 mRNA³.
- **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*.
- **Neo:** The *neo* gene from Tn5 confers resistance to Kanamycin in *E. coli* and G418 in mammalian cells. The neo gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.
- **β Glo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.

1. Kim D.W. *et al.*, 1990. Use of human elongation factor 1 alpha promoter as a versatile and efficient expression system. *Gene*. 91(2):217-23. 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.

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 H₂O. 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 DH5 α .

Bacterial antibiotic selection

Kanamycin (not provided) is normally used for *E. coli* at a final concentration of 50 µg/ml in liquid or solid media.

Mammalian antibiotic selection

G418 is normally used at a concentration of 400 µg/ml. However, the optimal concentration needs to be determined for your cells.

RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116 cells	Competent <i>E. coli</i> cells	gt116-11
G418	Selection antibiotic	ant-gn-1

TECHNICAL SUPPORT

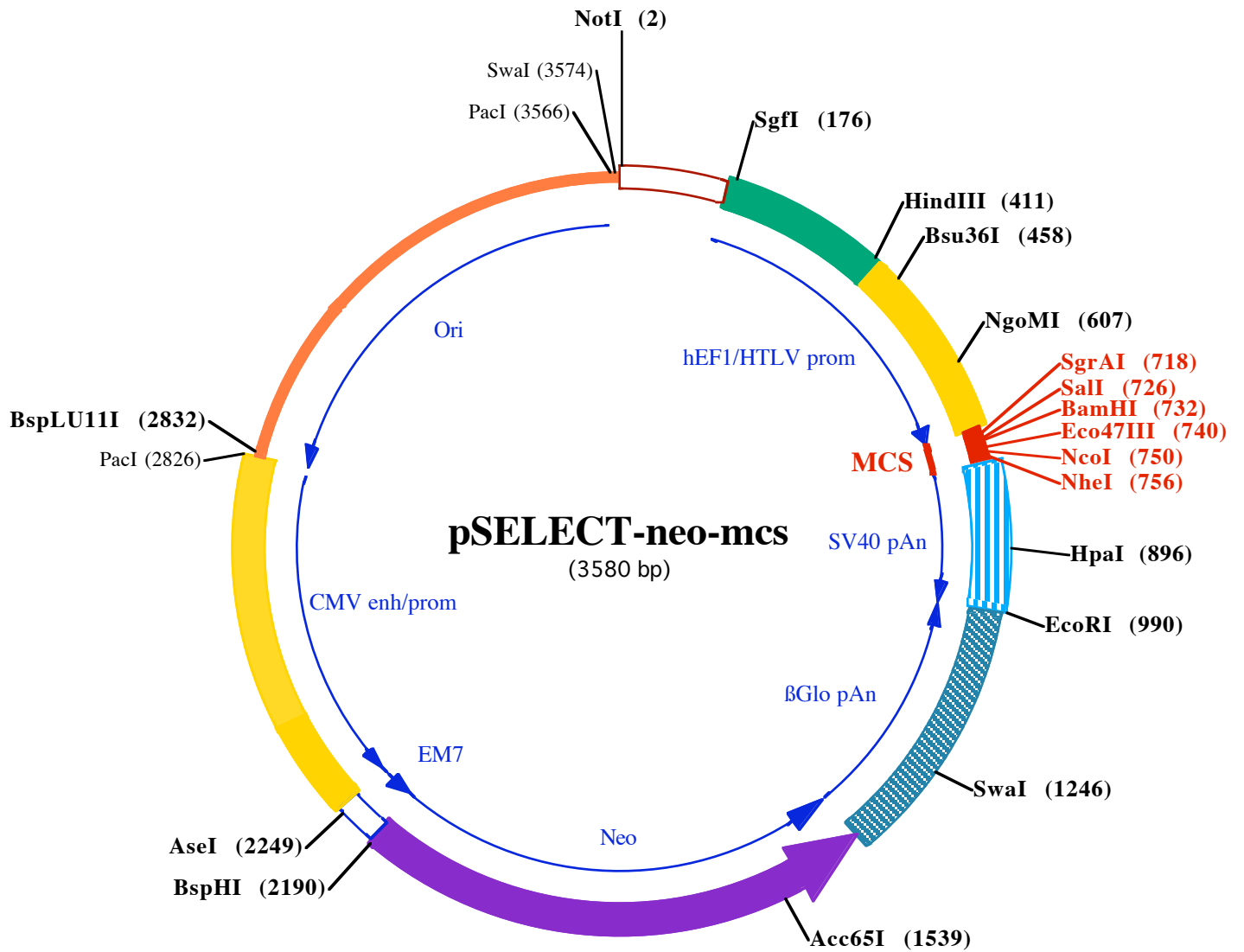
InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

E-mail: info@invivogen.com



100

NotI (2)
1 GCGGCCGAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACA

SgfI (176)
101 AAACAACTAGCAAATAGGCTGTCCCCAGTGAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCATCGCTCCGGTGCCCGTCAGTGGCA

HindIII (411) **Bsu36I (458)**
201 GAGCGACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTGCGCAATTGAACGGTGCCTAGAGAAGGTGGCGGGGTAAACTGGGAAAGTGATG
301 TCGTGTACTGGCTCCGCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCCCGTGAACGTTCTTTTTCCGAACGGGTTTGCCGCCAG

NgoMI (607)
401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACGCGCCCGCCCTACCTGAGGCCGCCATCCACGCCGGTGTAGTCCGCTTCTGCCGCT
501 CCCGCTGTGGTGCCTCCTGAAGTGCCTCCGCGCTTAGGTAAGTTTAAAGCTCAGGTGAGACCGGGCTTTGTCCGGCGCTCCCTTGAGGCTACCTA

SgrAI (718) **SalI (726)** **BamHI (732)** **Eco47III (740)** **NcoI (750)** **NheI (756)**
701 GGGCCCTACCTGAGATCACCGGCGTGTGACGGATCCAGCGCTCTCGAGCCATGGGTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAA

HpaI (896)
801 ACCACAAC TAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGAATAAACAAGTTAACA

EcoRI (990)
901 ACAACAATTGCATTCATTTTATGTTTCAGGTTCAAGGGGAGGTGTGGGAGTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTTCTAAAA

1001 TACAGCATAGCAAACTTTAACTCCAAATCAAGCCTCTACTTGAATCTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTTTGCCAATGTGC

1101 ATTAGTGTTTTGACGCTCACCTCTTTTATGGAGTTAAGATATAGTGTATTTTCCAAAGTTTGAAGTACTGCTCTTCATTTCTTTATGTTTTAAATGCA

SwaI (1246)
1201 CTGACCTCCACATTCCTTTTTAGTAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAAATCCAGATGC

1301 TCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTGGACTTAGGGAACAAAGGAACCTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTT

1401 AGAAGAAGCTCATCAAGAAGTCTGTAGAAGGCAATCTCTGGGAGTCAAGGGCTGCAATGCCATAGAGCACTAGGAACCTGTCTGCCACTCTCCCCCTAG
264 PhePheGluAspLeuLeuArgTyrPheAlaIleArgGlnSerAspProAlaAlaIleGlyTyrLeuValLeuPheArgAspAlaTrpGluGlyGlyLeu

Acc65I (1539)
1501 CTCTTCTGCTATGTCCTGGTTGCTAGGGCAATGTCCTGGTACCTGTGACCCACTCCAGCTGCCACAGTCTATGAAGCCAGAGAACCTTCCATTTTCA
231 GluGluAlaIleAspArgThrAlaLeuAlaIleAspGlnTyrArgAspAlaValGlyLeuArgGlyCysAspIlePheGlySerPheArgGlyAsnGluVal
1601 ACCATGATGTTGGGAAGGCAGGCATCCCATGAGTCAACTAGGTCCTCACCATCTGGCATGGATGCTTGGCCTGGCAAATAGTTCAGCAGGGGCCA
197 AlaMetIleAsnProLeuCysAlaAspGlyHisThrValValLeuAspGluGlyAspProMetSerAlaLysLeuArgAlaPheLeuGluAlaPProAlaLe
1701 GGCCCTGGTGTCTTCACTCAAGTCACTTGGTCCACCAGGCCAGCCTCATCTGGTCTGGCCCTCTCTATCTGTGCTTGGCTGGTGGTCAAAGGG
164 uGlyGlyHisSerGluGluAspLeuAspAspGlnAspValLeuGlyAlaGluMetArgThrArgAlaArgGluIleArgHisLysAlaGluHisAspPhePro
1801 GCAGGTGGCTGGTCAAGGGTGTGGAGTCTTCTCATGGCATCAGCCATGATTGACACTTCTCAGCTGGAGCTAGGTGAGAGGAAAGGAGGTCTGCCCA
131 CysThrAlaPProAspLeuThrHisLeuArgArgMetAlaAspAlaMetIleSerValLysGluAlaPProAlaLeuHisSerSerLeuLeuAspGlnGlyP
1901 GGCACCTCACCTAGTAGGAGCCAGTCCCTCCAGCTTCTGTGACCACATCAAGGACAGCTGCACAGGGGACCCAGTTGTTGCCAACAGGAGAGTCTGG
97 roValGluGlyLeuLeuLeuTrpAspArgGlyAlaGluThrValValAspLeuValAlaAlaCysProValGlyThrThrAlaLeuTrpSerLeuArgAl
2001 CAGCCTCATCTGGAGCTCATTGAGAGCCCCACTGAGGTCTGTCTTACAAAAGGACTGGCTGCTTGGGCTGAAAGTCTGAAAAGTCTGCATCAGA
64 AlaGluAspGlnLeuGluAsnLeuAlaGlySerLeuAspThrLysValPheLeuValProArgGlyGlyAlaSerLeuArgPheValAlaAlaAspSer

BspHI (2190)
2101 GCAACCAATGGTCTGCTGTGCCAGTATAGCCAAACAGTCTCTCAACCCAGGCAGCTGGAGAACCCTGCATGTAGGCCATCTTGTTCATCATGATGGCC
31 CysGlyIleThrGlnGlnAlaTrpAspTyrGlyPheLeuArgGluValTrpAlaAlaProSerGlyAlaHisLeuGlyAspGlnGluIleMet

AseI (2249)
2201 CTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTCACT

2301 AAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCCTACCGCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCCTTGAT

2401 TTACTAGTCAAAAACAACTCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTAAGTCCAAAA

2501 CCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAAGGTCATGTACTGGGCATAATGCCAGGGGGCCAT

2601 TTACCGTCATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGTACTGCAAGTGGGCGAGTTTACCCTAAATACTCCACCCATTGACGTC

2701 AATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATTATTGACGTCAATGGGGGGGGTCTGTTGGGCGGTGAGCCAGGGGGCCATTTACCGT

PacI (2826) **BspLU11I (2832)**
2801 AAGTTATGTAACGCTCGAGGTTAATTAAGAACATGTGAGCAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTCTGCGGTTTTCCATA

2901 GGCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAAGGTTGGCGAAACCCGACAGGACTATAAAGATACCAGGCTTTCCCCCTGGAAG

3001 CTCCCTCGTGGCTCTCTGTTCCGACCTGCGCTTACC GGATACCTGTCCGCTTTCTCCCTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGT

3101 AGGTATCTCAGTTCCGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCGACCGCTGCGCTTATCCGGTAAGTATCGTC

3201 TTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGTTCTT

3301 GAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGA

3401 TCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTT

3501 CTACGGGGTCTGACGCTCAGTGAACGAAAACACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA

PaI (3566) SwaI (3574)