

pSELECT-hygro-mcs

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

Catalog code: pseth-mcs

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

For research use only

Version 19A21-MM

PRODUCT INFORMATION

Contents

- 20 µg of pSELECT-hygro-mcs plasmid provided as lyophilized DNA
- 1 ml of Hygromycin B Gold at 100 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 Hygromycin B Gold 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 β-globin polyA terminates the transcription of the selectable marker.

pSELECT-hygro-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.

Hygromycin B usage:

This antibiotic can be used for *E. coli* at 50-100 µg/ml in liquid or solid media and at 50-500 µg/ml to select Hygromycin-resistant mammalian cells.

PLASMID FEATURES

First expression cassette

- **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) 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-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 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*.

- **Hygro:** Resistance to Hygromycin B is conferred by the *hph* gene from *E. coli* which encodes a phosphotransferase. The *hph* 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 transcription⁴.

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

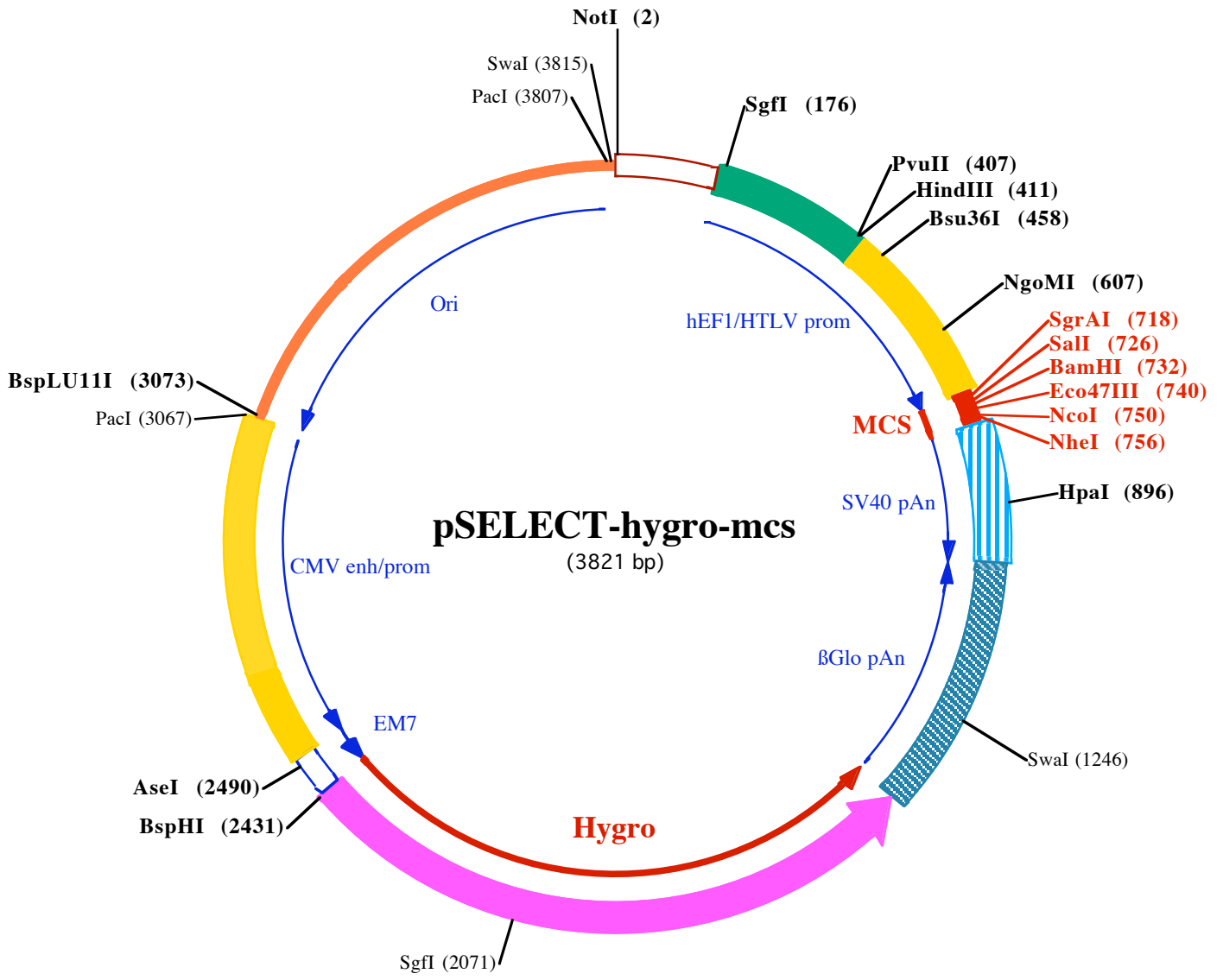
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100

NotI (2)
1 GCGGCCGAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAAACAAAACGAAACA
101 AAACAACTAGCAAAATAGGCTGTCCCAGTGAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCA
201 GAGCGCACATCGCCACAGTCCCGAGAAAGTTGGGGGAGGGTGCAGCAATTGAACGGTGCCTAGAGAAGGTGGCGCGGGTAAACTGGAAAGTGATG
301 TCGTGTACTGGCTCCGCCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTCGCAACGGGTTTGCGCCAC

HindIII (411)
PvuII (407) Bsu36I (458)
401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTCACGCGCCCGCCCTACTCGAGGCCCATCCACGCCGTTGAGTCGCGTTCTGCCGCT
501 CCCGCTGTGGTGCCTCTGAAGTGCCTCGCCGCTAGGTAAGTTTAAAGCTCAGGTGAGACGGGCTTTGTCCGGCGCTCCCTTGAGCCTACCTA

NgoMI (607)
601 GACTCAGCCGGCTCTCCACGCTTGCCTGACCCTGCTTCTCAACTCTACGCTTTGTTTCGTTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC

BamHI (732) NheI (756)
SgrAI (718) Sall (726) Eco47III (740) NcoI (750)
701 GCGCCTACTCGATACCGCGTGTGACGGATCCAGCGCTCTGCAGCCATGGGCTAGTGGCCAGACATGATAAGATACATTGATGAGTTGGACAA
801 ACCACAAC TAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAAACA
901 ACAACAATTGCATTCATTTTATGTTTCAGGTTCAAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAATTTCTAAAA
1001 TACAGCATAGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGC
1101 ATTAGCTGTTTGACGCTCACCTTCTTTCATGGAGTTAAGATATAGTGTATTTTCCCAAGTTTGAAGTACTAGCTCTTCATTTCTTTATGTTTTAAATGCA

HpaI (896)
1201 CTGACCTCCCACATTCCTTTTTAGTAAATATTGAGAAATAATTTAAATACATCATTGCAATGAAAAATAATGTTTTTTATTAGGCAGAATCCAGATGC
1301 TCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTCTAGCGAA

SwaI (1246)
1401 TTCTCGACTCATTCTTTGCCCTCGACGAGTGTGGGGCGTCGGTTTCCACTATCGGCGAGTACTTCTACACAGCCATCGTCCAGACGGCCGCTTC
342 ◀ • E K A R P R T S P R R N G S D A L V E V C G D T W V A A S R
1501 TCGGGCGATTTGTGTACGCCGACAGTCCCGGCTCCGGATCGACGATTGCGTCGCATCGACCCTGCGCCAAGCTGCATCATCGAAATTCGCGTCAAC
311 ◀ R A I Q T R G V T G A G S R V I A D C R G Q A W A A D D F N G D V
1601 CAAGCTCTGATAGAGTTGGTCAAGACCAATGCGGAGCATATACGCCGGAGCCGGCGATCTGCAAGCTCCGGATGCCTCCGCTCGAAGTAGCGCGTC
278 ◀ L S Q Y L Q D L G I R L M Y A R L R P S G A L E P H R R E F Y R T
1701 TGCTGCTCCATAAGCAACACCGCTCCAGAAGAAGATGTTGGCGACCTCGTATTGGGAATCCCCGAACATCGCTCGCTCCAGTCAATGACCGCTG
244 ◀ Q Q E M C A L W P R W F F I N A V E Y Q S D G F M A E S W D I V A T
1801 TTATGCGCCATTGTCGTGAGCATTGTTGGAGCGAAATCCGCTGACGAGGTGCCGACTTCCGGGAGTCCCTCGCCAAAGCATCAGCTCATC
211 ◀ I R G N D T L V N N S G F D A H V L H R V E P C D E A W L M L E D
1901 GAGAGCTGCGCGACGACGACTGACGGTGTCTCCATCACAGTTTGCAGTGATACACATGGGGATCAGCAATCGCGCATGAAATCACGCCATGTA
178 ◀ L A Q A V S A S V T D D M V T Q W H Y V H P D A I A C I F D R W T

SgfI (2071)
2001 GTGTATTGACCGATTCTTGGGTCCGAATGGGCCGAACCCGCTCGTCTGGCTAAGATCGGCCGACGATCGCATCCATGAGCTCCGCGACGGGTTGCA
144 ◀ T Y Q G G I G Q P G F P G F G S T Q S L D A A A I A D M L E A V P Q L
2101 GAACAGCGGGCAGTTCGTTTCAGGCAAGTTCGACCTGTGCACGGGGAGATGCAATAGGTGAGCTCTCGTGAATTTCCCAATGTC
111 ◀ V A P L E T E P L D Q L T V G Q A R R S I C Y T L S E S F E G I D
2201 AAGCACTTCCGAATCGGGAGCGCGCCGATGCAAAGTCCGATAAACATAACGATCTTTGTAGAAACCATCGGCGACGATTTTACCCGACGACATAT
78 ◀ L V E P I P L A A S A F H R Y V Y R D K Y F G D A C S N V R L V Y
2301 CCACGCCCTCATACGAAGCTGAAAGCACGAGATTTCTGCCCTCCGAGAGCTGCATCAGTCCGAGACGCTGTGCAACTTTTCGATCAGAAACTTCG
44 ◀ G R G G V D F S F A R S E E G E S L Q M L D S V S D F K E I L F K A

BspHI (2431) AseI (2490)
2401 CGACAGAGCTCGCGGTGAGTTCAGGCTTTTTCATGATGGCCCTCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAA
11 ◀ V S T A T L E P K K M
2501 AACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTCACTAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCCTACCGCCATTTGCGTCAA
2601 TGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCCTGTTGATTTACTAGTCAAAAACAACTCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGT
2701 GAGTCAAACCGCTATCCACGCCATTGATGTAAGTCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGT
2801 CCCATAAGGTCATGTAAGTGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGTACTGCTG

2901 CAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGG

PacI (3067) BspLU11I (3073)

3001 CGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCAGGTTAATTAAAGAACATGTGAGCAAAGGCCAGCAAAGGC

3101 CAGGAACCGTAAAAAGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAAC

3201 CCGACAGGACTATAAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCCTTC

3301 TCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCC

3401 CGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGG

3501 ATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGC

3601 TGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACCACCGCTGGTAGCGGTGGTTTTTTTGTGGCAAGCAGCAGATTAC

3701 GCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCT

PacI (3807) SwaI (3815)

3801 AGTTAATTAACATTTAAATCA