

pSELECT-blasti-mcs

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

Catalog code: psetb-mcs

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

For research use only

Version 20117-MM

PRODUCT INFORMATION

Contents

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

- 2 x 1 ml blasticidin 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 blasticidin 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-blasti-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 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 DH5α.

Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

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' - 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 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*.

- **Blasti:** Resistance to Blasticidin is conferred by the *bsr* gene from *Bacillus cereus*. The *bsr* 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

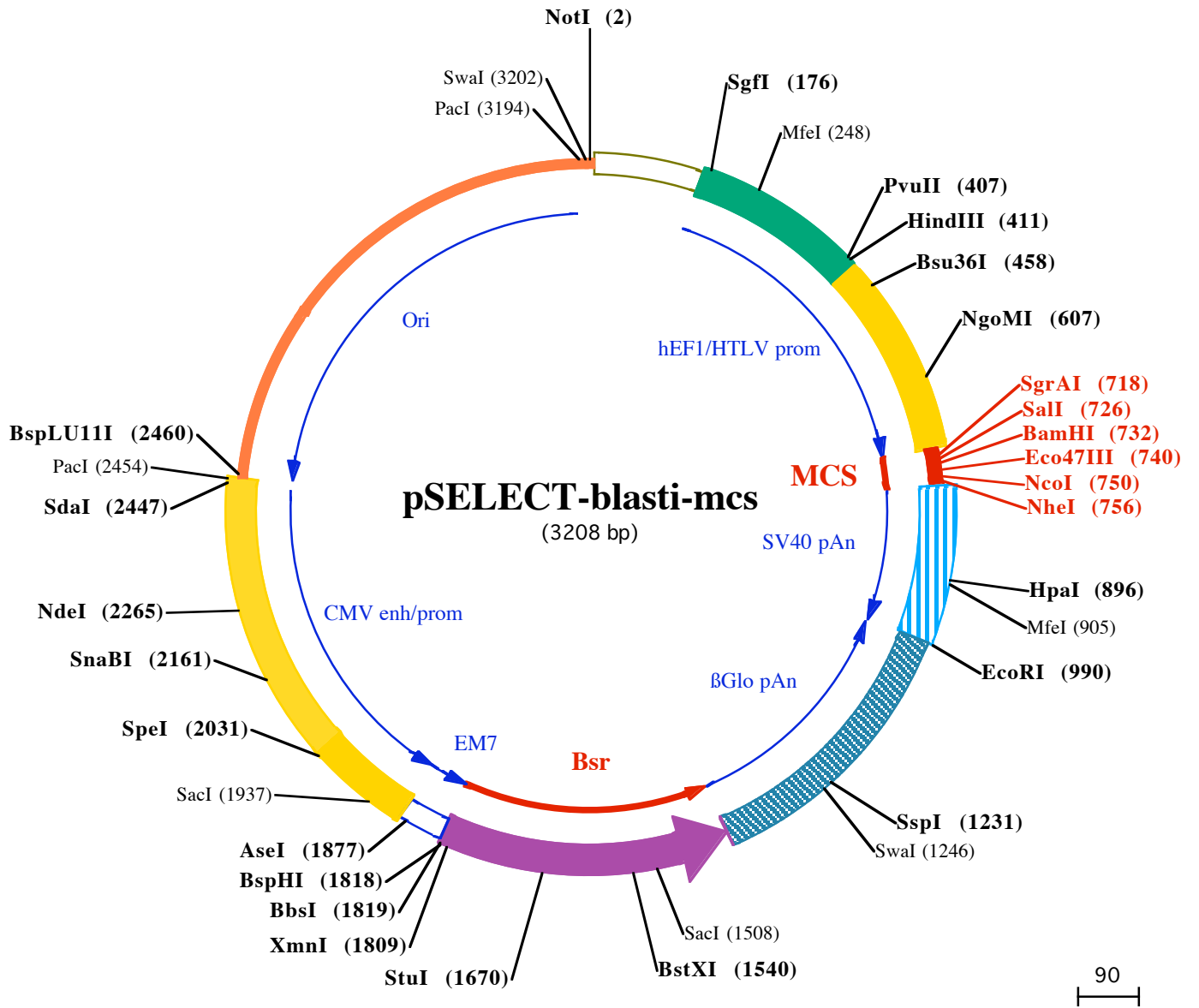
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NotI (2)
1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAAACAAAACGAAACA

SgfI (176)
101 AAACAACTAGCAAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCA

MfeI (248)
201 GAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCC TAGAGAAGGTGGCGGGGTAACTGGGAAAGTGATG

301 TCGTGTACTGGCTCCGCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCCGAACGGGTTTGCCGCCAG

HindIII (411)
PvuII (407)
401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACGCGCCCGCCCTACCTGAGGCCGCATCCACGCCGGTTGAGTCGCGTTTCTGCCGCTT

Bsu36I (458)
501 CCGCGTGTGGTGCCTCCTGAACTGCGTCCGCGTCTAGTAAAGTTTAAAGTCAAGTGCAGGTCGAGACCGGGCTTTGTCCGGCGCTCCCTTGAGGCTACCTA

NgoMI (607)
601 GACTCAGCCGGCTCTCCACGCTTTGCTGACCTGCTTCAACTCTACGTTCTTTGTTTCTGTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC

SgrAI (718) **SalI (726)** **Eco47III (740)** **NheI (756)**
701 GCGCGCTACCTGAGATCaccggtgtcgcagcggatccagcgtctgcagCCATGGCTAGCTGCCAGACATGATAAGATACATTGATGAGTTTGGACAA

HpaI (896)
801 ACCACAAC TAGAATGCAGTGAATAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAGTTAAACA

MfeI (905) **EcoRI (990)**
901 ACAACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAATGTGGTATGGAATCTAAAAA

1001 TACAGCATAGCAAAACCTTAACTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGC

1101 ATTAGCTGTTGACGCTCACCTTCTTTCATGGAGTTTAAAGATAGTGTATTTCCAAAGTTTGAAC TAGCTCTTCAATTTCTTTATGTTTTAAATGCA

SspI (1231) **SwaI (1246)**
1201 CTGACCTCCCACATTCCTTTTTAGTAAAATATTGAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAAATCCAGATGC

1301 TCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGAACCTTAAATGAAAATGGACAGCAAGAAGCGAGCTTCTAGCTTTT

1401 AGTTCCTGGTACTTTCAGGGGATGAGTTCCTCAATGGTGGTTTTGACCAGCTTGCATTCTCAATGAGCACAAAGCAGTCAGGAGCATAGTCAGA
1404 AsnArgThr TyrLysLeuProl IeLeuGI uGI uI IeThr Thr LysVal IeLeuLysGI yAsnMetuGI uI IeLeuVal PheCysAspP roAI aTyrAspSer

SacI (1508) **BstXI (1540)**
1501 GATGAGCTCTTCGCACATGCCACAGGGGCTGACCACCTGATGGATCTGTCCACCTCATCAGAGTAGGGGTGCCTGACAGCCACAATGGTGTCAAAGTCC

1074 I IeLeuGI uArgCysMe tGI yCysP roSer Val IeVal ArgI IeSer ArgAspVal GI uAspSer TyrProHi sArgValAI aVal I IeThrAspPheAspL

StuI (1670)
1601 TTCTGCCGTTGCTCACAGCAGACCAATGGCAATGGCTTACGACAGACAGTACCCTGCCAATGTAGGCCTCAATGTGGCAGCAGAGATGATCTCCC

734 ysGI nGI yAsnSer ValAI aSer GI yI IeAI aI IeAI aGI uAI aCysVal Thr ValArgGI yI IeTyrAl aGI uI IeHi sValAI aSer I IeI IeGI uGI

1701 CAGTCTTGGTCTGATGGCCGCCGACATGGTGTGTTGTCTCATAGAGCATGGTGTCTTCTCAGTGCCGACCTCCACCAGCTCCAGATCTCGCTG

404 yThr LysThr ArgI IeAI aAI aGI yVal Hi sLysAsnAspGI uTyrLeuMe tThr I IeLysGI uThr AI aVal GI uVal IeLeuGI uLeuAspGI nGI n

BspHI (1818)
BbsI (1819)
XmnI (1809) **AseI (1877)**
1801 AGAGATGTTGAAGGCTTTCATGATGGCCCTCTATAGTGAGTCGTATTATACTATGCGATATACTATGCCGATGATTAATTGTCAAACAGCGTGGATG

74 Ser I IeAsnPheThr LysMet

SacI (1937)
1901 GCGTCTCCAGCTTATCTGACGGTTCCTAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCTACCGCCAATTTGCGTCAATGGGGCGGAGTTG

SpeI (2031)
2001 TTACGACATTTTGGAAAGTCCCCTGATTACTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCTGAGTCAAACCGCT

SnaBI (2161)
2101 ATCCACGCCATTGATGACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCAAT

NdeI (2265)
2201 GTA CTGGGCATAATGCCAGGCGGGCATTACCCTGATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGTACTGCCAAGTGGGCGAGTT

2301 TACCGTAAATACTCCACCCATTGACGTCAATGAAAGTCCCTATTGGCGTACTACTGGGAACATACGTCATTATTGACGTCAATGGGCGGGGCTGTTGG

PacI (2454) **SdaI (2447)** **BspLU11I (2460)**
2401 GCGGTCAGCCAGCGGGCCATTTACCCTAAGTTATGTAACGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGAACCGTAA

2501 AAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCTTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTAT

2601 AAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTCGGGAAG

2701 CGTGGCGCTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCTGCTCGCTCCAAGCTGGGCTGTGTGCAGCAACCCCGTTCAGCCCGAC

2801 CGCTGCGCTTATCCGTAACATCTGCTTGTAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGA

2901 GGTATGTAGGCGGTGTACAGAGTCTTGAAGTGGTGGCTAACTACGGTCACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTAC

3001 CTTCCGAAAAAGAGTTGGTAGCTTGTATCCGGCAAAACAAACCACCGCTGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCCGAGAAAAAAA

PaeI (3194) **SwaI (3202)**
3101 GGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACAT

3201 TTAATACA