

pSELECT-blasti-mcs

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

Catalog # psetb-mcs

For research use only

Version # 04A08-MT

PRODUCT INFORMATION

Content:

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

Storage and Stability:

Product is shipped at room temperature.

Lyophilized DNA should be resuspended upon receipt and stored at -20°C. Lyophilized DNA is stable 12 months at -20°C. Resuspended DNA is stable more than one year at -20°C. Avoid repeated freeze-thaw cycles.

Store *E. coli* Fast-Media® Blas 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 (#psirna3g:21-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 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*.

• **Blasti:** Resistance to Blasticidin S 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.

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.

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® Blas can be ordered separately (#fas-bl-l (liquid), #fas-bl-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

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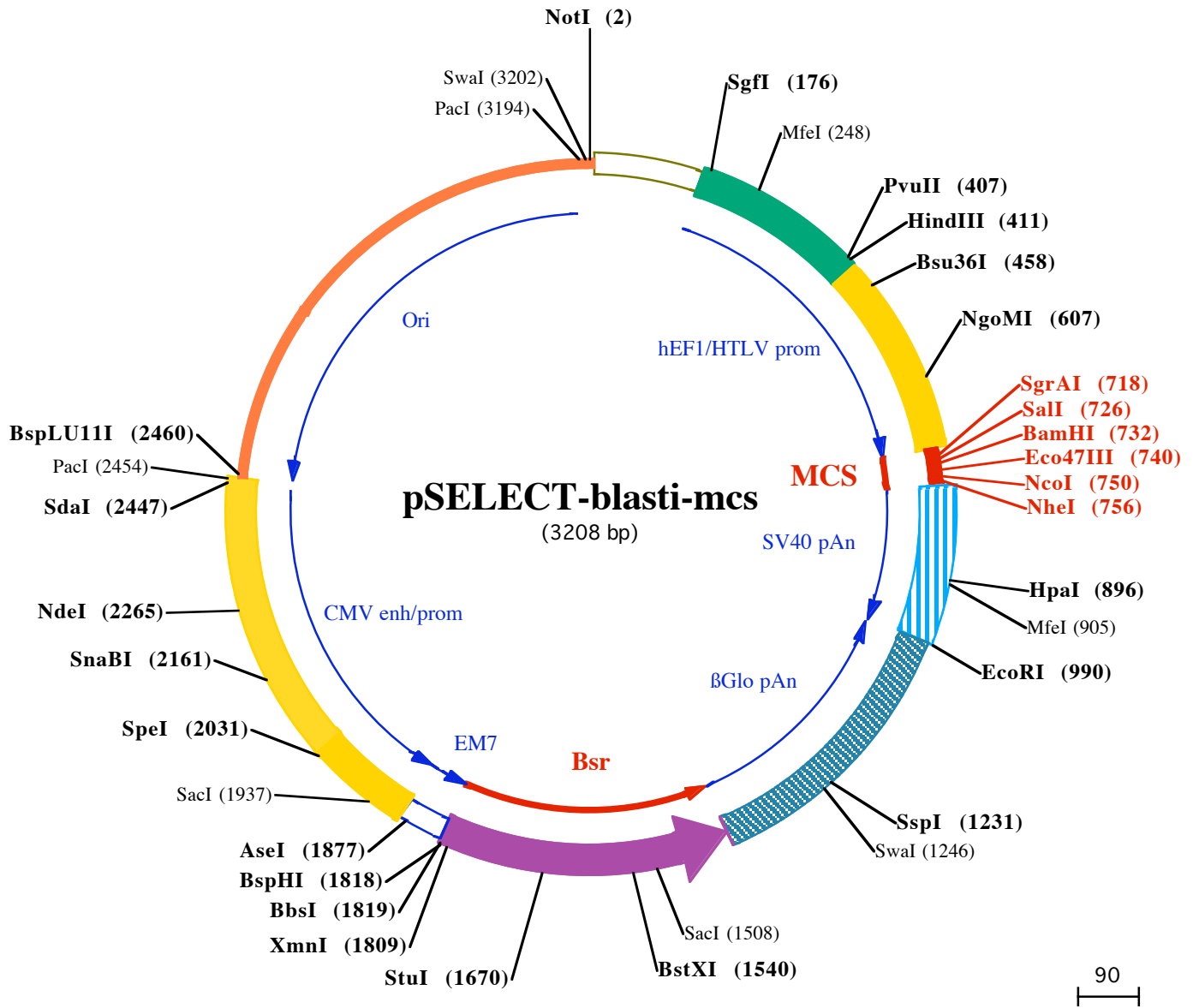
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NotI (2)
1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGGTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAAACAAAACGAAACA

SgfI (176)
101 AAACAACTAGCAAAATAGGCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCA

MfeI (248)
201 GAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCC TAGAGAAGGTGGCGGGGTAAACTGGGAAAGTGATG

301 TCGTGTACTGGCTCCGCTTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAG

HindIII (411)
PvuII (407)
401 AACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACGCGCCCGCCCTACCTGAGGCCGCATCCACGCCGGTTGAGTCGCGTTTCTGCCGCTT

Bsu36I (458)
501 CCGCGTGTGGTGCCTCCTGAACTGCGTCCGCGTCTAGTAAAGTTAAAGTCAAGTGCAGGTCGAGACGGGCTTTGTCCGGCGCTCCCTTGAGGCTACCTA

NgoMI (607)
601 GACTCAGCCGGCTCTCCACGCTTTGCTGACCTGCTTCAACTCTACGTTCTTTGTTTCTGTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACC

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

HpaI (896)
801 ACCACAAC TAGAATGCAGTGA AAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAGTTAAACA

MfeI (905) **EcoRI (990)**
901 ACAACAATTGCATTCA TTTTATGTTTCAGGTT CAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAATGTGGTATGGAATCTAAAAA

1001 TACAGCATAGCAAAAC TTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGC

1101 ATTAGCTGTTG CAGCCTCACCTTCTTCATGGAGTTTAAAGATAGTGTATTTTCCAAAGTTTGAAC TAGCTCTTCATTTCTTTATGTTTTAAATGCA

SspI (1231) **SwaI (1246)**
1201 CTGACCTCCCACATTC CTTTTTAGTAAAATATT CAGAAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAAATCCAGATGC

1301 TCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGAACCTTTAATAGAAATGGACAGCAAGAAGCGAGCTTCTAGCTTTT

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

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

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 AGAGATGTTGAAGGTCTTCATGATGGCCCTCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACAGCGTGGATG

74 Ser I IeAsnPheThr LysMet

SacI (1937)
1901 GCGTCTCCAGCTTATCTGACGGTTCCTAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCTACCGCCAATTTGCGTCAATGGGGCGGAGTTG

SpeI (2031)
2001 TTACGACATTTTGGAAAGTCCC GTTGATTACTAGTCAAAAACAACTCCCATTGACGTCAATGGGGTGGAGACTTGAAATCCCCGTGAGTCAAACCGCT

SnaBI (2161)
2101 ATCCACGCCCAATTGATGACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCAAT

NdeI (2265)
2201 GTA CTGGGCATAATGCCAGGCGGGCCATTACC GTTATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGTACTGCAAGTGGGCGAGTT

2301 TACCGTAAATACTCCACCCATTGACGTCAATGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGTCGTTGG

PacI (2454) **SdaI (2447)** **BspLU11I (2460)**
2401 GCGGTCAGCCAGCGGGCCATTTACC GTAAGTTATGTAACGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGAACCGTAAAC

2501 AAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCTTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTAT

2601 AAAGATACCAGGCGTTTCCCTCGAAGCTCCCTCGTGCCTCTCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTCGGGAAG

2701 CGTGGCGCTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCTGCTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCCGAC

2801 CGCTGCGCTTATCCGTAAC TATCGTCTT GAGTCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGA

2901 GGTATGTAGGCGGTGTACAGAGTCTTGAAGTGGTGGCTAACTACGGTCACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTAC

3001 CTTGGA AAAAGAGTTGGTAGCTTGTATCCGGCAAAACCAACCCTGGTAGCGGTGTTTTTTGTTTGAAGCAGCAGATTACGCCGAGAAAAAAA

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

3201 TTAATACA