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 20I17-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 ß-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 H_2O. 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 promoter[1] 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[2]. 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[3].
- **ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

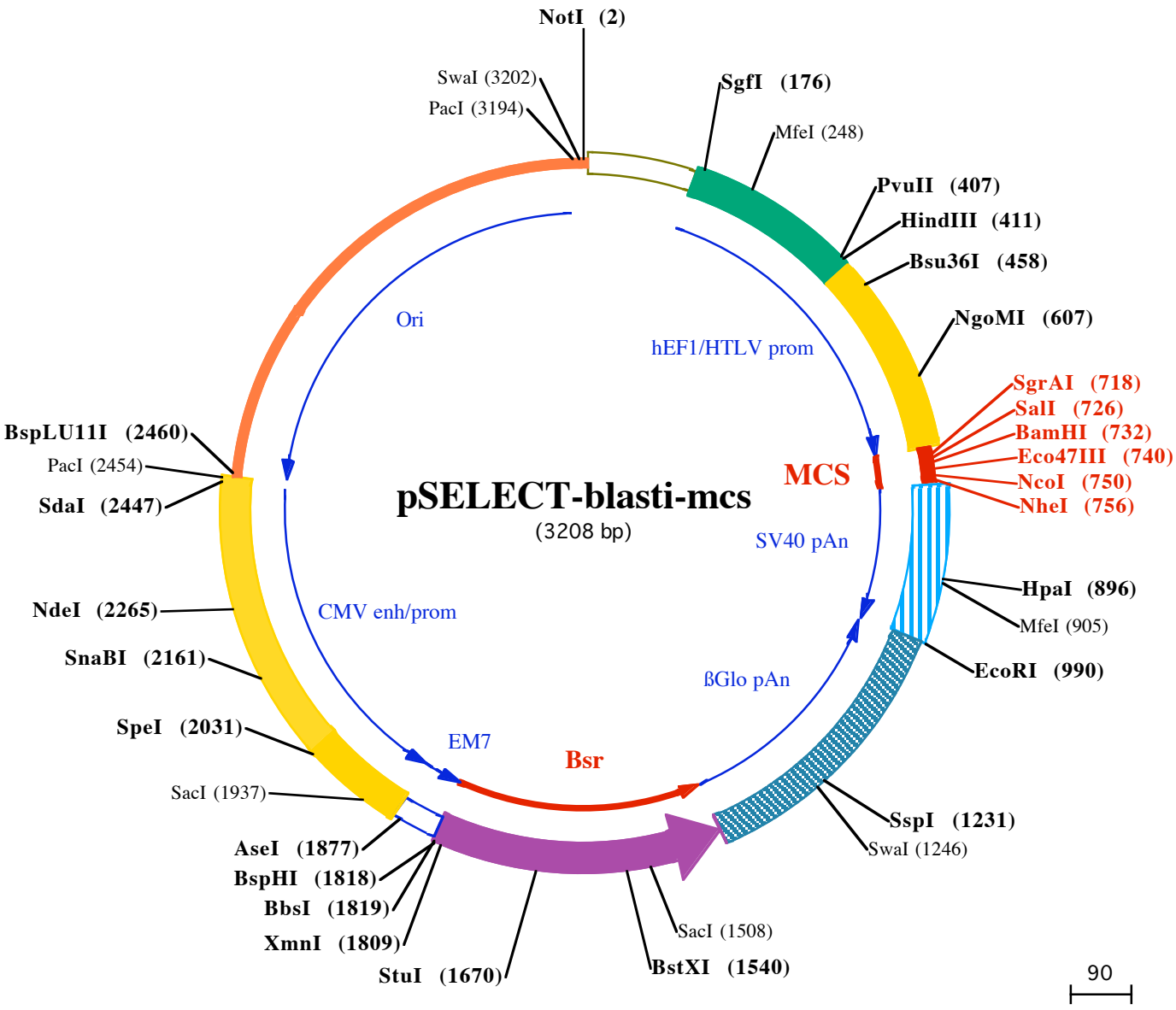
Second expression cassette

- **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 transcription[4].

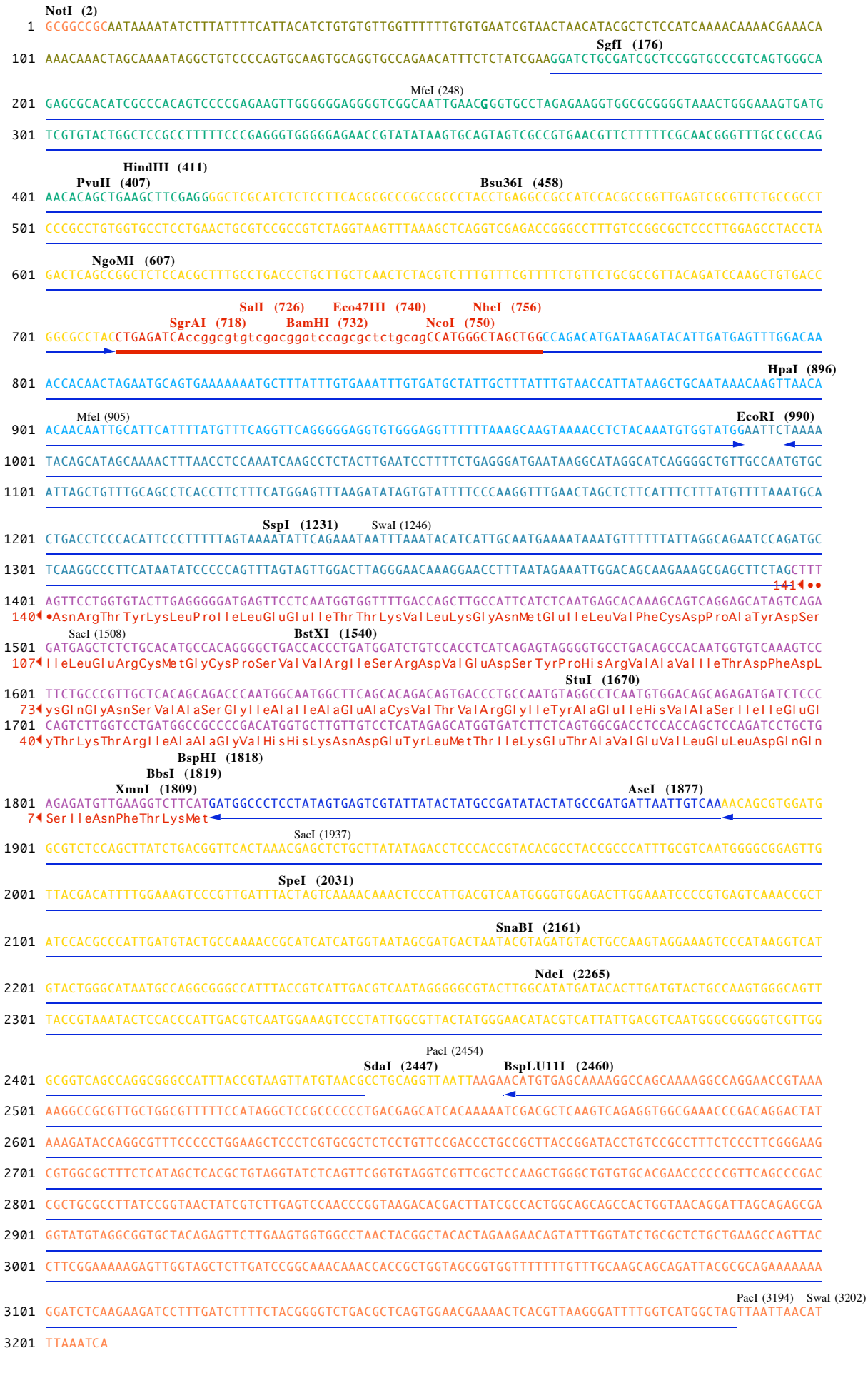
References

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
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


NotI (2)
SwaI (3202)
PacI (3194)
SgfI (176)
MfeI (248)
PvuII (407)
HindIII (411)
Bsu36I (458)
NgoMI (607)
SgrAI (718)
SalI (726)
BamHI (732)
Eco47III (740)
NcoI (750)
NheI (756)
HpaI (896)
MfeI (905)
EcoRI (990)
SspI (1231)
SwaI (1246)
SacI (1508)
BstXI (1540)
StuI (1670)
XmnI (1809)
BbsI (1819)
BspHI (1818)
AseI (1877)
SacI (1937)
SpeI (2031)
SnaBI (2161)
NdeI (2265)
SdaI (2447)
PacI (2454)
BspLU11I (2460)
pSELECT-blasti-mcs
(3208 bp)
Ori
hEF1/HTLV prom
MCS
SV40 pAn
ßGlo pAn
Bsr
EM7
CMV enh/prom
90

InvivoGen


NotI (2)
SgfI (176)
MfeI (248)
HindIII (411)
PvuII (407)
Bsu36I (458)
NgoMI (607)
SalI (726)
Eco47III (740)
NheI (756)
SgrAI (718)
BamHI (732)
NcoI (750)
HpaI (896)
MfeI (905)
EcoRI (990)
SspI (1231)
SwaI (1246)
SacI (1508)
BstXI (1540)
StuI (1670)
BspHI (1818)
BbsI (1819)
XmnI (1809)
AseI (1877)
SacI (1937)
SpeI (2031)
SnaBI (2161)
NdeI (2265)
PacI (2454)
SdaI (2447)
BspLU11I (2460)
PacI (3194)
SwaI (3202)