pSELECT-zeo-LacZ

A LacZ Reporter Gene System Selectable with Zeocin™

Catalog code: psetz-lacz

For research use only

Version 20K30-MM

PRODUCT INFORMATION

Content:

- 20 µg of **pSELECT-zeo-LacZ** plasmid provided as lyophilized DNA
- 1 ml of Zeocin™ (100 mg/ml)

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 for 3 months at -20˚C. Resuspended DNA is stable more than one year at -20˚C.

Store Zeocin™ at 4 °C or at -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-LacZ plasmids can be used as control vectors for cloning of an open reading frame, as the LacZ gene is flanked by two unique restriction sites: Nco I at the 5' end that encompasses the Start codon, and Nhe I at the 3'end.

pSELECT-LacZ can serve as a gene reporter system for the study of eukaryotic gene expression and regulation. The *E. coli lacZ* gene encoding β-galactosidase is the classical histochemical reporter gene. β-Galactosidase catalyzes the hydrolysis of X-Gal producing a blue precipitate that can be easily visualized under a microscope.

PLASMID FEATURES

First expression cassette

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1a (EF-1a) 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-1a promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1a core promoter to enhance stability of RNA.

• **LacZΔCpG gene:** a humanized and CpG-free allele of the LacZ gene. This CpG-free gene is ten times more active than the wild-type gene in mammalian cells. It can be used for *in vitro* or *in vivo* applications.

• **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.*

• **Zeo:** Resistance to Zeocin™ is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus* The *Sh ble* gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.

• **ßGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

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 other commonly used laboratory *E. coli* strains, such as DH5α.

Zeocin™ usage

This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

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.

RELATED PRODUCTS

Product	Catalog Code
ChemiComp GT116	gt116-11
Zeocin™	ant-zn-1

TECHNICAL SUPPORT
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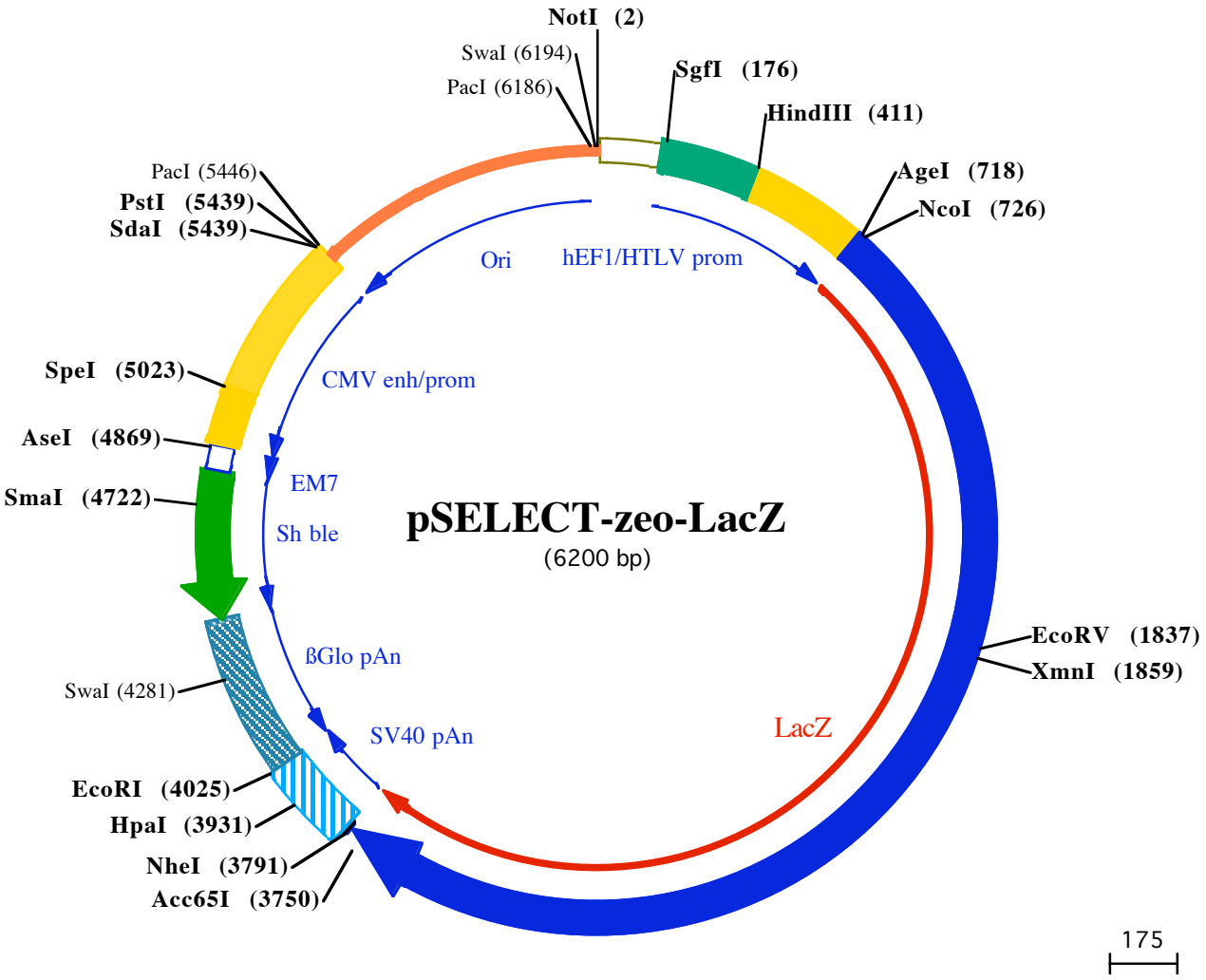
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PacI (6186)
SgfI (176)
HindIII (411)
AgeI (718)
NcoI (726)
PacI (5446)
PstI (5439)
SdaI (5439)
Ori
hEF1/HTLV prom
SpeI (5023)
CMV enh/prom
AseI (4869)
EM7
SmaI (4722)
Sh ble
pSELECT-zeo-LacZ
(6200 bp)
EcoRV (1837)
XmnI (1859)
ßGlo pAn
SwaI (4281)
SV40 pAn
LacZ
EcoRI (4025)
HpaI (3931)
NheI (3791)
Acc65I (3750)
175

InvivoGen


NotI (2)
SgfI (176)
HindIII (411)
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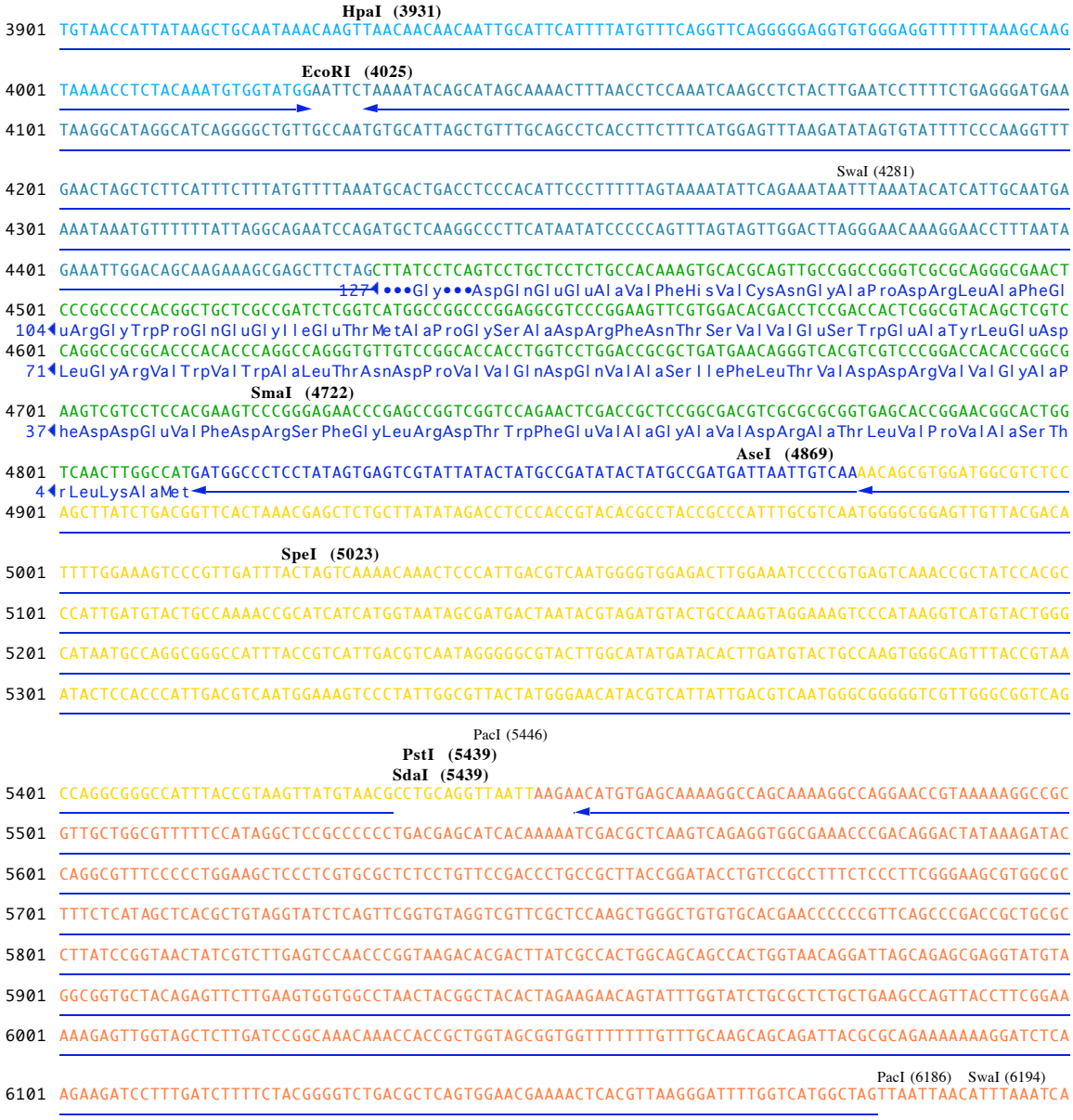
Acc65I (3750)
NheI (3791)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