pSELECT-hygro-LacZ

A LacZ-expression plasmid selectable with Hygromycin

Catalog code: pseth-lacz

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

For research use only

Version 19A21-MM

PRODUCT INFORMATION

Contents

- 20 µg of pSELECT-hygro-LacZ 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 ß-globin polyA terminates the transcription of the selectable marker.

pSELECT-LacZ plasmids can be used as control vectors or 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.

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 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.
- **LacZ:** The *E. coli lacZ* gene codes for the enzyme β-galactosidase which catalyzes the hydrolysis of the substrate X-Gal to produce a blue color that is easily visualized under a microscope.
- **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.*
- **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*.
- **ßGlo 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.

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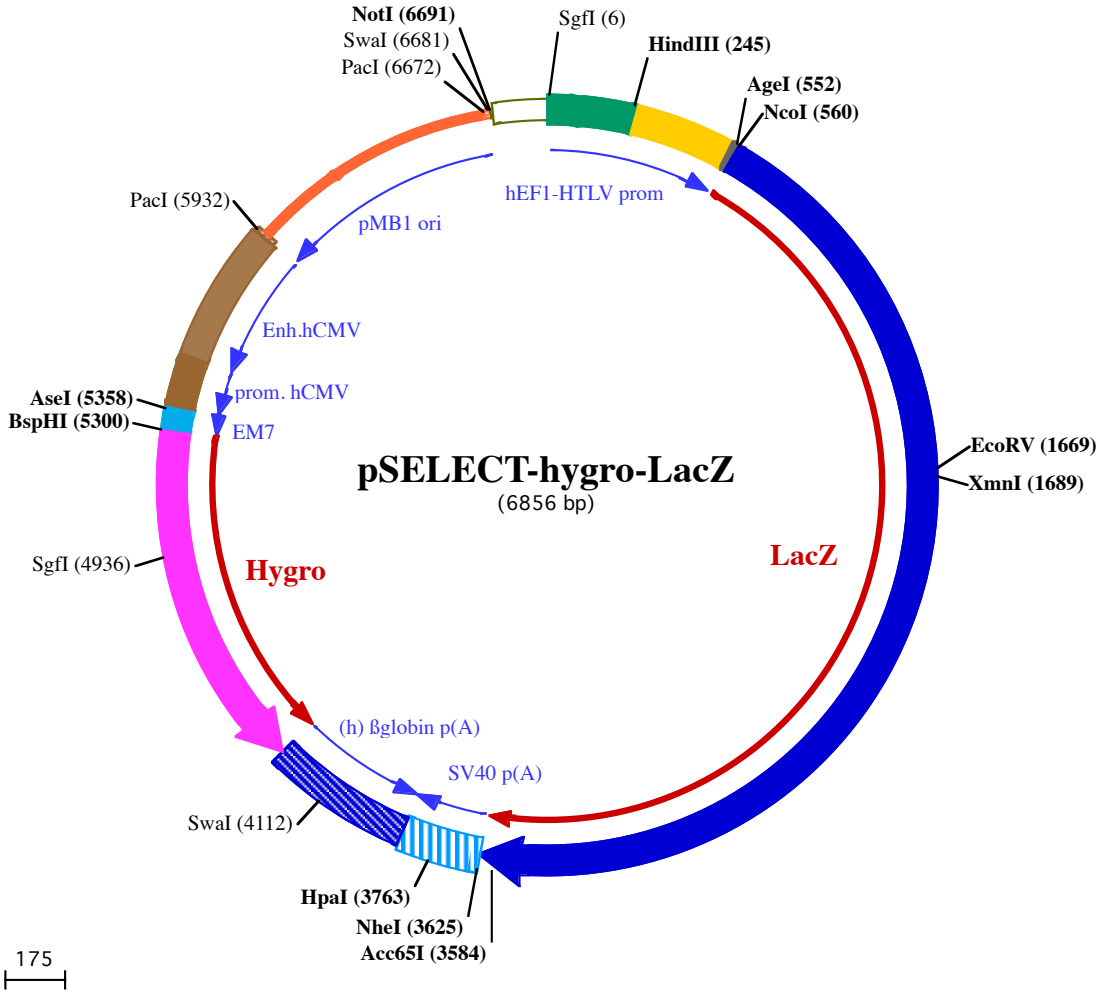
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SwaI (6681)
PacI (6672)
SgfI (6)
HindIII (245)
AgeI (552)
NcoI (560)
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pMB1 ori
PacI (5932)
Enh.hCMV
prom. hCMV
EM7
AseI (5358)
BspHI (5300)
pSELECT-hygro-LacZ
(6856 bp)
EcoRV (1669)
XmnI (1689)
SgfI (4936)
Hygro
LacZ
(h) ßglobin p(A)
SV40 p(A)
SwaI (4112)
HpaI (3763)
NheI (3625)
Acc65I (3584)
175

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SgfI (6)
1    GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA

101  GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

                                                     HindIII (245)
201  GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC

301  GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401  GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT

                                                            AgeI (552) NcoI (560)
501  TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCACCATGGACCCTGTTGTGCTGCAAAGGAGAGACTGGGAGAA
                                                                   1▶ M  D  P  V  V  L  Q  R  R  D  W  E  N
601  CCCTGGAGTGACCCAGCTCAACAGACTGGCTGCCCACCCTCCCTTTGCCTCTTGGAGGAACTCTGAGGAAGCCAGGACAGACAGGCCCAGCCAGCAGCTC
13▶   P  G  V  T  Q  L  N  R  L  A  A  H  P  P  F  A  S  W  R  N  S  E  E  A  R  T  D  R  P  S  Q  Q  L
701  AGGTCTCTCAATGGAGAGTGGAGGTTTGCCTGGTTCCCTGCCCCTGAAGCTGTGCCTGAGTCTTGGCTGGAGTGTGACCTCCCAGAGGCTGACACTGTTG
47▶  R  S  L  N  G  E  W  R  F  A  W  F  P  A  P  E  A  V  P  E  S  W  L  E  C  D  L  P  E  A  D  T  V
801  TGGTGCCCAGCAACTGGCAGATGCATGGCTATGATGCCCCCATCTACACCAATGTCACCTACCCCATCACTGTGAACCCCCCTTTTGTGCCCACTGAGAA
80▶ V  V  P  S  N  W  Q  M  H  G  Y  D  A  P  I  Y  T  N  V  T  Y  P  I  T  V  N  P  P  F  V  P  T  E  N
901  CCCCACTGGCTGCTACAGCCTGACCTTCAATGTTGATGAGAGCTGGCTGCAAGAAGGCCAGACCAGGATCATCTTTGATGGAGTCAACTCTGCCTTCCAC
113▶  P  T  G  C  Y  S  L  T  F  N  V  D  E  S  W  L  Q  E  G  Q  T  R  I  I  F  D  G  V  N  S  A  F  H
1001 CTCTGGTGCAATGGCAGGTGGGTTGGCTATGGCCAAGACAGCAGGCTGCCCTCTGAGTTTGACCTCTCTGCCTTCCTCAGAGCTGGAGAGAACAGGCTGG
147▶  L  W  C  N  G  R  W  V  G  Y  G  Q  D  S  R  L  P  S  E  F  D  L  S  A  F  L  R  A  G  E  N  R  L
1101 CTGTCATGGTGCTCAGGTGGTCTGATGGCAGCTACCTGGAAGACCAAGACATGTGGAGGATGTCTGGCATCTTCAGGGATGTGAGCCTGCTGCACAAGCC
180▶ A  V  M  V  L  R  W  S  D  G  S  Y  L  E  D  Q  D  M  W  R  M  S  G  I  F  R  D  V  S  L  L  H  K  P
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1301 GACTACCTGAGAGTCACAGTGAGCCTCTGGCAAGGTGAGACCCAGGTGGCCTCTGGCACAGCCCCCTTTGGAGGAGAGATCATTGATGAGAGAGGAGGCT
247▶  D  Y  L  R  V  T  V  S  L  W  Q  G  E  T  Q  V  A  S  G  T  A  P  F  G  G  E  I  I  D  E  R  G  G
1401 ATGCTGACAGAGTCACCCTGAGGCTCAATGTGGAGAACCCCAAGCTGTGGTCTGCTGAGATCCCCAACCTCTACAGGGCTGTTGTGGAGCTGCACACTGC
280▶ Y  A  D  R  V  T  L  R  L  N  V  E  N  P  K  L  W  S  A  E  I  P  N  L  Y  R  A  V  V  E  L  H  T  A
1501 TGATGGCACCCTGATTGAAGCTGAAGCCTGTGATGTTGGATTCAGAGAAGTCAGGATTGAGAATGGCCTGCTGCTGCTCAATGGCAAGCCTCTGCTCATC
313▶  D  G  T  L  I  E  A  E  A  C  D  V  G  F  R  E  V  R  I  E  N  G  L  L  L  L  N  G  K  P  L  L  I
                                                                            EcoRV (1669)         XmnI (1689)
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380▶ N  A  V  R  C  S  H  Y  P  N  H  P  L  W  Y  T  L  C  D  R  Y  G  L  Y  V  V  D  E  A  N  I  E  T  H
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513▶  S  I  K  K  W  L  S  L  P  G  E  T  R  P  L  I  L  C  E  Y  A  H  A  M  G  N  S  L  G  G  F  A  K
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747▶  G  N  K  R  W  Q  F  N  R  Q  S  G  F  L  S  Q  M  W  I  G  D  K  K  Q  L  L  T  P  L  R  D  Q  F
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780▶ T  R  A  P  L  D  N  D  I  G  V  S  E  A  T  R  I  D  P  N  A  W  V  E  R  W  K  A  A  G  H  Y  Q  A
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813▶  E  A  A  L  L  Q  C  T  A  D  T  L  A  D  A  V  L  I  T  T  A  H  A  W  Q  H  Q  G  K  T  L  F  I
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847▶  S  R  K  T  Y  R  I  D  G  S  G  Q  M  A  I  T  V  D  V  E  V  A  S  D  T  P  H  P  A  R  I  G  L
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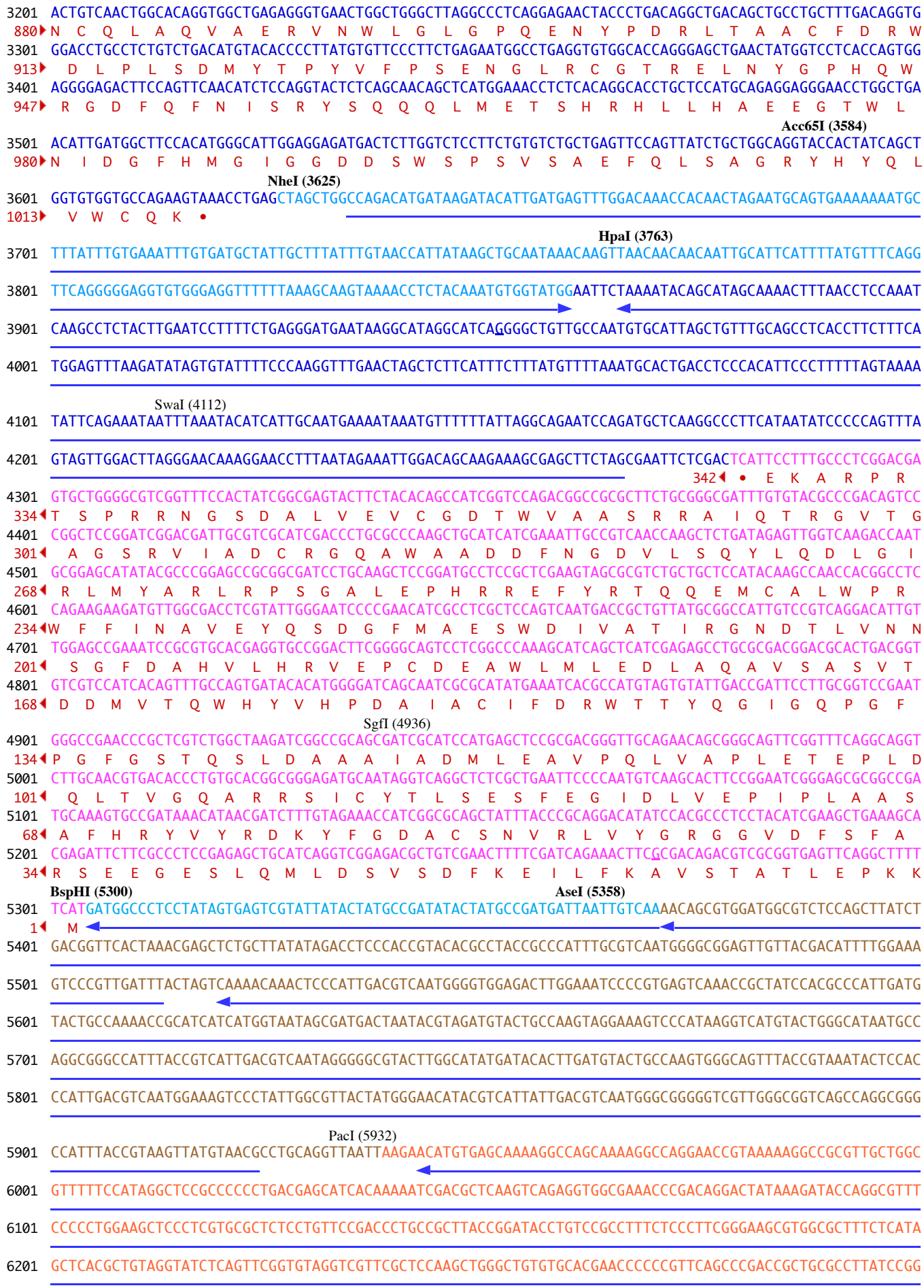
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                                                    PacI (6672)  SwaI (6681)  NotI (6691)
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