

pSELECT-CHA-zeo

Plasmid for the expression of HA-C-terminal tagged proteins

Catalog # psetz-cha

For research use only

Version 20K30-MM

PRODUCT INFORMATION

Content:

- 20 µg of pSELECT-CHA-zeo plasmid provided as lyophilized DNA
- 3' b n' qh' \ gqel p' I * 322' b i b n'

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 β-globin polyA terminates the transcription of the selectable marker.

pSELECT-Tag is a new family of expression plasmids designed to generate tagged proteins in order to facilitate their detection and/or purification. pSELECT-Tag features three well-known tags: the green fluorescent protein (GFP) gene, the human influenza hemagglutinin (HA) epitope and the polyhistidine (His) tag. pSELECT-Tag plasmids allow to add the tag either at the N or C terminus of the protein of interest.

C-terminal tag: the tag is cloned downstream of a multiple cloning site and followed by a Stop codon.

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' - Age I, Sal I, Eco47III, BamHI, Nco I - 3'

Each restriction site is compatible with many other enzymes.

• **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*.

• **Sh ble gene** confers zeocin resistance. 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*.

• **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.

CLONING STRATEGY

For expression of a tagged protein, it is important to ensure to clone your gene-of-interest into the correct reading frame. In general it is recommended to use Age I/Bam HI restriction site combination for cloning into plasmids with the C-Tag. For the plasmids with C-Tag, check whether the start codon of your gene of interest is in the correct reading frame with the C-Tag.

Note: The Bam HI restriction site is compatible with Bgl II.



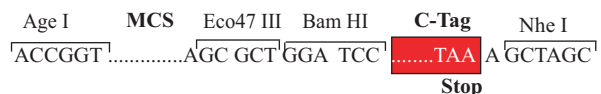
If it is not possible to use the Age I restriction site, it is possible to use another restriction site such as Sal I, upstream of Bam HI.



If it is not possible to use the Bam HI restriction site, it is possible to use another restriction site such as Nco I.



Alternatively, blunt end cloning can be achieved using the Eco 47 III site, which is upstream of the Bam HI site. Blunt end PCR fragments can be obtained using T4 DNA polymerase or Klenow.



TECHNICAL SUPPORT

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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 in 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:

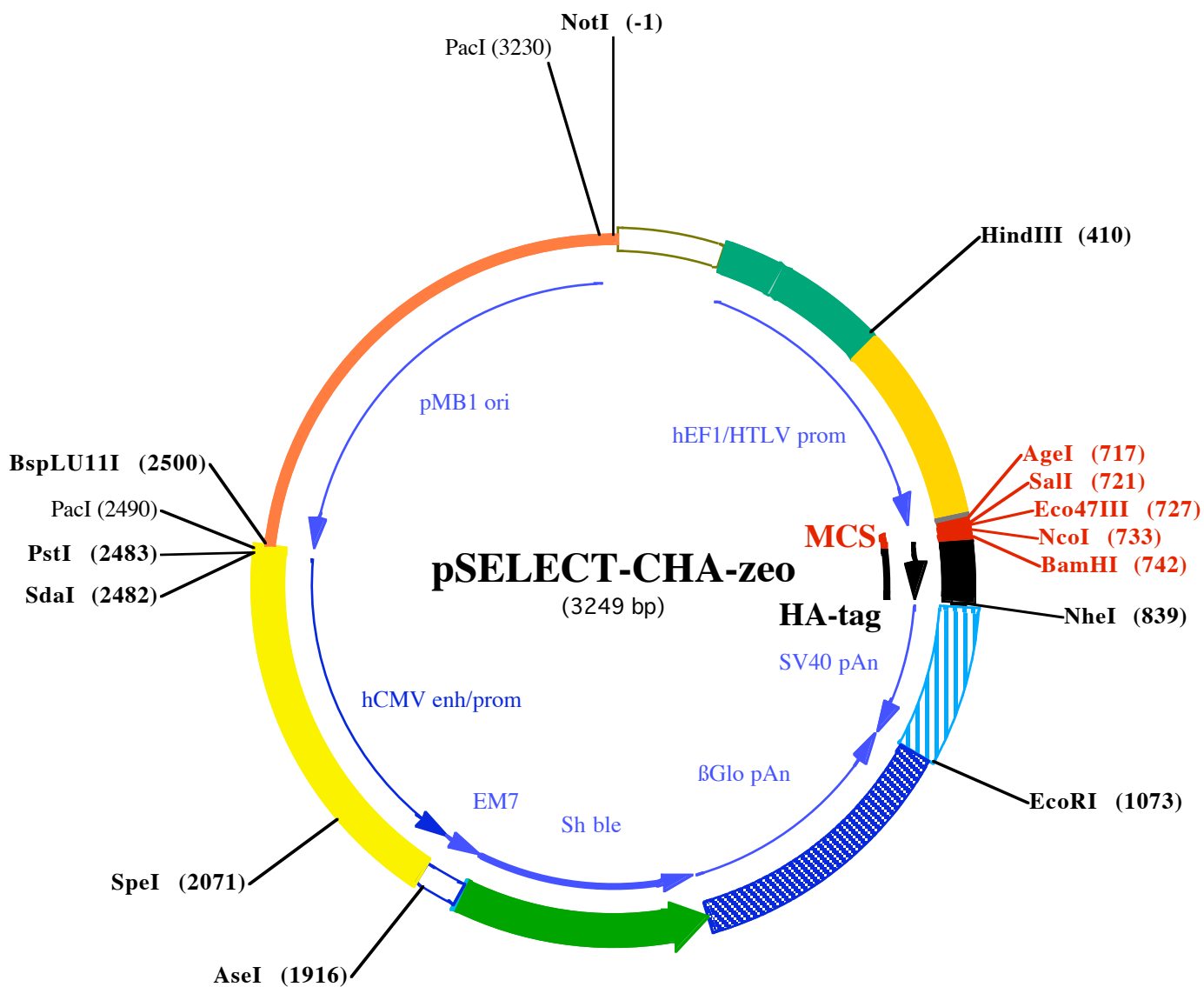
1. Kim, D.W. *et al.* (1990). *Gene* 2: 217-223.
2. Takebe, Y. *et al.* (1988). *Mol. Cell Biol.* 1: 466-472.
3. Carswell, S., and Alwine, J.C. (1989). *Mol. Cell Biol.* 10: 4248-4258.
4. Yu J & Russell JE. (2001). *Mol Cell Biol*, 21(17):5879-88.

RELATED PRODUCTS

Product	Catalog Code
pSELECT-NGFP-blasti	psetb-ngfp
pSELECT-CGFP-blasti	psetb-cgfp
pSELECT-CGFP-zeo	psetz-cgfp
pSELECT-NGFP-zeo	psetz-ngfp
pSELECT-NHA-blasti	psetb-nha
pSELECT-NHA-zeo	psetz-nha
pSELECT-CHA-blasti	psetb-cha
pSELECT-CHis-blasti	psetb-chis
pSELECT-NHis-zeo	psetz-nhis
pSELECT-CHis-zeo	psetz-chis

TECHNICAL SUPPORT

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NotI (-1)

1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATAC
75 GCTCTCCATCAAAAACAAAACGAAAACAAAACAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAG
149 AACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCC

223 CCGAGAAGTTGGGGGAGGGGTCGGCAATTGAACGGTGCCTAGAGAAGGTGGCGGGGTAAACTGGGAAAGT

297 GATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAA

HindIII (410)

371 CGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTACACGGC

445 CCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCC

519 TGAAGTGCCTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAG

593 CCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGTCAACTCTACGTCTTTGTTTCGTTTT

SalI (721) NcoI (733)

AgeI (717) Eco47III (727)

667 CTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCGACAGCGCTCCATGGC

BamHI (742)

741 TCGGATCCATCCCTATGATGTGCCAGACTATGCTGGCTATCCATATGATGTTCTGATTATGCTGGATACCCT

1 Gl ySer TyrProTyrAspVal P roAspTyrAl aGl yTyrP roTyrAspVal P roAspTyrAl aGl yTyrP ro

NheI (839)

815 TATGATGTGCCAGACTATGCCTAAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCA

25 TyrAspVal P roAspTyrAl a •••

889 CAACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATA

963 AGCTGCAATAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGAGGT

EcoRI (1073)

1037 TTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAACTTTAACCTCC

1111 AAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGC

1185 ATTAGCTGTTTGAGCCTCACCTTCTTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAAGTAGCT

1259 CTTCAATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTTAGTAAAATATTCAGAAAATAATTTAA

1333 ATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCC

1407 CCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAG

1481 CTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGTTCGCGCAGGGCGAACTCCCGC

125 AspGlnGluAlaValPheHisValCysAsnGlyAlaProAspArgLeuAlaPheGluArgG

1555 CCCACGGCTGCTCGCCGATCTCGGTCTGCGCGCCCGGAGGCGTCCCGAAGTTCGTGGACACGACCTCCGA

102 IlyTrpProGlnGluGlyIleGluThrMetAlaProGlySerAlaAspArgPheAsnThrSerValValGluSer

1629 CCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTGTCCGGCACCACTGGTCTT

78 TrpGluAlaTyrLeuGluAspLeuGlyArgValTrpValTrpAlaLeuThrAsnAspProValValGluAspGln

1703 GGACCGCGCTGATGAACAGGGTCACGTCTGCCGACCACACCGGCAAGTCTCTCCACGAAGTCCCGGGAG

53 nValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspAspGluValPheAspArgSerP

1777 AACCCGAGCCGGTCCGTCAGAAGTCCGCGCAGCTCGCGCGCGGTGAGCACCGGAACGGCACTGGT

28 heGlyLeuArgAspThrTrpPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThr

AseI (1916)

1851 CAACTTGGCCATGATGGCCCTCCTATAGTGAGTCGATTATACTATGCCGATATACTATGCCGATGATTAATTG

4 LeuLysAlaMet

1925 TCAAACAGCGTGGATGGCGTCTCCAGCTATCTGACGGTTCACATAACGAGCTCTGCTTATATAGACCTCCCA

1999 CCGTACACGCCTACCGCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTA
 2073 CTAGTCAAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCA
 2147 CGCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGT
 2221 AGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGG
 2295 GCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGT
 2369 CAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGG

PacI (2490)

PstI (2483)

SdaI (2482)

BspLU11I (2500)

2443 CGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCGAGGTTAAATAAGAACATGTGAGCAAAA
 2515 GGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGA
 2589 GCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCC
 2663 CTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCCTTTCTCCCTTCG
 2737 GGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTGCTCCAAGCTGGG
 2811 CTGTGTGCACGAACCCCCGTTAGCCCCGACCCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGG
 2885 TAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCT
 2959 ACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAA
 3033 GCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTT
 3107 TTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCT
 3181 GACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATC A

PacI (3230)