pSELECT-hASC-GFP

A mammalian expression plasmid encoding a human ASC::GFP fusion protein

Catalog code: psetz-hascgfp

http://www.invivogen.com/gfp-inflammasome-genes

For research use only

Version 20L01-MM

PRODUCT INFORMATION

Contents

• 20 µg of pSELECT-hASC-GFP plasmid provided as lyophilized DNA
• 1 ml of ZeocinÊ (100 mg/ml)

Storage and stability

• Product is shipped at room temperature.
• Lyophilized DNA should be stored at -20°C.
• Resuspended DNA should be stored at -20°C and is stable for 1 year.
• Store ZeocinÊ at 4 ÁC or at -20 ÁC. The expiry date is specified on the product label.

Quality control

• Plasmid construct has been confirmed by restriction analysis and sequencing.
• Plasmid DNA has been purified by ion exchange chromatography.

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 variation in selection markers for obtaining stable transfectants. pSELECT plasmids contain two expression cassettes: the first one drives the expression of the gene of interest, and the second one drives the expression of a large choice of dominant selectable markers for both *E. coli* and mammalian cells. Each cassette terminates with a strong polyadenylation signal (polyA) 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 selection marker.

pSELECT-hASC-GFP is a mammalian expression vector containing the human ASC gene fused at its 3' end to the green fluorescent protein (GFP) gene. This plasmid is selectable in bacteria and mammalian cells with Zeocin™. Expression of ASC::GFP in cells equipped with inflammasome components allows the visual monitoring of ASC specks, a hallmark of inflammasome activation. During inflammasome formation, ASC::GFP polymerizes to form a macromolecular complex that can be imaged using time-lapse confocal or high-resolution fluorescence microscopy. In most cells, only one speck forms upon inflammasome activation.

The same plasmid is available with the GFP gene alone as a control. This control plasmid is called pSELECT-CGFP-zeo (cat. code: psetz-cgfp).

PLASMID FEATURES

First expression cassette

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter 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. 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.
• **hASC::GFP** encodes a 48.9 kDa fusion protein in which GFP is fused via a six-amino-acid linker at the C terminus of the human ASC protein to avoid interfering with ASC functionality. The N-terminal pyrin domain of ASC is important for self-dimerization of the protein and for its recruitment by upstream molecules such as pyrin or cryopyrin. This fusion protein absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).
• **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
• **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.

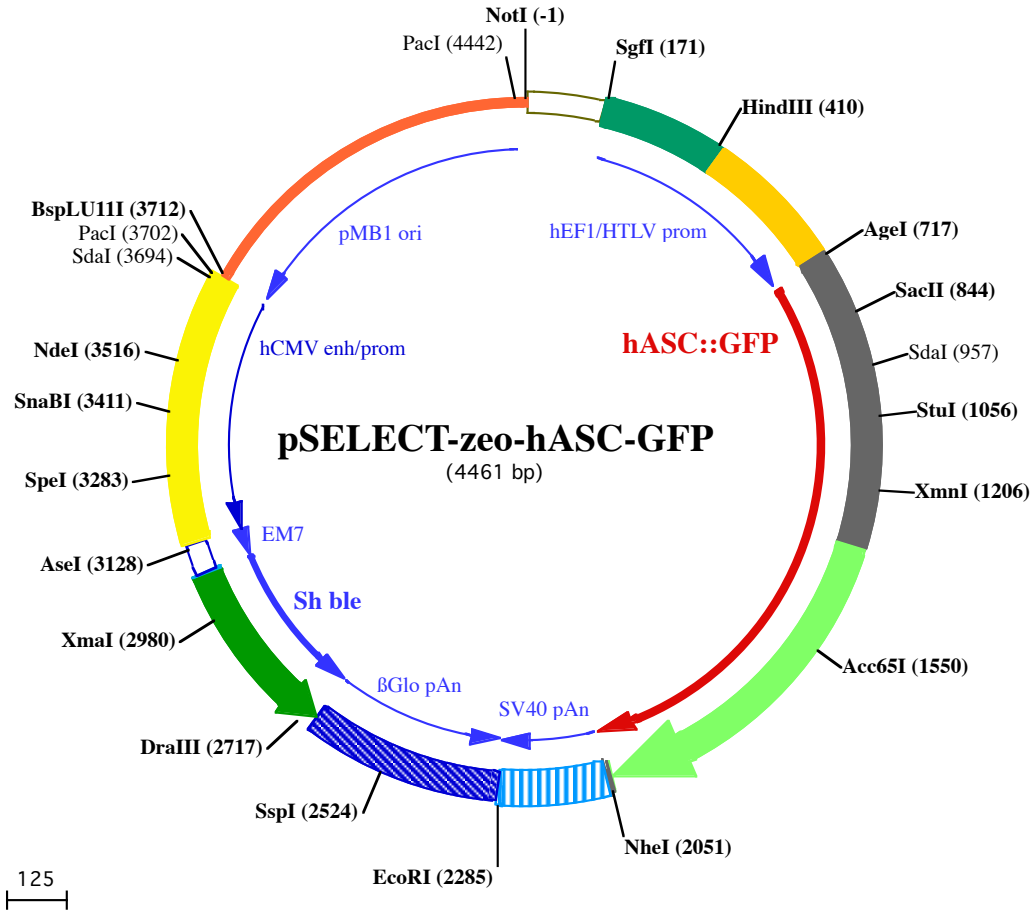
Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT116 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.

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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NheI (2051)
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NdeI (3516)
BspLU11I (3712)
PacI (3702)
SdaI (3694)
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hEF1/HTLV prom
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hASC::GFP
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Sh ble
ßGlo pAn
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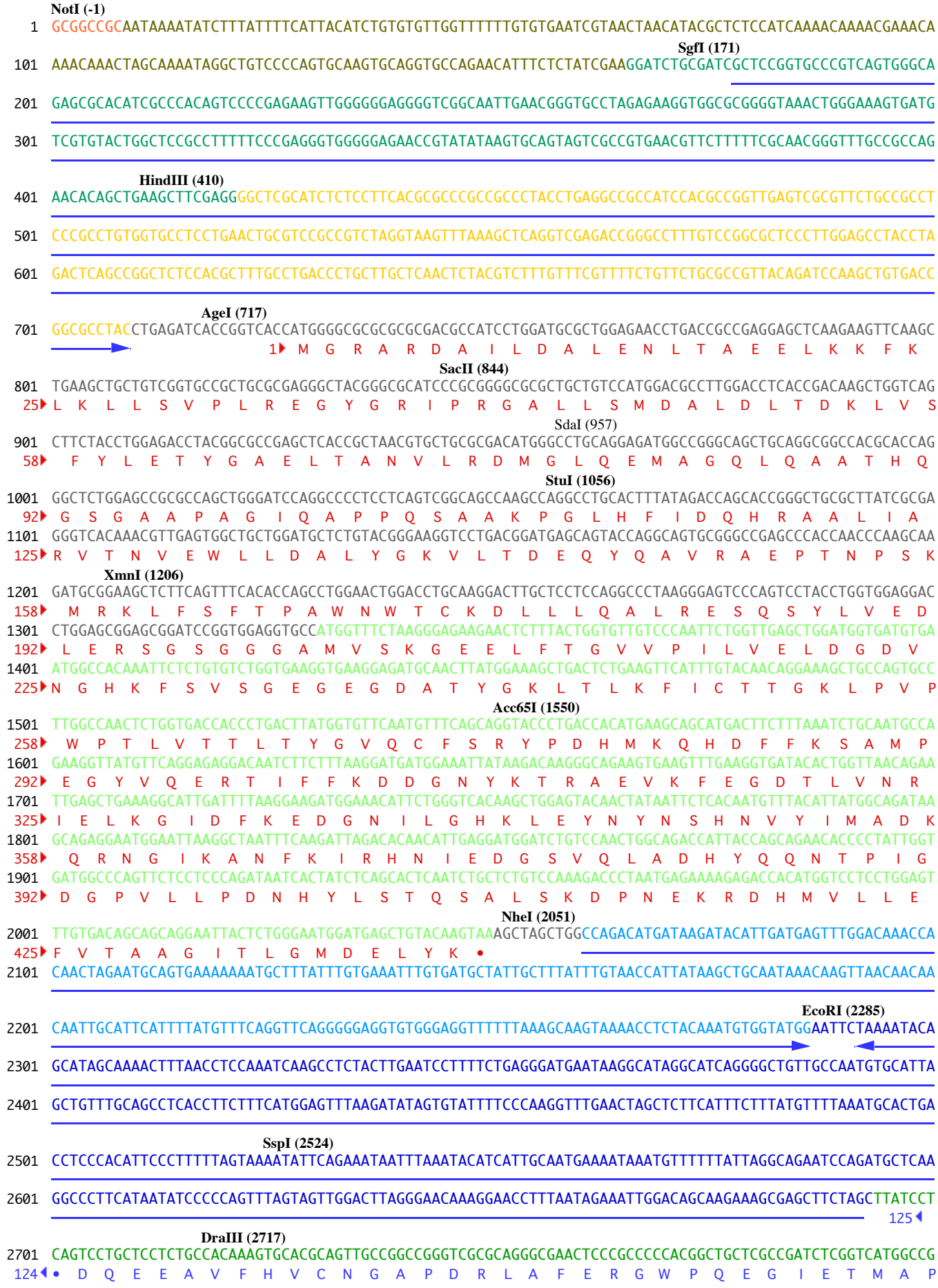
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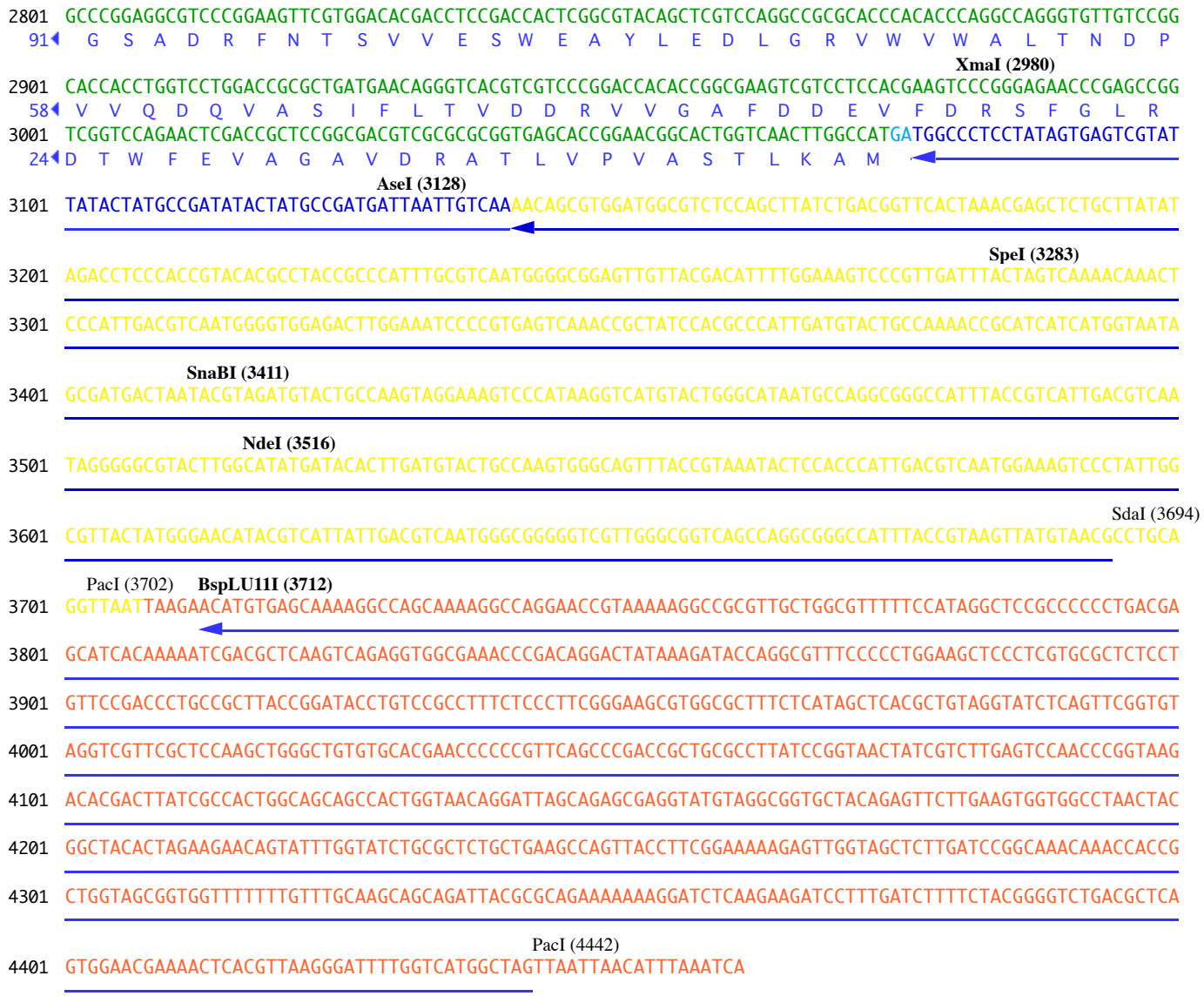
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NdeI (3516)
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