

pUNO3-⟨Gene⟩

Expression vector containing a fully sequenced open reading frame

Catalog code: puno3-⟨gene⟩

For research use only

Version 18J04-MM

PRODUCT INFORMATION

Contents:

- 20 µg of lyophilized plasmid DNA
- 1 ml 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 full-length ORF sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

GENERAL PRODUCT USE

- Obtaining a gene to subclone into another vector. The gene of interest is flanked by two unique restriction sites allowing its convenient excision. These restriction sites are compatible with other restriction sites contained in multiple cloning sites, thus facilitating subcloning.
- Stable gene expression in mammalian cells. pUNO3 plasmids can be used directly in transfection experiments both *in vitro* and *in vivo*. pUNO3 plasmids contain the hygromycin-resistance gene (*hph*) driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. This allows the amplification of the plasmid in *E. coli*, as well as the selection of stable clones in mammalian cells using the same selective antibiotic. pUNO3 allows high levels of expression and secretion (where applicable) of the gene product.

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

• **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter¹ and the 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α utilizes a type 2 promoter that encodes for a «house keeping» gene. It is expressed at high levels in all cell cycles and lower levels during G0 phase. The promoter is also non-tissue specific; it is highly expressed in all cell types. The R segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat² has been coupled to the EF-1α promoter to enhance stability of DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency possibly through mRNA stabilization.

• **ORF:** pUNO3 provides an intronless ORF from the ATG to the stop codon, fully-sequenced, and typically flanked by convenient cloning sites for easy subcloning.

Typically, the 5' end of the ORF contains a unique NcoI, BspHI, BspLU11I, or SphI site encompassing the ATG Start codon. When this 5' cloning site is not unique, another restriction (e.g. AgeI) is added a few bases upstream of the ATG.

The 3' end of the ORF contains a unique NheI site (or compatible site) after the Stop codon.

- AgeI is compatible with XmaI, BspEI, NgoMIV and SgrAI.
- NcoI is compatible with BspHI and BspLU11I.
- NheI is compatible with XbaI, SpeI, and AvrII.

• **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA³.

• **pMB1 ori** is a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

• **CMV promoter & enhancer** drives the expression of the hygromycin resistance in mammalian cells.

• **Hygro (hygromycin B resistance gene):** 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 promoter/enhancer in tandem with the bacterial EM7 promoter. Therefore, hygromycin B can be used to select stable mammalian cells transfectants and *E. coli* transformants.

• **Human beta-Globin polyA** is a strong polyadenylation (pAn) signal placed downstream of *hph*. The use of beta-globin pAn minimizes interference⁴ and possible recombination events with the SV40 polyadenylation signal.

1. Kim DW. *et al.*, 1990. Use of the human elongation factor 1α promoter as a versatile and efficient expression system. *Gene* 91(2):217-23. 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.* 8(1):466-72. 3. Carswell S. & Alwine JC., 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol Cell Biol.* 9(10):4248-58. 4. Yu J. & Russell JE., 2001. Structural and functional analysis of an mRNP complex that mediates the high stability of human β-globin mRNA. *Mol Cell Biol.* 21(17):5879-88.

TECHNICAL SUPPORT

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