pMONO-hygro-mcs

Single expression cassette plasmid for the expression of one gene of interest

Catalog code: pmonoh-mcs

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

For research use only

Version 19A03-MM

PRODUCT INFORMATION

Contents

- 20 µg of pMONO-hygro-mcs plasmid provided as lyophilized 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 sequencing.
- Plasmid DNA was purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE

pMONO 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 choice of selectable markers. pMONO plasmids contain a unique transcription unit that drives the expression of the gene of interest and the selectable marker through an internal ribosome entry site (IRES). This dual gene expression system ensures that stable clones express the gene of interest. Transcription of the expression cassette is efficiently terminated by the late SV40 polyadenylation signal (polyA).

Note: The use of the late SV40 polyA allows you to silence your gene of interest by using the ready-made psiRNA-SV40pA (#psirna3gz21-sv40pa), a plasmid expressing a short hairpin siRNA targeting the late SV40 polyA.

PLASMID FEATURES

- **SV40/FerH/mEF1α:** pMONO plasmids feature a composite ferritin promoter that confers strong and constitutive expression in a wide range of mammalian cells. The promoter is composed of the ferritin heavy chain (FerH) core promoter[1] fused at its 5' end to the SV40 enhancer, and at its 3' end to the intron-containing 5'UTR of the mouse elongation factor 1 alpha gene. This composite promoter yields similar levels of expression as the CMV promoter in all cell lines tested.
- **MCS:** The multiple cloning site contains the following restriction sites:

5' - Age I, EcoR V, BamH I, Mlu I, Cla I, Sal I, Avr II - 3'

Each restriction site is unique and compatible with many other enzymes, increasing the cloning options.

- **FMDV IRES:** The internal ribosome entry site of the Foot and Mouth Disease Virus enables the translation of two open reading frames from one mRNA with high levels of expression[2].
- **Hygro:** Resistance to Hygromycin B is conferred by the *hph* gene from *E. coli* which encodes a phosphotransferase. In mammalian cells, the *hph* gene is transcribed from the composite ferritin promoter as a polycistronic mRNA and translated through the FMDV IRES. In *E. coli*, *hph* is transcribed from the bacterial EM7 promoter.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **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.

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.

References

1. Eisenstein RS. & Munro HN. 1990. Translational regulation of ferritin synthesis by iron. Enzyme 44(1-4):42-58. **2. Ramesh N *et al.* 1996.** High-titer bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. Nucleic Acids Res. 24(14):2697-700. **3. Carswell S. & Alwine JC. 1989.** Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol. Cell Biol. 10: 4248-4258.

TECHNICAL SUPPORT
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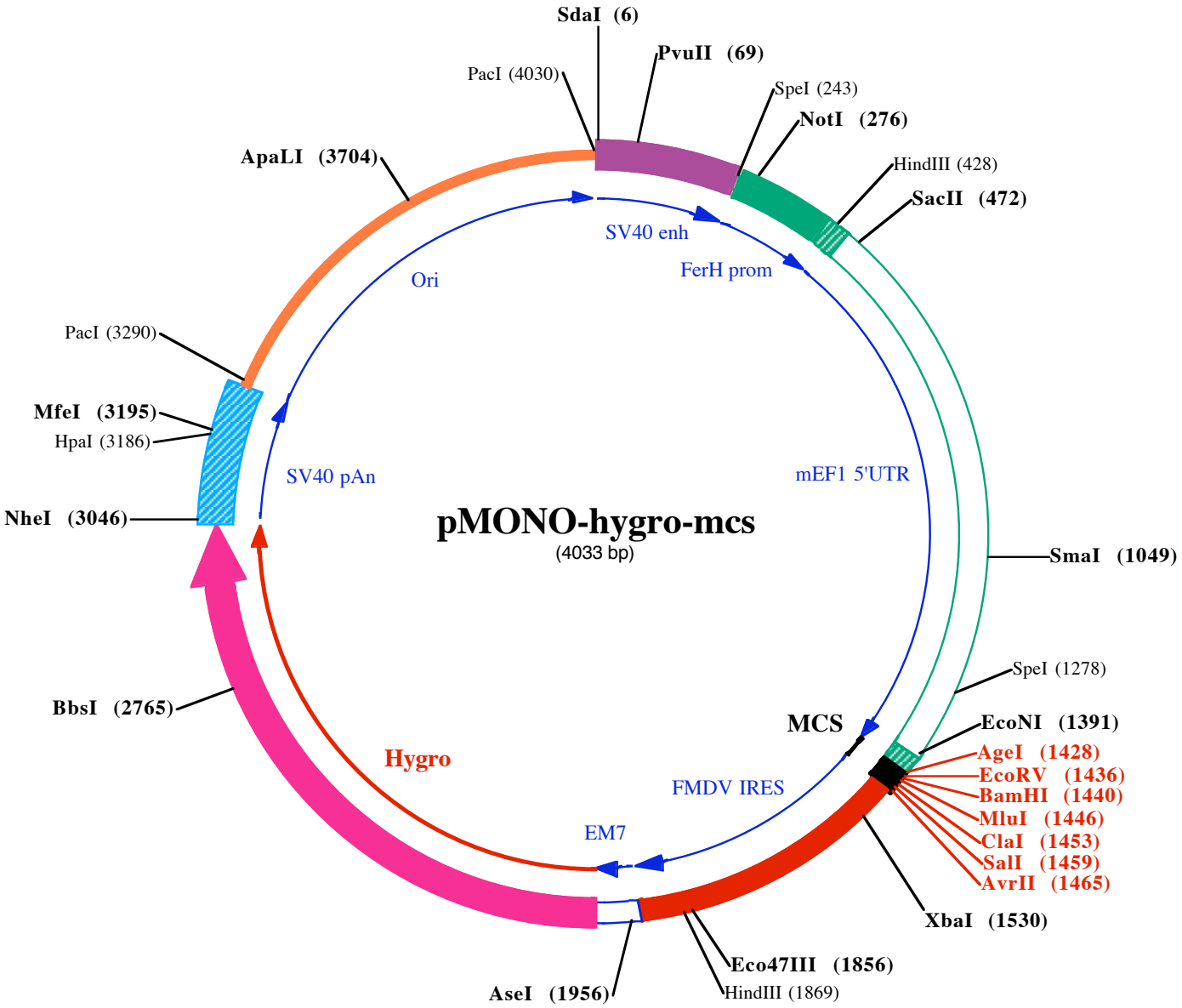
SdaI (6)
PvuII (69)
PacI (4030)
SpeI (243)
NotI (276)
ApaLI (3704)
HindIII (428)
SacII (472)
SV40 enh
FerH prom
Ori
PacI (3290)
MfeI (3195)
HpaI (3186)
SV40 pAn
mEF1 5'UTR
NheI (3046)
pMONO-hygro-mcs
(4033 bp)
SmaI (1049)
SpeI (1278)
BbsI (2765)
MCS
EcoNI (1391)
Hygro
AgeI (1428)
EcoRV (1436)
BamHI (1440)
FMDV IRES
MluI (1446)
ClaI (1453)
EM7
SalI (1459)
AvrII (1465)
XbaI (1530)
Eco47III (1856)
AseI (1956)
HindIII (1869)
100

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SdaI (6)
PvuII (69)
SpeI (243)
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EcoNI (1391)
EcoRV (1436) MluI (1446) SalI (1459)
AgeI (1428) BamHI (1440) ClaI (1453) AvrII (1465)
XbaI (1530)
Eco47III (1856) HindIII (1869)
AseI (1956)
BbsI (2765)
NheI (3046)
HpaI (3186) MfeI (3195)


PacI (3290)
3201 CATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAAATGTTAATTAACTAGCCA
3301 TGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAAT
3401 CTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGA
3501 GCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGT
3601 TACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGG
ApaLI (3704)
3701 TTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAG
3801 GCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTC
3901 GCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTT
PacI (4030)
4001 TTGCTGGCCTTTTGCTCACATGTTCTTAATTAA