# pMONO-neo-mcs

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

Catalog code: pmonon-mcs

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

For research use only

Version 19L13-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of pMONO-neo-mcs plasmid provided as lyophilized DNA

### 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 at least 1 year at -20°C.

### 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].
- **Neo:** The *neo* gene from Tn5 confers resistance to Kanamycin in *E. coli* and G418 in mammalian cells. In mammalian cells, the *neo* gene is transcribed from the composite ferritin promoter as a polycistronic mRNA and translated through the FMDV IRES. In *E. coli*, *neo* 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.

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

## 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 other commonly used laboratory *E. coli* strains, such as DH5α.

### Bacterial antibiotic selection

Kanamycin (not provided) is normally used for *E. coli* at a final concentration of 50 µg/ml in liquid or solid media.

### Mammalian antibiotic selection

G418 is normally used at a concentration of 400 µg/ml. However, the optimal concentration needs to be determined for your cells.

## RELATED PRODUCTS

| Product | Description | Cat. Code |
|---|---|---|
| ChemiComp GT116 cells | Competent *E. coli* cells | gt116-11 |
| G418 | Selection antibiotic | ant-gn-1 |

TECHNICAL SUPPORT
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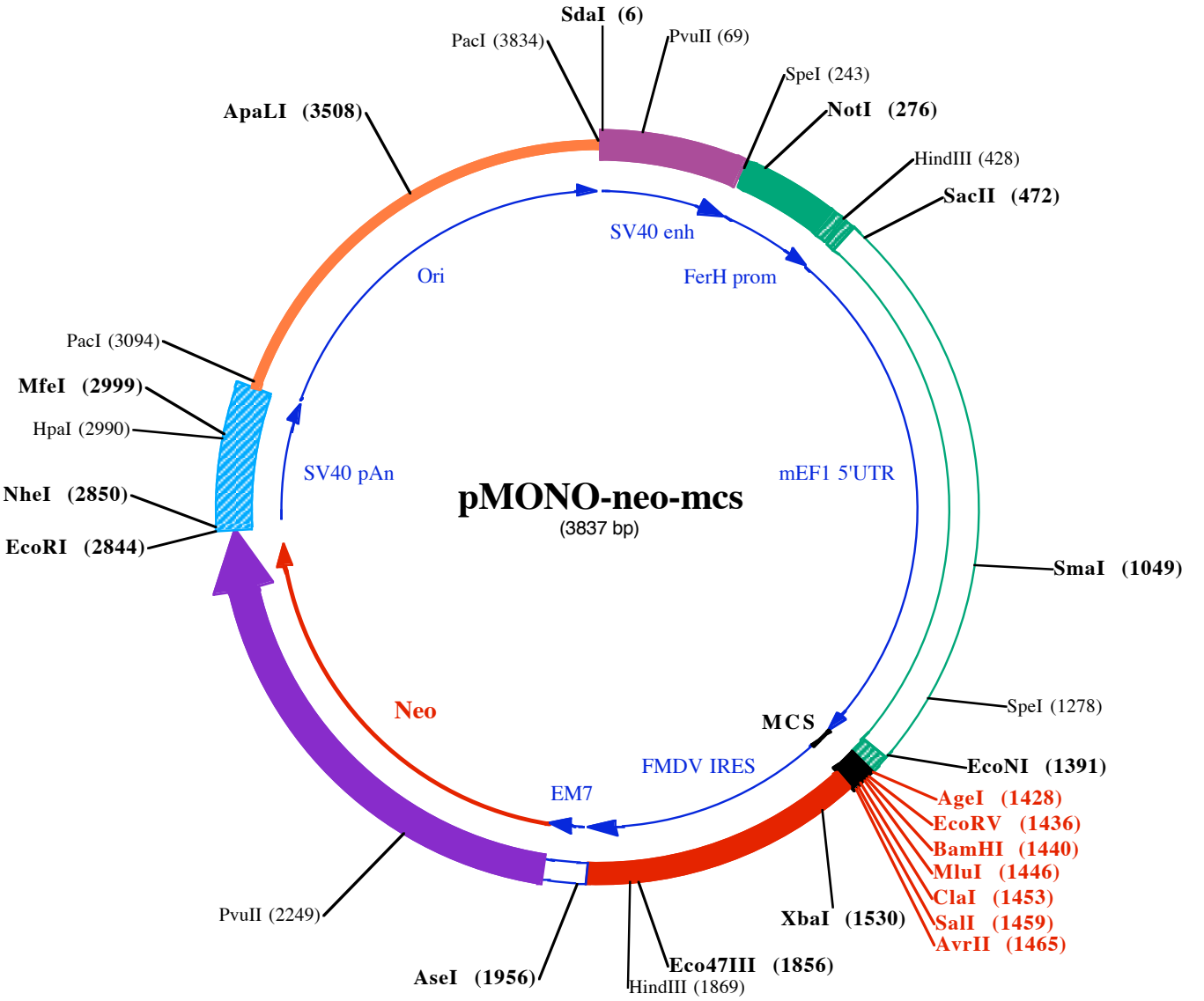
SdaI (6)
PacI (3834)
PvuII (69)
SpeI (243)
NotI (276)
ApaLI (3508)
HindIII (428)
SacII (472)
SV40 enh
Ori
FerH prom
PacI (3094)
MfeI (2999)
HpaI (2990)
SV40 pAn
mEF1 5'UTR
NheI (2850)
pMONO-neo-mcs
(3837 bp)
EcoRI (2844)
SmaI (1049)
Neo
SpeI (1278)
MCS
EcoNI (1391)
FMDV IRES
AgeI (1428)
EM7
EcoRV (1436)
BamHI (1440)
MluI (1446)
ClaI (1453)
PvuII (2249)
XbaI (1530)
SalI (1459)
AvrII (1465)
Eco47III (1856)
AseI (1956)
HindIII (1869)
100

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AseI (1956)
PvuII (2249)
NheI (2850)
EcoRI (2844)
HpaI (2990) MfeI (2999)
PacI (3094)


3101 GCCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCG
3201 TAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAG
3301 CAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATC
3401 CTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGG
ApaLI (3508)
3501 GGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAG
3601 AAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGG
3701 TTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGG
PacI (3834)
3801 CCTTTTGCTGGCCTTTTGCTCACATGTTCTTAATTAA