

pMONO-hygro-GFP

A GFP-expression plasmid selectable with Hygromycin

Catalog code: pmonoh-gfp

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

For research use only

Version 19A03-MM

PRODUCT INFORMATION

Contents

- 20 µg of pMONO-hygro-gfp 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.

pMONO-GFP plasmids feature a new allele of the GFP gene called LGFP. They can be used as control vectors or for cloning of an open reading frame, as the LGFP gene is flanked by two unique restriction sites: Bsp HI at the 5' end that encompasses the Start codon, and Avr II at the 3' end.

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 promoter¹ 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.
- **LGFP:** This red-shifted variant of the jellyfish GFP gene encodes a green fluorescent protein that absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).
- **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 expression².

- **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 mRNA³.
- **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

InvivoGen USA (Toll-Free): 888-457-5873

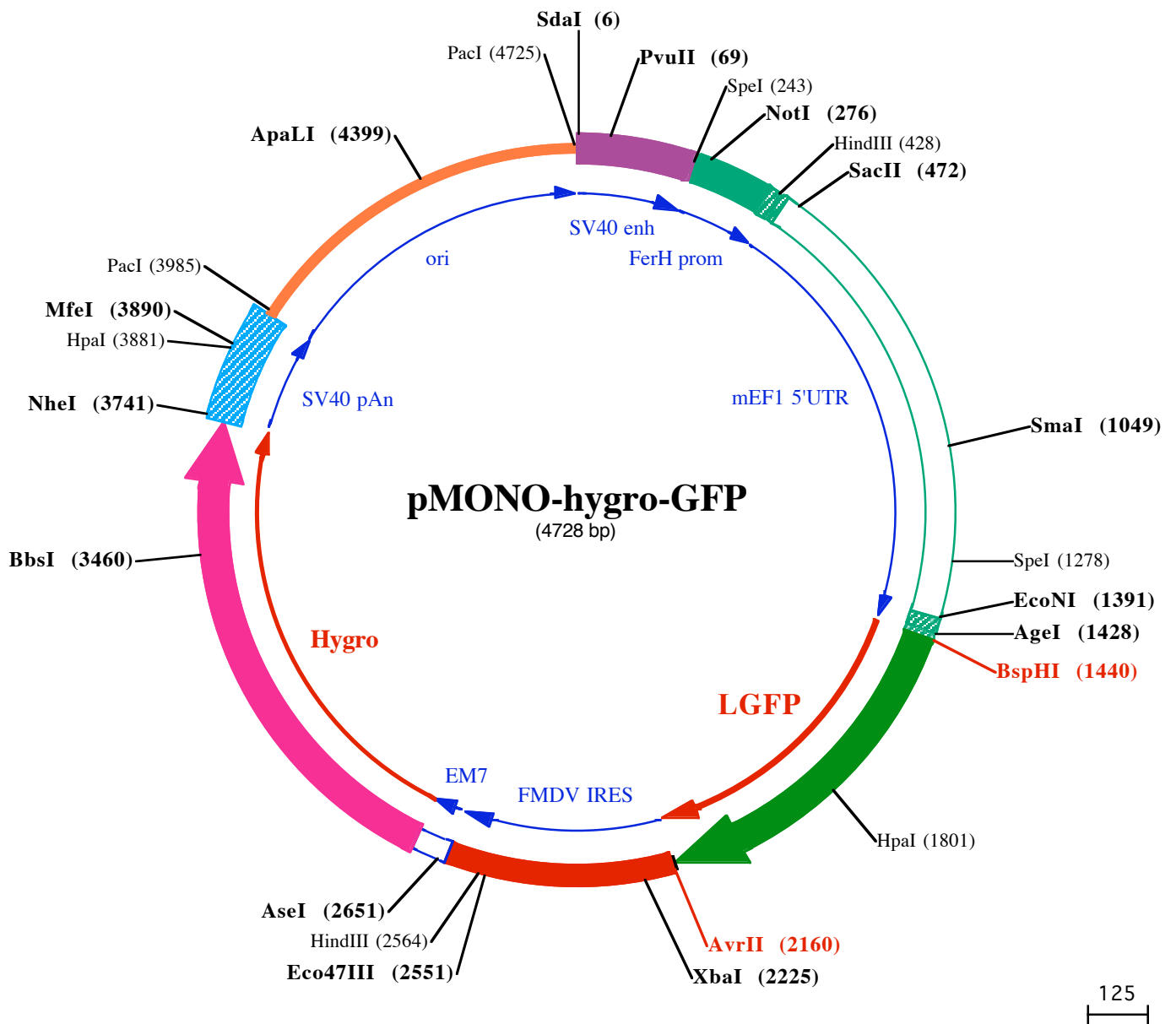
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SdaI (6) **PvuII (69)**

1 CCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACCTGGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGCAGTTAGGGTGTGGAA

101 AGTCCCAGGCTCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGAAAGTCCCAGGCTCCCAGCAGGCAGAAG

SpeI (243) **NotI (276)**

201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCACTAGTTCGCCAGAGCGCGGAGGGCCTCCAGCGGCCGCCCTCCCCACAGCAGGG

301 GCGGGTCCCAGCCACCGAAGGAGCGGGCTCGGGCGGGCGGCTGATTGGCCGGGGCGGCTGACGCCGACGGCTATAAGAGACCACAAGCG

HindIII (428) **SacII (472)**

401 ACCCGCAGGGCCAGACGTTCTTCGCCGAAGCTTGCCTCAGAACGCAGGTGAGGGCGGGGTGTGGCTTCGCGGGCCGCCAGCTGGAGTCTGCTCCG

501 AGCGGGCGGGCCCGCTGTCTGTCGGCGGGATTAGCTGCGAGCATTCCCGCTTCGAGTTGCGGGCGGCGGGAGGCAGAGTGCAGGCTAGCGGCAA

601 CCCCGTAGCCTCGCTCGTGTCCGGCTTGTAGCCCTAGCGTGTGTCCGCGCCGCCCGCGTGTACTCCGGCCGACTCTGGTCTTTTTTTTTTTTGT

701 GTTGTTCCTGCTGCTTCGATTGCCGTTACGAAATAGGGGCTAACAAAGGGAGGGTGGGGCTTGTCTGCCCGAGCCGGAGAGGTCATGGTTGGG

801 GAGGAATGGAGGACAGGAGTGGCGCTGGGGCCCGCCGCTTCGAGACATGTCCGACGCCACCTGGATGGGCGAGGCTGGGGTTTTTCCGAAG

901 CAACCAGGCTGGGGTTAGCTGCCGAGGCCATGTGGCCCGACCCGCGACGATCTGGCTTGGCGGCGCGCTTGCCTGCCTCCTAACTAGGGTGA

SmaI (1049)

1001 GGCCATCCGTCGGCACCAGTTGCGTGCCTGAAAGATGGCCGCTCCGGGCCCTGTTGCAAGGAGCTCAAATGGAGGACGGCGAGCCGGTGGAGC

1101 GGGCGGTGAGTCACCCACAAAAGGAGGGCCTGGTCCCTACCGGCTGCTGCTTCTGTGACCCCGTGGTCTATCGGCCGAATAGTCACCTCGG

SpeI (1278)

1201 GCTTTTGAGCACGGCTAGTCGCGGGGGGGAGGGGATGTAATGGCGTTGGAGTTTGTTCACATTTGGTGGGTGGAGACTAGTCAGGCCAGCCTGGCGCT

EcoNI (1391)

1301 GGAAGTCATTTTTGAATTTGCTCCCTTGAGTTTTGAGCGGAGCTAATTCTGGGCTTCTTAGCGGTTCAAAGGTATCTTTAAACCCTTTTTAGGTGT

AgeI (1428) **BspHI (1440)**

1401 TGTGAAACCACCGTAATTCAAAGCAACCGTGCAGCTCAGCAAGGAGGAGAAGAACTTTACTGGTGTGTCCAACTTCTGGTTGAGCTGGATGG

1501 TGATGTGAATGGCCACAAATCTCTGTGTGTTGAAAGTGAAGGAGATGCAACTTATGAAAGCTGACTCTGAAGTTCATTTGTACAACAGGAAAGCTG

1601 CCAGTGCCTTGGCCAACCTCTGGTGACCACCTGACTTATGGTGTCAATGTTTCAGCAGGTACCCTGACCACATGAAGCAGCATGACTCTTTAAATCTG

1701 CAATGCCAGAAGTTATGTTCCAGGAGGACAATCTTCTTTAAGGATGATGAAATTATAAGACAAGGGCAGAAGTGAAGTTTGAAGGTGATACACTGGT

1801 TAACAGAATTGAGCTGAAAGCATTGATTTAAGGAAGATGAAACATTCTGGTCCACAGCTGGAGTACAACATAATTCTCACAATGTTTACATTATG

1901 GCAGATAAGCAGAAGAATGGAATTAAGTTAATTTCAAGATTAGACACAACATTTGAGGATGGATCTGTCCAACCTGGCAGACCATACCAGCAGAACCCC

2001 CTATTGGTATGGCCAGTTCTCTCCAGATAATCACTATCTCCGACTCAATCTGCTCTGTCCAAAGACCCTAATGAGAAAAGAGACCACATGGTCTCT

2101 CCTGGAGTTGTGACAGCAGCAGGAATTACTCTGGGAATGGATGAGCTGTACAAGTAAACCTAGGAGCAGGTTTCCCAATGACACAAAACGTGCAACT

2201 GAAACTCCGCTGGTCTTTCCAGGTCTAGAGGGGTAACACTTTGACTGCGTTTGGTCCACGCTCGATCCACTGGCGAGTGTTAGTAACAGCACTGTTG

XbaI (2225)

2301 CTTCTGAGCGGACATGACGGCCGTGGAACTCCTCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCACAGGGCCCGTCATGTGTCAACCC

2401 CAGCACGGCGACTTACTGCGAAACCCACTTTAAAGTGACATTGAAACTGGTACCACACACTGGTACAGGCTAAGGATGCCCTCAGGTACCCGAGG

Eco47III (2551) HindIII (2564)

2501 TAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCTGAATAGGTGACCGGAGGTGCGCA

AseI (2651)

2601 CCTTTCCTTTGCAATTACTGACCTATGAATACAACCTGACTGTTTGAACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGG

2701 AGGGCCACCTGAAGAAACCTGAACTGACAGCAACTTCTGTTGAGAAGTTTCTCATTGAAAAATTTGATTCTGTTTCTGATCTCATGCAGCTGTCTGAAG

2801 GTGAAGAAAGCAGAGCCTTTTCTTTGATGTTGGAGGAAGAGTTATGTTCTGAGGGTCAATTCCTGTGCTGATGGTTTTTACAAGACAGATATGTTTA

2901 CAGACACTTTGCTCTGCTGCTGCTGCAATCCAGAAGTTCTGGACATTTGAGAATTTCTGAATCTCTCACTACTGCATCAGCAGAAGAGCACAAGGA

3001 GTCACCTCCAGGATCTCCCTGAACTGAGCTGCCAGCTGTTCTGCAACCTGTTGCTGAAGCAATGGATGCCATTGCGAGCAGCTGATCTGAGCCAACT

3101 CTGGATTTGGTCTTTTGGTCCCAAGGCATTGGTCACTACCACTTTGGAGGATTTCAATTTGTCCATTGCTGATCTCTATCTATCTGTCAGCAG

3201 TGTGATGGATGACACAGTTTCTGCTTCTGTTGCTCAGGCACTGGATGAACCTCATGCTGTGGCAGAAGATTGCTCTGAAGTCAGACACTGGTCCATGCT

164 r ValMetAspAspThr Val SerAl aSer ValAl aGl nAl aLeuAspGl uLeuMetLeuTrpAl aGl uAspCysP roGl uVal l ArgHi sLeuVal l Hi sAl a

3301 GATTTTGAAGCAACAATGTTCTGACAGACAATGGCAGAATCACTGCAGTCAATTGACTGGTCTGAAGCCATGTTTGGAGATTCTCAATATGAGGTTGCCA
198▶ AspPheGlySerAsnAsnValLeuThrAspAsnGlyArgIleThrAlaValIleAspTrpSerGluAlaMetPheGlyAspSerGlnTyrGluValAla

BbsI (3460)

3401 ACATTTTTTTTTGGAGACCTGGCTGGCTTGCATGGAACAACAACAAGATATTTTGAAGAAGACACCCAGAAGCTGGCTGGTCCCCAGACTGAGAGC
231▶ snllePhePheTrpArgProTrpLeuAlaCysMetGluGlnGlnThrArgTyrPheGluArgArgHisProGluLeuAlaGlySerProArgLeuArgAla

3501 CTACATGCTCAGAATTGGCCTGGACCAACTGTATCAATCTCTGGTTGATGGAACTTTGATGATGCTGCTGGGCACAAGGAAGATGTGATGCCATTGTG
264▶ aTyrMetLeuArgIleGlyLeuAspGlnLeuTyrGlnSerLeuValAspGlyAsnPheAspAspAlaAlaTrpAlaGlnGlyArgCysAspAlaIleVal

3601 AGGTCTGGTCTGGAAGCTGTTGGAAGAACTCAAATTGCAAGAAGTCTGCTGCTGTTGGACTGATGGATGTGTTGAAGTCTGGCTGACTCTGGAAACA
298▶ ArgSerGlyAlaGlyThrValGlyArgThrGlnIleAlaArgArgSerAlaAlaValTrpThrAspGlyCysValGluValLeuAlaAspSerGlyAsnAla

NheI (3741)

3701 GGAGACCTCCACAAGACCCAGAGCCAAAGGAATGAATCTGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACACTAGAATG
331▶ r gArgProSerThrArgProArgAlaLysGlu•••

HpaI (3881) MfeI (3890)

3801 CAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAACAAACAACAAATTGCATTC

PaeI (3985)

3901 ATTTTATGTTTCAGGTTCAAGGGGAGGTGTGGGAGGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAAATGTTAATTAAGTCCATGACC
▶

4001 AAAATCCCTTAACGTGAGTTTTCTGTTCCACTGAGCGTACAGCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCT

4101 GCTTGCAAACAAAAAACACCCTACCAGCGGTGTTTGTGGCCGATCAAGAGCTACCAACTCTTTTCCGAAGGTAAGTGGCTTCAGCAGAGCGCA

4201 GATACCAAACTACTGTTCTTAGTGTAGCCGTAGTTAGGCCACCACCTCAAGAAGTCTGTAGCACCCTACATACCTCGCTCTGCTAATCCTGTTACCA

ApaI (4399)

4301 GTGGCTGCTGCCAGTGGCGATAAGTCGTGCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGCTGAACGGGGGTTCTGT

4401 GCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCGAAGGGAGAAAGCGGGA

4501 CAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCAGGGAGCTTCCAGGGGAAACGCTGGTATCTTTATAGTCTGTGCGGTTTCGCCAC

4601 CTCTGACTTGAGCGTCGATTTTTGTGATGCTGTCAGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTACGGTTCCTGGCCTTTTGCT

PaeI (4725)

4701 GGCCTTTGCTCACATGTTCTTAATTAA
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