pMONO-blasti-GFP
A GFP-expression plasmid selectable with Blasticidin
Catalog code: pmonob-gfp
https://www.invivogen.com/pmono-blasti

For research use only
Version 20F16-MM

PRODUCT INFORMATION
Contents
- 20 μg of pMONO-blasti-gfp plasmid provided as lyophilized DNA
- 2 x 1 ml blasticidin at 10 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 blasticidin 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 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.
- **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 expression[2].
- **Blasti:** Resistance to Blasticidin is conferred by the *bsr* gene from *Bacillus cereus*. The *bsr* gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.
- **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.

Blasticidin usage
Blasticidin should be used at 25-100 μg/ml in bacteria and 1-30 μg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

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.

RELATED PRODUCTS

Product	Description	Cat. Code
ChemiComp GT116 cells	Competent *E. coli* cells	gt116-11
Blasticidin	Selection antibiotic	ant-bl-05

InvivoGen
www.invivogen.com

SdaI (6)
PvuII (69)
PacI (4127)
SpeI (243)
NotI (276)
ApaLI (3801)
HindIII (428)
SacII (472)
PacI (3387)
MfeI (3292)
HpaI (3283)
NheI (3143)
EcoRI (3137)
SmaI (1049)
Blasti
SpeI (1278)
EcoNI (1391)
AgeI (1428)
BspHI (1440)
XmnI (2711)
AseI (2651)
HindIII (2564)
Eco47III (2551)
HpaI (1801)
XbaI (2225)
AvrII (2160)

SV40 enh
ori
FerH prom
SV40 pAn
mEF1 5'UTR
pMONO-blasti-GFP
(4130 bp)
EM7
FMDV IRES
LGFP

110

InvivoGen
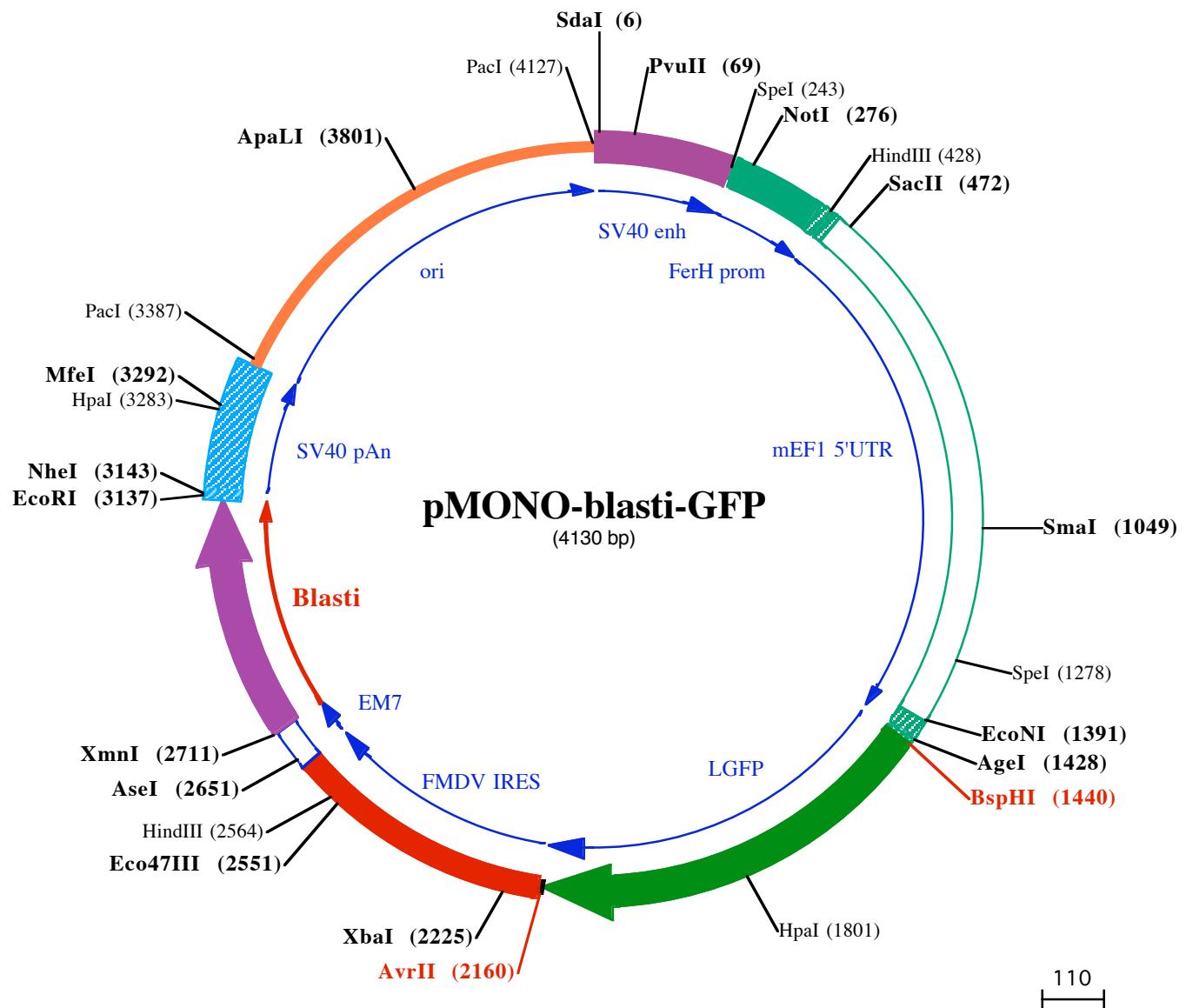
SdaI (6)   PvuII (69)
   1 CCTGCAGGGCCTGAAATAACCTCTGAAAGAGGAACTTGGTTAGGTACCTTCTGAGGCTGAAAGAACCAGCTGTGGAATGTGTGTCAGTTAGGGTGTGGAA

 101 AGTCCCCAGGCTCCCCAGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGGCAGAAG

                                                SpeI (243)   NotI (276)
 201 TATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCACTAGTTCCGCCAGAGCGCGCGAGGGCCTCCAGCGGCCGCCCCTCCCCCACAGCAGGG

 301 GCGGGGTCCCGCGCCCACCGGAAGGAGCGGGCTCGGGGCGGGCGGCGCTGATTGGCCGGGGCGGGCCTGACGCCGACGCGGCTATAAGAGACCACAAGCG

                         HindIII (428)   SacII (472)
 401 ACCCGCAGGGCCAGACGTTCTTCGCCGAAGCTTGCCGTCAGAACGCAGGTGAGGGGCGGGTGTGGCTTCCGCGGGCCGCCGAGCTGGAGGTCCTGCTCCG

 501 AGCGGGCCGGGCCCCGCTGTCGTCGGCGGGGATTAGCTGCGAGCATTCCCGCTTCGAGTTGCGGGCGGCGCGGGAGGCAGAGTGCGAGGCCTAGCGGCAA

 601 CCCCGTAGCCTCGCCTCGTGTCCGGCTTGAGGCCTAGCGTGGTGTCCGCGCCGCCGCCGCGTGCTACTCCGGCCGCACTCTGGTCTTTTTTTTTTTTGTT

 701 GTTGTTGCCCTGCTGCCTTCGATTGCCGTTCAGCAATAGGGGCTAACAAAGGGAGGGTGCGGGGCTTGCTCGCCCGGAGCCCGGAGAGGTCATGGTTGGG

 801 GAGGAATGGAGGGACAGGAGTGGCGGCTGGGGCCCGCCCGCCTTCGGAGCACATGTCCGACGCCACCTGGATGGGGCGAGGCCTGGGGTTTTTCCCGAAG

 901 CAACCAGGCTGGGGTTAGCGTGCCGAGGCCATGTGGCCCCAGCACCCGGCACGATCTGGCTTGGCGGCGCCGCGTTGCCCTGCCTCCCTAACTAGGGTGA

                                            SmaI (1049)
1001 GGCCATCCCGTCCGGCACCAGTTGCGTGCGTGGAAAGATGGCCGCTCCCGGGCCCTGTTGCAAGGAGCTCAAAATGGAGGACGCGGCAGCCCGGTGGAGC

1101 GGGCGGGTGAGTCACCCACACAAAGGAAGAGGGCCTGGTCCCTCACCGGCTGCTGCTTCCTGTGACCCCGTGGTCCTATCGGCCGCAATAGTCACCTCGG

                                                                         SpeI (1278)
1201 GCTTTTGAGCACGGCTAGTCGCGGCGGGGGGGAGGGGATGTAATGGCGTTGGAGTTTGTTCACATTTGGTGGGTGGAGACTAGTCAGGCCAGCCTGGCGCT

                                                                      EcoNI (1391)
1301 GGAAGTCATTTTTGGAATTTGTCCCCTTGAGTTTTGAGCGGAGCTAATTCTCGGGCTTCTTAGCGGTTCAAAGGTATCTTTTAAACCCTTTTTTAGGTGT

            AgeI (1428)   BspHI (1440)
1401 TGTGAAAACCACCGCTAATTCAAAGCAACCGGTCGCACGTCATGAGCAAGGGAGAAGAACTCTTTACTGGTGTTGTCCCAATTCTGGTTGAGCTGGATGG
                                              1▶MetSerLysGlyGluGluLeuPheThrGlyValValProIleLeuValGluLeuAspGl

1501 TGATGTGAATGGCCACAAATTCTCTGTGTCTGGTGAAGGTGAAGGAGATGCAACTTATGGAAAGCTGACTCTGAAGTTCATTTGTACAACAGGAAAGCTG
     20▶yAspValAsnGlyHisLysPheSerValSerGlyGluGlyGluGlyAspAlaThrTyrGlyLysLeuThrLeuLysPheIleCysThrThrGlyLysLeu

1601 CCAGTGCCTTGGCCAACTCTGGTGACCACCCTGACTTATGGTGTTCAATGTTTCAGCAGGTACCCTGACCACATGAAGCAGCATGACTTCTTTAAATCTG
     54▶ProValProTrpProThrLeuValThrThrLeuThrTyrGlyValGlnCysPheSerArgTyrProAspHisMetLysGlnHisAspPhePheLysSerA

                                                                                              HpaI (1801)
1701 CAATGCCAGAAGGTTATGTTCAGGAGAGGACAATCTTCTTTAAGGATGATGGAAATTATAAGACAAGGGCAGAAGTGAAGTTTGAAGGTGATACACTGGT
     87▶laMetProGluGlyTyrValGlnGluArgThrIlePhePheLysAspAspGlyAsnTyrLysThrArgAlaGluValLysPheGluGlyAspThrLeuVa

1801 TAACAGAATTGAGCTGAAAGGCATTGATTTTAAGGAAGATGGAAACATTCTGGGTCACAAGCTGGAGTACAACTATAATTCTCACAATGTTTACATTATG
     120▶lAsnArgIleGluLeuLysGlyIleAspPheLysGluAspGlyAsnIleLeuGlyHisLysLeuGluTyrAsnTyrAsnSerHisAsnValTyrIleMet

1901 GCAGATAAGCAGAAGAATGGAATTAAGGTTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAACTGGCAGACCATTACCAGCAGAACACCC
     154▶AlaAspLysGlnLysAsnGlyIleLysValAsnPheLysIleArgHisAsnIleGluAspGlySerValGlnLeuAlaAspHisTyrGlnGlnAsnThrP

2001 CTATTGGTGATGGCCCAGTTCTCCTCCCAGATAATCACTATCTCCGCACTCAATCTGCTCTGTCCAAAGACCCTAATGAGAAAAGAGACCACATGGTCCT
     187▶roIleGlyAspGlyProValLeuLeuProAspAsnHisTyrLeuArgThrGlnSerAlaLeuSerLysAspProAsnGluLysArgAspHisMetValLe

                                                               AvrII (2160)
2101 CCTGGAGTTTGTGACAGCAGCAGGAATTACTCTGGGAATGGATGAGCTGTACAAGTAAACCTAGGAGCAGGTTTCCCCAATGACACAAAACGTGCAACTT
     220▶uLeuGluPheValThrAlaAlaGlyIleThrLeuGlyMetAspGluLeuTyrLys•••

                            XbaI (2225)
2201 GAAACTCCGCCTGGTCTTTCCAGGTCTAGAGGGGTAACACTTTGTACTGCGTTTGGCTCCACGCTCGATCCACTGGCGAGTGTTAGTAACAGCACTGTTG

2301 CTTCGTAGCGGAGCATGACGGCCGTGGGAACTCCTCCTTGGTAACAAGGACCCACGGGGCCAAAAGCCACGCCCACACGGGCCCGTCATGTGTGCAACCC

2401 CAGCACGGCGACTTTACTGCGAAACCCACTTTAAAGTGACATTGAAACTGGTACCCACACACTGGTGACAGGCTAAGGATGCCCTTCAGGTACCCCGAGG

                  Eco47III (2551)   HindIII (2564)
2501 TAACACGCGACACTCGGGATCTGAGAAGGGGACTGGGGCTTCTATAAAAGCGCTCGGTTTAAAAAGCTTCTATGCCTGAATAGGTGACCGGAGGTCGGCA

                            AseI (2651)
2601 CCTTTCCTTTGCAATTACTGACCCTATGAATACAACTGACTGTTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACTCACTATAGG

            XmnI (2711)
2701 AGGGCCACCATGAAGACCTTCAACATCTCTCAGCAGGATCTGGAGCTGGTGGAGGTCGCCACTGAGAAGATCACCATGCTCTATGAGGACAACAAGCACC
                      1▶MetLysThrPheAsnIleSerGlnGlnAspLeuGluLeuValGluValAlaThrGluLysIleThrMetLeuTyrGluAspAsnLysHisH

2801 ATGTCGGGGCGGCCATCAGGACCAAGACTGGGGAGATCATCTCTGCTGTCCACATTGAGGCCTACATTGGCAGGGTCACTGTCTGTGCTGAAGCCATTGC
     31▶isValGlyAlaAlaIleArgThrLysThrGlyGluIleIleSerAlaValHisIleGluAlaTyrIleGlyArgValThrValCysAlaGluAlaIleAl

2901 CATTGGGTCTGCTGTGAGCAACGGGCAGAAGGACTTTGACACCATTGTGGCTGTCAGGCACCCCTACTCTGATGAGGTGGACAGATCCATCAGGGTGGTC
     64▶aIleGlySerAlaValSerAsnGlyGlnLysAspPheAspThrIleValAlaValArgHisProTyrSerAspGluValAspArgSerIleArgValVal

3001 AGCCCCTGTGGCATGTGCAGAGAGCTCATCTCTGACTATGCTCCTGACTGCTTTGTGCTCATTGAGATGAATGGCAAGCTGGTCAAAACCACCATTGAGG
     98▶SerProCysGlyMetCysArgGluLeuIleSerAspTyrAlaProAspCysPheValLeuIleGluMetAsnGlyLysLeuValLysThrThrIleGluG

NheI (3143)

EcoRI (3137)

3101 AACTCATCCCCCTCAAGTACACCAGGAACTAAACCTGAATTCGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAA

131▶ luLeuIleProLeuLysTyrThrArgAsn•••

HpaI (3283)   **MfeI (3292)**

3201 TGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCAT

PacI (3387)

3301 TCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAAATGTTAATTAACTAGCCATGA

3401 CCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTG

3501 CTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCG

3601 CAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTAC

3701 CAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTC

ApaLI (3801)

3801 GTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCG

3901 GACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCC

4001 ACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTG

PacI (4127)

4101 CTGGCCTTTTGCTCACATGTTCTTAATTAA