pBROAD3-mcs

An optimized vector for mouse and rat transgenesis

Catalog # pbroad3

For research use only

Version # 05A12-SV

PRODUCT INFORMATION

Content:

- 20 µg of pBROAD3-mcs provided as lyophilized DNA

Storage and Stability:

- Products are shipped at room temperature.
- Lyophilized DNA is stable for 1 year when stored at -20˚C
- Resuspended DNA is stable for 6 months at -20˚C.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Plasmid DNA were purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE

The pBROAD3-mcs plasmid was designed for expression of a transgene in virtually all tissues of transgenic mice and rats. This feature is brought by the ROSA promoter. The murine ROSA26 promoter was initially identified by random retroviral gene trapping in mouse embryonic stem cells[1]. This high CpG content promoter was shown to drive ubiquitous expression of the human placental alkaline phosphatase and enhanced green fluorescent protein during embryonic and postnatal development in mouse and rat[2].

A multiple cloning site (MCS) has been added downstream of the ROSA promoter for convenient cloning of your gene of interest. The MCS contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning. Furthermore, the *E. coli* region is flanked on either side by the well cutting 8 bp-recognizing restriction enzyme *Pa*c I that enables linearization and easy excision of the *E. coli* region.

PLASMID FEATURES

• **mROSA prom:** This TATA-less promoter was found to be very effective *in vitro* in a very broad range of mammalian cell lines. The strength of the murine ROSA promoter is ascribed to the 10 potential Sp1 sites found within the CpG island extending from the core promoter to the first half of 5'untranslated region (5'UTR), the highest number of Sp1 sites ever recorded in any natural promoter. The 5'UTR contains an engineered intron of 350 bp which increases the transcription of the transgene[3].

• **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription[4].

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

• **Amp:** The ampicillin resistance gene allows the selection of transformed *E. coli* carrying a pBROAD plasmid.

• **MCS:** The multiple cloning site contains the following restriction sites: *Age* I, *Bsp* LU11I, *Bgl* II, *Hind* III, *Eco* RI, *and Msc* I

Age I is compatible with *Bsp* EI and *Sgr* AI.
Bsp LU11I is compatible with *Bsp* HI and *Nco* I.
Bgl II is compatible with *Bam* HI, *Bst* YI and *Bcl* I.
Eco RI is compatible with *Apo* I and *Mfe* I

EXPERIMENTAL OUTLINE

Clone your transgene into pBROAD mcs
∇
Select and isolate recombinant pBROAD
∇
Linearize recombinant pBROAD with Pac I
∇
Purify *Pac* I/*Pac* I fragment containing your transgene
∇
Prepare DNA for microinjection
∇
Generate transgenic lines

METHODS

Plasmid resuspension:

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1µg/µl, resuspend the DNA in 20µl of sterile H_2O. Store resuspended plasmid at -20°C.

Pac I linearization of recombinant pBROAD:

1- Digest 10 µg recombinant pBROAD3 plasmid with 1 to 5 units of *Pac* I restriction enzyme.

Note: *Pac I may be purchased from New England Biolabs and used at 0.1-0.5 unit per µg plasmid DNA.*

2- Incubate at 37°C for 1-2 hours.

3- Purify the fragment containing the ROSA26 prom-transgene-βGlo pAn cassette by agarose gel following your usual protocol.

References:

1. Zambrowicz BP. et al. 1997. Disruption of overlapping transcripts in the ROSA beta geo 26 gene trap strain leads to widespread expression of beta-galactosidase in mouse embryos and hematopoietic cells. Proc Natl Acad Sci USA. 94:3789-94.
2. Kisseberth WC. et al. 1999. Ubiquitous expression of marker transgenes in mice and rats. Dev Biol.214:128-38.
3. Brinster RL. et al. 1988. Introns increase transcriptional efficiency in transgenic mice. Proc Natl Acad Sci USA 85(3):836-40
4. Yu J, Russell JE. 2001. Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA. Mol Cell Biol. 21(17):5879-88.

TECHNICAL SUPPORT
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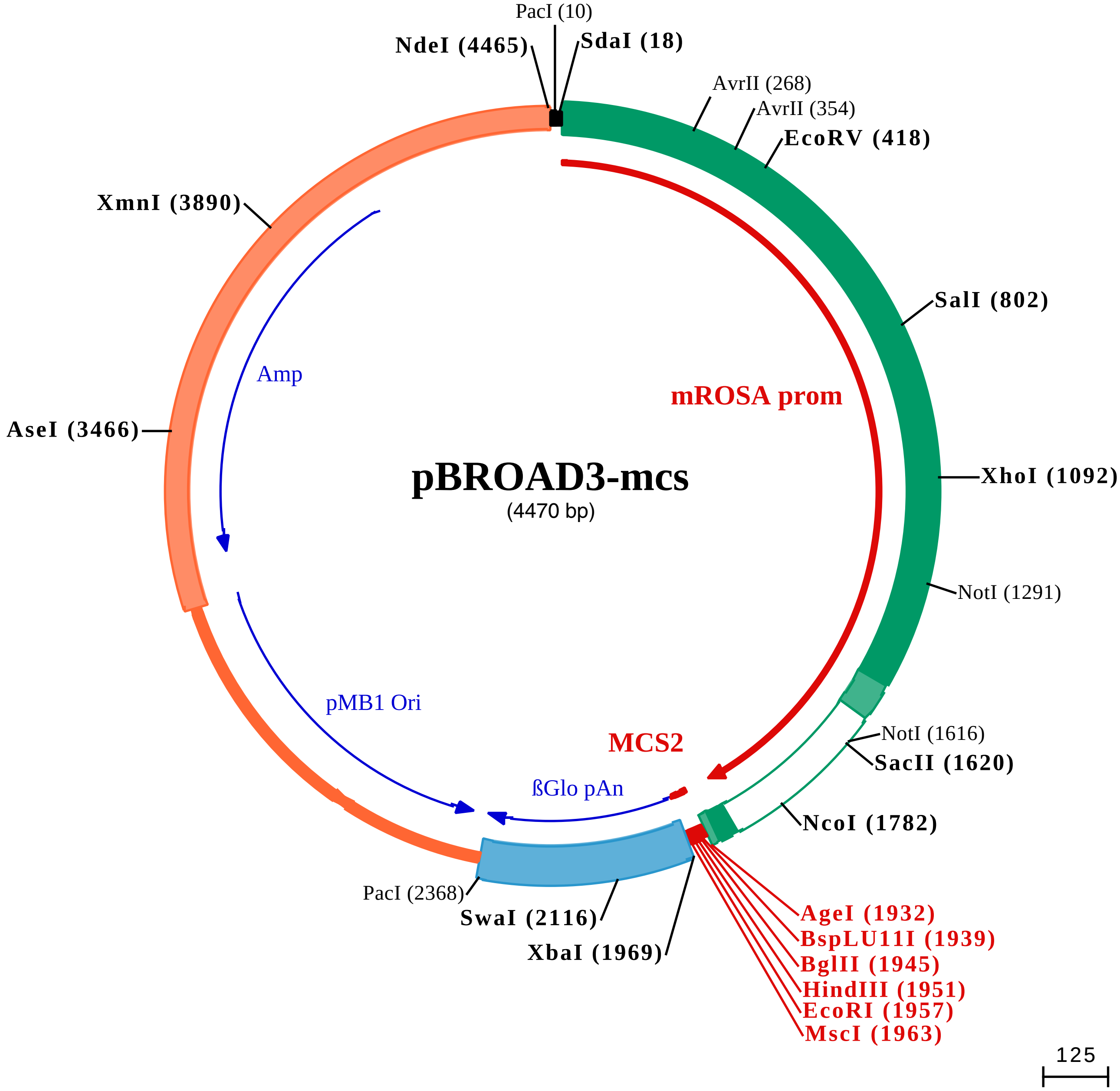
pBROAD3-mcs
(4470 bp)
PacI (10)
NdeI (4465)
SdaI (18)
AvrII (268)
AvrII (354)
EcoRV (418)
SalI (802)
XhoI (1092)
NotI (1291)
NotI (1616)
SacII (1620)
NcoI (1782)
AgeI (1932)
BspLU11I (1939)
BglII (1945)
HindIII (1951)
EcoRI (1957)
MscI (1963)
XbaI (1969)
SwaI (2116)
PacI (2368)
AseI (3466)
XmnI (3890)
mROSA prom
MCS2
ßGlo pAn
pMB1 Ori
Amp
125

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              PacI (10)  SdaI (18)
   1 GATCTCGACTTAATTAACCTGCAGGTGAAGACGTTACACAAGTAACATGAGAAAGCAGAAAATGCAGGTCATCCACGCACCCCTGACCCAGGCCAGCAGG

 101 GCGGGCTGCAGCATCAGTACACAGGAGAAAGATCCTTATTCCTAAGAATGAGAAAGGCAAAGGCGCCCGATAGAATAAATTAGCATAGAAGGGGCTTTCC

                                                                              AvrII (268)
 201 CAGGAGTTAAAACTTTCCTTCTGAGCGATTACCTACTAAAACCAGGGCTTTTGCCCACTACCATTTACCTAGGATCTTGGCTTGCACGGATTCATAGGGG

                                                                 AvrII (354)
 301 CATATCCCTCCCCCTCTTCTTTAGAGTCGTTCTTAAAAGATCGCTCTCCACGCCCTAGGCAGGGAAAACGACAAAATCTGGCTCAATTCCAGGCTAGAAC

                EcoRV (418)
 401 CCTACAAATTCAACAGGGATATCGCAAGGATACTGGGGCATACGCCACAGGGAGTCCAAGAATGTGAGGTGGGGGTGGCGAAGGTAATGTCTTTGGTGTG

 501 GGAAAAGCAGCAGCCATCTGAGATAGGAACTGGAAAACCAGAGGAGAGGCGTTCAGGAAGATTATGGAGGGGAGGACTGGGCCCCCACGAGCGACCAGAG

 601 TTGTCACAAGGCCGCAAGAACAGGGGAGGTGGGGGGCTCAGGGACAGAAAAAAAAGTATGTGTATTTTGAGAGCAGGGTTGGGAGGCCTCTCCTGAAAAG

 701 GGTATAAACGTGGAGTAGGCAATACCCAGGCAAAAAGGGGAGACCAGAGTAGGGGGAGGGGAAGAGTCCTGACCCAGGGAAGACATTAAAAAGGTAGTGG

      SalI (802)
 801 GGTCGACTAGATGAAGGAGAGCCTTTCTCTCTGGGCAAGAGCGGTGCAATGGTGTGTAAAGGTAGCTGAGAAGACGAAAAGGGCAAGCATCTTCCTGCTA

 901 CCAGGCTGGGGAGGCCCAGGCCCACGACCCCGAGGAGAGGGAACGCAGGGAGACTGAGGTGACCCTTCTTTCCCCCGGGGCCCGGTCGTGTGGTTCGGTG

                                                                                                  XhoI (1092)
1001 TCTCTTTTCTGTTGGACCCTTACCTTGACCCAGGCGCTGCCGGGGCCTGGGCCCGGGCTGCGGCGCACGGCACTCCCGGGAGGCAGCGAGACTCGAGTTA

1101 GGCCCAACGCGGCGCCACGGCGTTTCCTGGCCGGGAATGGCCCGTACCCGTGAGGTGGGGGTGGGGGGCAGAAAAGGCGGAGCGAGCCCGAGGCGGGGAG

                                                                                                 NotI (1291)
1201 GGGGAGGGCCAGGGGCGGAGGGGGCCGGCACTACTGTGTTGGCGGACTGGCGGGACTAGGGCTGCGTGAGTCTCTGAGCGCAGGCGGGCGGCGGCCGCCC

1301 CTCCCCCGGCGGCGGCAGCGGCGGCAGCGGCGGCAGCTCACTCAGCCCGCTGCCCGAGCGGAAACGCCACTGACCGCACGGGGATTCCCAGTGCCGGCGC

1401 CAGGGGCACGCGGGACACGCCCCCTCCCGCCGCGCCATTGGCCTCTCCGCCCACCGCCCCACACTTATTGGCCGGTGCGCCGCCAATCAGCGGAGGCTGC

1501 CGGGGCCGCCTAAAGAAGAGGCTGTGCTTTGGGGCTCCGGCTCCTCAGAGAGCCTCGGCTAGGtaggggatcgggactctggcgggagggcggcttggtg

                  SacII (1620)
              NotI (1616)
1601 cgtttgcggggatgggcggccgcggcaggccctccgagcgtggtggagccgttctgtgagacagccgggtacgagtcgtgacgctggaaggggcaagcgg

                                                                                          NcoI (1782)
1701 gtggtgggcaggaatgcggtccgccctgcagcaaccggagggggagggagaagggagcggaaaagtctccaccggacgcggccatggctcgggggggggg

1801 gggcagcggaggagcgcttccggccgacgtctcgtcgctgattggcttcttttcctcccgccgtgtgtgaaaacacaattgtactaaccttcttctcttt

                                                     HindIII (1951)
                                          BspLU11I (1939)         MscI (1963)
                                   AgeI (1932)   BglII (1945)  EcoRI (1957)  XbaI (1969)
1901 cctctcctgacagGTGTGAAACAGGAAGAGAACCGGTGACATGTAGATCTAAGCTTGAATTCTGGCCATCTAGAAGCTCGCTTTCTTGCTGTCCAATTTC

2001 TATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTT

              SwaI (2116)
2101 TCATTGCAATGATGTATTTAAATTATTTCTGAATATTTTACTAAAAAGGGAATGTGGGAGGTCAGTGCATTTAAAACATAAAGAAATGAAGAGCTAGTTC

2201 AAACCTTGGGAAAATACACTATATCTTAAACTCCATGAAAGAAGGTGAGGCTGCAAACAGCTAATGCACATTGGCAACAGCCCCTGATGCCTATGCCTTA

                                                                              PacI (2368)
2301 TTCATCCCTCAGAAAAGGATTCAAGTAGAGGCTTGATTTGGAGGTTAAAGTTTTGCTATGCTGTATTTTAATTAAAAACCCGCTTCGGCGGGTTTTTTTA

2401 TGCATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAA

2501 ATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCT

2601 GCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGC

2701 TCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTAT

2801 CGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAG

2901 AAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGT

3001 GGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAA
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3101 ACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATA

3201 TGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTC

287◀ • • • TrpHisLysIleLeuSerAlaGlyIleGluAlaIleGlnArgAsnArgGluAspMetThrAlaGlnSerGlyThrT

3301 GTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAA

260◀ hrTyrIleValValIleArgSerProLysGlyAspProGlyLeuAlaAlaIleIleGlyArgSerGlyArgGluGlyAlaGlySerLysAspAlaIlePh

AseI (3466)

3401 ACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTC

227◀ eTrpGlyAlaProLeuAlaSerArgLeuLeuProGlyAlaValLysAspAlaGluMetTrpAspIleLeuGlnGlnArgSerAlaLeuThrLeuLeuGlu

3501 GCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAACGA

194◀ GlyThrLeuLeuLysArgLeuThrThrAlaMetAlaValProMetThrThrAspArgGluAspAsnProIleAlaGluAsnLeuGluProGluTrpArgA

3601 TCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCAC

160◀ spLeuArgThrValHisAspGlyMetAsnHisLeuPheAlaThrLeuGluLysProGlyGlyIleThrThrLeuLeuLeuAsnAlaAlaThrAsnAspSe

3701 TCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATA

127◀ rMetThrIleAlaAlaSerCysLeuGluArgValThrMetGlyAspThrLeuHisLysGluThrValProSerTyrGluValLeuAspAsnGlnSerTyr

XmnI (3890)

3801 GTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCT

94◀ HisIleArgArgGlyLeuGlnGluGlnGlyAlaAspIleArgSerLeuValAlaGlyCysLeuLeuValLysPheThrSerMetMetProPheArgGluG

3901 TCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCA

60◀ luProArgPheSerGluLeuIleLysGlySerAsnLeuAspLeuGluIleTyrGlyValArgAlaGlyLeuGlnAspGluAlaAspLysValLysValLe

4001 GCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATA

27◀ uThrGluProHisAlaPheValProLeuCysPheAlaAlaPhePheProIleLeuAlaValArgPheHisGlnIleSerMet

4101 TTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGA

4201 AAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATG

4301 ACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGG

NdeI (4465)

4401 TGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATG