

pBROAD2-mcs

An optimized vector for mouse and rat transgenesis

Catalog # pbroad2

For research use only

Version # 05F15-SV

PRODUCT INFORMATION

Content:

- 20 µg of pBROAD2-mcs provided as lyophilized DNA

Storage and Stability:

- Products are shipped at room temperature.
- Lyophilized DNA should be resuspended upon receipt and stored at -20°C (see Methods).
- Resuspended DNA is stable more than one year 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 pBROAD2-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 cells¹. 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 rat². The ROSA promoter cloned into pBROAD2-mcs is the human counterpart of the murine Rosa 26 promoter.

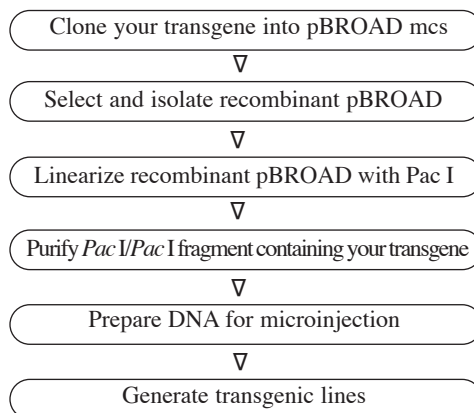
A multiple cloning site (MCS) has been added downstream of the ROSA promoter for convenient cloning of your gene of interest. The MCS contains several restriction sites that are compatible with many other enzymes, thus facilitating cloning. Furthermore, the *E. coli* region is flanked on either side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

PLASMID FEATURES

- **hROSA prom:** This TATA-less promoter, highly homologous to the murine promoter, was found to be very effective *in vitro* in a very broad range of mammalian cell lines. The strength of the human 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 higher number of Sp1 sites never recorded in any natural promoter. The 5'UTR contains an engineered intron of 1200 bp which increases the transcription of the transgene³.
- **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.
- **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*, *Nco I*, *Bgl II*, *Hind III*, *Sal I*, and *Eco RV*.
Age I is compatible with *Bsp EI* and *Sgr AI*.
Nco I is compatible with *Bsp HI* and *Bsp LU11I*.
Bgl II is compatible with *Bam HI*, *Bst YI* and *Bcl I*.
Sal I is compatible with *Ava I* and *Xho I*.

EXPERIMENTAL OUTLINE



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 H₂O. Store resuspended plasmid at -20°C.

Pac I linearization of recombinant pBROAD:

1- Digest 10 µg recombinant pBROAD2 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 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

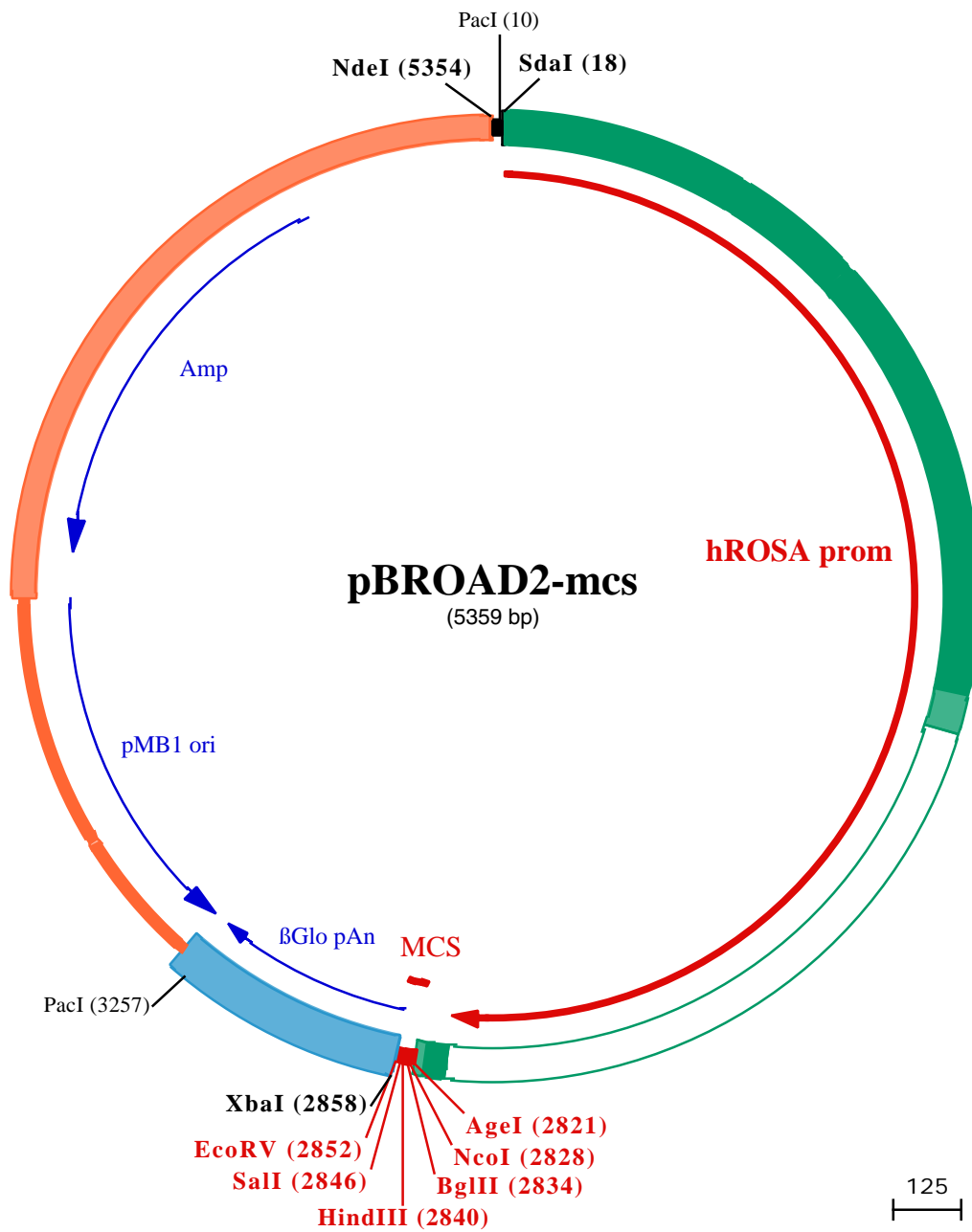
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PacI (10) SdaI (18)

1 GATCTCGACTTAATTAACCTGCAGGTGAATCATTGCACAAGTAACATGAGAAAAGCAGAAAATGCAGGTCATACACGCCACCCCTGACCAGACCAGCAGAG

101 CTGACTGCAGCATCCATATCCAAGAGAAAAGACCCTGACGCCAAGAAGTGAGACAAGCAAGGACTCTATAGAATCAATTAGCATAGAAGGGCTTTCCC

201 AACAGTTTAACTTTCCCTCTCATGCGATTTACCTACTTGAACCAGGGCTCTTTCTACACTCTCTTACATTTCCGACTTACACGCAGAGGGAAAGAGA

301 ATTCATAAAGGGAATATTTTTCTGCTTTGAAGATATTCTCACAAAGATCGTTCTCCACGCCAAGGCAAGTAAAACGACACAATCTGGCTCAACTCCAGG

401 CTCGAACCTACACATTCAACGAGGCTATCTCAGACACGCTGTGGCACACGCCACGGGGAGCCAGAAAACGTGTGGTGGGGGTGGCGAAGGTAATGCCTT

501 TGGGAAGCAGCCATCTGAGGTGGGAAGCCAGAAAACGAGAGGGAAGCGCTCCAGGAAGATTACGGAGGGGAGATCGCGGCCCCAGAGCGATCAGAGTTG

601 TCTGTACAAGGCCGCGAGAACGGGGTAGGGAGTGGGGATCGGGGAGAGAAAAAAGTATGCCTGTGTATTTGAGCGGAGGGCAGCAAGAGGCCTGT

701 CCTCATTGAAAAGTAAACGTGGAGTAGGCAGTTCCAGGAAAGGGGTGAAGAGGCGTTGGGGAGGGGAAGCGTCTGACCCAGGAAAAACATGAAAG

801 GGGGAGTTGGTTCGCTAGATTAGAGGGGATCTCTCTCCCTGGGAAAATGGGGTGTGCAACGGTGTGTGCAAGCGCGGAGGGGGGTGAGAAGTGCCA

901 GCATCTCTTAAGAGCTTGGGAGGGCCAGGCCACGACCCAAGGAGAGCGAGCGGGGAGACGGAGGAGGTGACCCTTCCCTCCCTGGGGCCCGATC

1001 GTGAGGTTGGTCTCTTTTCTGTCGGACCCTTACCTTGTCCAGGCGCTGCCGGGGCTGGGCCCGGCTGCCGCGCACGGCACTCCCGGAGGGCGCACG

1101 GACTCGAGTTAGGCCAACCGCGGCCACGGCCTTCTGGCCGGGAATGGCCCTACCCGTGAGGTGGGGGTGGGGGCAGAAAGCGGAGCGAGCCAA

1201 AGCGGGGAGGGGGGAGGGCCAGGAAAGAGGGGGCCGGCACTACTGTGTTGGCGGACTGGCGGACTGGGGCTGCGTGAAGTCTCTGAGCGCAGGGC

1301 GCGCGCGGCCGCCCTCCCGGCGAGCGGGCGGGCGGGCGGGCGGGCGGGCGGGCAGTCACTCAGCCCGTCCCGAGCGGAAACGCCACTGACCCG

1401 ACGGGGATTCAGCGCCGGCGCCAGGGGCACCCGGGACACGCCCTCCCGCGCGCCATTGGCCTCTCCGCCACCGCCCGCACCCATTGGCCCACT

1501 CGCCGCAATCAGCGGAAGCCGCCGGGGCGCCTAGAGAAGAGGCTGTGCTTCGGCGCTCCGGCTCTCAGAGACCTCGGCTAGgt aggggagcggaac

1601 tctggtgggaggggaggtgcggtgactgggggatgggtggctagggggcgctctggtggcttgcgggggtgcctttcccgagggaagtgcggaaca

1701 taatglttgttacgttgggagggaaaggggtggctggatgcaggcgaggaggagggcccgccctgcggaaccggagggggagggagaagggagcggaaaa

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1901 aacacaaatggcgtattctggttggagtaagctcctgtcagttacaccgtcgggagtagcagccgcttagcgactctcgcgttccccctgggtgggg

2001 cgggtaggtaggtggggtgtagagatgctgggtgtcgggpcggcccgccctcctgcggcgaggaggagggtcagtgaaattggctctggcgggcgct

2101 cctcccaccctccccttcttcgggggagtcggtttaccgcccctgcttctctcgacacctgattggctgtcgaagctgtgggaccgggccccttctgct

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2601 ggggagtgccgcaatacctttatgggagttcttctgctgcctcccctcttgaaggaccgcccctgggctggaagaagccctccctccttctcctcgcg

2701 tgatctcgtcatcgctccatgtcgagtcgcttctcgattatggcgggattcttttgcctagacaattgtactaaccttcttcttcttctcctcctgac

NcoI (2828) HindIII (2840) EcoRV (2852)

AgeI (2821) BglIII (2834) SalI (2846) XbaI (2858)

2801 agGTGTGAAACAGGAAGAGAACCGGTGCCATGGAGATCTAAGCTTGTGACGATATCTCTAGAAGCTCGCTTTCTTGCTGTCCAATTTCTATTAAGGTT

2901 CCTTTGTTCCCTAAGTCCAACCTACTAACTGGGGATATTATGAAGGCCTTGGACATCTGGATTCTGCCTAATAAAAAACATTTATTTTATTGCAATG

3001 ATGTATTTAAATATTTCTGAATATTTACTAAAAAGGGAATGTGGGAGTCAAGTGCATTTAAAACATAAAGAAATGAAGAGCTAGTTCAAACCTTGGGA

3101 AAATACACTATATCTTAACTCCATGAAAGAAGGTGAGGCTGCAAACAGCTAATGCACATTGGCAACAGCCCCTGATGCCTATGCCTTATTATCCCTCA

3201 GAAAAGGATTCAAGTAGAGGCTTGATTGGAGGTTAAAGTTTGCTATGCTGATTTAATTAATAAAACCCGCTTCGGCGGGTTTTTTTATGCATGTGAGC

3301 AAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGCCCGCTTGTGCGGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAGAAAAATCGACGCTCA

3401 AGTCAGAGGTGGCGAAACCCGACAGGACTATAAGATACCAGGCGTTTCCCCTGGAAGCTCCCTCGTGGCTCTCTGTTCGACCCTGCCGTTACCG

3501 GATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGG
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