

pBROAD2 Kit

An optimized vector for mouse and rat transgenesis

Catalog code: kbroad2

For research use only

Version 20L07-MM

PRODUCT INFORMATION

Content:

- 20 µg of pBROAD2-mcs provided as lyophilized DNA
- 20 µg of pBROAD2-LacZnls 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). Lyophilized DNA is stable 3 months at -20°C. 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 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 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

• **hROSAprom:** 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.

• **MCS (pBROAD2-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

• **LacZ-ΔCpG NLS (pBROAD2-LacZnls):** The *E. coli lacZ* gene codes for the enzyme β -galactosidase which catalyzes the hydrolysis of the substrate X-Gal to produce a blue color that is easily visualized under a microscope. A nuclear localization signal of SV40 large T has been inserted in the 5' end of the *lacZ* gene to allow the targeting of the chimeric protein to the nucleus. To reduce the immunogenicity of this bacterial gene, InvivoGen has engineered a synthetic *lacZnls* gene that is entirely free of CpG motifs, whereas the wild type *lacZ* gene contains 298 CpG dinucleotides.

• **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.

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

TECHNICAL SUPPORT

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

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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 pBROAD2:

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.

Plasmid amplification and cloning

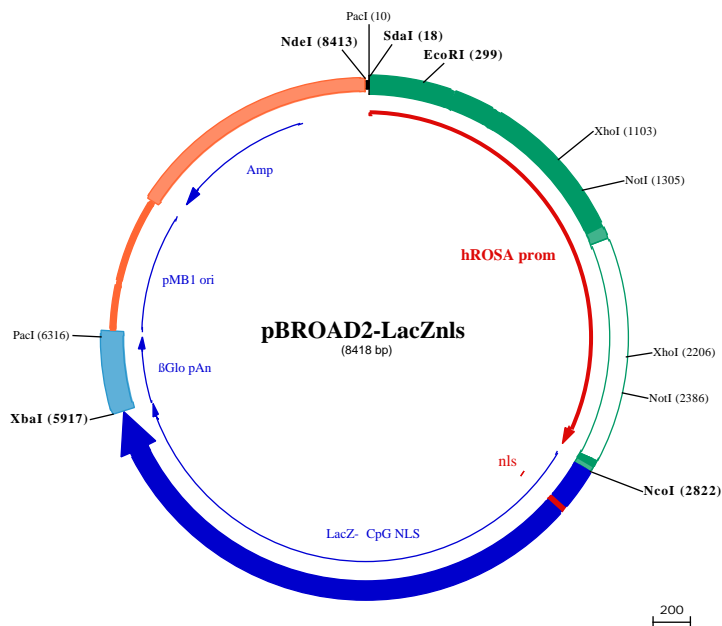
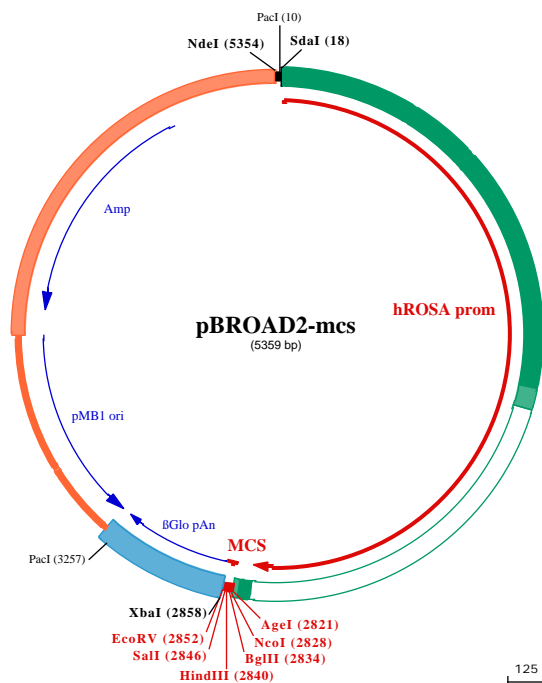
Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5α.

Ampicillin usage

Ampicillin (not provided) can be used for *E. coli* at 50-100 µg/ml in liquid or solid media.

References:

1. Zambrowicz BP, et al. 1997. Disruption of overlapping transcripts in the ROSA26 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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pBROAD2-mcs sequence

PacI (10) SdaI (18)

1 GATCTCGACTTAATTAACCTGCAGGTGAATCATTGCACAAGTAACATGAGAAAGCAGAAAATGCAGGTCATACACGCCACCCCTGACCCAGACCAGCAGAG
101 CTGACTGCAGCATCCATATCCAAGAGAAAGACCCTGACGCCCAAGAAGTGAGACAAGCAAGGACTCTATAGAATCAATTAGCATAGAAGGGCTTTCCC
201 AACAGTTTAACTTTCCCTCTCATGCGATTTACCTACTTGAACCAGGGCTCTTTCTACACTCTCTTACATTCCCGACTTACACGCAGAGGGAAAGAGA
301 ATTCATAAAGGGAATATTTTTCTGCTTTGAAGATATTCTCACAAGATCGTTCTCCACGCCAAGGCAAGTAAAACGACACAATCTGGCTCAACTCCAGG
401 TCGAACCTACACATTCAACGAGGCTATCTCAGACACGCTGTGGCACACGCCACGGGGAGCCAGAAAACGTGTGGTGGGGTGGCGAAGGTAATGCCTT
501 TGGGAAGCAGCCATCTGAGGTGGGAAGCCAGAAAACGAGAGGGAAGGCGTCCAGGAAGATTACGGAGGGGAGATCGCGGCCCCAGAGCGATCAGAGTTG
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NcoI (2828) HindIII (2840) EcoRV (2852)
AgeI (2821) BglII (2834) SalI (2846) XbaI (2858)
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NdeI (5354)

pBROAD2-LacZnls sequence

PacI (10) SdaI (18)

EcoRI (299)

XhoI (1103)

NotI (1305)

XhoI (2206)

NotI (2386)

NotI (2822)

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326 rLeul leGluAlaGluAlaCysAspValGlyPheArgGluValArgI leGluAsnGlyLeuLeuLeuAsnGlyLysProLeuLeul leArgGlyVal
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4801 ATGGTGGCCTGGATGGCAAGCCTCTGGCTTCTGGTGGAGTGCCTCTGGATGTGGCCCTCAAGGAAAGCAGCTGATTGAACCTGCCTGAGCTGCCTCAGC
660 MetValAlaLeuAspGlyLysProLeuAlaSerGlyGluValProLeuAspValAlaProLeuAspGlnProPheProAlaValProLysTrpSerI leLy
4901 CAGAGTCTGCTGGACAACCTGTGGCTAACAGTGGGTTAGCCCAATGCAACAGCTGGTCTGAGGAGCCACATCTCTGCATGGCAGCAGTGGAG
693 roGluSerAlaGlyGlnLeuTrpLeuThrValArgValValGlnProAsnAlaThrAlaTrpSerGluAlaGlyHisI leSerAlaTrpGlnGlnTrpAr
5001 GCTGGCTGAGAACCTCTCTGTGACCTGCCTGCTGCCTCTCATGCCATCCCTCACCTGACAACATCTGAAATGGACTTCTGATTGAGCTGGGCAACAAG
726 gLeuAlaGluAsnLeuValThrLeuProAlaAlaSerHisAlal leProHisLeuThrThrSerGluMetAspPheCysI leGluLeuGlyAsnLys
5101 AGATGGCAGTTCACACAGGCACTGTGGCTTCTCTCAGATGTGATGGAGACAAGAAGCAGCTCCTCACCCCTCAGGAGCAATTCACAGGGCTC
760 ArgTrpGlnPheAsnArgGlnSerGlyPheLeuSerGlnMetTrpl leGlyAspLysLysGlnLeuLeuThrProLeuArgAspGlnPheThrArgAlaP
5201 CTCTGGACAATGACATTGGAGTGTCTGAGGCCACCAGGATTGACCCAAATGCTTGGGTGGAGAGGTGGAAGGCTGCTGGACACTACCAGGCTGAGGCTGC
793 roLeuAspAsnAspl leGlyValSerGluAlaThrArgI leAspProAsnAlaTrpValGluArgTrpLysAlaAlaGlyHisTyrGlnAlaGluAlaAl
5301 CTGCTCCAGTGCACAGCAGACCCCTGGCTGATGCTGTCTGATCACCACAGCCATGCTTGGCAGCACAAGGCAAGACCCTGTTTATCAGCAGAAAG
826 aLeuLeuGlnCysThrAlaAspThrLeuAlaAspAlaValLeul leThrThrAlaHisAlaTrpGlnHisGlnGlyLysThrLeuPheI leSerArgLys
5401 ACCTACAGGATTGATGGCTCTGGACAGATGGCAATCACAGTGGATGTGGAGGTTGCCTCTGACACACCTCACCTGCAAGGATTGGCTGAACTGTCAAC
860 ThrTyrArgI leAspGlySerGlyGlnMetAlal leThrValAspValGluValAlaSerAspThrProHisProAlaArgI leGlyLeuAsnCysGlnL
5501 TGGCACAGGTGGCTGAGAGGGTGAACCTGGCTGGGCTTAGGCCCTCAGGAGAACTACCCTGACAGGCTGACAGCTGCCTGCTTTGACAGGTGGGACCTGCC
893 euAlaGlnValAlaGluArgValAsnTrpLeuGlyLeuGlyProGlnGluAsnTyrProAspArgLeuThrAlaAlaCysPheAspArgTrpAspLeuPr
5601 TCTGCTGACATGTACACCCTTATGTCTCCCTTCTGAGAATGGCCTGAGGTGGCCAGGAGCTGAACTATGGCTCACCAGTGGAGGAGAC
926 oLeuSerAspMetTyrThrProTyrValPheProSerGluAsnGlyLeuArgCysGlyThrArgGluLeuAsnTyrGlyProHisGlnTrpArgGlyAsp
5701 TTCCAGTTCACATCTCCAGTACTCTCAGCAACAGCTCATGAAACCTCTCACAGGCACCTGCCTCATGAGAGGAGGAACCTGGCTGAACATTGATG
960 PheGlnPheAsnl leSerArgTyrSerGlnGlnGlnLeuMetGluThrSerHisArgHisLeuLeuHisAlaGluGluGlyThrTrpLeuAsnl leAspG
5801 GCTTCCACATGGGCATTGGAGGAGATGACTCTGGTCTCCTTCTGTCTGCTGAGTCCAGTATCTGCTGGCAGGTACCCTATCAGCTGGTGTGGT
993 lyPheHisMetGlyI leGlyGlyAspSerTrpSerProSerValSerAlaGluPheGlnLeuSerAlaGlyArgTyrHisTyrGlnLeuValTrpCy

XbaI (5917)

5901 CCAGAAGTAAACCTAATCTAGAAGCTCGCTTTCTTGGCTGTCCAATTTCTATTAAGGTTCCCTTTGTTCCCTAAGTCCAACCTACTAACTGGGGATATTA
1026 sGlnLys•••
6001 TGAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAATGATGTATTTAAATTTTCTGAATATTTTACTAAAAAGGAA
6101 TGTGGGAGGTCACTGCATTTAAAAACATAAAGAAATGAAGAGCTAGTTCAAACCTTGGGAAAATACACTATATCTTAAACTCCATGAAAGAAGGTGAGGCT
6201 GCAAACAGCTAATGCACATTGGCAACAGCCCTGATGCCTATGCCTTATTCATCCCTCAGAAAAGGATTCAAGTAGAGGCTTGATTTGGAGGTTAAAGTT
PacI (6316)
6301 TTGCTATGCTGATTTTAAATTAATAAAACCCGCTTCGGCGGGTTTTTTTATGCATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCT
6401 TGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAAACCCGACAGGACTATAAAGATACCA
6501 GGGCTTTCCCTGGAAAGCTCCCTCGTGGCTCTCTGTTCGAGCCTGCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTT
6601 TCTCATAGCTCAGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGCTCAGCCGACCGCTGCGCCT
6701 TATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGG
6801 CCGTGTACAGAGTCTTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAA
6901 AGAGTTGGTAGCTCTTGATCCGGCAAACAAACCCGCTGGTAGCGGTGGTTTTTTTGTGGCAAGCAGCAGATTACCGCGAGAAAAAAGGATCTCAAG

7001 AAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTA
7101 GATCCTTTTAAATTA AAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATC
7201 TCAGCGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAA
7301 TGATACCGGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGC
7401 CTCCATCCAGTCTATTAATGTTGCCGGGAAGCTAGAGTAAGTAGTTCCGCCAGTTAATAGTTTGGCACAAGTGTGGCATTGCTACAGGCATCGTGGTG
7501 TCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCT
7601 TCGGTCTCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTTACTGTCATGCCATCCGTAAG
7701 ATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCACTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCTCAATACGGGATAATACC
7801 GCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGAAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGT
7901 AACCCACTCGTGACCCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAAT
8001 AAGGGCGACACGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTATCAGGGTATTGTCTCATGAGCGGATACATATTTGAA
8101 TGTATTTAGAAAAATAACAAATAGGGTTCCGGCGACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATA
8201 AAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCT
8301 GTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGGCTCAGCGGTTGTTGGCGGTTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTA
8401 CTGAGAGTGCACCATATG

NdeI (8413)