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 DNAis stable 3 months at -20˚C. Resuspended DNAis 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 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]. 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 ROSApromoter 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 transgene[3].

• **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

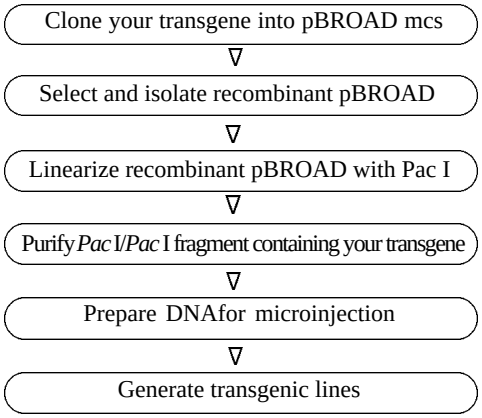
• **LacZ-ΔCpG NLS (pBROAD2-LacZnls):** The *E. coli lacZ* gene codes for the enzyme β-galactosidase which catalyzes the hydrolysis of the substrate X-Gal to produce a blue color that is easily visualized under a microscope. A nuclear localization signal of SV40 large T has been inserted in the 5'end 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.

• **ßGlo 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.

EXPERIMENTAL OUTLINE



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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 1µg/µl, resuspend the DNA in 20µl of sterile H_2O. Store resuspended plasmid at -20°C.

Pac I linearization of recombinant pBROAD2:

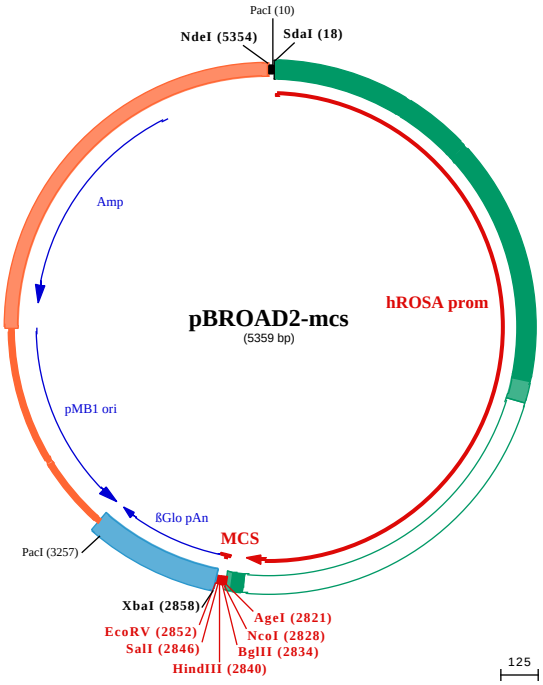
1- Digest 10 µg recombinant pBROAD2 plasmid with 1 to 5 units of *Pac* I restriction enzyme.
Note: *Pac I may be purchased from New England Biolabs and used at 0.1-0.5 unit per µg plasmid DNA.*
2- Incubate at 37°C for 1-2 hours.
3- Purify the fragment containing the ROSA26 prom-transgene-ßGlo pAn cassette by agarose gel following your usual protocol.

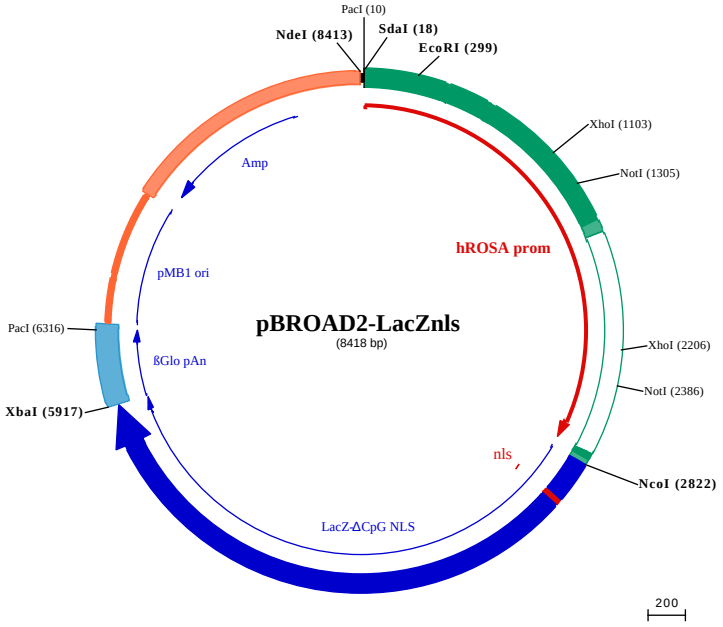
Plasmid amplification and cloning

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 ROSAbeta 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 USA85(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.

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pBROAD2-mcs sequence

PacI (10) **SdaI (18)**

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 101 CTGACTGCAGCATCCATATCCAAGAGAAAGACCACTGACGCCCAAGAAGTGAGACAAGCAAGGACTCTATAGAATCAATTAGCATAGAAGGGGCTTTCCC
 201 AACAGTTTAACTTTCCCTCTCATGCGATTTACCTACTTGAACCAGGGCTCTTTCCTACACTCCTCTTCACATTCCCGACTTACACGCAGAGGGAAAGAGA
 301 ATTCATAAAGGGAATATTTTTCTGCCTTTGAAGATATTCTCACAAGATCGTTCTCCACGCCCAAGGCAAGTAAAACGACACAATCTGGCTCAACTCCAGG
 401 CTCGAACCCTACACATTCAACGAGGCTATCTCAGACACGCTGTGGCACACGCCACGGGGAGCCAGAAAACGTGTGGTGGGGGTGGCGAAGGTAATGCCTT
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NcoI (2828) HindIII (2840) EcoRV (2852)
AgeI (2821) BglII (2834) SalI (2846) XbaI (2858)

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pBROAD2-LacZnls sequence

PacI (10) **SdaI (18)**

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 401 CTCGAACCCTACACATTCAACGAGGCTATCTCAGACACGCTGTGGCACACGCCACGGGGAGCCAGAAAACGTGTGGTGGGGGTGGCGAAGGTAATGCCTT

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 801 GGGGAGTTGGGTCGCCTAGATTAGAGGGGGATCTCTCTCCCTGGGAAAATGGGGTGTTGCAACGGTGTGTGCAAGGCGGCGAGGGGGGTGAGAAGTGGCA

 901 GCATCCTCCTAAGAGCTTGGGGAGGGCCAGGCCCACGACCCAAGGAGAGCGAGCGCGGGGAGACGGAGGAGGTGACCCTTCCCTCCCCTGGGGCCCGATC

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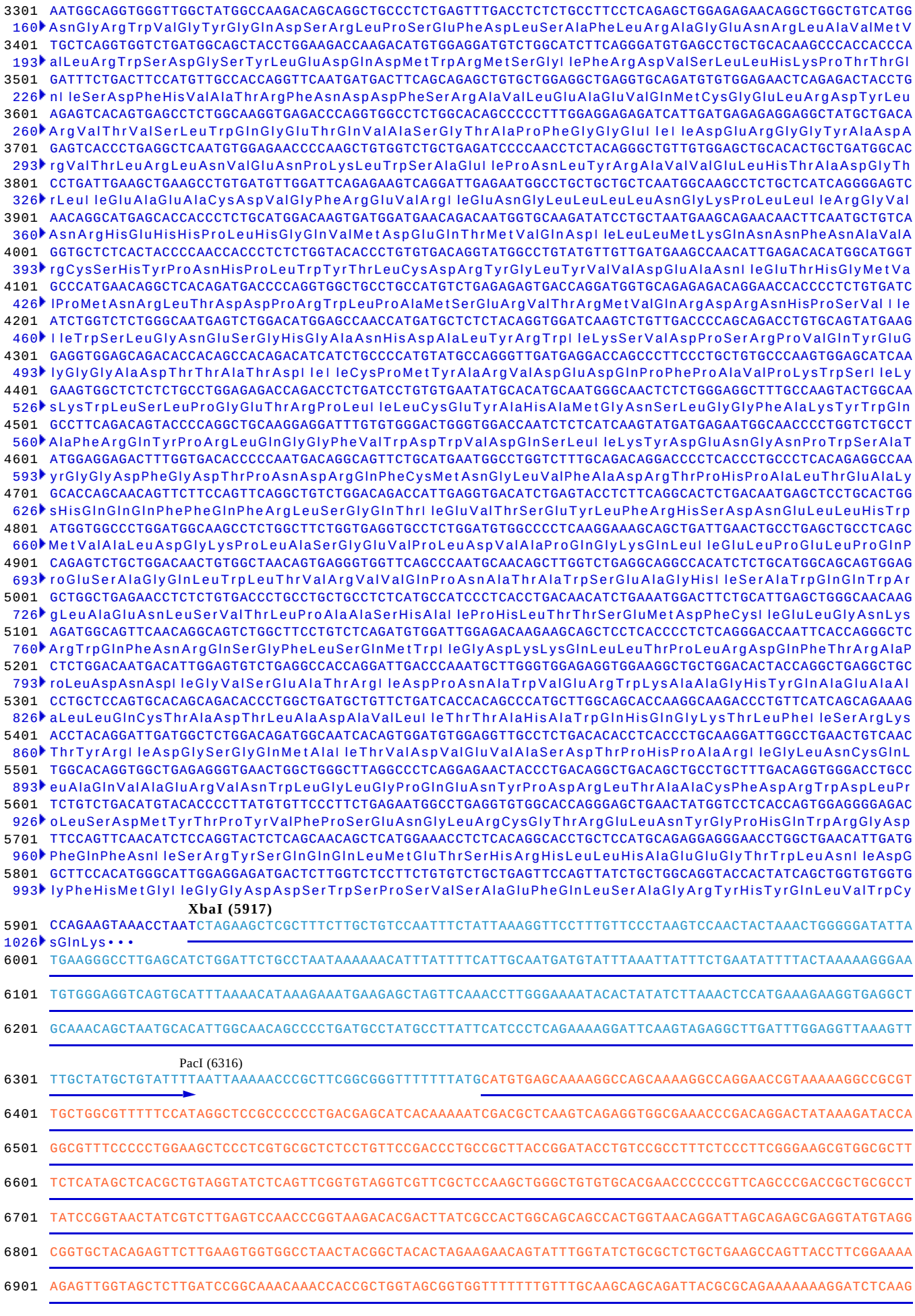
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PacI (6316)

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NdeI (8413)

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