

pBROAD3-LacZ

An optimized vector for mouse and rat transgenesis

Catalog # pbroad3-lacz

For research use only

Version # 03B04-MT

PRODUCT INFORMATION

Content:

- 20 µg of pBROAD3-LacZ 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 was purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE

The pBROAD3-LacZ plasmid is designed for the expression of *E. coli* β-galactosidase gene 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. pBROAD3-LacZ expresses a new chemically synthesized gene (*LacZΔCpGNLS*) deprived of CpGs to eliminate interferences of CpG methylation on gene expression. 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.

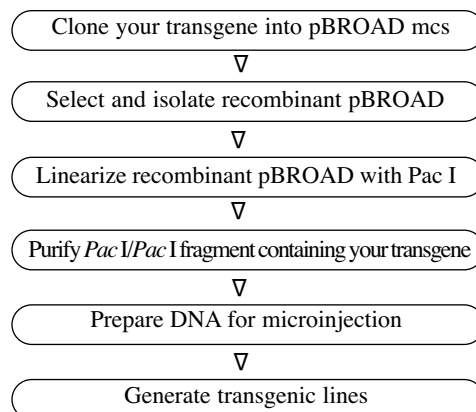
PLASMID FEATURES

- **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 transgene³.

- **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.

- **βGlob 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



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 H₂O. 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.

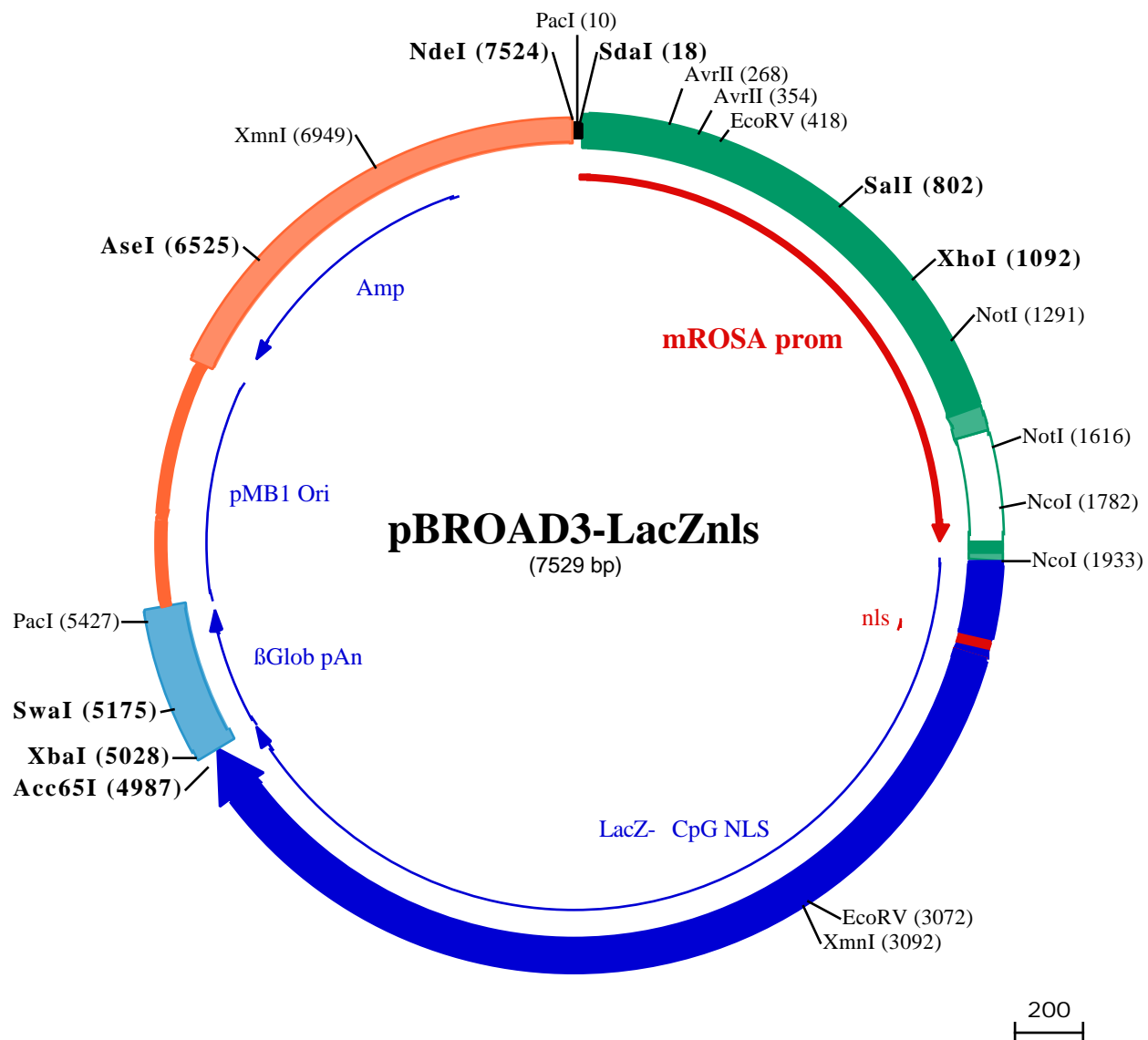
3- Purify the fragment containing the ROSA26 prom-transgene-βGlo pAn cassette by agarose gel following your usual protocol.

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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PacI (10) SdaI (18)
1 GATCTCGACTTAATTAActgcaGGTGAAGACGTTACACAAGTAACATGAGAAAGCAGAAAATGCAGGTCATCCACGCACCCTGACCCAGGCCAGCAGG
101 CCGGGCTGCAGCATCAGTACACAGGAGAAAGATCCTTATTCTTAAGAATGAGAAAGGCAAGGCCCGCATAGAATAAATTAGCATAGAAGGGCTTTCC
AvrII (268)
201 CAGGAGTTAAACTTTCTTCTGAGCGATTACCTACTAAAACCAGGGCTTTTGGCCACTACCATTTACCTAGGATCTTGGCTTGCACGGATTTCATAGGGG
AvrII (354)
301 CATATCCCTCCCCTCTTCTTTAGAGTCGTTCTTAAAAGATCGCTCTCCACGCCCTAGGCAGGGAAAACGACAAAATCTGGCTCAATTCAGGCTAGAAC
EcoRV (418)
401 CCTACAAATTCAACAGGGATATCGCAAGGATACTGGGGCATAACCCACAGGGAGTCCAAGAATGTGAGGTGGGGGTGGCGAAGGTAATGTCTTTGGTGTG
501 GGAAAAGCAGCAGCCATCTGAGATAGGAAGTGGAAAACCAGAGGAGAGGCGTTCAGGAAGATTATGGAGGGAGGACTGGGCCCCACGAGCGACCAGAG
601 TTGTCACAAGGCCGAAGAACAGGGGAGGTGGGGGCTCAGGGACAGAAAAAAGTATGTGTATTTTGGAGAGCAGGTTGGGAGGCTCTCCTGAAAAG
701 GGTATAAACGTGGAGTAGGAATACCCAGGCAAAAAGGGAGACCAGAGTAGGGGAGGGGAAGAGTCTGACCCAGGAAGACATTA AAAAGGTAGTGG
SalI (802)
801 GGTGACTAGATGAAGGAGAGCCTTTCTCTGCGCAAGAGCGGTGCAATGGTGTGTAAGGTAGCTGAGAAGCAGAAAAGGGCAAGCATCTTCTGCTA
901 CCAGGCTGGGGAGGCCAGGCCACGACCCGAGGAGAGGGAACGAGGGAGACTGAGGTGACCTTTTCCCGGGGGCCGGTGTGTGGTTCGGTG
XhoI (1092)
1001 TCTCTTTTCTGTTGGACCTTACCTTGACCCAGGCGCTGCCGGGGCTGGGCCCGGGTGCAGCGCACGGCACTCCCGGGAGGCAGCGAGACTCGAGTTA
1101 GGCCCAACCGGGCCACGGCGTTCCTGCGCGGAATGGCCCTACCCGTGAGGTGGGGTGGGGGCAGAAAAGCGGAGCGAGCCCGAGCGGGGAG
NotI (1291)
1201 GGGAGGGCCAGGGCGGAGGGGCCGCACTACTGTGTTGGCGGACTGGCGGACTAGGGTGCCTGAGTCTCTGAGCGCAGCGGGGGCGGGCCGCC
1301 CTCCCCGGCGGGCAGCGGGCCAGCGGGCAGCTCACTAGCCCGCTGCCGAGCGGAAACGCCACTGACCGCAGGGGATTCCAGTGCCGGCGC
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NotI (1616)
1601 cgtttgccccgatggcgcccgccaggccctccgagcgtggtggagccgttctgtgagacagccgggtacgagtcgtgacgctggaaggggcaagcgg
NcoI (1782)
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1801 gggcagcggaggagcgttccggccgacgtctcgtcgctgattggcttcttttctcccgcgtgtgtgaaaacacaattgactaacctcttctcttt
NcoI (1933)
1901 cctctcctgacagGTGTGAAACAGGAAGAGAACCATGGACCTGTTGTGCTGCAAAGGAGAGACTGGGAGAACCCTGGAGTGACCCAGCTCAACAGACTG
1▶MetAspProValValLeuGlnArgArgAspTrpGluAsnProGlyValThrGlnLeuAsnArgLeu
2001 GCTGCCACCCTCCCTTTGCCTCTTGAGGAAGTCTGAGGAAGCCAGGACAGACAGGCCAGCCAGCAGCTCAGGCTCTCAATGGAGAGTGGAGGTTTG
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EcoRV (3072) XmnI (3092)
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856 leSerArgLysThrTyrArgI leAspGlySerGlyGlnMetAlal leThrValAspValGluValAlaSerAspThrProHisProAlaArgI leGlyLe
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956 rpArgGlyAspPheGlnPheAsnI leSerArgTyrSerGlnGlnGlnLeuMetGluThrSerHisArgHisLeuLeuHisAlaGluGlyThrTrpLe

Acc65I (4987)

4901 GAACATTGATGGCTTCCACATGGGCATTGGAGGAGATGACTCTTGGTCTCCTTCTGTGCTGCTGAGTTCAGTTATCTGCTGGCAGGTACCACATACG
989 uAsnI leAspGlyPheHisMetGlyI leGlyGlyAspAspSerTrpSerProSerValSerAlaGluPheGlnLeuSerAlaGlyArgTyrHisTyrGln

XbaI (5028)

5001 CTGGTGTGGTGCCAGAAGTAAACCTAATCTAGAAGCTCGCTTTCTTGTCTGCCAATTTCTATTAAAGGTTCCCTTTGTTCCCTAAGTCCAACCTACTAACT
1023 LeuValTrpCysGlnLys•••

SwaI (5175)

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PacI (5427)

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2874 ••• TrpHisLysI leLeuSe
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AseI (6525)

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XmnI (6949)
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NdeI (7524)
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