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 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].

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 transgene[3].

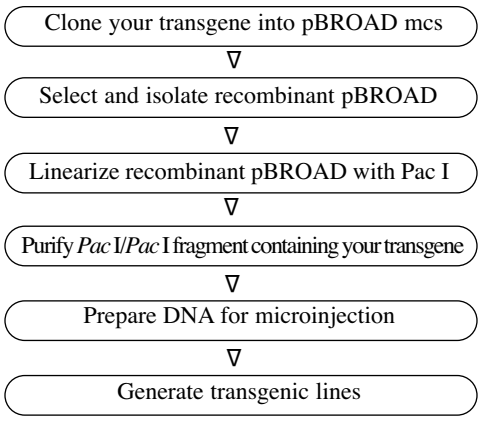
• **LacZ-ΔCpG NLS (pBROAD2-LacZnls):** The *E. coli lacZ* gene codes for the enzyme β-galactosidase which catalyzes the hydrolysis of the substrate X-Gal to produce a blue color that is easily visualized under a microscope. A nuclear localization signal of SV40 large T has been inserted in the 5' end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



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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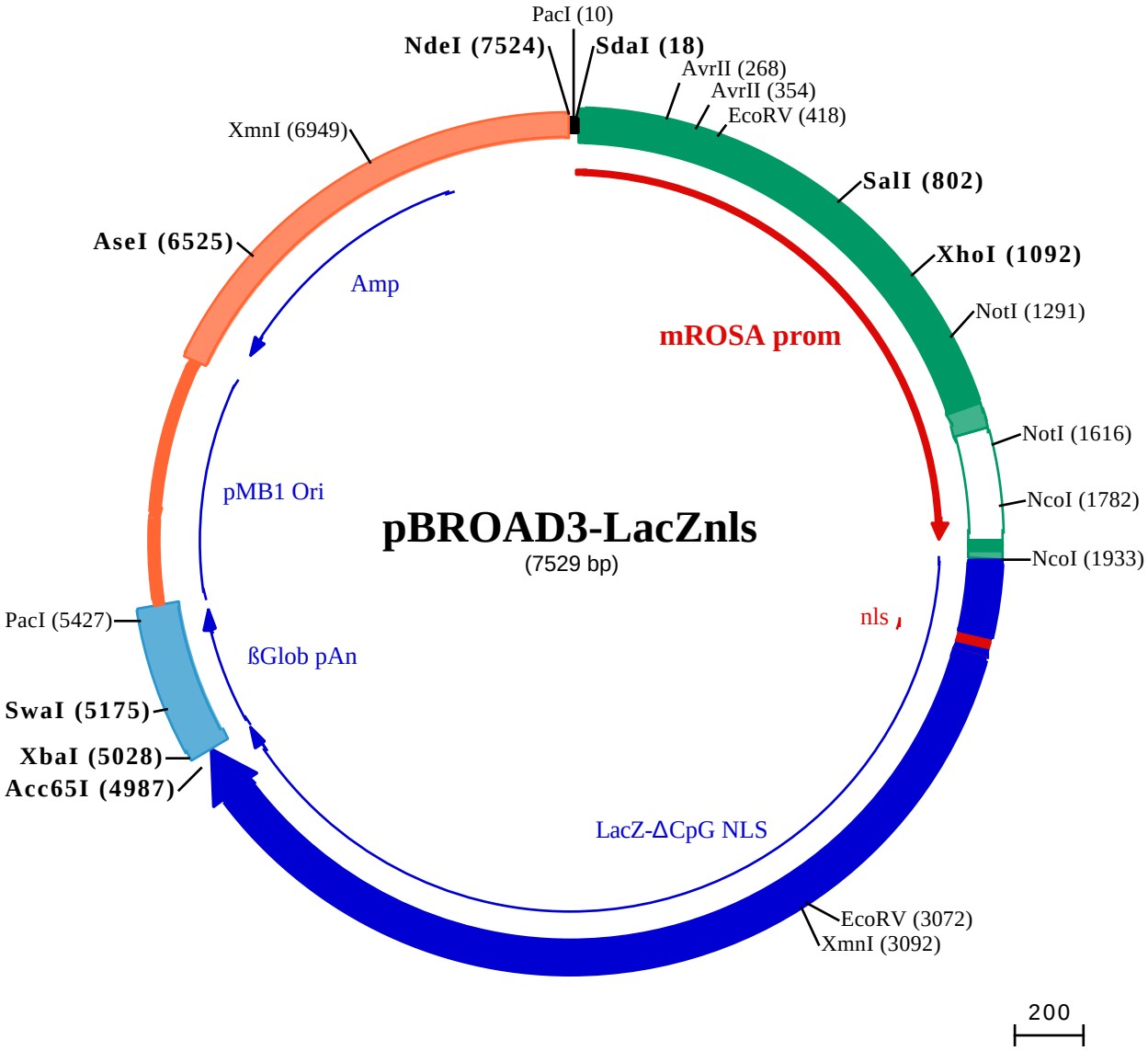
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NdeI (7524)
SdaI (18)
AvrII (268)
AvrII (354)
EcoRV (418)
XmnI (6949)
SalI (802)
AseI (6525)
XhoI (1092)
Amp
NotI (1291)
mROSA prom
NotI (1616)
pMB1 Ori
NcoI (1782)
pBROAD3-LacZnls
(7529 bp)
NcoI (1933)
PacI (5427)
nls
ßGlob pAn
SwaI (5175)
XbaI (5028)
Acc65I (4987)
LacZ-ΔCpG NLS
EcoRV (3072)
XmnI (3092)
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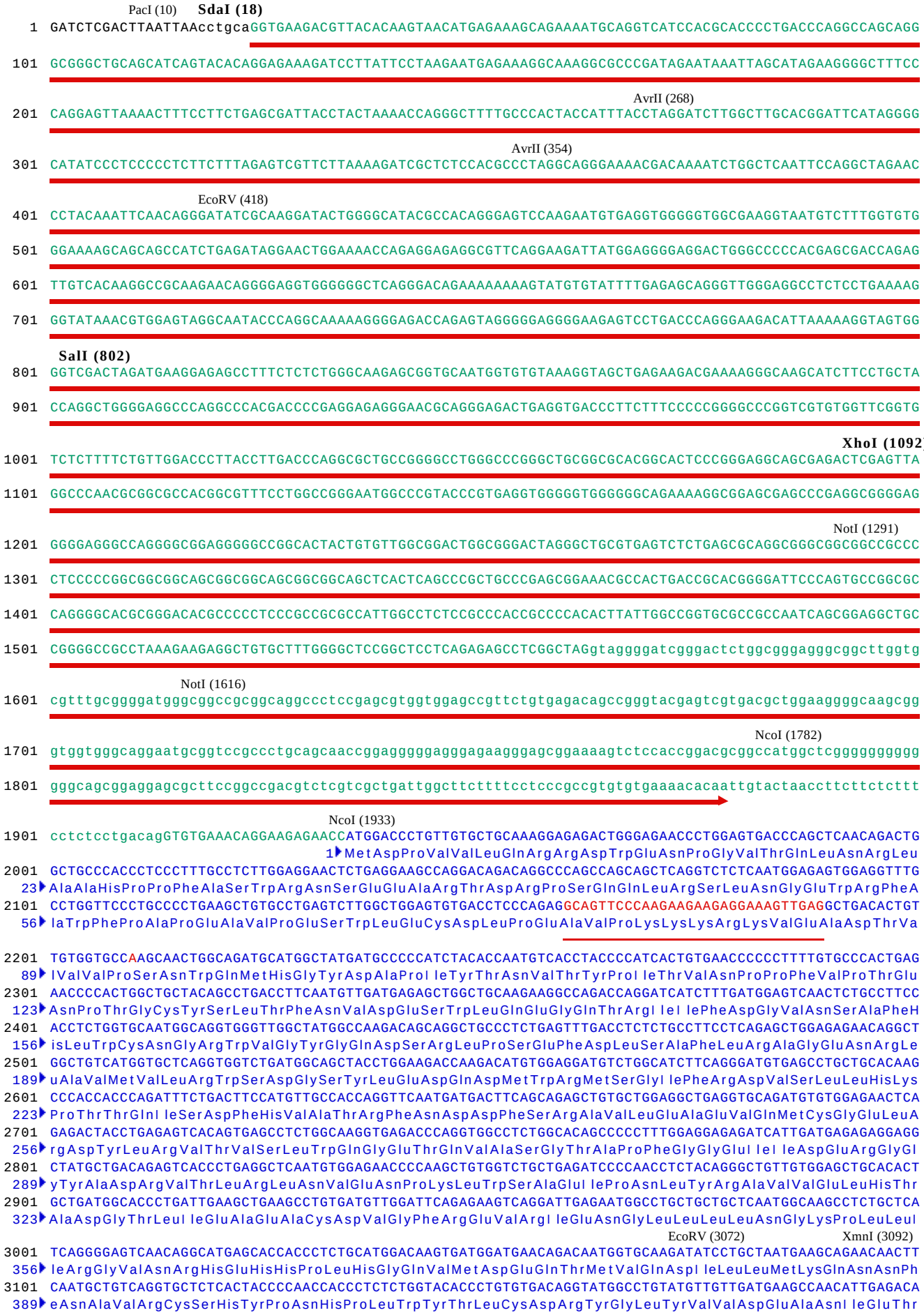
InvivoGen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XbaI (5028)
SwaI (5175)
PacI (5427)
AseI (6525)


6801 CCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATAC
113 lyAspThrLeuHisLysGluThrValProSerTyrGluValLeuAspAsnGlnSerTyrHisI leArgArgGlyLeuGlnGluGlnGlyAlaAspI leAr
XmnI (6949)
6901 GGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATC
80 gSerLeuValAlaGlyCysLeuLeuValLysPheThrSerMetMetProPheArgGluGluProArgPheSerGluLeuI leLysGlySerAsnLeuAsp
7001 CAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCA
47 LeuGluI leTyrGlyValArgAlaGlyLeuGlnAspGluAlaAspLysValLysValLeuThrGluProHisAlaPheValProLeuCysPheAlaAlaP
7101 AAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGAT
13 hePheProI leLeuAlaValArgPheHisGlnI leSerMet
7201 ACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGAC
7301 ATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTC
7401 ACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAG
NdeI (7524)
7501 AGCAGATTGTACTGAGAGTGCACCATATG