**pBROAD3-LacZ**

An optimized vector for mouse and rat transgenesis

Catalog # pbroads-lacz

For research use only

Version # 03B04-MT

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**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. 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.

pBROAD3-LacZ expresses a new chemically synthesized gene (LacZ∆CpG NLS) 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 ever recorded in any natural promoter. The 5'UTR contains an engineered intron of 350 bp which increases the transcription of the transgene.

- **LacZ-∆CpG 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.

- **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

**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:**

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**TECHNICAL SUPPORT**
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CCATCCGTAAAGATGCTTTTCTGTAGCTACCTCAACAAAGTCATAGTATGCTGAGCTCAGCAGGCGACCGAGTTGCTCTTGCCCGGCGTCAATAC

GGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATC

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