The vector sequence has been compiled using the information from sequence databases, published literature, and other sources, together with partial sequences obtained by Evrogen. This vector has not been completely sequenced.

**Vector description**

pBrUSLEE-N is a mammalian expression vector encoding green fluorescent protein BrUSLEE. The vector allows generation of fusions to the BrUSLEE N-terminus and expression of BrUSLEE or fusions alone in eukaryotic (mammalian) cells.

BrUSLEE codon usage is optimized for high expression in mammalian cells (humanized) [Haas et al. 1996]. To increase mRNA translation efficiency, Kozak consensus translation initiation site is generated upstream of the BrUSLEE coding sequence [Kozak 1987]. Multiple cloning site (MCS) is located between CMV IE promoter and BrUSLEE coding sequence.

The vector backbone contains immediate early promoter of cytomegalovirus (P<sub>CMV IE</sub>) for protein expression, SV40 origin for replication in mammalian cells expressing SV40 T-antigen, pUC origin of replication for propagation in E. coli, and fl origin for single-stranded DNA production. SV40 polyadenylation signals (SV40 poly A) direct proper processing of the 3'-end of the reporter mRNA. SV40 early promoter (P<sub>SV40</sub>) provides neomycin resistance gene (Neo<sup>+</sup>) expression to select stably transfected eukaryotic cells using G418. Bacterial promoter (P) provides kanamycin resistance gene expression (Kan<sup>r</sup>) in E. coli. Kan<sup>r</sup>/Neo<sup>+</sup> gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals.

**Generation of BrUSLEE fusion proteins**

A localization signal or a gene of interest can be cloned into MCS of the vector. It will be expressed as a fusion to the BrUSLEE N-terminus when inserted in the same reading frame as BrUSLEE and no in-frame stop codons are present. The inserted sequence should contain an initiating ATG codon. BrUSLEE-tagged fusions retain fluorescent properties of the native protein allowing fusion localization in vivo. Unmodified vector will express BrUSLEE when transfected into eukaryotic (mammalian) cells.

**Note:** The plasmid DNA was isolated from dam<sup>+</sup>-methylated E. coli. Therefore some restriction sites are blocked by methylation. If you wish to digest the vector using such sites you will need to transform the vector into a dam<sup>-</sup> host and make fresh DNA.

**Exposure in mammalian cells**

pBrUSLEE-N vector can be transfected into mammalian cells by any known transfection method. CMV promoter provides strong, constitutive expression of pBrUSLEE or its fusions in eukaryotic cells. If required, stable transformants can be selected using G418 [Gorman 1985].

**Propagation in E. coli**

Suitable host strains for propagation in E. coli include DH5α, HB101, XL1-Blue, and other general purpose strains. Plasmid incompatibility group is pMB1/ColE1. The vector confers resistance to kanamycin (30 µg/ml) to E. coli hosts. Copy number in E. coli is about 500.