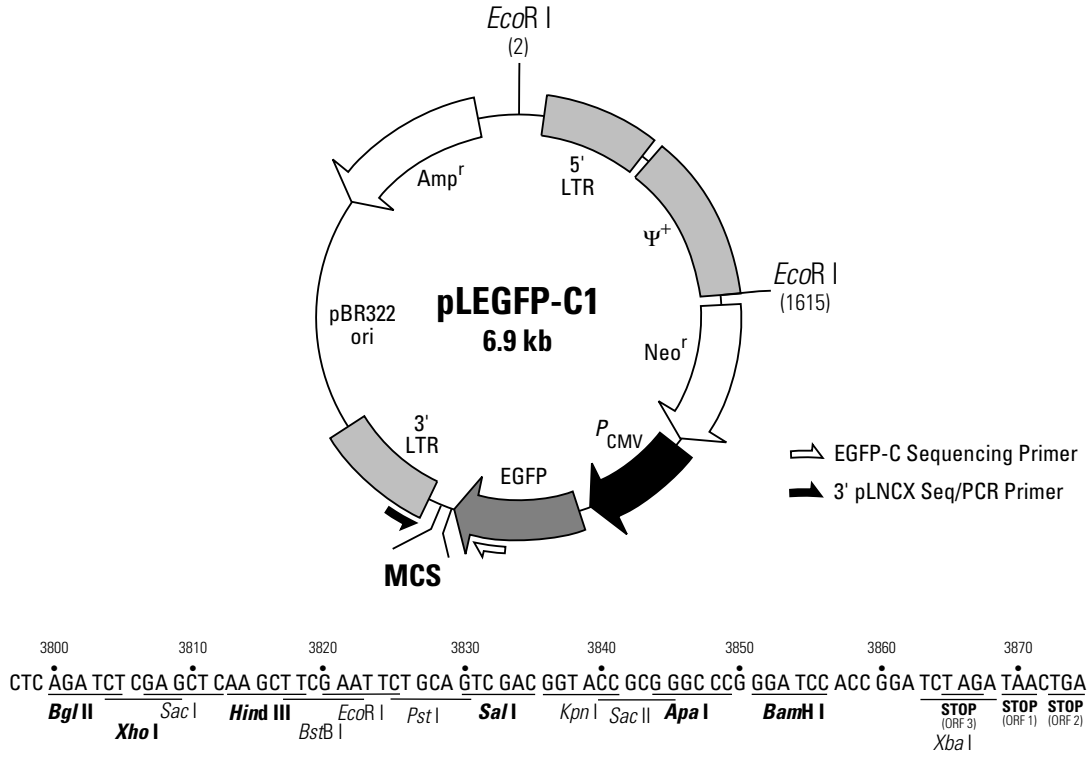

Restriction Map and Multiple Cloning Site (MCS) of pLEGFP-C1 Retroviral Vector. Unique restriction sites are in bold. The *Bst*B I is not unique, although it appears only once in the attached sequence file. As noted below, the vector sequence was created from compiled sequences, but the vector has not been completely sequenced.

Description

pLEGFP-C1 facilitates retroviral delivery and expression of the enhanced green fluorescent protein (EGFP, 1–4) or carboxy-terminal fusions of EGFP to a protein of interest. The retroviral elements in pLEGFP-C1 are derived from a Moloney murine leukemia virus (MoMuLV; 5–8).

EGFP is a red-shifted variant of wild-type green fluorescent protein (GFP) of the jellyfish *Aequorea victoria*. EGFP has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) EGFP includes the GFPmut1 variant (1) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (2). Upstream sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (3) to further increase the translation efficiency in eukaryotic cells.

Upon transfection into a packaging cell line, pLEGFP-C1 can transiently express, or integrate and stably express, a transcript containing Ψ^+ (the extended viral packaging signal), the neomycin selectable marker, and either EGFP or a fusion of EGFP to a gene of interest cloned into the multiple cloning site downstream of the EGFP gene. The 5' viral LTR in this vector contains a viral promoter that controls expression of the neomycin resistance (Neo^r) gene for antibiotic selection in eukaryotic cells. EGFP or an EGFP fusion is expressed from the human cytomegalovirus (CMV) immediate early promoter (P_{CMV}). pLEGFP-C1 also includes the pBR322 origin of replication and *E. coli* Amp^r gene for propagation and antibiotic selection in bacteria.

(PR29958; published 03 October 2002)

Use

pLEGFP-C1 or derivatives espressing EGFP fusions to your gene of interest can be transfected into any of BD Biosciences Clontech packaging cell lines (see **www.bdbiosciences.com** for a complete list). pLEGFP-C1 transcripts produced in the packaging cell contain the Ψ^+ (psi) RNA packaging signal, the neomycin gene, transcription and processing elements, and the gene of interest. pLEGFP-C1 does not contain the structural *gag*, *pol*, and *env* genes necessary for retroviral particle formation and replication; however, these genes are stably integrated in the packaging cell genome. Once in the cell, RNA from the vector is packaged into high-titer, infectious, replication-incompetent retroviral particles (9–12). That is, these retroviral particles can infect target cells and transmit the EGFP or EGFP-fusion gene, but cannot replicate within these cells since the cells lack the viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation.

Location of features

- 5' MoMuLV LTR: 145–733
- Ψ^+ (extended packaging signal): 803–1612
- Neomycin resistance gene (Neo^r): 1656–2450
- Immediate early CMV promoter (P_{CMV}): 2468–3057
- Enhanced green fluorescent protein (EGFP) gene: 3074–3790
  - Kozak consensus translation initiation site: 2931–2941
  - Start codon (ATG): 3074–3076;
  - Last EGFP codon: 3788–3790
  - Stop codon of the protein expressed by the native vector: 3869–3871
    - (Note that the version of EGFP encoded by this vector differs slightly from the original EGFP due to the extra amino-acids at the C-terminus)
  - Stop codons downstream of the MCS: 3865–3867; 3869–3871; 3873–3875;
    - (Note that stop codons may be introduced by inserts)
  - Insertion of Val at position 2: 3077–3079
  - GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 3355–3360
  - His-231 to Leu mutation (A→T): 3768
- Multiple Cloning Site: 3799–3868
- 3' MoMuLV LTR: 3938–4531
- pBR322 plasmid replication region
  - Site of replication initiation: 5068
- Ampicillin resistance gene (β-lactamase): 6687–5827

Sequencing primer locations

- EGFP-C Sequencing Primer (#6478-1 [3727–3748]):
  - 5'-CATGGTCCTGCTGGAGTTCGTG-3'
- 3' primer pLNCX Seq/PCR Primer (#K1060-F; [3960–3935]):
  - 5'-ACCTACAGGTGGGGTCTTTCATTCCC-3'
  - (The 5' primer in this set anneals upstream of the EGFP sequence and is not useful for analyzing derivatives of pLEGFP-C1.)

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pBR322
- Copy number: low

NOTE: The viral supernatants produced by this retroviral vector could, depending on your cloned insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant retrovirus. Appropriate NIH, regional, and institutional guidelines apply.

References:

1. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. *Clontechniques* (April 1996) **XI** (2):2–3.
5. Coffin, J. M. & Varmus, H. E., Eds. (1996) *Retroviruses* (Cold Spring Harbor Laboratory Press, NY).
6. Ausubel, F. M., *et al.* (1994) *Current Protocols in Molecular Biology* (Greene Publishing Associates, Inc. & John Wiley & Sons, Inc.).
7. Miller, A. D. & Rosman, G. J. (1989) *BioTechniques* **7**:980–990.
8. *Clontechniques* (April 1997) **XII** (2):8–9.
9. Mann, R., *et al.* (1983) *Cell* **33**:153–159.
10. Miller, A. D. & Buttimore, C. (1986) *Mol. Cell. Biol.* **6**:2895–2902.
11. Morgenstern, J. P. & Land, H. (1990) *Nucleic Acids Res.* **18**:3587–3590.
12. Miller, A. D. & Chen, F. (1996) *J. Virol.* **70**:5564–5571.