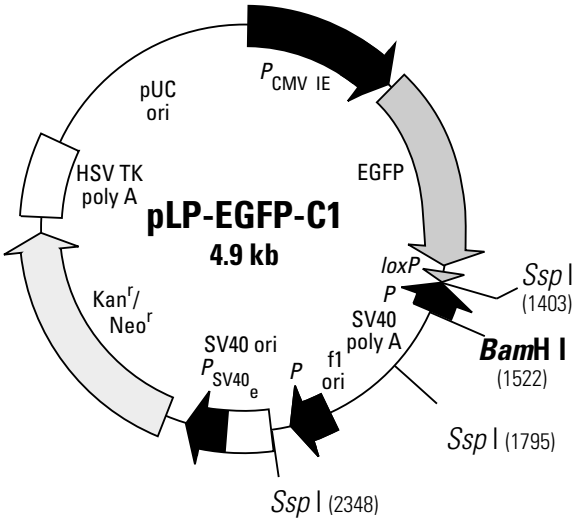

Map of pLP-EGFP-C1 Vector. Unique restriction sites are shown in bold.

Description

pLP-EGFP-C1 Acceptor Vector is designed to be used with BD Creator™ Cloning Kits to rapidly generate a reporter construct expressing a fusion between the protein of interest and enhanced green fluorescent protein (EGFP). Instead of a multiple cloning site (MCS), pLP-EGFP-C1 contains the *loxP* sequence from the P1 bacteriophage (1). In the presence of Cre Recombinase, the *loxP* site allows rapid transfer of a gene of interest from any Creator System donor vector into pLP-EGFP-C1 through Cre-mediated recombination (1). Genes cloned into the donor vector in frame with the *loxP* site will automatically be in frame with EGFP when transferred to pLP-EGFP-C1. The target gene should be cloned into the donor vector so that it is in frame with the upstream *loxP* site with no intervening in-frame stop codons.

The gene of interest is expressed from the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) as a C-terminal fusion to EGFP. EGFP is a red-shifted variant of wild-type GFP (2–4), which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The pLP-EGFP-C1 backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neo^r), consisting of the SV40 early promoter, the neomycin/ kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418 (5). A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

pLP-EGFP-C1 also contains a bacterial promoter adjacent to the *loxP* site. This promoter drives expression of the chloramphenicol resistance gene, which is transferred from the donor vector in conjunction with the gene of interest. The separation of the promoter and the coding sequence on the two parent vectors (pLP-EGFP-C1 and the donor vector), ensures that only recombinant pLP-EGFP-C1 vectors containing the transferred fragment in the correct orientation will be propagated in the presence of chloramphenicol. The inclusion of sucrose in the medium provides further selection against the parent donor vector.

Use

The EGFP fusion protein expressed from pLP-EGFP-C1 can be used to monitor gene expression and protein localization for the gene of interest. Fusions to the C terminus of EGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The

recombinant EGFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (5). pLP-EGFP-C1 can also be used simply to express EGFP in a cell line of interest (e.g., as a transfection marker).

EGFP fluorescence can be observed in living cells by microscopy. Please refer to the Living Colors® User Manual (PT2040-1) provided with this vector for additional information on detection of EGFP.

Location of Features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560
  Transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- Enhanced green fluorescent protein gene
  Start codon (ATG): 613–615; Stop codon: 1408–1410
  Insertion of Val at position 2: 616–618
  GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 805–810
  His-231 to Leu mutation (A→T): 1307
  Last amino acid in wild-type GFP: 1327–1329
- *loxP* site: 1360–1393
- Bacterial promoter: 1394–1520
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1681–1686 &1710 –1715; mRNA 3' ends: 1719 & 1731
- f1 single-strand DNA origin: 1778–2233 (Packages the noncoding strand of EGFP.)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2295–2300; –10 region: 2318–2322
  Transcription start point: 2330
- SV40 origin of replication: 2574–2709
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2407–2478 & 2479–2550
  21-bp repeats: 2554–2574, 2575–2595, & 2597–2617
  Early promoter element: 2630–2636
  Major transcription start points: 2626, 2664, 2670 & 2675
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2758–2760; stop codon: 3550–3552
  G→A mutation to remove *Pst* I site: 2940
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3286
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3788–3793 & 3801–3806
- pUC plasmid replication origin: 4137–4780

Primer Locations

- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: `~500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Sauer, B. (1994) *Curr. Opin. Biotechnol.* **5**:521–527.
2. Prasher, D. C., *et al.* (1992) *Gene* **111**:229–233.
3. Chalfie, M., *et al.* (1994) *Science* **263**:802–805.
4. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* **341**:277–280..
5. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.