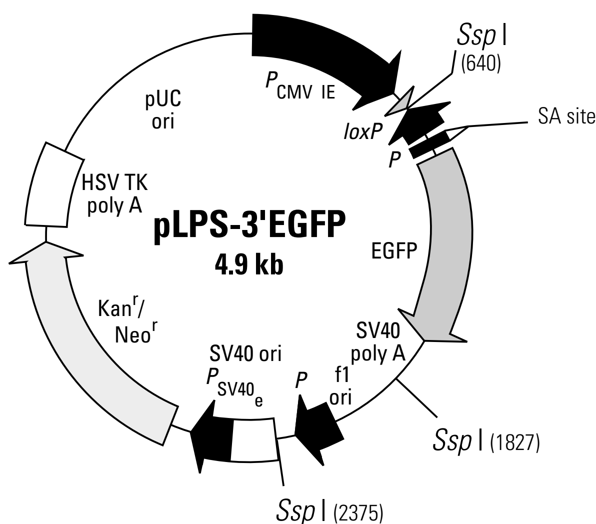

Map of pLPS-3'EGFP Vector. Unique restriction sites are shown in bold.

Description

pLPS-3'EGFP Acceptor Vector is designed to be used with BD Biosciences Clontech pDNR-Dual donor vector to rapidly generate reporter constructs expressing a fusion between the 3' terminal of a protein of interest and enhanced green fluorescent protein (EGFP). Instead of a multiple cloning site (MCS), pLPS-3'EGFP 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 pDNR-Dual into pLPS-3'EGFP through Cre-mediated recombination (1). Genes cloned into the pDNR-Dual in frame with the splice donor (SD) site will automatically be in frame with EGFP when transferred to pLPS-3'EGFP. The target gene should be cloned into the pDNR-Dual so that it is in frame with the SD site with no intervening in-frame stop codons.

pLPS-3'EGFP contains a splice acceptor (SA) site immediately upstream of EGFP. When the recombinant construct is expressed in mammalian cells, EGFP is expressed from the immediate early promoter of cytomegalovirus ($P_{CMV\ IE}$) as a 3' fusion to the gene of interest, through intron splicing. 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 pLPS-3'EGFP 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.

pLPS-3'EGFP 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 (pLPS-3'EGFP and the donor vector), ensures that only recombinant pLPS-3'EGFP 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.

(PR29990; published 04 October 2002)

Use

The EGFP fusion protein expressed from pLPS-3'EGFP can be used to monitor gene expression and protein localization for the gene of interest. Fusions to the N-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.

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$\rightarrow$G mutation to remove *Sac* I site: 569
- *loxP* site: 597–630
- Bacterial promoter: 632–757
- Splice acceptor (SA) site: 784–835
- Enhanced green fluorescent protein gene (without start codon): 846–1559
  GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 1032–1037
  His-231 to Leu mutation (A$\rightarrow$T): 1534
  Last amino acid in wild-type GFP: 1554–1556
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1713–1718 &1742 –1747; mRNA 3' ends: 1751 & 1763
- f1 single-strand DNA origin: 1810–2265 (Packages the noncoding strand of EGFP.)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2327–2332; –10 region: 2350–2354
  Transcription start point: 2362
- SV40 origin of replication: 2606–2741
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2439–2510 & 2511–2582
  21-bp repeats: 2586–2606, 2607–2627, & 2629–2649
  Early promoter element: 2662–2668
  Major transcription start points: 2658, 2696, 2702 & 2707
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2790–2792; stop codon: 3582–3584
  G$\rightarrow$A mutation to remove *Pst* I site: 2972
  C$\rightarrow$A (Arg to Ser) mutation to remove *Bss*H II site: 3318
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3820–3825 & 3833–3838
- pUC plasmid replication origin: 4169–4812

Primer Location

- EGFP-N Sequencing Primer (#6479-1): 906–886

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 Tj /F3 1 Tf 0.285 0 Tpj /F10 1 1Il61 1.2569 -1.125 TD 0.6 1.995 0 Tw77:229*
4.

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.

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