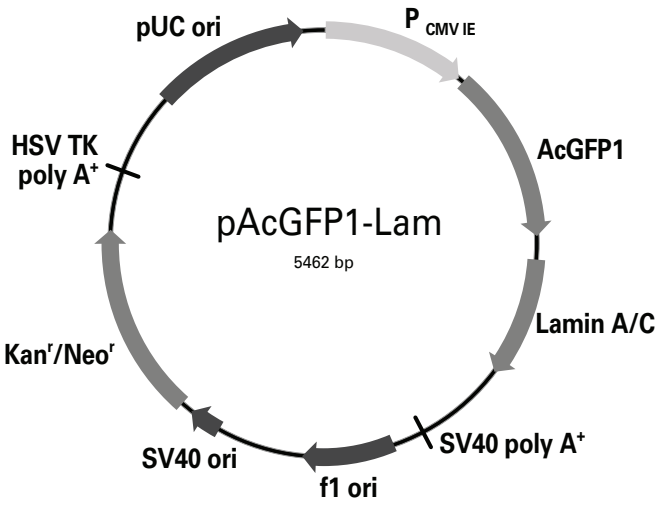
Vector Information

pAcGFP1-Lam Vector Information PT3936-5

Sold as part of Catalog No. 630458



Map of pAcGFP1-Lam Vector. All restriction sites shown are unique.

Description

pAcGFP1-Lamin encodes a green fluorescent protein (GFP) from *Aequorea coerulescens* (excitation maximum = 475 nm; emission maximum = 505 nm) and the gene encoding the human nuclear lamin (1). SV40 polyadenylation signals downstream of the AcGFP1-Lamin fusion direct proper processing of the 3'end of the AcGFP1-Lamin mRNA.

AcGFP1 contains silent mutations that create an open reading frame comprised almost entirely of optimized human codons. These changes increase the translational efficiency of the AcGFP1 mRNA and consequently the expression of AcGFP1 in mammalian and plant cells.

The vector backbone also contains an SV40 origin for replication in any mammalian cell line that expresses 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. A bacterial promoter upstream of this cassette drives expression of the gene encoding kanamycin resistance in *E. coli.* The pAcGFP1-Lamin backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

The pAcGFP1-Lamin is provided in the Matchmaker™ Chemiluminescent Co-IP Vector Set (Cat. No. 630458) as a negative bait control construct. In mammalian cells, this vector expresses the AcGFP1-Lamin fusion protein—the AcGFP1 serves as a tag, through which a polyclonal antibody against AcGFP1 can be used for immunoprecipitation, while lamin serves as the bait for the capture of interacting proteins via coimmunoprecipitation. Because lamin does not interact with SV40 largeT antigen (2), in lysates of cells cotransfected with pAcGFP1-Lamin and pProLabel-T, there is no ProLabel activity detected in the Co-IP of this control for non-interacting pairs of proteins. The fluorescence from AcGFP1-Lamin fusion protein also allows for easy detection of fusion expression and localization *in vivo*, as well as offering a mode for determining transfection efficiency.

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Location of features

- P CMV ie
  Start: 1 End: 589
- AcGFP1
  Start: 613 End: 1329
- Lamin A/C
  Start: 1426 End: 1920
- SV40 early poly A
  Start: 2281 End: 2331
- f1 ori
  Start: 2378 End: 2833
- SV40 ori
  Start: 3174 End: 3309
- Kan R/Neo R
  Start: 3358 End: 4150
- Kanamycin/neomycin resistance gene:
  Neomycin phosphotransferase coding sequences:
  start codon (ATG): 2629–2631
  stop codon: 3421–3423
  G to A mutation to remove Pst I site: 2811
  C to A mutation to remove BssH II site: 3157
- HSV TK poly A
  Start: 4388 End: 4406
- pUC ori
  Start: 4737 End: 5380

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 JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Sturman, N. et al. (1998) *J. Stuct. Biol.* **122**(1–2) 42–66.
2. Matchmaker Co-IP Kit (April 1999) Clontechniques XIV(2): 14–15.
3. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II.* Ed. D.M. Glover. (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 Clontech. This vector has not been completely sequenced.

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CMV Sequence

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