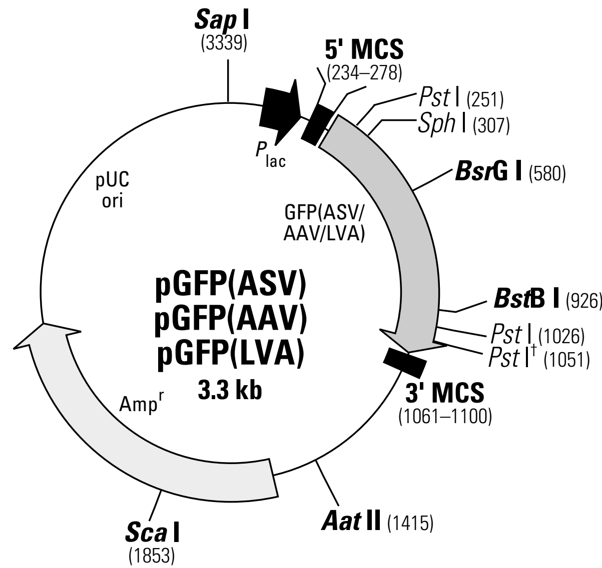
pGFP(ASV/AAV/LVA) Vector Information PT3207-5

GenBank Accession #s: Submissions in progress. Catalog #K6002-1



Restriction Map and Multiple Cloning Sites (MCS) of pGFP(ASV), pGFP(AAV), or pGFP(LVA). Unique restriction sites are in bold. † The *Pst* I site at position #1051 is present in pGFP(AAV) only.

Description:

pGFP(ASV), pGFP(AAV), and pGFP(LVA) Vectors (created by Dr. Jens Bo Andersen) carry destabilized variants of the gene encoding the *Aequorea victoria* green fluorescent protein (GFP). In contrast to wild-type GFP or GFPmut3.1, these dGFP variants have short half-lives in bacteria due to the presence of a C-terminal tail that targets the protein for degradation by specific bacterial proteases (1, 2). These vectors are therefore suitable for use as *in vivo* reporters of gene expression in bacteria (1). The last three amino acids of the destabilizing tail (ASV, AAV, or LVA) impart a specific half-life to each variant. In *E. coli*, GFP(ASV) has a half-life of 110 minutes; GFP(AAV) a half-life of 60 minutes; and GFP(LVA), a half-life of 40 minutes. **Note that half-lives will vary significantly from host to host; these stated half-lives should be considered merely as guidelines with which to design initial experiments.**

All three destabilized variants contain the GFPmut3b mutations (Ser-65 to Gly & Ser-72 to Ala) that result in a brighter chromophore and increase the efficiency of protein folding and chromophore formation at 37°C (1, 4). Each variant was constructed from the GFPmut3.1 parent gene; both the parent and the variants have an excitation maximum at 501 nm, an emission maximum at 511 nm, and are minimally excited by UV light (4). They also contain a Ser-2 to Arg substitution which creates an *Sph* I site at the initiating ATG codon (1). Within the vector, the destabilized GFP (dGFP) coding regions and the ribosome binding site from pJBA27 (2) are located between the two MCSs of the pUC19 derivative pPD16.43 (2, 5). The 5' MCS lies immediately upstream of a synthetic ribosome binding site (RBSII), followed by the dGFP start codon; the 3' MCS lies downstream from the dGFP stop codon. Each dGFP gene was inserted in frame with the *lac*Z initiation codon from pUC19 so that in *E. coli,* dGFP is expressed from the *lac* promoter as a fusion with several additional amino acids,

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including the first five amino acids of the *lacZ* protein. Note, however, that if you excise the dGFP coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., non-fusion) dGFP protein. The pUC19 backbone of pGFP(ASV, AAV, or LVA) provides a high copy number origin of replication and ampicillin resistance gene for propagation in *E. coli*.

Use:

pGFP (ASV, AAV, or LVA) provides a convenient source of a dGFP gene for insertion into other bacterial constructs as a reporter for gene expression. Each dGFP gene can be excised using sites in the flanking MCSs; alternatively, the dGFP coding sequences can be amplified by PCR. The vectors can also be used directly to express the destabilized GFP protein in *E. coli* from the *lac* promoter. Note that the attached sequence file is for pGFP(AAV) only. The sequences of the three vectors differ only in the regions of their destabilization tails.

Location of features:

- *lac* promoter: 95–178
  - CAP binding site: 111–124
  - –35 region: 143–148; –10 region: 167–172
  - Transcription start point: 179
  - *lac* operator: 179–199
- *lac*Z–dGFP fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; stop codon: 1057–1059
- 5' MCS: 234–278
- Synthetic Ribosome Binding Site (RBSII): 286–294
- GFP(ASV, AAV, or LVA) gene
  - Start codon (ATG): 304–306; stop codon: 1057–1059
  - Ser-2 to Arg mutation (A→C) to create *Sph* I site: 307–309
  - GFPmut3.1 chromophore mutations (Ser-65 to Gly: 496–498; Ser-72 to Ala: 517–519)
  - Amino acids from GFPmut3.1: 304–1017
  - Destabilizing tail: 1018–1056
    - GFP(ASV) RPAANDENY**ASV** (no *Pst* I site at position #1051)
    - GFP(AAV) RPAANDENY**AAV** (introduces a *Pst* I site at position #1051)
    - GFP(LVA) RPAANDENY**LVA** (no *Pst* I site at position #1051)
- 3' MCS: 1061–1100
- Ampicillin resistance gene
  - Promoter: –35 region: 1476–1481; –10 region: 1499–1504
  - Transcription start point: 1511
  - Ribosome binding site: 1534–1538
  - β-lactamase coding sequences:
    - Start codon (ATG): 1546–1548; stop codon: 2404–2406
    - β-lactamase signal peptide: 1546–1614
    - β-lactamase mature protein: 1615–2403
- pUC plasmid replication origin: 2554–3197

Primer locations:

- M13 Reverse Sequencing Primer (#6430-1):210–225
- GFP-N Sequencing Primer (#6476-1): 367–346
- GFP-C Sequencing Primer (#6477-1): 957–979

Propagation in *E. coli*:

- Recommended host strain: JM109
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts
- *E. coli* replication origin: pUC
- Copy number: ≈500; plasmid incompatibility group: pMB1/ColE1

References:

1. Andersen, J. B., *et al.* (1998) *Appl. Environ. Microbiol.* **64**:2240–2246.
2. Keiler, K. C., *et al.* (1996) *Science* **271**:990–993.
3. Keiler, K. C. & Sauer, R. T. (1996) *J. Biol. Chem.* **271**:2589–2593.
4. Cormack, B. P., *et al.* (1996) *Gene* **173:** 33–38.
5. Fire, A., *et al.* (1990) *Gene* **93**:189–198.

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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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