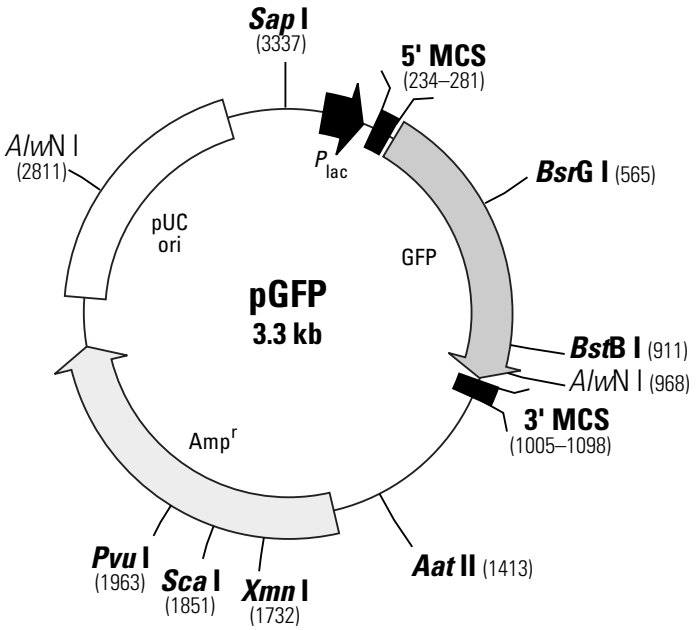
Vector Information

pGFP Vector Information PT2039-5

GenBank Accession No.: U17997 Cat. No. 632370



5' MCS

LacZ

ATG ACCATGATTACGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGA

*Hin*d III *Sph* I *Pst* I *Sal* I *Xba* I

GFP

GGATCCCCGGGTACCGGTAGAAAAA **ATG AGT**

*Bam*H I *Xma* I *Sma* I *Kpn* I *Age* I *Asp*718 I

3' MCS

GFP stop

TAG CATTCGTAGAATTCCAACTGAGCGCCGGTCGCTACCATTACCAA

Bsm I *Eco*R I

CTTGTCTGGTGTCAAAAATAATAGGCCTACTAGTCGGCCGTACGGGCCC

Stu I *Spe* I *Eag* I *BsiW* I *Bsp*120 I *Apa* I

Restriction Map and Multiple Cloning Site (MCS) of pGFP. Unique restriction sites are in bold.

Description:

pGFP carries the complete GFP coding sequence derived from the GFP cDNA by PCR (1, 2). This PCR product was cloned between the two MCSs of the pUC19 derivative pPD16.43 (2, 5). The 5' MCS lies immediately upstream from the GFP start codon; the 3' MCS lies downstream from the GFP stop codon. The GFP gene was inserted in frame with the *lac*Z initiation codon from pUC19 so that in *E. coli,* GFP is expressed from the *lac* promoter as a fusion with several additional amino acids, including the the first five amino acids of the *lacZ* protein. Note, however, that if you excise the GFP coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., non-fusion) GFP protein. The pUC19 backbone of pGFP provides a high copy number origin of replication and ampicillin resistance gene for propagation in *E. coli* .

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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–green fluorescent protein (GFP) fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; stop codon: 1003–1005
- 5' MCS: 234–281
- Green fluorescent protein gene
  - Start codon (ATG): 289–291; stop codon: 1003–1005
  - GFP fluorescent chromophore: 481–489
- 3' MCS: 1005–1098
- Ampicillin resistance gene
  - Promoter: –35 region: 1474–1479; –10 region: 1497–1502
  - Transcription start point: 1509
  - Ribosome binding site: 1532–1536
  - β-lactamase coding sequences:
    - Start codon (ATG): 1544–1546; stop codon: 2402–2404
    - β-lactamase signal peptide: 1544–1612
    - β-lactamase mature protein: 1613–2401
- pUC plasmid replication origin: 2552–3195

Primer location:

- GFP-N Sequencing Primer (#6476-1): 352–331
- GFP-C Sequencing Primer (#6477-1): 942–964

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. Prasher, D. C., *et al.* (1992) *Gene* **111**:229–233.
2. Chalfie, M., *et al.* (1994) *Science* **263**:802–805.
3. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* **341**:277–280.
4. Wang, S. & Hazelrigg, T. (1994) *Nature* **369**:400–403.
5. Fire, A., *et al.* (1990) *Gene* **93**:189–198.