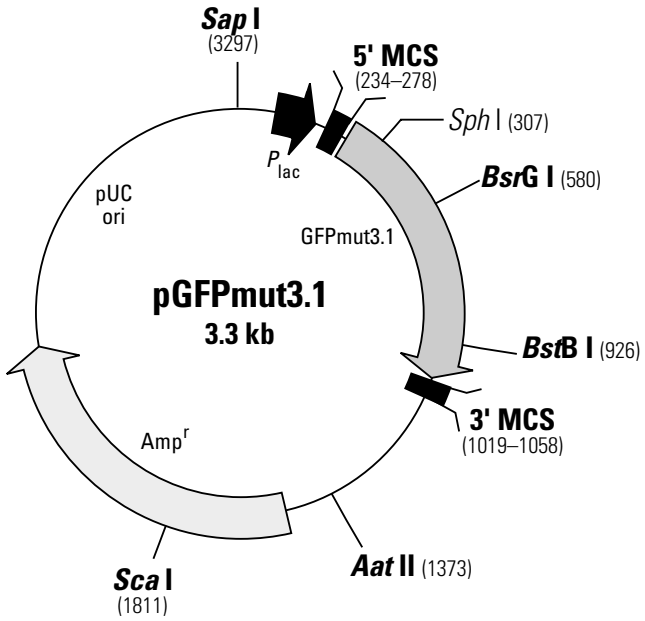
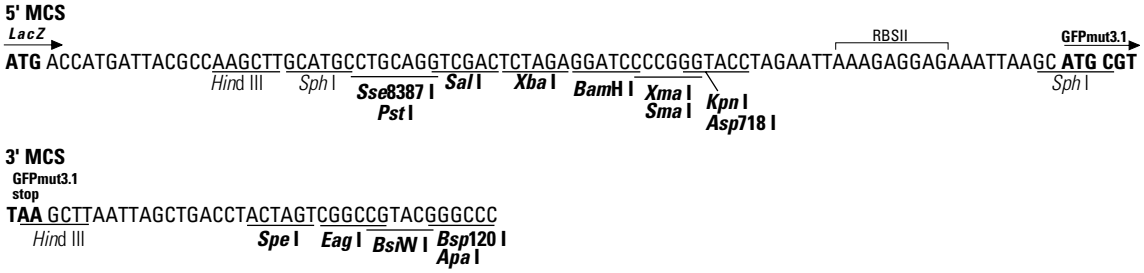
GenBank Accession #: Submission in progress. Catalog #6039-1





Restriction Map and Multiple Cloning Site (MCS) of pGFPmut3.1. Unique restriction sites are in bold.

Description:

pGFPmut3.1 (created by Dr. Jens Bo Andersen) contains the GFPmut3.1 variant of the *Aequorea victoria* green fluorescent protein (GFP). This variant gives very bright green fluorescence when expressed in bacteria (1, 2) because it contains the GFPmut3b mutations (Ser-65 to Gly and Ser-72 to Ala) that increase the efficiency of protein folding and chromophore formation at 37°C (1). The GFPmut3.1 fluorophore has an excitation maximum at 501 nm, an emission maximum at 511 nm, and is minimally excited by UV light (1). The GFPmut3.1 gene also contains a Ser-2 to Arg substitution which creates an *Sph* I site at the initiating ATG codon (2). In pGFPmut3.1, the GFP coding regions and the ribosome binding site from pJBA27 (2) are located between the two MCSs of the pUC19 derivative pPD16.43 (3). The 5' MCS lies immediately upstream of a synthetic ribosome binding site (RBSII), followed by the GFPmut3.1 start codon; the 3' MCS lies downstream from the GFPmut3.1 stop codon. The GFPmut3.1 gene was inserted in frame with the *lac*Z initiation codon from pUC19 so that in *E. coli,* GFPmut3.1 is expressed from the *lac* promoter as a fusion with several additional amino acids, including the first five amino acids of the *lacZ* protein. Note, however, that if you excise the GFPmut3.1 coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., non-fusion) GFPmut3.1 protein. The pUC19 backbone of pGFPmut3.1 provides a high copy number origin of replication and ampicillin resistance gene for propagation in *E. coli.*

(PR93601; published 18 March 1999)

Use:
pGFPmut3.1 provides a convenient source of the GFPmut3.1 gene for insertion into other bacterial constructs as a reporter for gene expression. The GFPmut3.1 gene can be excised using sites in the flanking MCSs; alternatively, the GFPmut3.1 coding sequences can be amplified by PCR. pGFPmut3.1 can also be used directly to express the GFPmut3.1 protein in *E. coli* from the *lac* promoter.

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–GFPmut3.1 fusion protein expressed in *E. coli*
  - Ribosome binding site: 206–209
  - Start codon (ATG): 217–219; stop codon: 1018–1020
- 5' MCS: 234–278
- Synthetic Ribosome Binding Site (RBSII): 286–294
- GFPmut3.1 gene
  - Start codon (ATG): 304–306; stop codon: 1018–1020
  - 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)
- 3' MCS: 1019–1058
- Ampicillin resistance gene
  - Promoter: –35 region: 1434–1439; –10 region: 1457–1462
  - Transcription start point: 1469
  - Ribosome binding site: 1492–1496
  - β-lactamase coding sequences:
    - Start codon (ATG): 1504–1506; stop codon: 2362–2364
    - β-lactamase signal peptide: 1504–1572
    - β-lactamase mature protein: 1573–2361
- pUC plasmid replication origin: 2512–3155

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. Cormack, B. P., *et al.* (1996) *Gene* **173:** 33–38.
2. Andersen, J. B., *et al.* (1998) *Appl. Environ. Microbiol.* **64**:2240–2246.
3. Fire, A., *et al.* (1990) *Gene* **93**:189–198.

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