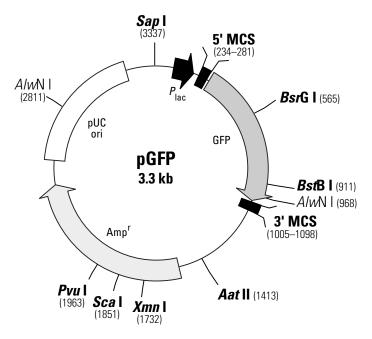
GenBank Accession No.: U17997 Cat. No. 632370



5' MCS

ATG ACCATGATTACGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGA

Hind III Sph I Pst I Sal I Xba I

GGATCCCCGGGTACCGGTAGAAAA ATG AGT

BamH | Xma | Kpn | Age | Sma | Asp718 |

3' MCS GFP

CTTGTCTGGTGTCAAAAATAAT<u>AGGCCTACTAGTCGGCCG</u>TAC<u>GGGCCC</u>

Stu | Spe | Eag | BsiW | Bsp120 |

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



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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 *lacZ* 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*. GFP excitation maxima = 395 nm. and the emission maxima = 509 nm.

(032113)

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

· lacZ-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.

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This document has been reviewed and approved by the Clontech Quality Assurance Department.