



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

Description:

pGFPuv carries the “cycle 3” variant of GFP described by Cramer *et al.* (1). This gene was cloned between the two MCSs of the pUC19 derivative pPD16.43 (2). The GFPuv gene can be easily excised from pGFPuv. Alternatively, the GFPuv coding sequence can be amplified by PCR. The GFPuv gene was inserted in frame with the *lacZ* initiation codon from pUC19 so that a β-galactosidase-GFPuv fusion protein is expressed from the *lac* promoter in *E. coli*. Note, however, that if you excise the GFPuv coding sequence using a restriction site in the 5' MCS, the resulting fragment will encode the native (i.e., non-fusion) GFPuv protein. The pUC backbone of pGFPuv provides a high copy number origin of replication and ampicillin resistance gene for propagation in *E. coli*.

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*-GFPuv fusion protein expressed in *E. coli*
 - Ribosome binding site: 206–209
 - Start codon (ATG): 217–219; Stop codon: 1003–1005
- 5' MCS: 234–281
- GFPuv gene
 - Start codon (ATG): 289–291; Stop codon: 1003–1005
 - GFP chromophore: 481–489
 - wt GFP cDNA sequences (3): 289–454
 - Synthetic GFP gene with "cycle 3" mutations from pBAD-GFPuv (1): 455–1007
 - Cycle 3 mutation F99S (T→C): 584
 - Cycle 3 mutation M153T (T→C): 7Cycle 3
 - Cycle 3 mutation V163A (T→C): 776
 - Cycle 3 silent mutation in L137 (T→C): 699
 - Cycle 3 silent mutation in T225 (A→T): 963
 - Q80R mutation (A→G) (4): 527
 - Arg codons optimized for *E. coli*: R73 (AgA→CgT): 505–507, R96 (AgA→CgC): 574–576, R12(AgA→CgT): 652–654, R168 (AgA→CgC): 790–792, R1215 (AgA→CgT): 931–933
 - Silent mutations (CccA→TccG) creating *BspE* I site: 510 & 513
 - Silent mutation (A→G) creating *Mlu* I site: 612
 - Silent mutations (TtGgaA→CtCgaG) creating *Xho* I site: 709, 711 & 714
 - Silent mutations (AG→TC) creating *BamH* I site: 811–812
 - Silent mutation (C→G) creating *Sal* I site: 894
 - Silent mutations (ActA→GctC) creating *Sac* I site: 993 & 996
 - Silent mutation in S72 (A→C): 504
- 3' MCS: 107–1091
- Ampicillin resistance gene
 - Promoter: –35 region: 1467–1472; –10 region: 1490–1495
 - Transcription start point: 1502
 - Ribosome binding site: 1525–1529
 - β-lactamase coding sequences:
 - Start codon (ATG): 1537–1539; Stop codon: 2395–2397
 - β-lactamase signal peptide: 1537–1605
 - β-lactamase mature protein: 1606–2394
- pUC plasmid replication origin: 2545–3188

Primer Location:

- GFP-N Sequencing Primer: 331–352
(Note: The GFP-C Sequencing Primer cannot be used with pGFPuv.)

Propagation in *E. coli*:

- Recommended host strain: JM109 or DH5α
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) on *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References:

1. Cramer, A., *et al.* (1996) *Nature Biotechnol.* **14**:315–319.
2. Fire, A., *et al.* (1990) *Gene* **93**:189–198.
3. Prasher, D. C., *et al.* (1992) *Gene* **111**:229–233.
4. Chalfie, M., *et al.* (1994) *Science* **263**:802–805.

