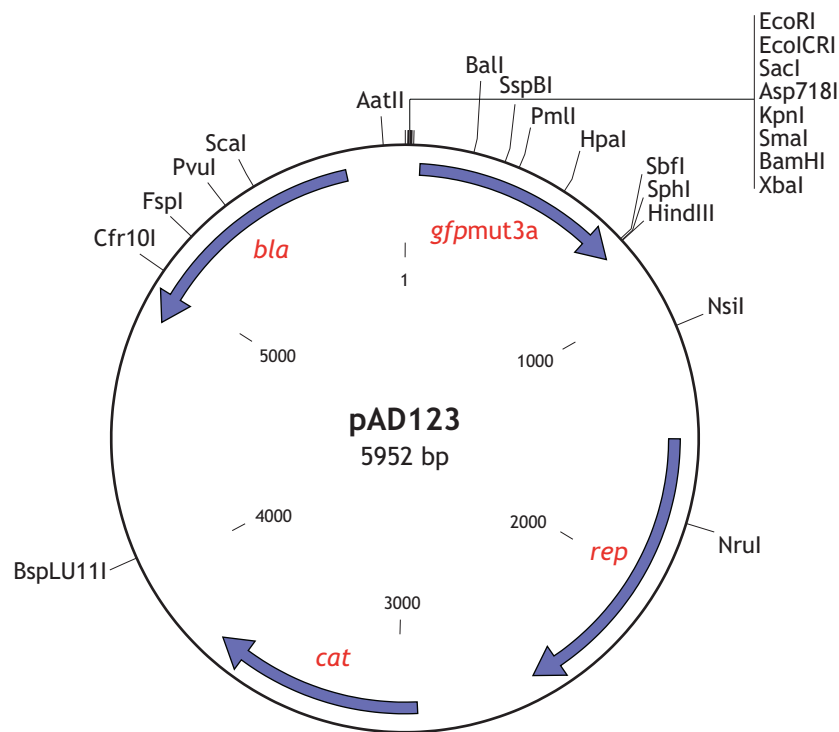


New Gram-Positive – *E. coli* Shuttle Vector for Construction of Green Fluorescent Protein Fusions



BGSC Accession: ECE165 (see also ECE166)

Original Code: DH5 α (pAD123)

Reference: Dunn, A. K., and J. Handelsman. 1999. A vector for promoter trapping in *Bacillus cereus*. *Gene* **226**:297-305

Sequence: Not in database; available from BGSC at <http://www.bgsc.org/sequences/pAD123.htm>

Features:

- gfpmut3a* promoter-less gene encoding a variant of Green Fluorescent Protein from plasmid pFPV25 Valdivia, R. H. and S. Falkow. 1997. *Science* **277**:2007-2011
- rep* Replication initiation protein from cryptic rolling circle plasmid pTA1060 (GenBank U32380) from *Bacillus subtilis* "natto".
- cat* encodes chloramphenicol acetyl transferase; selectable in either *E. coli* or *B. subtilis* (chloramphenicol 5 μ g/ml)
- bla* encodes β -lactamase; selectable in *E. coli* only (ampicillin 100 μ g/ml)

Description: pAD123 is a shuttle vector, replicating in *E. coli* from the pBR322 origin and in *Bacillus* from the pTA1060 origin. A promoter-containing fragment, inserted in the correct orientation within the multiple cloning site upstream from *gfpmut3a*, allows for expression of a Green Fluorescent Protein variant.

Construction: pAD123 was constructed from portions of the plasmids pKK232-8(*bla* and pBR322 origin), pHP13 (*cat* and pTA1060 origin) and pFPV25 (promoterless *gfpmut3a*).

Use: Insertion of a functional promoter upstream of *gfpmut3a* allows the expression of a mutant GFP that has been optimized for use in fluorescence-activated cell sorting, with an optimal excitation wavelength of 498 nm. This technique has great power to isolate sets of promoters that are active under specific environmental or physiological conditions. This shuttle vector should replicate in a wide variety of Gram-positive organisms along with *E. coli*. Plasmid pAD43-25 (in our strain ECE166) contains a 1 kb *Bacillus cereus* chromosomal DNA insert in pAD123 that allows for high-level constitutive expression of GFP during vegetative growth.

Our thanks to Anne K. Dunn for donating pAD123 and pAD43-25 to the BGSC Collection!