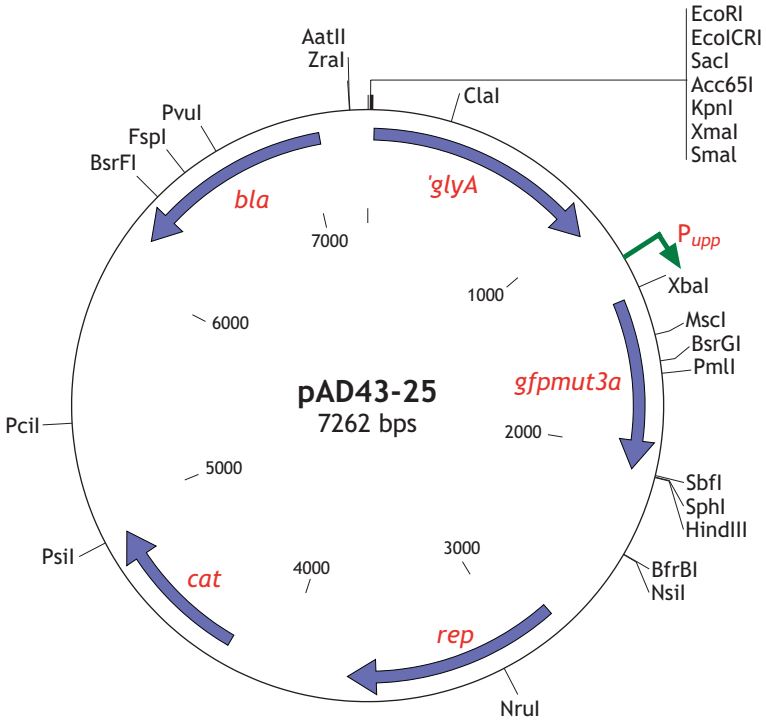
Bacillus Genetic Stock Center

New Product Announcement *February 2003*

New Gram-Positive — *E. coli* Shuttle Vector With Constitutive Expression of Green Fluorescent Protein



BGSC Accession: ECE166 (see also ECE165) **Orignal Code:** DH5α(pAD43-25)

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/pAD43-25.htm

Features:

*gfp*mut3a	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 μg/ml)
bla	encodes β-lactamase; selectable in *E. coli* only (ampicillin 100 μg/ml)
'glyA	last 1010 bp of the *Bacillus cereus* structural gene for glycine/serine hydroxymethyltransferase
P_{upp}	constitutive promoter from the *Bacillus cereus upp* (uracil phosphoribosyltransferase) gene

Description: pAD43-25 is a shuttle vector, replicating in *E. coli* from the pBR322 origin and in *Bacillus* from the pTA1060 origin. A chromosomal fragment from *Bacillus cereus* UW85, containing the first 21 bp of the *upp* gene and all of its upstream regulatory regions, allows for high-level constitutive expression of a Green Fluorescent Protein variant.

Construction: pAD43-25 was constructed by ligating chromosomal DNA *Sau3*A fragments from UW85 into the pAD123 BamHI site and screening for clones that express high levels of GFP in *Bacillus cereus* during vegetative growth.

Use: The placement of the *B. cereus* UW85 *upp* promoter upstream of *gfp*mut3a allows for constitutive 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 shuttle vector should replicate in a wide variety of Gram-positive organisms along with *E. coli*. Plasmid pAD43-25 should serve as a useful marker for quantifying or sorting cells in a wide variety of applications.

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