Bacillus Genetic Stock Center

New Product Announcement *April 2002*

New Fluorescent Protein Tagging Vectors

The *Bacillus* Genetic Stock Center is pleased to offer 16 new vectors designed for constructing fluorescent protein fusions. Three of the vectors come from the laboratory of Wolfgang Schumann at the University of Bayreuth, Germany, while the remaining 13 come from Peter Lewis at the University of Newcastle, Australia. We thank these researchers for their generosity in making their vectors available to the Gram-positive genetics community through the BGSC.

In choosing an appropriate vector, please take note that each contains one of six fluorescent tagging sequences. Three encode mutant GFP with altered excitation maxima. GFP+ and GFPmut1 have red-shifted excitation maxima so that they will appear brighter with conventional FITC filters. GFPuv is excited in the UV range, allowing it to be detected separately from other GFP variants. The cyan and yellow proteins (CFP and YFP) are altered in their emission maxima, making them easily distinguishable from GFP in doubly-labeled cells. The gene encoding dsRed, also easily distinguishable from the other markers, was isolated from a different organism than was the GFP gene. This set of vectors, then, should allow double or even triple labeling of Gram-positive cells.

The table below highlights other differences as well: whether the tag is fused to the N-terminus or C-terminus of the expressed protein; whether the construct integrates into the chromosome by single recombination at the cloned locus or by double recombination at the *B. subtilis amyE* locus; and whether the construct contains an inducible promoter for the expression of either the gene fusion (at *amyE*) or any downstream markers (at the chromosomal locus). Finally, please note that the Schumann vectors are freely available, while the Lewis vectors are available only to non-profit users. For-profit users should inquire at peter.lewis@newcastle.edu.au for a materials transfer agreement.

Strain	Plasmid	Tag	Fused	Integrates	Marker	Size	Features	Restrictions	Ref
ECE149	pMutin-GFP+	GFP+	C-ter	Insert	Em	6.2	IPTG-inducible expression of downstream genes	None	(2)
ECE150	pMutin-CFP	CFP	C-ter	Insert	Em	6.2	IPTG-inducible expression of downstream genes	None	(2)
ECE151	pMutin-YFP	YFP	C-ter	Insert	Em	6.2	IPTG-inducible expression of downstream genes	None	(2)
ECE152	pSG1151	GFPmut1	C-ter	Insert	Cm	4.6		Academic	(3)
ECE153	pSG1154	GFPmut1	C-ter	*amyE*	Sp	7.6	Fusion is xylose inducible	Academic	(3)
ECE154	pSG1156	GFPuv	C-ter	Insert	Cm	4.6		Academic	(3)
ECE155	pSG1164	GFPmut1	C-ter	Insert	Cm	4.8	Xylose-inducible expression of downstream genes	Academic	(3)
ECE156	pSG1170	GFPuv	C-ter	Insert	Cm	6.7	IPTG-inducible expression of downstream genes	Academic	(3)
ECE157	pSG1186	CFP	C-ter	Insert	Cm	4.6		Academic	(1)
ECE158	pSG1187	YFP	C-ter	Insert	Cm	4.6		Academic	(1)
ECE159	pSG1190	CFP	N-ter	*amyE*	Sp	7.6	Fusion is xylose inducible	Academic	(1)
ECE160	pSG1191	YFP	N-ter	*amyE*	Sp	7.6	Fusion is xylose inducible	Academic	(1)
ECE161	pSG1192	CFP	C-ter	*amyE*	Sp	7.6	Fusion is xylose inducible	Academic	(1)
ECE162	pSG1193	YFP	C-ter	*amyE*	Sp	7.6	Fusion is xylose inducible	Academic	(1)
ECE163	pSG1194	dsRed	C-ter	Insert	Cm	4.6		Academic	(1)
ECE164	pSG1729	GFPmut1	N-ter	*amyE*	Sp	7.6	Fusion is xylose inducible	Academic	(3)

References:

1. **Feucht, A., and P. J. Lewis.** 2001. Improved plasmid vectors for the produciton of multiple fluorescent protein fusions in *Bacillus subtilis*. Gene **264:**289-297.
2. **Kaltwasser, M., T. Wiegert, and W. Schumann.** 2002. Construction and Application of Epitope- and Green Fluorescent Protein-Tagging Integration Vectors for *Bacillus subtilis*. Appl. Environ. Microbiol. **68:**2624-2628.
3. **Lewis, P. J., and A. L. Marston.** 1999. GFP vectors for controlled expression and dual labelling of protein fusions in *Bacillus subtilis*. Gene **227:**101-109.