Bacillus Genetic Stock Center

New Product Announcement *February 2009*

NOVEL INDUCIBLE PROTEIN DEGRADATION VECTORS FOR *BACILLUS SUBTILIS*

In the post-genomics era, one critical need is to identify the functions of novel proteins predicted by sequence data. Simple gene knockouts remain a powerful tool for creating loss of function mutants, and the BGSC offers a variety of integration vectors to facilitate their construction. For many studies, however, other kinds of tools are required. If one is analyzing an essential gene, for example, a knockout insertion is by definition not possible. What is needed is a system to regulate the activity of the gene or its product, a switch to turn it off or on at will so that the phenotypic effects can be observed in real time.

Most such tools rely on the inducible expression of the gene of interest. A fusion, usually created in an integration vector, typically places the gene under the control of an inducible promoter. When the inducer is present, the gene is expressed; when it is withdrawn, the gene product is no longer synthesized. Normal turnover gradually depletes the concentration of the protein, and the null phenotype is eventually observed. These types of tools, such as the pMUTIN series of integration vectors, have provided invaluable insights into protein function. The transcriptional depletion strategy is not without drawbacks, however. Protein turnover can be slow, and the time required to reduce the protein concentration to the threshold for expressing a null phenotype can be significant.

A novel system, relying on inducible protein degradation rather than inducible expression, has been developed by Kevin Griffith and Alan Grossman at the Massachusetts Institute of Technology. This strategy cleverly makes use of the naturally occurring protein degradation system widely occurring among bacteria, in which nascent peptides "stuck" in stalled ribosomes are tagged with a signal, called *ssrA*, marking the peptide for degradation with the host ClpXP protease. Adaptor proteins, called SspB, can interact with the tag to tether it more efficiently to the protease. The Griffth and Grossman system makes use of *ssrA* and SspB components from either *Escherichia coli* or *Caulobacter crescentus* along with the native *B. subtilis* ClpXP protease to facilitate inducible protein degradation in *B. subtilis*.

First, one must tag the 3' end of the gene of interest by fusing about 500 bases in frame with *ssrA* in one of the pKG series of vectors (see Table 1). Plasmids pKG1268 and pKG1520 are integration vectors that tag the gene with a modified *E. coli ssrA*, with selection for chloramphenicol and kanamycin, repsectively. Similarly, the gene can be tagged with a modified *C. crescentus ssrA* sequence using integrative vectors pKG1522 (kanamycin selection) or pKG1523 (chloramphenicol selection). Other vectors allow the user to tag the protein with an epitope for easier detection and isolation, along with the *ssrA* tag (see Table 1). All the vectors have an XbaI site upstream and in-frame with the tag and a stop codon, with an SphI site immediately downstream from the tag.

Second, one must integrate the vector containing the tagged gene into a specially designed *B. subtilis* host. These strains each have the *sspB* gene from *E. coli* or *C. crescentus* under control of an ITPG-inducible or xylose-inducible promoter. In the absence of inducer, the protein of interest accumulates in the cell normally. When the inducer is added, however, the protein is rapidly degraded by the *B. subtilis* ClpX. In some cases, significant degradation can be observed in only a few minutes. One such strain (1A880) has both a xylose-inducible *E. coli sspB* and an IPTG-inducible *C. crescentus sspB.* In this host, two proteins can be degraded either individually or simultaneously, and the combined effect of their loss can be observed.

We are excited to make the strains and plasmids listed in Table 1 available to the *Bacillus* genetics community and thank the Grossman lab for donating them to the BGSC.

REFERENCE:

Griffith, K. L. and A. D. Grossman. 2008. Inducible protein degradation in *Bacillus* subtilis using heterologous peptide tags and adaptor proteins to target substrates to the protease ClpXP. Mol. Microbiol. **70:**1012-25.

Table 1. Strains and plasmids facilitating inducible degradation of proteins in *Bacillus subtilis*

BGSC №	Original	Description*	Notes
1A875	KG844	*amyE::*{Pspank-(Ec)*sspB spc*}	IPTG-inducible expression of *E. coli* SspB adaptor
1A876	KG1030	*amyE::*{Pspank-(Cc)*sspB spc*}	IPTG-inducible expression of *C. crescentus* SspB adaptor
1A877	KG1096	*lacA::*{Pxyl-(Ec)*sspB tet*}	Xylose-inducible expression of *E. coli* SspB adaptor
1A878	KG1098	*amyE::*{Pspank(T-7A)-(Ec)*sspB spc*}	IPTG-inducible expression of *E. coli* SspB; reduced basal expression
1A879	KG1100	*amyE::*{Pspank(T-35C)-(Ec)*sspB spc*}	IPTG-inducible expression of *E. coli* SspB; reduced basal expression
1A880	KG1133	*lacA::*{Pxyl-(Ec)*sspB tet*} *amyE::*{Pspank-(Cc)*sspB spc*}	IPTG-inducible expression of *C. crescentus* SspB; xylose-inducible expression of *E. coli* SspB
ECE206	DH5α(pKG1268)	pGEM*cat*-(Ec)ssrA*	For fusing *E. coli* ssrA tag to 3' end of gene; chloramphenicol selection
ECE207	DH5α(pKG1520)	pGEM*kan*-(Ec)ssrA*	For fusing *E. coli* ssrA tag to 3' end of gene; kanamycin selection
ECE208	DH5α(pKG1522)	pGEM*kan*-(Cc)ssrA(A2D)-ALGG	For fusing *C. crescentus* ssrA tag to 3' end of gene; kanamycin selection
ECE209	DH5α(pKG1523)	pGEM*cat*-(Cc)ssrA(A2D)-ALGG	For fusing *C. crescentus* ssrA tag to 3' end of gene; chloramphenicol selection
ECE210	DH5α(pKG1524)	pGEM*cat*-HA-(Ec)ssrA*	For fusing HA epitope with *E. coli* ssrA tag to 3' end of gene
ECE211	DH5α(pKG1525)	pGEM*cat*-his_6-(Ec)ssrA*	For fusing His_6 epitope with *E. coli* ssrA tag to 3' end of gene
ECE212	DH5α(pKG1526)	pGEM*cat*-myc-(Ec)ssrA*	For fusing Myc epitope with *E. coli* ssrA tag to 3' end of gene
ECE213	DH5α(pKG1527)	pGEM*cat*-his_6-(Cc)ssrA*	For fusing His_6 epitope with *C. crescentus* ssrA tag to 3' end of gene
ECE214	DH5α(pKG1528)	pGEM*cat*-myc-(Cc)ssrA*	For fusing Myc epitope with *C. crescentus* ssrA tag to 3' end of gene

*All *B.* subtilis strains were constructed in the JH642(=BGSC 1A96 and) and therefore carry the auxotrophic markers *pheA1* and *trpC2.*