- *Financial Contributions:* The BGSC relies on corporate strain sales and contributions to purchase equipment and undertake special projects not covered by the NSF grant. The Ohio State University Development Fund has a separate account for the BGSC. Contributions are tax deductible to the full extent of the law. Please contact us if you wish to make such a contribution.

How to order cultures

There are several ways to place orders with or request information from the BGSC:

- E-mail: zeigler.1@osu.edu
- Internet: www.bgsc.org
- Phone: (+1) 614-292-5550
- FAX: (+1) 614-292-3206
- Mail: Daniel R. Zeigler, Ph.D.
  Department of Biochemistry
  The Ohio State University
  484 West Twelfth Avenue
  Columbus, OH 43210 (USA)

Academic, government, and other non-profit users need only specify the strains, plasmids, or phages they are require and supply a full shipping address. For-profit corporate users should also supply a purchase order number or procurement card number and expiration date (Visa, MasterCard, and American Express accepted). We make every effort to respond to all inquiries within one working day of receipt, together with an estimated delivery date if an order has been placed.

Pricing information—*Please note the changes!*

- *Academic, Government, and Non-Profit Users—*

  Not-for-profit users are requested to pay a $195 yearly subscription fee. This subscription entitles the user to receive up to 20 strains over a twelve-month period. Alternatively, a user may purchase individual strains at $35 each. If you are lacking inresearch support, please contact us to inquire about a fee waiver for a particular order.

- *For-profit Corporate Users—*

  Users may purchase cultures as needed at $135 per item. Shipping via UPS is included for all domestic orders and for international orders of two or more items. A pre-paid service plan is available for $1950, entitling the user to receive up to 50 cultures within the next twelve calendar months at no additional cost. UPS delivery is included (maximum of five express deliveries per year on international shipments).

Integration Vectors—Quick Reference

Vector			Integration		Select	Upstream	Fusion		Downstream
Name	Strain	Map	Locus	Portion	with	Promoter	Tag	ORF's	Promoter
pBGSC6	ECE22	Detail	Cloned locus	Plasmid	Cm				
pCP115	ECE19	Detail	Cloned locus	Plasmid	Cm				
pMutin4	ECE139	Detail	Cloned locus	Plasmid	Em		β-Gal	C-term	Pspac
pDG364	ECE46	Detail	*B. sub amyE*	Cassette	Cm				
pMLK83	ECE103	Detail	*B. sub amyE*	Cassette	Nm		β-Glu	C-term	
pDG1661	ECE112	Detail	*B. sub amyE*	Cassette	Cm		β-Gal	C-term	
pDG1662	ECE113	Detail	*B. sub amyE*	Cassette	Cm				
pDG1728	ECE114	Detail	*B. sub amyE*	Cassette	Sp		β-Gal	C-term	
pDG1730	ECE115	Detail	*B. sub amyE*	Cassette	Sp				
pDG1663	ECE116	Detail	*B. sub thrC*	Cassette	Em		β-Gal	C-term	
pDL	ECE144	Detail	*B. sub amyE*	Cassette	Cm		BgaB	C-term	
pDK	ECE143	Detail	*B. sub amyE*	Cassette	Km		BgaB	C-term	
pDG1664	ECE117	Detail	*B. sub thrC*	Cassette	Em				
pDG1729	ECE118	Detail	*B. sub thrC*	Cassette	Sp		β-Gal	C-term	
pDG1731	ECE119	Detail	*B. sub thrC*	Cassette	Sp				
pAX01	ECE137	Detail	*B. sub lacA*	Cassette	Em	Pxyl			
pA-spac	ECE138	Detail	*B. sub lacA*	Cassette	Em	Pspac			
pPolHis1	ECE120	Detail	*B. sub rpoC*	Plasmid	Sp		His_6	C-term	
pSAS144	ECE142	Detail	*B. sub dif*	Plasmid	Cm				
pMUTIN-Flag	ECE146	Detail	Cloned locus	Plasmid	Em		FLAG	C-term	Pspac
pMUTIN-cMyc	ECE147	Detail	Cloned locus	Plasmid	Em		cMyc	C-term	Pspac
pMUTIN-HA	ECE148	Detail	Cloned locus	Plasmid	Em		HA	C-term	Pspac
pMUTIN-GFP+	ECE149	Detail	Cloned locus	Plasmid	Em		GFP+	C-term	Pspac
pMUTIN-CFP	ECE150	Detail	Cloned locus	Plasmid	Em		CFP	C-term	Pspac
pMUTIN-YFP	ECE151	Detail	Cloned locus	Plasmid	Em		YFP	C-term	Pspac
pSG1151	ECE152	Detail	Cloned locus	Plasmid	Cm		GFPmut1	C-term	
pSG1156	ECE154	Detail	Cloned locus	Plasmid	Cm		GFPuv	C-term	
pSG1186	ECE157	Detail	Cloned locus	Plasmid	Cm		CFP	C-term	
pSG1187	ECE158	Detail	Cloned locus	Plasmid	Cm		YFP	C-term	
pSG1194	ECE163	Detail	Cloned locus	Plasmid	Cm		dsRed	C-term	
pSG1164	ECE155	Detail	Cloned locus	Plasmid	Cm		GFPmut1	C-term	Pxyl
pSG1170	ECE156	Detail	Cloned locus	Plasmid	Cm		GFPuv	C-term	Pspac
pSG1154	ECE153	Detail	*B. sub amyE*	Cassette	Sp	Pxyl	GFPmut1	C-term	
pSG1192	ECE161	Detail	*B. sub amyE*	Cassette	Sp	Pxyl	CFP	C-term	
pSG1193	ECE162	Detail	*B. sub amyE*	Cassette	Sp	Pxyl	YFP	C-term	
pSG1729	ECE164	Detail	*B. sub amyE*	Cassette	Sp	Pxyl	GFPmut1	N-term	
pSG1190	ECE159	Detail	*B. sub amyE*	Cassette	Sp	Pxyl	CFP	N-term	
pSG1191	ECE160	Detail	*B. sub amyE*	Cassette	Sp	Pxyl	YFP	N-term	

Selected Methods

β-Galactosidase Assays

Permeabilization with lysozyme:

1. Measure the OD_{595} of the culture. Centrifuge 1.0 ml of culture 1 min at top speed in a microcentrifuge.
2. Discard supernatant; wash pellet in 0.5 ml of ice-cold 25 mM Tris·HCl, pH 7.4, centrifuging as before.
3. Discard supernatant; freeze pellet in dry ice/ethanol bath. The pellet can be stored at -20°C until the assay.
4. Suspend the pellet in 0.64 ml Z buffer (below). Add 0.16 ml of lysozyme stock (made up 2.5 mg/ml in Z buffer on day of use). Vortex briefly. Incubate at 37°C for 5 min.
5. Add 8 µl of 10% Triton X100. Vortex briefly. Store on ice.
6. Pre-warm the extract for 2-5 min in a 30°C water bath. Add 0.2 ml ONPG solution (4.0 mg/ml *o*-Nitrophenyl-β-D-galactoside in Z buffer). Note the time. Incubate at 30°C.
7. When the color is clearly yellow, stop the reaction by adding 0.4 ml of 1 M Na_2CO_3. Record the time.
8. Centrifuge the sample for 5 min a microcentrifuge. Read the A_{420} of the supernatant against a blank prepared from 0.8 ml Z buffer, 0.2 ml ONPG, and 0.4 ml Na_2CO_3.

Unit Definition: $(1000 \times A_{420})/(\text{reaction time (min)} \times OD_{595})$

Permeabilization with toluene:

1. Measure the OD_{595} of the culture. Centrifuge 1.0 ml of culture 1 min at top speed in a microcentrifuge.
2. Discard the supernatant and freeze the pellet at -70°C.
3. Add 1.0 ml of Z buffer to the frozen pellet. Add one drop (10 µl) toluene and vortex for 15 s.
4. Pre-warm the extract for 2-5 min in a 30°C water bath. Add 0.2 ml ONPG solution (4.0 mg/ml *o*-Nitrophenyl-β-D-galactoside in Z buffer). Note the time. Incubate at 30°C.
5. When the color is clearly yellow, stop the reaction by adding 0.5 ml 1 M Na_2CO_3. Record the time.

Unit Definition: $1000 \times (A_{420}\text{-}(1.75 \times OD_{550})/(\text{reaction time (min)} \times OD_{595})$ (Miller Units)

Assay with MUG (4-Methylumbelliferyl-β-D-galactopyranoside):

1. Centrifuge 0.5 ml of culture by a 1-min centrifugation at top speed in a microcentrifuge.
2. Suspend the cell pellet in 0.6 ml of Z buffer containing lysozyme (200 µg/ml) and Dnase I (100 µg/ml)
3. Add 0.2 ml of MUG (40 µg/ml in Z buffer). Incubate at 30°C for 40 min.
4. Stop the reaction by adding 0.4 ml 1 M Na_2CO_3.
5. Centrifuge the sample for 5 min a microcentrifuge.
6. Measure the fluorescence of the supernatant using excitation at 365 nM and emission at 450 nm.

Unit Definition: 1 unit catalyzes the production of 1 pmol of MUG per min. Calibrate the fluorometer with standard solutions of MUG.

Z-buffer:

$Na_2HPO_4 \cdot 7H_2O$	60 mM
NaH_2PO_4	40 mM
KCl	10 mM
$MgSO_4 \cdot 7H_2O$	1 mM
*β-mercaptoethanol, pH 7.0	50 mM

*add on day of use

References:

Cutting, S. M. and P. B. Vander Horn. 1990. Genetic Analysis, p. 27-74. *In* C. R. Harwood and S. M. Cutting (ed.), Molecular Biological Methods for *Bacillus.* John Wiley, Chichester.

Return to Table of Contents

Thermostable β-Galactosidase Assay

Follow the directions for the standard β-Galactosidase assay, increasing incubation temperature to 55°C.

Unit Definition: One unit hydrolyzes 1 nmol of substrate per min.

References:

Yuan, G. and S.-L. Wong. 1995. Regulation of *groE* expression in *Bacillus subtilis:* the involvement of the σ^A-like promoter and the roles of the inverted repeat sequence (CIRCE). J. Bacteriol. **177:**5427-5433.

Hirata, H., T. Fukazawa, S. Negoro, and H. Okada. 1986. Structure of a β-galactosidase gene of Bacillus stearothermophilus. J. Bacteriol. **166:**722-727.

Amylase Production Screening

To test for the production of α-amylase:

1. Inoculate transformant colonies to be tested as small patches onto a rich medium (LB or TBAB) supplemented with 1% soluble starch (Sigma Catalog No. S5651 or other similar product). Grow overnight at 30-37°C.
2. Flood the surface of the plate with 5 ml of Gram's iodine stain (a solution of 0.5% w/v iodine and 1% potassium iodide, Sigma HT90-2 or similar product). Incubate at room temperature for 1 min.
3. Decant the stain and observe plates for clear haloes around the colony. Colonies without significant zones of clearing are amylase negative, and therefore presumed to be *amy* knockouts.

References:

Cutting, S. M. and P. B. Vander Horn. 1990. Genetic Analysis, p. 27-74. *In* C. R. Harwood and S. M. Cutting (ed.), Molecular Biological Methods for *Bacillus.* John Wiley, Chichester.

β-Glucuronidase Assay

Follow the directions for the β-Galactosidase assay of lysozyme-permeabilized cells on the previous page. Substitute *p*-nitrophenyl β-D-glucuronidide (4 mg/ml) for the ONPG solution in the protocol.

Unit Definition: Karow and Piggot (1995) expressed specific activity is as nmol *p*-nitrophenyl β-D-glucuronidide hydrolyzed per minute per mg of bacterial dry weight. Others might find it more convenient to define units in terms of OD_{595} of original culture rather than bacterial dry weight.

References:

Karow, M. L. and P. J. Piggot. 1995. Construction of *gusA* transcriptional fusion vectors for *Bacillus subtilis* and their utilization for studies of spore formation. Gene **163:**69-74.

Nicholson, W. L. and P. Setlow. 1990. Sporulation, germination and outgrowth, p. 391-429. *In* C. R. Harwood and S. M. Cutting (ed.), Molecular Biological Methods for *Bacillus.* John Wiley, Chichester.

B. subtilis Competent Cell Preparation and Transformation

Competence development in *B. subtilis* is one of several stationary phase processes triggered by a nutritional downshift. Since the pioneering work of Anagnostopolous and Spizizen, a number of protocols for preparing competent *B. subtilis* cells have appeared. The following method, modified by Ron Yasbin from protocols developed in the Frank Young lab at Rochester, was used routinely in Stan Zahler's wonderful bacterial genetics course at Cornell. This protocol assumes that you use a spectrophotometer that accepts 16×125 mm test tubes. If your spectrophotometer, like mine, works only with cuvettes, simply increase the culture volume to 10 or 20 ml in a 250-ml Erlenmeyer flask.

1. Streak recipient strain on one-half of a Tryptose Blood Agar Base plate. Incubate overnight (18 hr) at 37°C.
2. Inoculate a few colonies into 4.5 ml of Medium A in a 16×125 mm test tube that lacks visible scratches. Mix the contents of the tube thoroughly. Read its optical density at 650 nm in the spectrophotometer. Adjust the OD_{650} to be 0.1-0.2, maintaining the volume at 4.5 ml.
3. Incubate at 37°C with vigorous aeration. Read the OD_{650} every 20 min, plotting OD_{650} against time on semi-log paper. After a brief lag, the OD should increase logarithmically—that is, they should fall on a straight line. Note the point at the culture leaves log growth—the graph points fall below the straight line. In *B. subtilis* genetics, this point is known as t_0. It should take 60-90 minutes of incubation and occur at OD_{650}=0.4-0.6.
4. Continue incubation for 90 minutes after the cessation of log growth (t_{90}). Transfer 0.05 ml of this culture into 0.45 ml of pre-warmed Medium B in a 16×125 mm test tube. Set up one tube for each transformation you intend to perform, plus an extra for a DNA-less control.
5. Incubate the diluted cultures at 37°C with vigorous aeration for 90 min. At this point, the cultures should be highly competent.
6. Add 1 µg of DNA to the competent cells and incubate at 37°C with aeration for 30 minutes.
7. Plate aliquots of the transformed cells onto selective agar.

10× Medium A base:

Yeast extract	10 g
Casamino acids	2 g
Distilled water	to 900 ml
Autoclave, then add:	
50% glucose, filter-sterilized	100 ml

10× Bacillus salts

$(NH_4)_2SO_4$	20 g
$K_2HPO_4 \cdot 3H_2O$	183 g
KH_2PO_4	60 g
Na^+citrate	10 g
$MgSO_4 \cdot 7H_2O$	2 g
Water	to 1000 ml

Medium A

Sterile water	81 ml
10× Medium A base	10 ml
10× Bacillus salts	9 ml

Medium B

Medium A	10 ml
50 mM $CaCl_2 \cdot 2H_2O$	0.1 ml
250 nM $MgCl_2 \cdot 6H_2O$	0.1 ml

References:

Yasbin, R. E., G. A. Wilson, and F. E. Young. 1975. Transformation and transfection in lysogenic strains of *Bacillus subtilis:* Evidence for selective induction of prophage in competent cells. J. Bacteriol. **121:**296-304.

Zahler, S. A. (personal communication)

Purification of His_6-tagged *Bacillus subtilis* RNA Polymerase

The plasmid pPolHis1, when integrated into the *Bacillus subtilis* chromosome, tags the RNA polymerase α subunit with a poly-histidine tail. Under the proper conditions, the entire holoenzyme can be rapidly purified using this tag.

1. Grow cells to OD_{600}= 0.8 in 2 liters of LB medium containing 50 µg spectinomycin.
2. Harvest cells by centrifugation and suspend them in 10 ml of buffer 1 (below) with 2.5 mM imidazole.
3. Disrupt the cell suspension at 4°C by two passages through a French press at 70,000 kPa.
4. Centrifuge 20 min at 5,000 rpm in a GSA rotor. Keep the supernatant.
5. Add the supernatant to 4 ml of a Ni^{++}-NTA superflow matrix (Qiagen) previously equilibrated with buffer 1 with 2.5 mM imidazole. Incubate at 4°C for 1 hr.
6. Pack the resin into a disposable column, then wash with 15 buffer 1 with 20 mM imidazole.
7. Elute the His-tagged RNA polymerase with 5 ml of buffer 1 with 300 mM imidazole.

At this stage, the RNA polymerase activity can be assayed and used in *in vitro* transcription experiments.

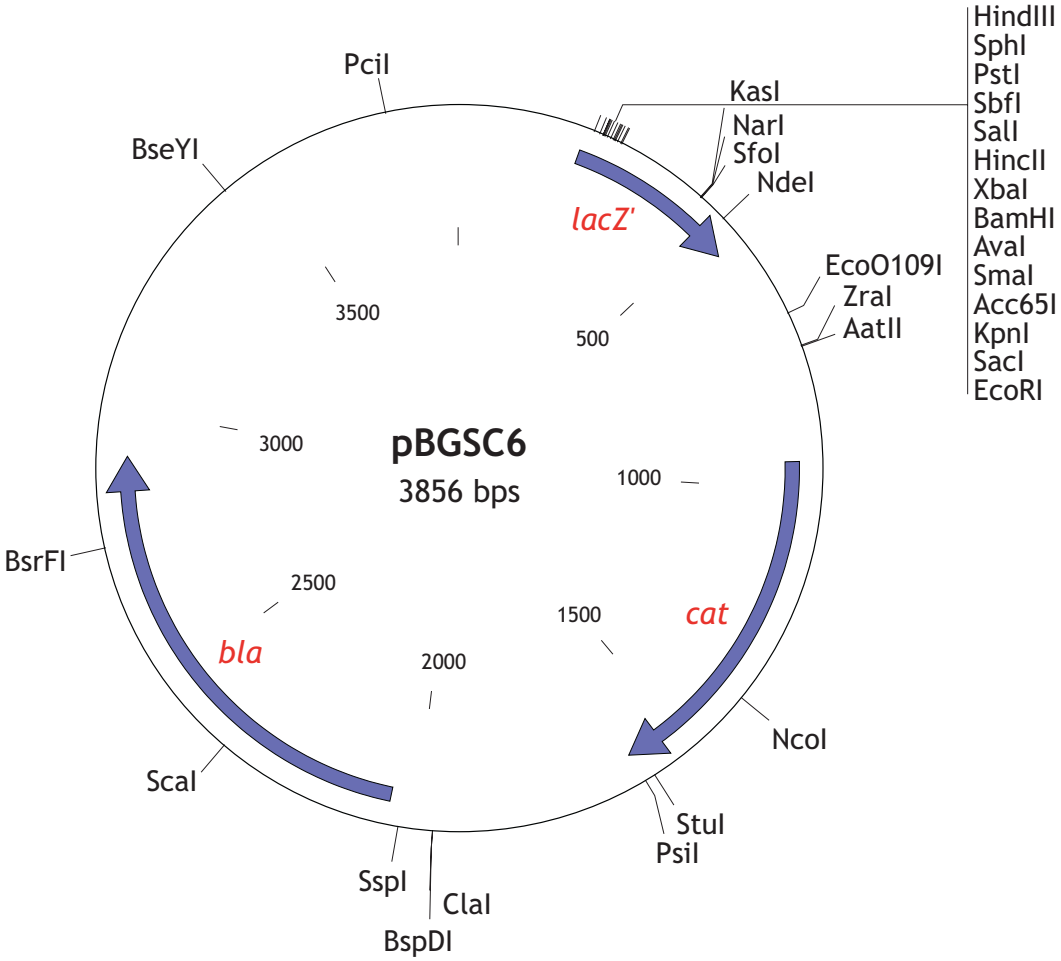
Buffer 1

Tris HCl, pH 8.0	10 mM
NaCl	0.1 M
Glycerol	5%
β-mercaptoethanol	1 mM
PMSF	1 mM

References:

Schyns, G. and C. P. Moran, Jr. (personal communication)

Integration Vector Maps



BGSC Accession: ECE22 **Orignal Code:** ECE22

Reference: D.R. Zeigler, unpublished. For an example of use, see Fajardo-Cavazos, P., *et al*. 1993. J. Bacteriol. 175:1635-1744.

Sequence: GenBank DQ483056 (Note: previous restriction map and sequence have been corrected in this release.)

Features:

lacZ'	β-lactamase α-subunit
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)

Description: Integration vector; integrates by Campbell-type recombination between cloned insert and chromosome.

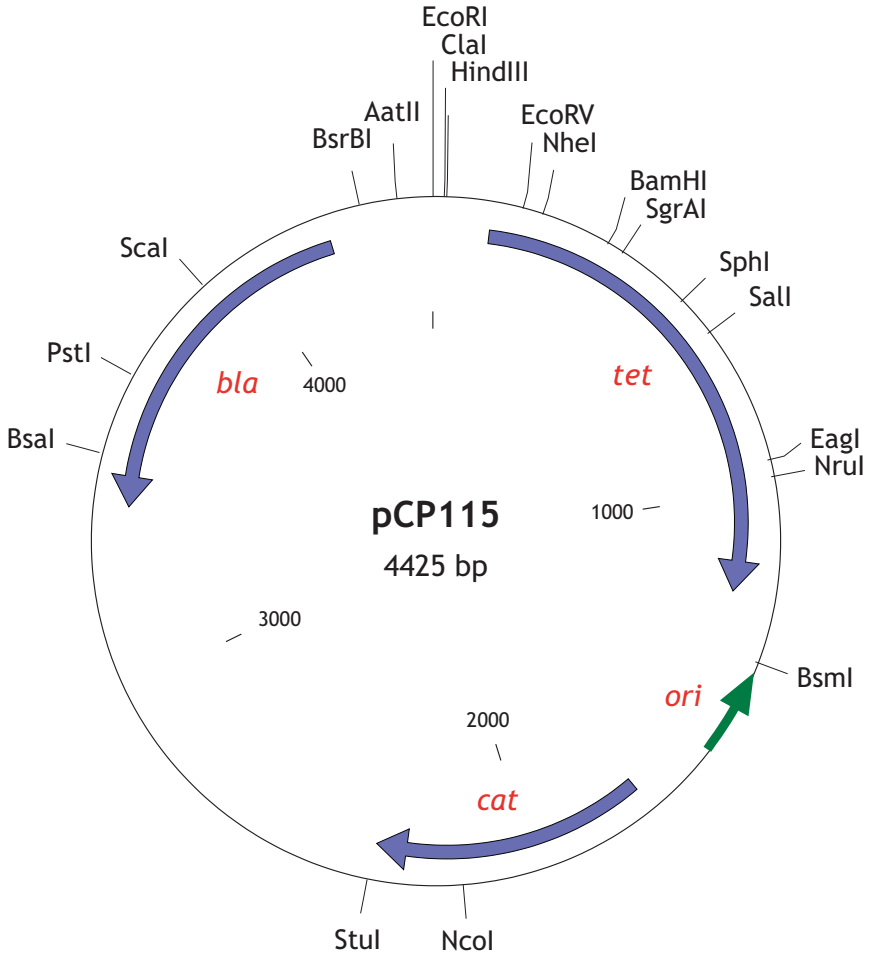
Construction: Insertion of the DraI fragment from pC194, bearing the *cat* gene, into the SspI site of pUC19.

Use: pBGSC6 is a very simple, general-purpose integration vector for Gram-positive organisms. An insert is cloned into the multiple cloning site. The blue-white system on X-gal plates or red-white on MacConkey agar detects the presence of the insert. The insert-bearing plasmid is used to transform the target organism with selection for chloramphenicol resistance. The plasmid integrates into the chormosome by homologous recombination with the cloned insert.

Recipient strains: pBGSC6 should work with any transformable bacterial species that can express *cat* but not support replication of pUC19. It has been used primarily with *Bacillus subtilis*, but should work with other organisms equally well.

Protocols: *B. subtilis* competent cell preparation and transformation

Integration Vector Maps (*continued*)



BGSC Accession: ECE19 **Orignal Code:** BNN45(pCP115)

Reference: Price, C.W. and R.H. Doi. 1985. Mol. Gen. Genet. 201:88-95

Sequence: Not in database; not available from BGSC

Features:

tet	encodes tetracycline resistance protein; selectable in *E. coli* only (tetracycline 50 μg/ml)
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)

Description: Integration vector; integrates by Campbell-type recombination between cloned insert and chromosome.

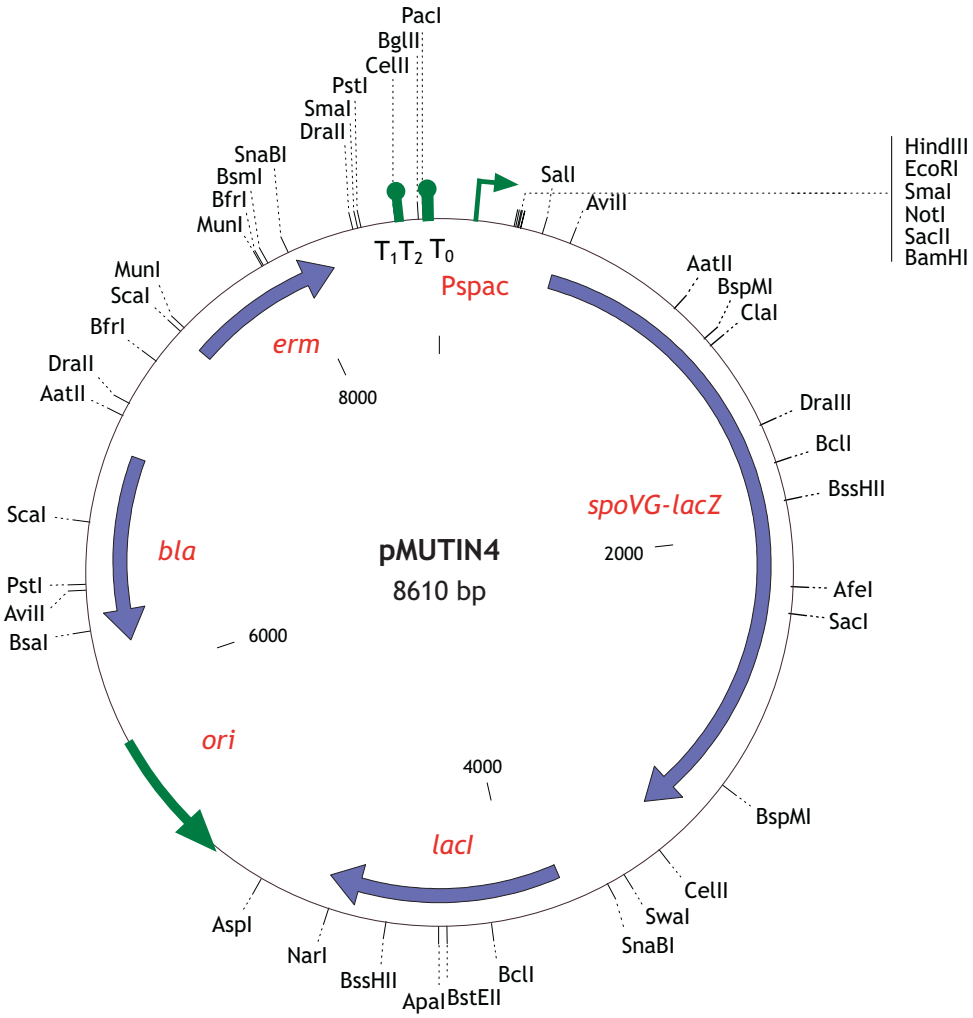
Construction: Composed of components of the vectors pBR327 (*tet, bla, ori*) and pC194 (*cat*).

Use: pCP115 is a simple, general-purpose integration vector for Gram-positive organisms. An insert is cloned into an available restriction site lying within either *bla* or *tet*. Insertional inactivation of antibiotic resistance detects the presence of the insert. The insert-bearing plasmid is used to transform the target organism with selection for chloramphenicol resistance. The plasmid integrates into the chormosome by homologous recombination with the cloned insert.

Recipient strains: pCP115 should work with any transformable bacterial species that can express *cat* but not support replication of pBR322-like plasmids. It has been used exclusively with *Bacillus subtilis,* but should work with other organisms equally well.

Protocols: *B. subtilis* competent cell preparation and transformation.

Integration Vector Maps (*continued*)



BGSC Accession: ECE139 **Orignal Code:** MC1061(pMUTIN4)

Reference: **Vagner, V., E. Dervyn and S. D. Ehrlich.** 1998. A vector for systematic gene inactivation in *Bacillus subtilis*. Microbiology **144:**3097-3104.

Sequence: not available in databases; the sequence of a nearly identical sister plasmid, pMUTIN2, is available at GenBank AF072806, however.

Features:

spoVG-lacZ	*E. coli lacZ* gene, fused to the *B. subtilis* 168 *spoVG* ribosome binding site
Pspac	hybrid promoter, inducible by IPTG
lacI	encodes *lac* repressor, with modified ribosome binding site for Gram-positive expression
erm	encodes rRNA adenine N-6-methyltransferase; selectable in *B. subtilis* (erythromycin 0.3 μg/ml)
bla	encodes β-lactamase; selectable in *E. coli* only (ampicillin 100 μg/ml)

Description: Integration vector designed for systematic inactivation of coding sequences discovered in genomic sequences.

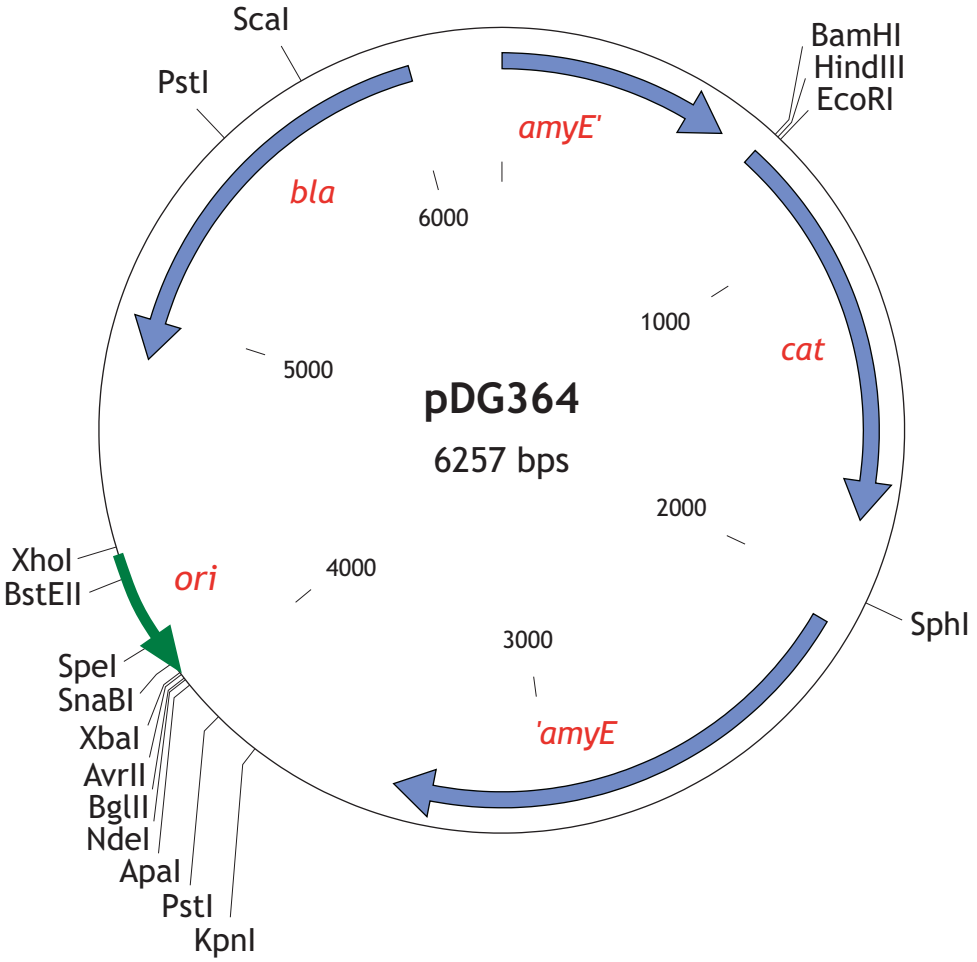
Construction: A pVE6023 *Xba*I-*Bam*HI fragment containing *erm* was joined with a pDG148 *Eco*RI fragment containing *bla*, the ColE1 origin, the *E. coli lacI* gene, and the Pspac promoter. A pJM783 BamHI-DraI fragment containing *spoVG-lacZ* was inserted into the blunted *Sph*I site of the construct. Terminators from λ and *E. coli rrnB* were amplified and inserted between *erm* and Pspac. The "oid" operator was amplified and inserted between Pspac and *spoVG-lacZ*. Finally, a multiple cloning site was inserted after pSpac.

Use: The plasmid is designed to integrate by Campbell-type insertion into the chromosomal locus of a cloned coding sequence. The net effect is to divide the coding sequence from its natural regulatory regions. The coding sequence is placed under the control, allowing for comparison of phenotypes in the presence and absence of induction. The unknown gene's promoter and regulatory regions are fused to the *spoVG-lacZ* gene, allowing for analysis of expression patterns via theβ-galactosidase reporter.

Recipient strains: Has been used in *B. subtilis,* but should perform well in any transformable Gram-positive host.

Protocols: *B. subtilis* competent cell preparation and transformation; β-galactosidase assay

Integration Vector Maps (*continued*)



BGSC Accession: ECE46 **Orignal Code:** TG1(pDG364)

Reference: Stragier, P. (unpublished); see Cutting, S. M. and P. B. Vander Horn. 1990. Genetic Analysis. In: Harwood, C. R. and S. M. Cutting, eds. Molecular Biology Methods for *Bacillus,* p. 53-54. John Wiley and Sons, Chichester.

Sequence: not available in databases; not available from BGSC.

Features:

amyE'...'amyE	5' and 3' parts of *B. subtilis* 168 α-amylase gene
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 50 μg/ml)
spc	encodes spectinomycin adenyltransferase; selectable in either *E. coli* or *B. subtilis* (spectinomycin 100 μg/ml)

Description: Promotes ectopic integration into the *amyE* locus of 1A771, replacing the resident MLS resistance cassette. Integrants are Cm^R MLS^S.

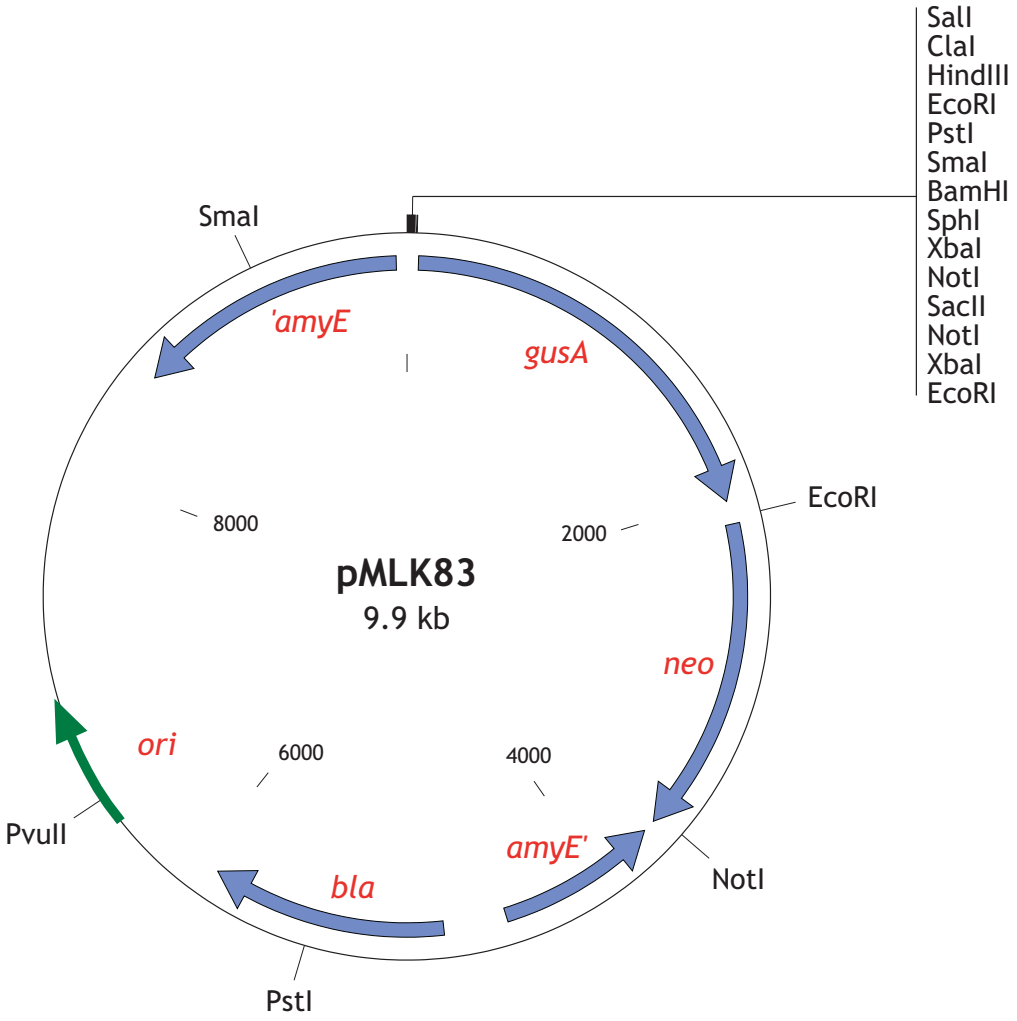
Construction: pDG268 with a 3 kb fragment containing *lacZ* deleted.

Use: The plasmid is designed to integrate a cloned insert into the *Bacillus subtilis* 168 chromosome at the *amyE* locus. The user inserts the fragment of interest into the multiple cloning site. The plasmid is transformed into a special *B. subtilis* host (see below), with selection for chloramphenicol resistance. Transformants are screened for sensitivity to erythromycin (indicating that the resident *amyE* locus has been replaced). The second-generation vectors derived from pDG364 (such as pDG1661) make it simpler to demonstrate that a Campbell-type insertion has not occurred.

Recipient strains: Although any recombination-proficient *B. subtilis* 168 derivative will serve as a recipient, 1A771, with an *erm*-insertion in its chromosomal *amyE* locus, allows for rapid screening for marker replacements. If another host is used, the user may need to confirm that integration has been at the proper locus by screening for loss of amylase production in the transformed cell.

Protocols: *B. subtilis* competent cell preparation and transformation; amylase production screening

Integration Vector Maps (*continued*)



BGSC Accession: ECE103 **Orignal Code:** DH5α(pMLK83)

Reference: **Karow, M. L, and P. J. Piggot**. 1994. Construction of *gusA* transcriptional fusion vectors for *Bacillus subtilis* and their utilization for studies of spore formation. Gene **163:**69-74.

Sequence: not available in databases; not available from BGSC.

Features:

amyE'...'amyE	5' and 3' parts of *B. subtilis* 168 α-amylase gene
neo	encodes ; selectable in either *E. coli* or *B. subtilis* (neomycin or kanamycin 5 μg/ml)
bla	encodes β-lactamase; selectable in *E. coli* only (ampicillin 50 μg/ml)
gusA	encodesβ-glucuronidase reporter

Description: Promotes ectopic integration into the *B. subtilis amyE* locus; allows for fusions with a β-glucuronidase reporter..

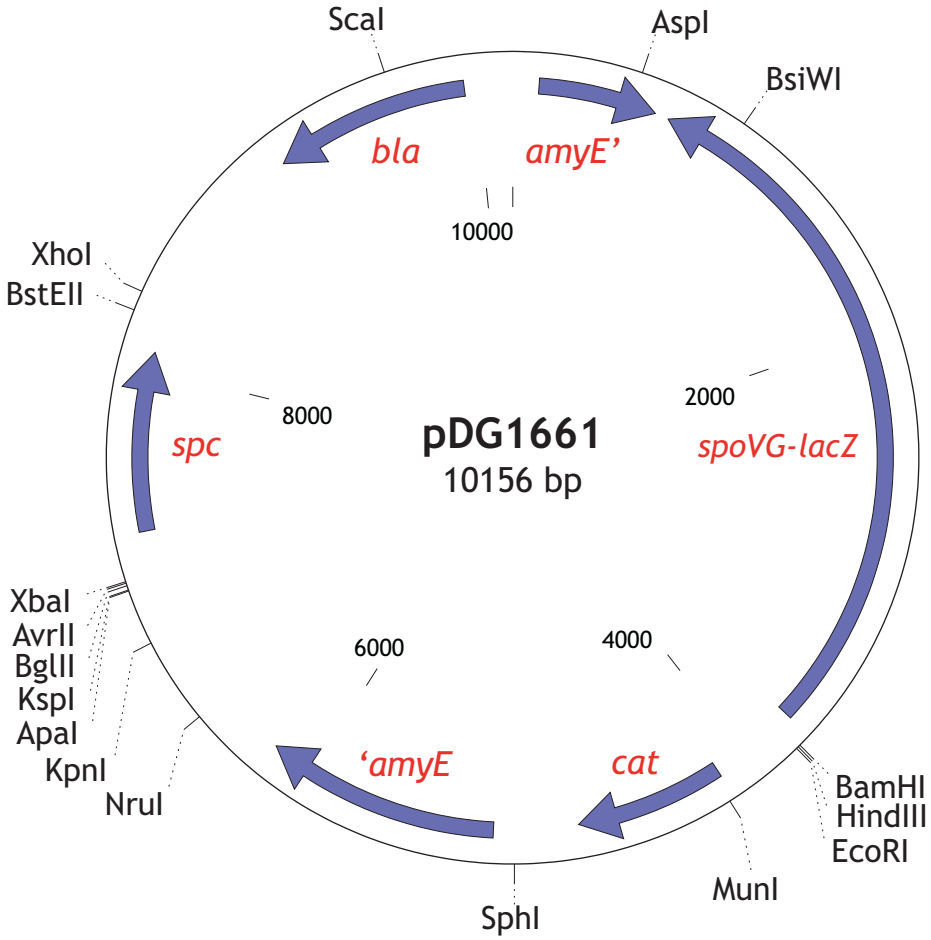
Construction: The *lacZ* gene was removed from pDH32. A *neo* cassette from pBEST501 was inserted into the SalI site of the resulting construct. A *gusA* cassette, formed by modifying the ribosome binding site of the gene in pRAJ275 (Clontech) and adding a multiple cloning site, was inserted to produce pMLK83.

Use: The plasmid is designed to create a *gusA* gene fusion and then to integrate it into the *Bacillus subtilis* 168 chromosome at the *amyE* locus. The user inserts the fragment of interest into the multiple cloning site. The plasmid is transformed into a special *B. subtilis* host (see below), with selection for neomyhcin or kanamycin resistance. Transformants are screened for sensitivity to the resident antibiotic resistance gene in the host *amyE* locus (indicating that the host *amyE* locus has been replaced). Becauseβ-glucuronidase and β-galactosidase have non-overlapping activities, two gene fusions can be introduced into the same host and monitored separately.

Recipient strains: Although any recombination-proficient *B. subtilis* 168 derivative will serve as a recipient, 1A771, with an *erm*-insertion in its chromosomal *amyE* locus, and 1A772, with a *cat*-insertion, allow for rapid screening for marker replacements. If another host is used, the user may need to confirm that integration has been at the proper locus by screening for loss of amylase production in the transformed cell.

Protocols: *B. subtilis* competent cell preparation and transformation; β-glucuronidase assay; amylase production screening

Integration Vector Maps (*continued*)



BGSC Accession: ECE112 **Orignal Code:** TG1(pDG1661)

Reference: **Guérout-Fleury, A. M., N. Frandsen, P. Stragier**. 1996. Plasmids for ectopic integration in *Bacillus subtilis*. Gene **180:**57-61.

Sequence: GenBank U46196

Features:

amyE'...'amyE 5' and 3' segments of the *B. subitlis* 168 *amyE* gene
spoVG-lacZ *E. coli lacZ* coding sequence, fused to the ribosome binding site of *B. subtilis spoVG*.
spc encodes spectinomycin adenyltransferase; selectable in either *E. coli* or *B. subtilis* (spectinomycin100 μg/ml)
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 50 μg/ml)

Description: Promotes ectopic integration into the *B. subtilis amyE* locus; allows for fusions with a β-galactosidase reporter..

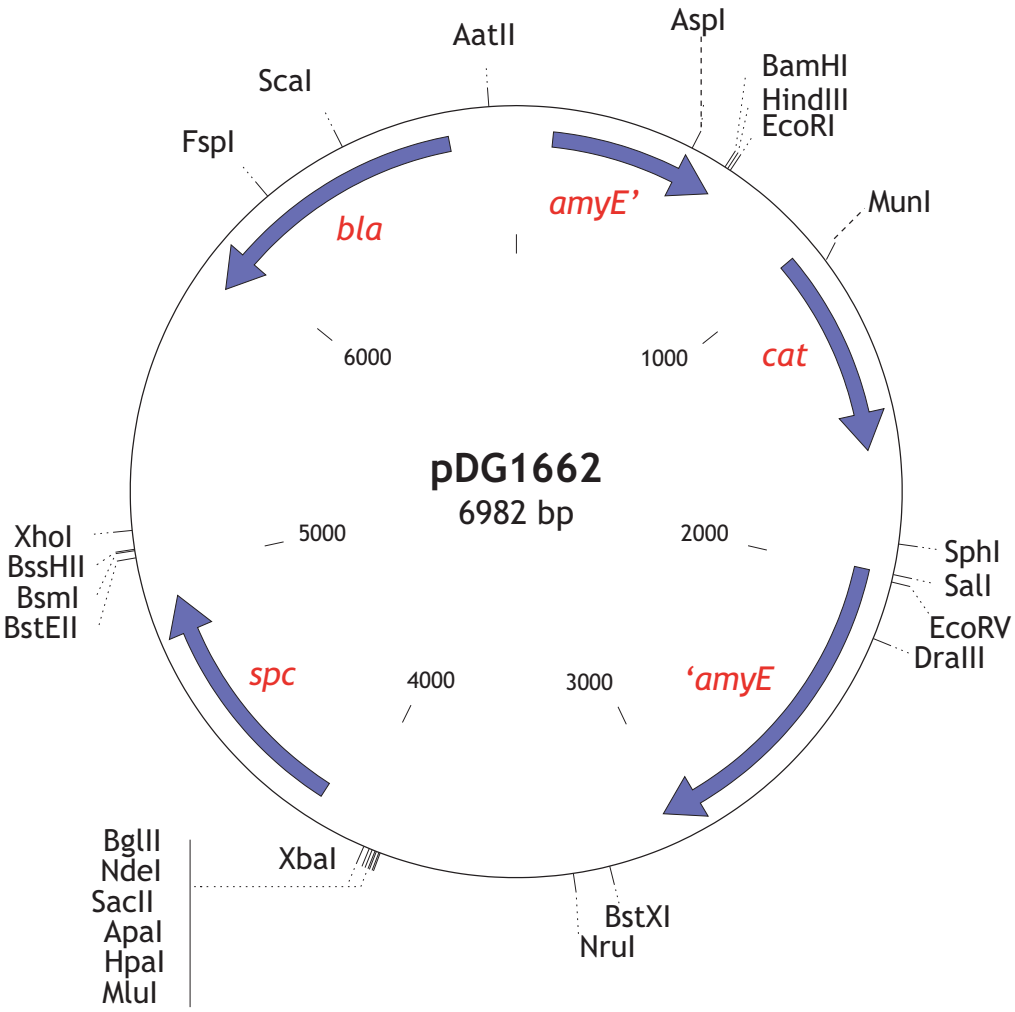
Construction: pDG268 with a spectinomycin cassette inserted into the non-integrating region of the plasmid.

Use: The plasmid is designed to integrate a *lacZ* fusion into the *Bacillus subtilis* 168 chromosome at the *amyE* locus. The user inserts the promoter-containing fragment of interest into the multiple cloning site to create a *lacZ* fusion. The plasmid is transformed into a special *B. subtilis* host (see below), with selection for chloramphenicol resistance. Transformants are screened for sensitivity to erythromycin (indicating that the resident *amyE* locus has been replaced) and for sensitivity to spectinomycin (indicating that a double-crossover recombination has occurred, rather than a Campbell-type insertion).

Recipient strains: Although any recombination-proficient *B. subtilis* 168 derivative will serve as a recipient, 1A771, with an *erm*-insertion in its chromosomal *amyE* locus, allows for rapid screening for marker replacements.

Protocols: *B. subtilis* competent cell preparation and transformation; β-galactosidase assay.

Integration Vector Maps (*continued*)



BGSC Accession: ECE113 **Orignal Code:** TG1(pDG1662)

Reference: Guérout-Fleury, A-M *et al.* (1996) Gene 180:57-61

Sequence: GenBank U46197

Features:

amyE'...'amyE	5' and 3' parts of *B. subtilis* 168 α-amylase gene
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 50 μg/ml)
spc	encodes spectinomycin adenyltransferase; selectable in either *E. coli* or *B. subtilis* (spectinomycin 100 μg/ml)

Description: Promotes ectopic integration into the *amyE* locus of 1A771, replacing the resident MLS resistance cassette. Integrants are Cm^R Sp^S MLS^S.

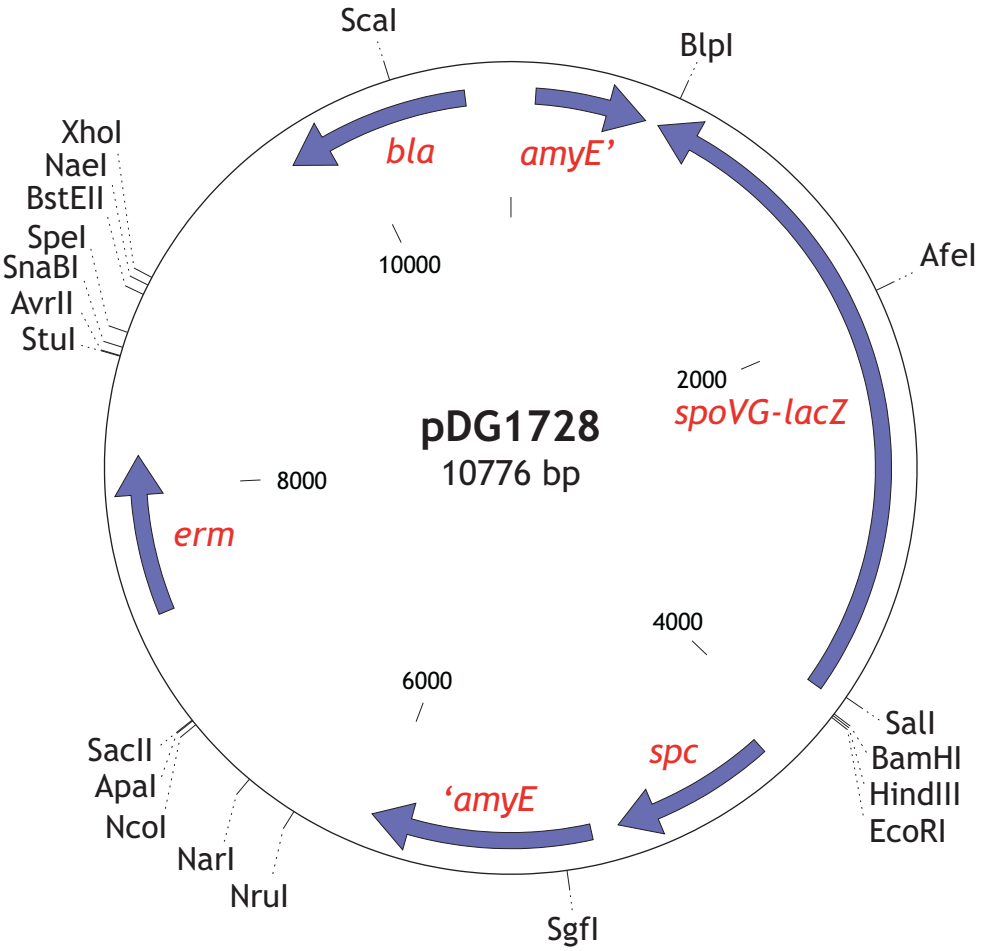
Construction: pDG268 with a spectinomycin cassette inserted into the non-integrating region of the plasmid.

Use: The plasmid is designed to integrate a cloned insert into the *Bacillus subtilis* 168 chromosome at the *amyE* locus. The user inserts the fragment of interest into the multiple cloning site. The plasmid is transformed into a special *B. subtilis* host (see below), with selection for chloramphenicol resistance. Transformants are screened for sensitivity to erythromycin (indicating that the resident *amyE* locus has been replaced) and for sensitivity to spectinomycin (indicating that a double-crossover recombination has occurred, rather than a Campbell-type insertion).

Recipient strains: Although any recombination-proficient *B. subtilis* 168 derivative will serve as a recipient, 1A771, with an *erm*-insertion in its chromosomal *amyE* locus, allows for rapid screening for marker replacements.

Protocols: *B. subtilis* competent cell preparation and transformation

Integration Vector Maps (*continued*)



BGSC Accession: ECE114 **Orignal Code:** TG1(pDG1728)

Reference: **Guérout-Fleury, A. M., N. Frandsen, P. Stragier**. 1996. Plasmids for ectopic integration in *Bacillus subtilis*. Gene **180:**57-61.

Sequence: GenBank U46198

Features:

amyE'...'amyE 5' and 3' segments of the *B. subitlis* 168 *amyE* gene
spoVG-lacZ *E. coli lacZ* coding sequence, fused to the ribosome binding site of *B. subtilis spoVG*.
spc encodes spectinomycin adenyltransferase; selectable in either *E. coli* or *B. subtilis* (spectinomycin100 μg/ml)
erm encodes rRNA adenine N-6-methyltransferase; selectable in *B. subtilis* (erythromycin 0.5 μg/ml, lincomycin 12.5 μg/ml)
bla encodes β-lactamase; selectable in *E. coli* only (ampicillin 50 μg/ml)

Description: Promotes ectopic integration into the *B. subtilis amyE* locus; allows for fusions with a β-galactosidase reporter.

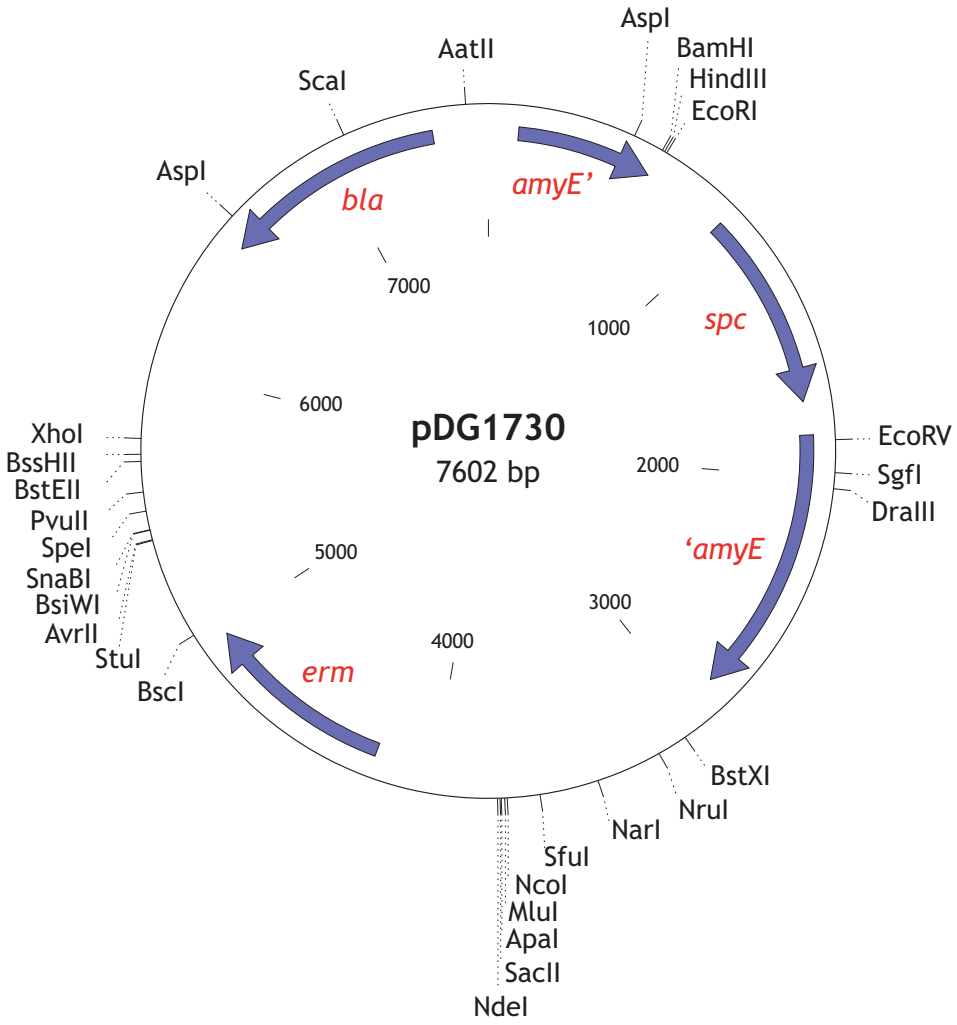
Construction: pDG1661 with a spectinomycin cassette substituted for *cat* in the integrative portion of the plasmid and *erm* substituted for *spc* in the non-integrative part.

Use: The plasmid is designed to integrate a *lacZ* fusion into the *Bacillus subtilis* 168 chromosome at the *amyE* locus. The user inserts the promoter-containing fragment of interest into the multiple cloning site to create a *lacZ* fusion. The plasmid is transformed into a special *B. subtilis* host (see below), with selection for spectinomycin resistance. Transformants are screened for sensitivity to chloramphenicol (indicating that the resident *amyE* locus has been replaced) and for sensitivity to erythromycin-lincomycin (indicating that a double-crossover recombination has occurred, rather than a Campbell-type insertion).

Recipient strains: Although any recombination-proficient *B. subtilis* 168 derivative will serve as a recipient, 1A772, witha *cat*-insertion in its chromosomal *amyE* locus, allows for rapid screening for marker replacements.

Protocols: *B. subtilis* competent cell preparation and transformation; β-galactosidase assay.

Integration Vector Maps (*continued*)



BGSC Accession: ECE115 **Orignal Code:** TG1(pDG1730)

Reference: Guérout-Fleury, A-M *et al.* (1996) Gene 180:57-61

Sequence: GenBank U46199

Features:

amyE'...'amyE	5' and 3' parts of *B. subtilis* 168 α-amylase gene
erm	encodes rRNA adenine N-6-methyltransferase; selectable in *B. subtilis* (erythromycin 0.5 μg/ml, lincomycin 12.5 μg/ml)
bla	encodes β-lactamase; selectable in *E. coli* only (ampicillin 50 μg/ml)
spc	encodes spectinomycin adenyltransferase; selectable in either *E. coli* or *B. subtilis* (spectinomycin 100 μg/ml)

Description: Promotes ectopic integration into the *amyE* locus of 1A772, replacing the resident Cm^R resistance cassette. Integrants are Sp^R Cm^S MLS^S.

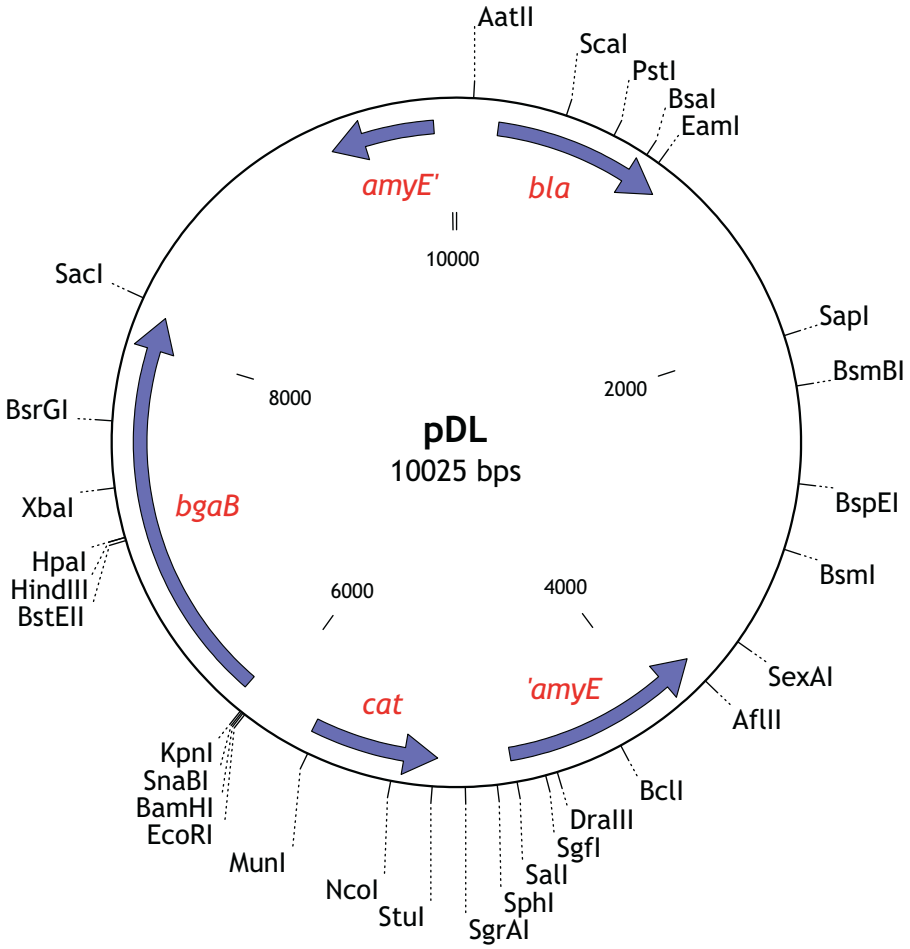
Construction: pDG268 with a spectinomycin cassette inserted into the non-integrating region of the plasmid.

Use: The plasmid is designed to integrate a cloned insert into the *Bacillus subtilis* 168 chromosome at the *amyE* locus. The user inserts the fragment of interest into the multiple cloning site. The plasmid is transformed into a special *B. subtilis* host (see below), with selection for spectinomycin resistance. Transformants are screened for sensitivity to chloramphenicol (indicating that the resident *amyE* locus has been replaced) and for sensitivity to erythromycin-lincomycin (indicating that a double-crossover recombination has occurred, rather than a Campbell-type insertion).

Recipient strains: Although any recombination-proficient *B. subtilis* 168 derivative will serve as a recipient, 1A772, witha *cat*-insertion in its chromosomal *amyE* locus, allows for rapid screening for marker replacements.

Protocols: *B. subtilis* competent cell preparation and transformation

Integration Vector Maps (*continued*)



BGSC Accession: ECE144 **Orignal Code:** DH5α(pDL)

Reference: **Yuan, G. and S. L. Wong.** 1995. Regulation of *groE* Expression in *Bacillus subtilis*: the Involvement of the sA-Like Promoter and the Roles of the Inverted Repeat Sequence (CIRCE) J. Bacteriol. **177:**5427.

Sequence: Not in database; available from BGSC at http://bacillus.biosci.ohio-state.edu/sequences/pDL.htm

Features:

bgaB	encodees thermostable β-galactosidase originally isolated from *Geobacillus stearothermophilus*
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)
amyE'-'amyE	5' and 3' ends, respectively, of the *B. subtilis* 168 *amyE* coding sequence

Description: Vector designed to fuse a sequence of choice to a thermostable reporter gene and to integrate the fusion into the *B. subtilis amyE* locus.

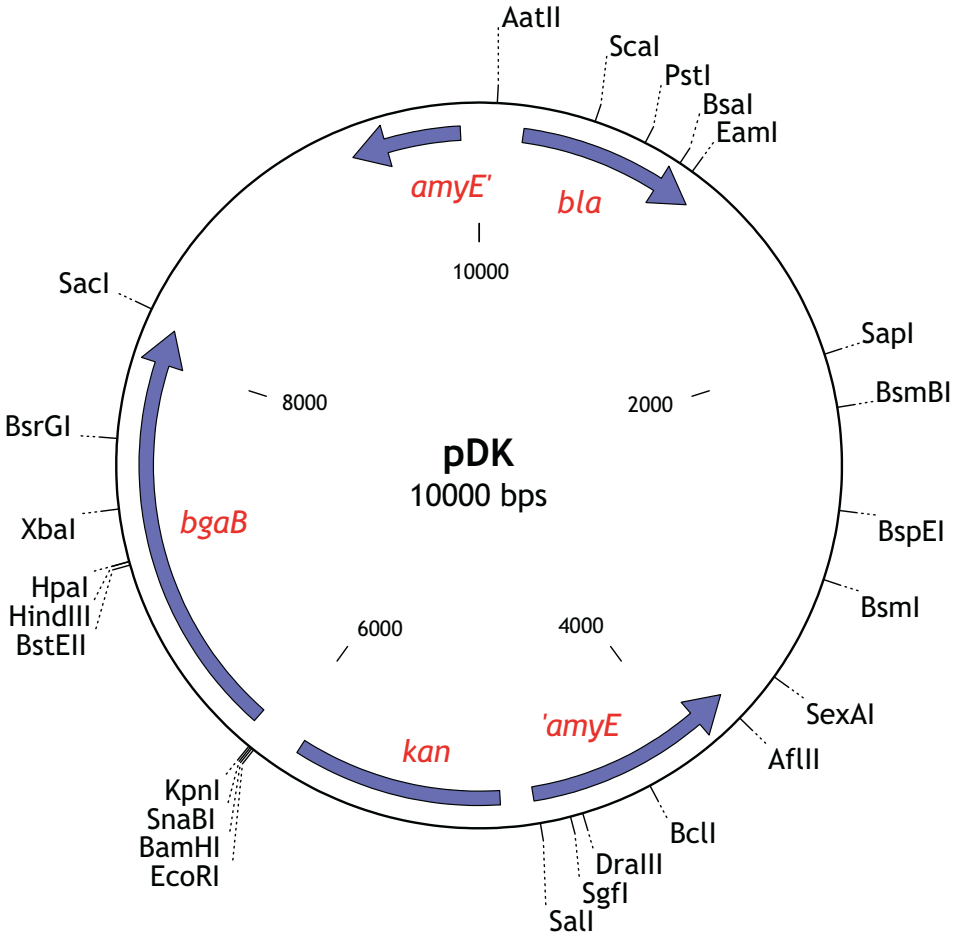
Construction: The promoterless *bgaB* coding sequence was amplified by PCR from *Geobacillus stearothermophilus* ATCC 7954, then inserted into the SnaBI-SacI backbone of pDH32/M (Kraus A., *et al*. 1994. J. Bacteriol. 176:3981-3992).

Use: A DNA fragment containing a gram-positive promoter and its regulatory region in inserted into the multiple cloning site upstream from *bgaB*. Upon transformation into *B. subtilis* 168 with selection for chloramphenicol resistance, colonies are recovered in which the resident *amyE* gene has been replaced by a cassette containing *cat* plus the *bgaB* fusion.

Recipient strains: Although any recombination-proficient *B. subtilis* 168 derivative will serve as a recipient, use of BGSC 1A771 or 1A772, with pre-existing antibiotic resistance cassettes in their chromosomal *amyE* loci, allows for rapid screening for marker replacements. If another host is used, the user may need to confirm that integration has been at the proper locus by screening for loss of amylase production in the transformed cell.

Protocols: *B. subtilis* competent cell preparation and transformation; amylase production screening; β-galactosidase assay.

Integration Vector Maps (*continued*)



BGSC Accession: ECE143 **Orignal Code:** DH5α(pDK)

Reference: **Yuan, G. and S. L. Wong.** 1995. Regulation of *groE* Expression in *Bacillus subtilis*: the Involvement of the sA-Like Promoter and the Roles of the Inverted Repeat Sequence (CIRCE) J. Bacteriol. **177:**5427.

Sequence: Not in database; not available from BGSC

Features:

bgaB	encodees thermostable β-galactosidase originally isolated from *Geobacillus stearothermophilus*
kan	encodes kanamycin adenyltransferase; selectable in either *E. coli* or *B. subtilis* (kanamycin or neomycin 5 μg/ml)
bla	encodes β-lactamase; selectable in *E. coli* only (ampicillin 100 μg/ml)
amyE'-'amyE	5' and 3' ends, respectively, of the *B. subtilis* 168 *amyE* coding sequence

Description: Vector designed to fuse a sequence of choice to a thermostable reporter gene and to integrate the fusion into the *B. subtilis amyE* locus.

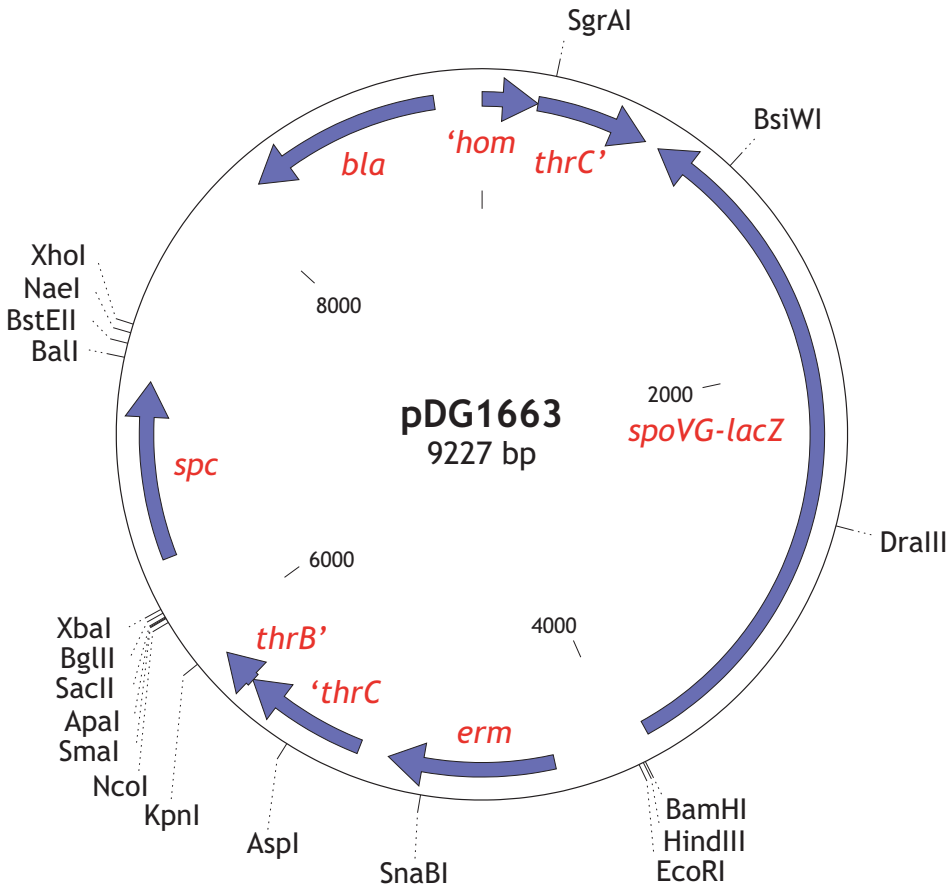
Construction: An EcoRI-SalI cassette carrying P43-*kan* was substituted for the EcoRI-SalI fragment of pDL.

Use: A DNA fragment containing a gram-positive promoter and its regulatory region in inserted into the multiple cloning site upstream from *bgaB*. Upon transformation into *B. subtilis* 168 with selection for kanamycin resistance, colonies are recovered in which the resident *amyE* gene has been replaced by a cassette containing *cat* plus the *bgaB* fusion.

Recipient strains: Although any recombination-proficient *B. subtilis* 168 derivative will serve as a recipient, use of BGSC 1A771 or 1A772, with pre-existing antibiotic resistance cassettes in their chromosomal *amyE* loci, allows for rapid screening for marker replacements. If another host is used, the user may need to confirm that integration has been at the proper locus by screening for loss of amylase production in the transformed cell.

Protocols: *B. subtilis* competent cell preparation and transformation; amylase production screening; β-galactosidase assay.

Integration Vector Maps (*continued*)



BGSC Accession: ECE116 **Orignal Code:** TG1(pDG1663)

Reference: **Guérout-Fleury, A. M., N. Frandsen, P. Stragier**. 1996. Plasmids for ectopic integration in *Bacillus subtilis*. Gene **180:**57-61.

Sequence: GenBank U46200

Features:

thrC'...'thrC	5' and 3' segments of the *B. subitlis* 168 *thrC* gene
'hom	3' end of the *B. subtilis* 168 *hom* gene (part of the *thrC* operon)
thrB'	5' end of the *B. subtilis* 168 *thrB* gene (part of the *thrB* operon)
spoVG-lacZ	*E. coli lacZ* coding sequence, fused to the ribosome binding site of *B. subtilis spoVG*.
spc	encodes spectinomycin adenyltransferase; selectable in either *E. coli* or *B. subtilis* (spectinomycin100 μg/ml)
erm	encodes rRNA adenine N-6-methyltransferase; selectable in *B. subtilis* (erythromycin 0.5 μg/ml, lincomycin 12.5 μg/ml)
bla	encodes β-lactamase; selectable in *E. coli* only (ampicillin 50 μg/ml)

Description: Promotes ectopic integration into the *B. subtilis thrC* locus; allows for fusions with a β-galactosidase reporter.

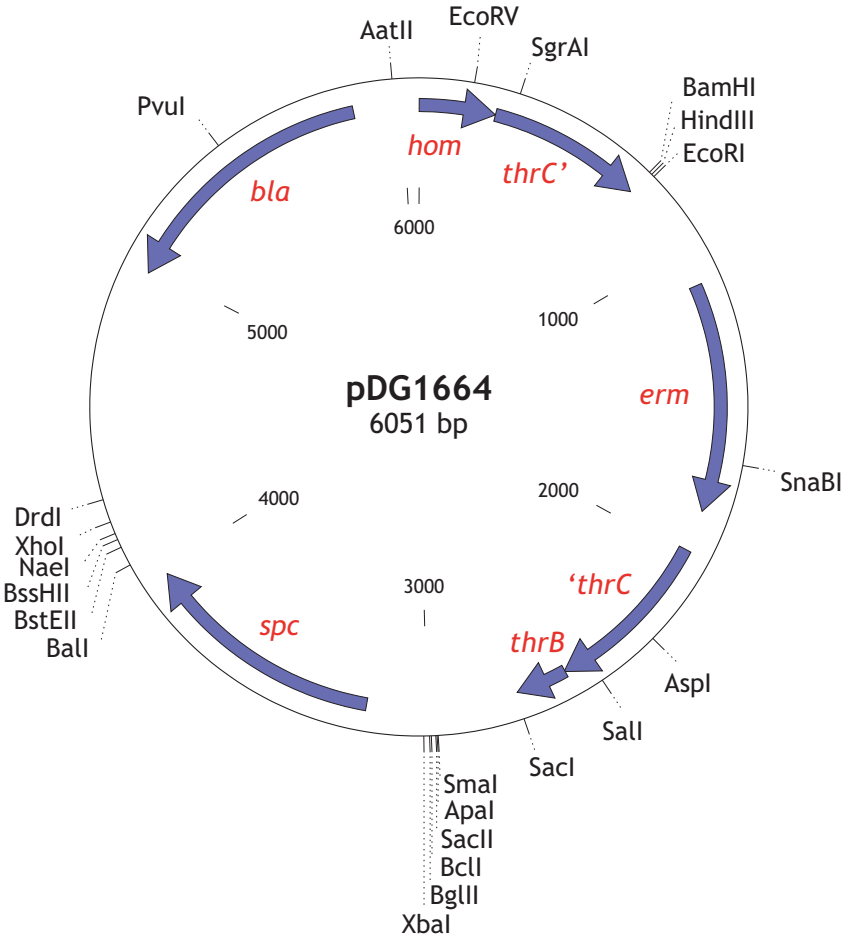
Construction: Composite of *hom-thrC* fragment from pSU11, hybrid *lacZ* fragment from pJM73, multiple cloning site from pDG268, *thrC-thrB* fragment from pSU11, multiple cloning site from pJRD184, *spc* cassette from pIC215, an origin-containing fragment from pSU11, and a *bla* fragment from pBR322

Use: The plasmid is designed to integrate a *lacZ* fusion into the *Bacillus subtilis* 168 chromosome at the *thrC* locus. The user inserts the promoter-containing fragment of interest into the multiple cloning site to create a *lacZ* fusion. The plasmid is transformed into a special *B. subtilis* host (see below), with selection for erythromycin-lincomycin resistance. Transformants are screened for sensitivity to chloramphenicol (indicating that the resident *thrC* locus has been replaced) and for sensitivity to spectinomycin (indicating that a double-crossover recombination has occurred, rather than a Campbell-type insertion).

Recipient strains: Although any recombination-proficient *B. subtilis* 168 derivative will serve as a recipient, 1A773, with a *cat*-insertion in its chromosomal *thrC* locus, allows for rapid screening for marker replacements.

Protocols: *B. subtilis* competent cell preparation and transformation; β-galactosidase assay.

Integration Vector Maps (*continued*)



BGSC Accession: ECE117 **Orignal Code:** TG1(pDG1664)

Reference: **Guérout-Fleury, A. M., N. Frandsen, P. Stragier**. 1996. Plasmids for ectopic integration in *Bacillus subtilis*. Gene **180:**57-61.

Sequence: GenBank U46201

Features:

thrC'...'thrC	5' and 3' segments of the *B. subitlis* 168 *thrC* gene
'*hom*	3' end of the *B. subtilis* 168 *hom* gene (part of the *thrC* operon)
thrB'	5' end of the *B. subtilis* 168 *thrB* gene (part of the *thrB* operon)
spc	encodes spectinomycin adenyltransferase; selectable in either *E. coli* or *B. subtilis* (spectinomycin100 μg/ml)
erm	encodes rRNA adenine N-6-methyltransferase; selectable in *B. subtilis* (erythromycin 0.5 μg/ml, lincomycin 12.5 μg/ml)
bla	encodes β-lactamase; selectable in *E. coli* only (ampicillin 50 μg/ml)

Description: Promotes ectopic integration into the *B. subtilis thrC* locus.

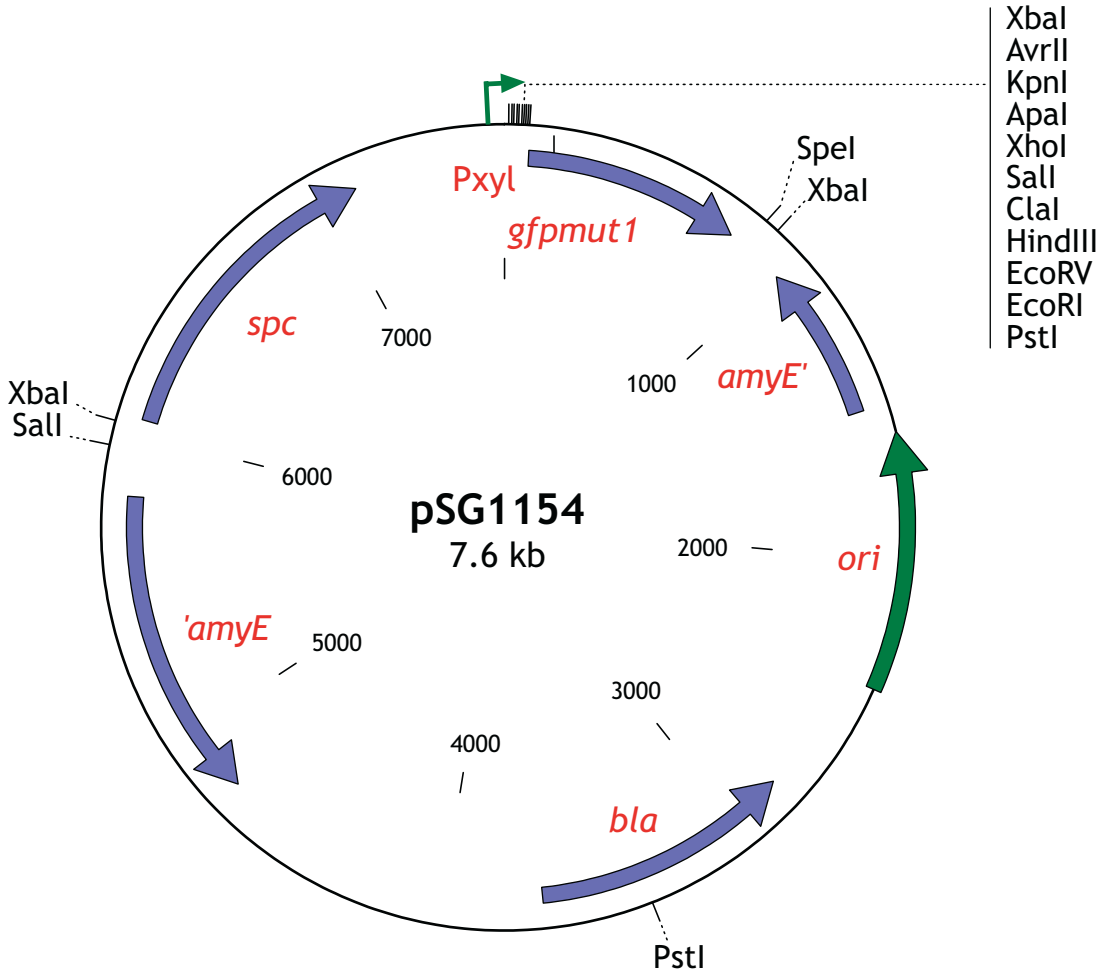
Construction: Composite of *hom-thrC* fragment from pSU11, hybrid *lacZ* fragment from pJM73, multiple cloning site from pDG268, *thrC-thrB* fragment from pSU11, multiple cloning site from pJRD184, *spc* cassette from pIC215, an origin-containing fragment from pSU11, and a *bla* fragment from pBR322.

Use: The plasmid is designed to integrate a cloned fragment into the *Bacillus subtilis* 168 chromosome at the *thrC* locus. The user inserts the promoter-containing fragment of interest into the multiple cloning site. The plasmid is transformed into a special *B. subtilis* host (see below), with selection for erythromycin-lincomycin resistance. Transformants are screened for sensitivity to chloramphenicol (indicating that the resident *thrC* locus has been replaced) and for sensitivity to spectinomycin (indicating that a double-crossover recombination has occurred, rather than a Campbell-type insertion).

Recipient strains: Although any recombination-proficient *B. subtilis* 168 derivative will serve as a recipient, 1A773, with a *cat*-insertion in its chromosomal *thrC* locus, allows for rapid screening for marker replacements.

Protocols: *B. subtilis* competent cell preparation and transformation.

Integration Vector Maps (*continued*)



BGSC Accession: ECE153 **Orignal Code:** DH5α(pSG1154)

Reference: **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.

Sequence: Not in database; not available from BGSC

Features:

gfmut-1	encodes highly fluorescent red-shifted GFP variant (single excitation peak at 488 nm, emits green light maximally at 507 nm).
spc	encodes spectinomycin adenyltransferase; selectable in either *E. coli* or *B. subtilis* (spectinomycin 50 μg/ml)
bla	encodes β-lactamase; selectable in *E. coli* only (ampicillin 100 μg/ml)
ori-ColE1	ColE1 origin of replication
amyE'-'amyE	5' and 3' ends, respectively, of the *B. subtilis* 168 *amyE* coding sequence
Pxyl	xylose-inducible promoter

Description: Vector designed to fuse GFP onto the C-terminus of any protein under the control of a xylose-inducible promoter and integrate the fusion into the *B. subtilis amyE* locus.

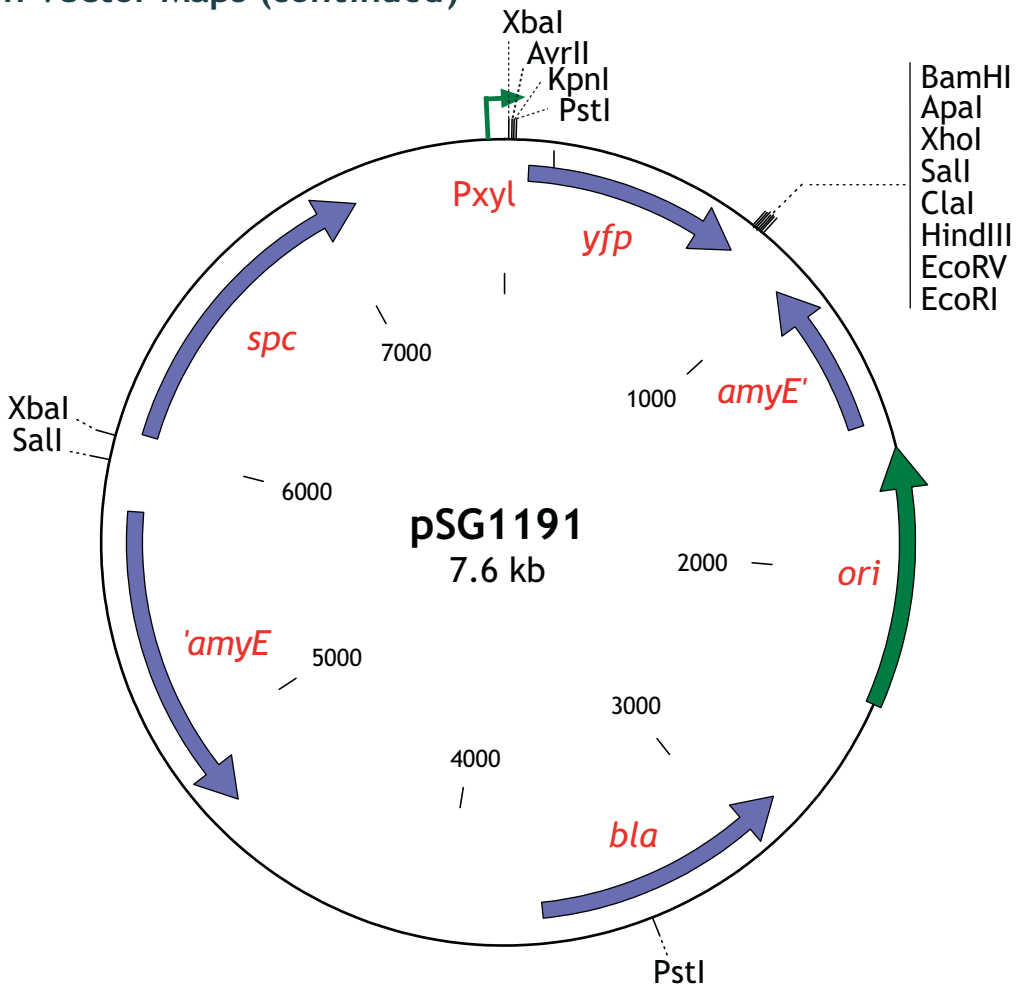
Construction: Derived from pJS2, which was in turn derived from the ectopically integrating expression vector, pMLK83. After unwanted restriction sites were removed from pJS2, a purified Pxyl-*gfpmut1* fragment from pSG1164 was inserted to produce pSG1154.

Use: The complete coding sequence of an ORF of interest is inserted upstream and in-frame with *gfpmut-1*. Transformation into *Bacillus subtilis* 168 with selection for spectinomycin resistance recovers integration mutants in which the target gene is fused to *gfp* and expression is driven from the xylose-inducible Pxyl promoter, and the fusion (plus the spectinomycin resistance cassette) is integrated into the host chromosomal *amyE* locus.

Recipient strains: Although any recombination-proficient *B. subtilis* 168 derivative will serve as a recipient, use of BGSC 1A771 or 1A772, with pre-existing antibiotic resistance cassettes in their chromosomal *amyE* loci, allows for rapid screening for marker replacements. If another host is used, the user may need to confirm that integration has been at the proper locus by screening for loss of amylase production in the transformed cell.

Protocols: *B. subtilis* competent cell preparation and transformation; amylase production screening

Integration Vector Maps (*continued*)



BGSC Accession: ECE160 **Orignal Code:** DH5α(pSG1191)

Reference: **Feucht, A. and P. J. Lewis.** 2001. Improved plasmid vectors for the production of multiple fluorescent protein fusions in *Bacillus subtilis*. Gene **264:**289-297.

Sequence: Not in database; not available from BGSC

Features:

yfp	encodes Yellow Fluorescent Protein (single excitation peak at 514 nm, emits yellow light maximally at 527 nm).
spc	encodes spectinomycin adenyltransferase; selectable in either *E. coli* or *B. subtilis* (spectinomycin 50 μg/ml)
bla	encodes β-lactamase; selectable in *E. coli* only (ampicillin 100 μg/ml)
ori-ColE1	ColE1 origin of replication
amyE'-'amyE	5' and 3' ends, respectively, of the *B. subtilis* 168 *amyE* coding sequence
Pxyl	xylose-inducible promoter

Description: Vector designed to fuse YFP onto the N-terminus of any protein under the control of a xylose-inducible promoter and integrate the fusion into the *B. subtilis amyE* locus.

Construction: To make pSG1191, the *yfp* coding sequence of pSG1187 was amplified with primers and the PCR product was inserted into the pSG1729 backbone.

Use: The complete coding sequence of an ORF of interest is inserted downstream and in-frame with *yfp*. Transformation into *Bacillus subtilis* 168 with selection for spectinomycin resistance recovers integration mutants in which the target gene is fused to *gfp* and expression is driven from the xylose-inducible Pxyl promoter, and the fusion (plus the spectinomycin resistance cassette) is integrated into the host chromosomal *amyE* locus.

Recipient strains: Although any recombination-proficient *B. subtilis* 168 derivative will serve as a recipient, use of BGSC 1A771 or 1A772, with pre-existing antibiotic resistance cassettes in their chromosomal *amyE* loci, allows for rapid screening for marker replacements. If another host is used, the user may need to confirm that integration has been at the proper locus by screening for loss of amylase production in the transformed cell.

Protocols: *B. subtilis* competent cell preparation and transformation; amylase production screening