

# Bacillus Genetic Stock Center

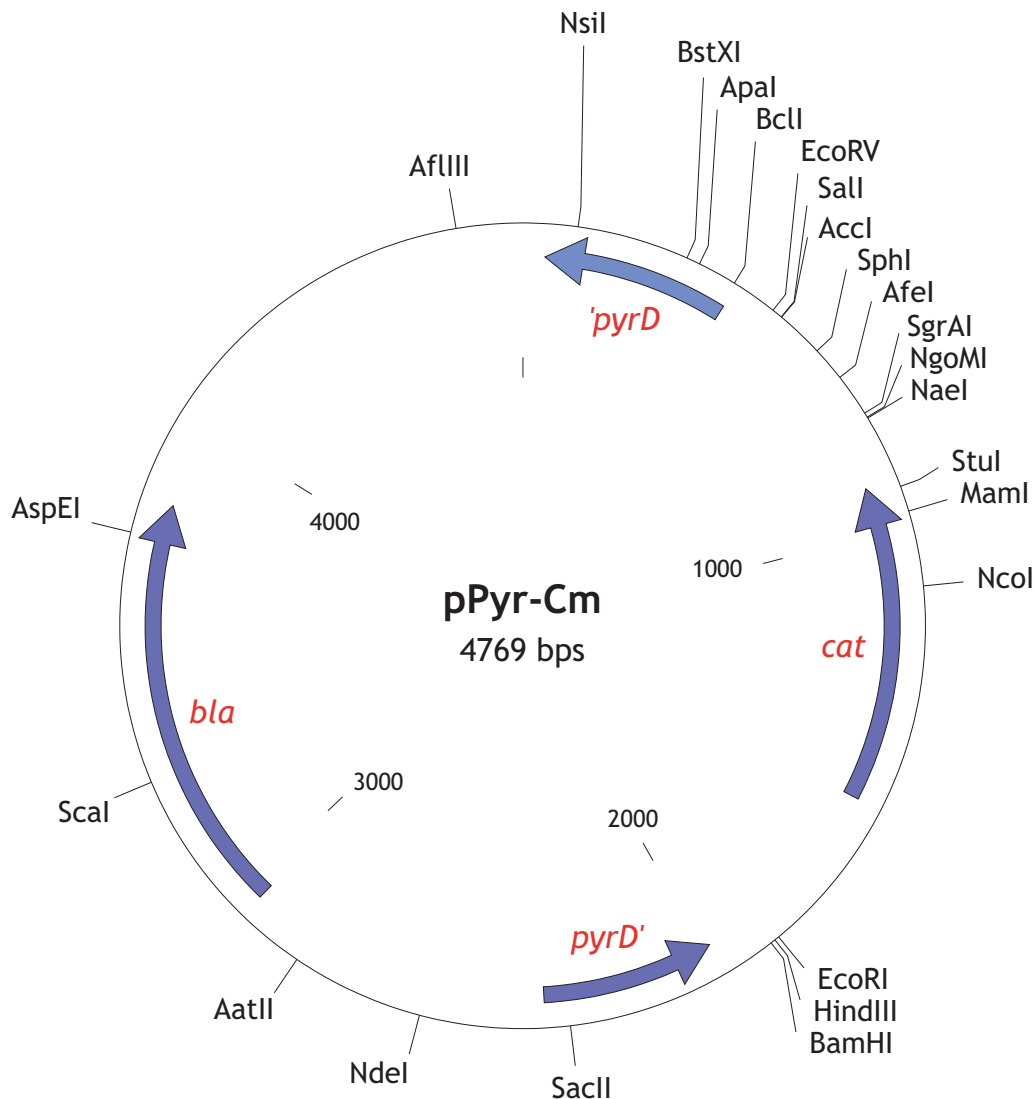
New Product Announcement

May 2004

## NEW ECTOPIC INTEGRATION VECTORS FOR *BACILLUS SUBTILIS*

Rebecca Middleton of the University of California, Berkeley, has generously donated to the BGSC collection a set of novel integration vectors. The vectors integrate into the *Bacillus subtilis* chromosome "ectopically," that is, at a locus targeted by homologous sequences within the vector itself, rather than by sequences within a cloned insert. Each vector contains an integration cassette consisting of the 5' and 3' ends of a non-essential chromosomal gene, interrupted by a selectable antibiotic resistance marker and a multiple cloning site. When the vectors are introduced into a host strain by transformation with selection for antibiotic resistance, a double-crossover event replaces the chromosomal locus with the plasmid-borne cassette, including any fragments that have been inserted into the cloning sites. The six plasmids within the collection allow the user to target any of three loci—*gltA*, *pyrD*, or *sacA*—with selection for either kanamycin or chloramphenicol resistance. The collection also includes six control strains in which the cassettes, without inserts, have been integrated into the chromosomal loci. The table below lists the twelve strains in the set. The pages that follow provide a restriction map and other key information about each vector. We thank Rebecca Middleton for her kindness in sharing these tools with the *Bacillus* genetics community!

BGSC №	Original	Description	Notes
1A806	RM35	Cm <i>pyrD::cat</i>	<i>B. subtilis</i> PY79 (1A747) with pPyr-Cm cassette integrated into <i>pyrD</i> gene; pyrimidine auxotroph
1A807	RM301	Km <i>pyrD::kan</i>	<i>B. subtilis</i> PY79 (1A747) with pPyr-Kan cassette integrated into <i>pyrD</i> gene; pyrimidine auxotroph
1A808	RM36	Cm <i>gltA::cat</i>	<i>B. subtilis</i> PY79 (1A747) with pGlt-Cm cassette integrated into <i>gltA</i> gene; glutamate or aspartate auxotroph
1A809	RM37	Km <i>gltA::kan</i>	<i>B. subtilis</i> PY79 (1A747) with pGlt-Kan cassette integrated into <i>gltA</i> gene; glutamate or aspartate auxotroph
1A810	RM58	Cm <i>sacA::cat</i>	<i>B. subtilis</i> PY79 (1A747) with pSac-Cm cassette integrated into <i>sacA</i> gene; unable to grow on sucrose as carbon source
1A811	RM182	Km <i>sacA::kan</i>	<i>B. subtilis</i> PY79 (1A747) with pSac-Kan cassette integrated into <i>sacA</i> gene; unable to grow on sucrose as carbon source
ECE170	RM30	DH5 $\alpha$ (pPyr-Cm)	<i>E. coli</i> DH5 $\alpha$ containing (pPyr-Cm), ectopic integration vector for inserting a <i>cat</i> cassette into the <i>B. subtilis pyrD</i> locus; go to <a href="#">map</a>
ECE171	RM57	DH5 $\alpha$ (pPyr-Kan)	<i>E. coli</i> DH5 $\alpha$ containing (pPyr-Kan), ectopic integration vector for inserting a <i>kan</i> cassette into the <i>B. subtilis pyrD</i> locus; go to <a href="#">map</a>
ECE172	RM33	DH5 $\alpha$ (pGlt-Cm)	<i>E. coli</i> DH5 $\alpha$ containing (pGlt-Cm), ectopic integration vector for inserting a <i>cat</i> cassette into the <i>B. subtilis gltA</i> locus; go to <a href="#">map</a>
ECE173	RM34	DH5 $\alpha$ (pGlt-Kan)	<i>E. coli</i> DH5 $\alpha$ containing (pGlt-Kan), ectopic integration vector for inserting a <i>kan</i> cassette into the <i>B. subtilis gltA</i> locus; go to <a href="#">map</a>
ECE174	RM53	DH5 $\alpha$ (pSac-Cm)	<i>E. coli</i> DH5 $\alpha$ containing (pSac-Cm), ectopic integration vector for inserting a <i>cat</i> cassette into the <i>B. subtilis sacA</i> locus; go to <a href="#">map</a>
ECE175	RM158	DH5 $\alpha$ (pSac-Kan)	<i>E. coli</i> DH5 $\alpha$ containing (pSac-Kan), ectopic integration vector for inserting a <i>kan</i> cassette into the <i>B. subtilis sacA</i> locus; go to <a href="#">map</a>



**BGSC Accession:** ECE170

**Original Code:** RM30

**Reference:** Middleton, R. (unpublished).

**Sequence:** GenBank accession number [AY464558](#)

**Features:**

- pyrD'*-*pyrD* end fragments of the *Bacillus subtilis* 168 *pyrD* (dihydroorotate dehydrogenase) 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 100 µg/ml)

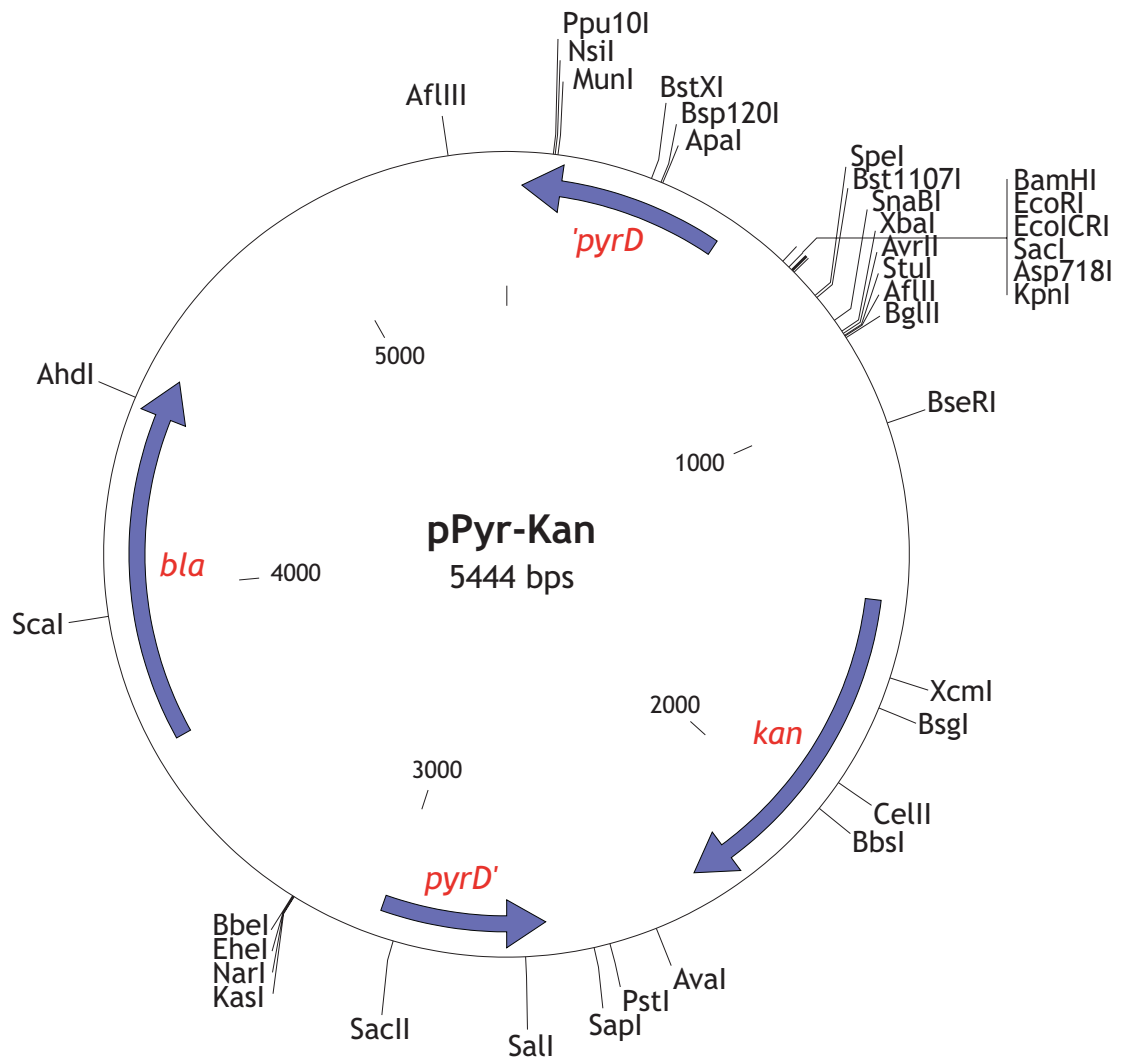
**Description:** Ectopic integration vector; cassette integrates by double recombination between plasmid and chromosomal *pyrD* sequences.

**Construction:** For the vector backbone, pUC18 was digested with the PvuII, and the resulting 2364 bp fragment was self-ligated. The 5' and 3' ends of *pyrD* were amplified from *B. subtilis* PY79 chromosomal DNA with *Ascl* restriction sites incorporated into the primers nearest the middle of the target gene. Recombinant PCR was then used to fuse the two portions of the genes, and the chimera was ligated into the PvuII site of the vector backbone. The *cat* gene and multiple cloning sites were amplified from pDG364 using oligonucleotides containing *Ascl* sites. The PCR products were then digested and cloned into the *Ascl* sites in the middle of the cloned *pyrD* chimera.

**Use:** The plasmid is designed to integrate a cloned insert into the *Bacillus subtilis* 168 chromosome at the *pyrD* locus. The user inserts the fragment of interest into any site lying between the *pyrD* fragments but outside *cat*. The plasmid is transformed into any *B. subtilis* 168 host (see below), with selection for chloramphenicol resistance. Transformants can be screened for pyrimidine auxotrophy on minimal media (indicating that the resident *pyrD* locus has been replaced).

**Recipient strains:** pPyr-Cm should work with any strain derived from *B. subtilis* 168.

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



**BGSC Accession:** ECE171

**Original Code:** RM57

**Reference:** Middleton, R. (unpublished).

**Sequence:** GenBank accession number [AY464559](https://www.ncbi.nlm.nih.gov/nuccore/AY464559)

**Features:**

- pyrD'*-*pyrD* end fragments of the *Bacillus subtilis* 168 *pyrD* (glutamate synthase, large subunit) gene
- kan* encodes kanamycin acetyl transferase; selectable in either *E. coli* or *B. subtilis* (kanamycin 5 µg/ml)
- bla* encodes β-lactamase; selectable in *E. coli* only (ampicillin 100 µg/ml)

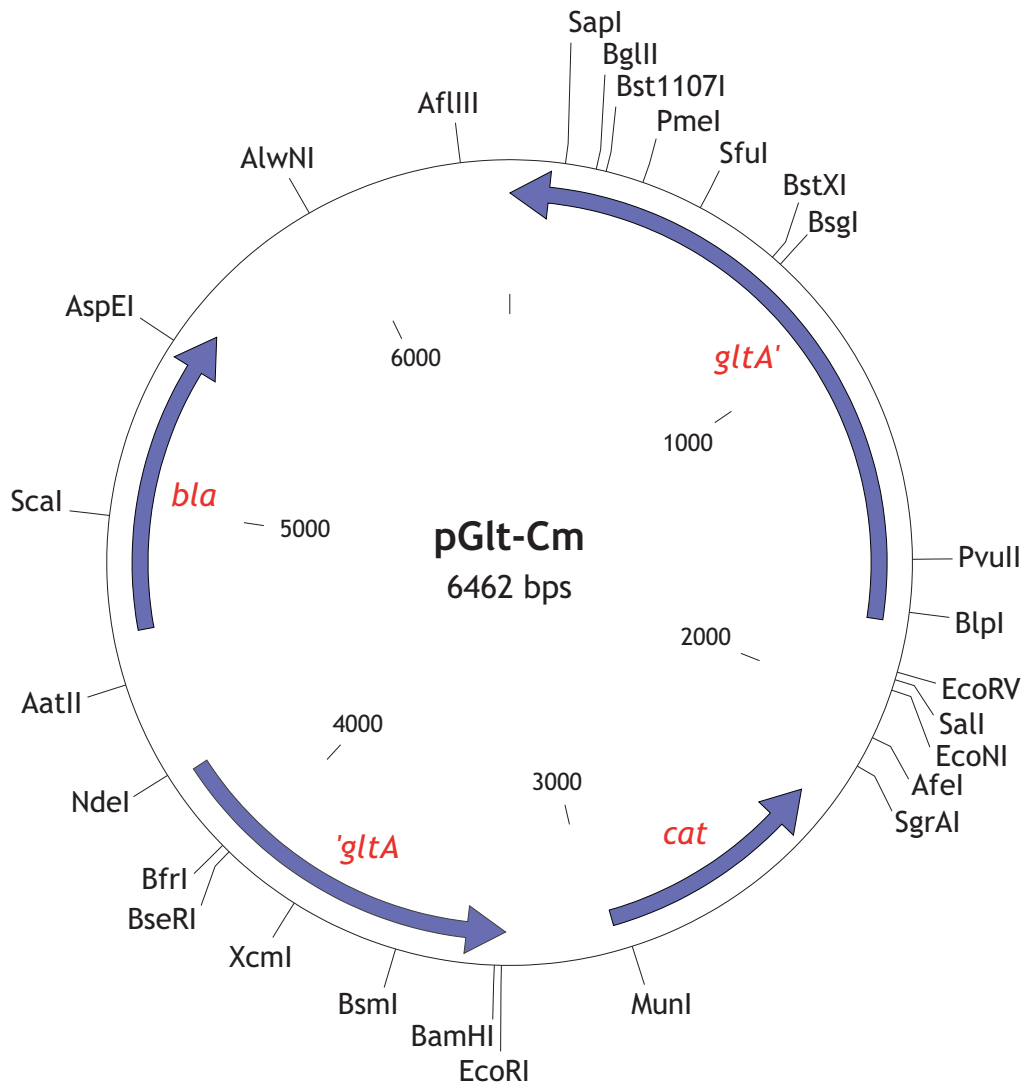
**Description:** Ectopic integration vector; cassette integrates by double recombination between plasmid and chromosomal *pyrD* sequences.

**Construction:** For the vector backbone, pUC18 was digested with the PvuII, and the resulting 2364 bp fragment was self-ligated. The 5' and 3' ends of *pyrD* were amplified from *B. subtilis* PY79 chromosomal DNA with *Ascl* restriction sites incorporated into the primers nearest the middle of the target gene. Recombinant PCR was then used to fuse the two portions of the genes, and the chimera was ligated into the PvuII site of the vector backbone. The *kan* gene and multiple cloning sites were amplified from pER82 using oligonucleotides containing *Ascl* sites. The PCR products were then digested and cloned into the *Ascl* sites in the middle of the cloned *pyrD* chimera.

**Use:** The plasmid is designed to integrate a cloned insert into the *B. subtilis* 168 chromosome at the *pyrD* locus. The user inserts the fragment of interest into any site lying between the *pyrD* fragments but outside *kan*. The plasmid is transformed into any *B. subtilis* 168 host (see below), with selection for kanamycin resistance. Transformants can be screened for pyrimidine auxotrophy on minimal media (indicating that the resident *pyrD* locus has been replaced).

**Recipient strains:** pPyr-Kan should work with any strain derived from *B. subtilis* 168.

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



**BGSC Accession:** ECE172

**Original Code:** RM33

**Reference:** Middleton, R. (unpublished).

**Sequence:** GenBank accession number [AY464560](#)

**Features:**

- gltA'*-*'gltA* end fragments of the *Bacillus subtilis* 168 *gltA* (glutamate syntase, large subunit) 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 100 µg/ml)

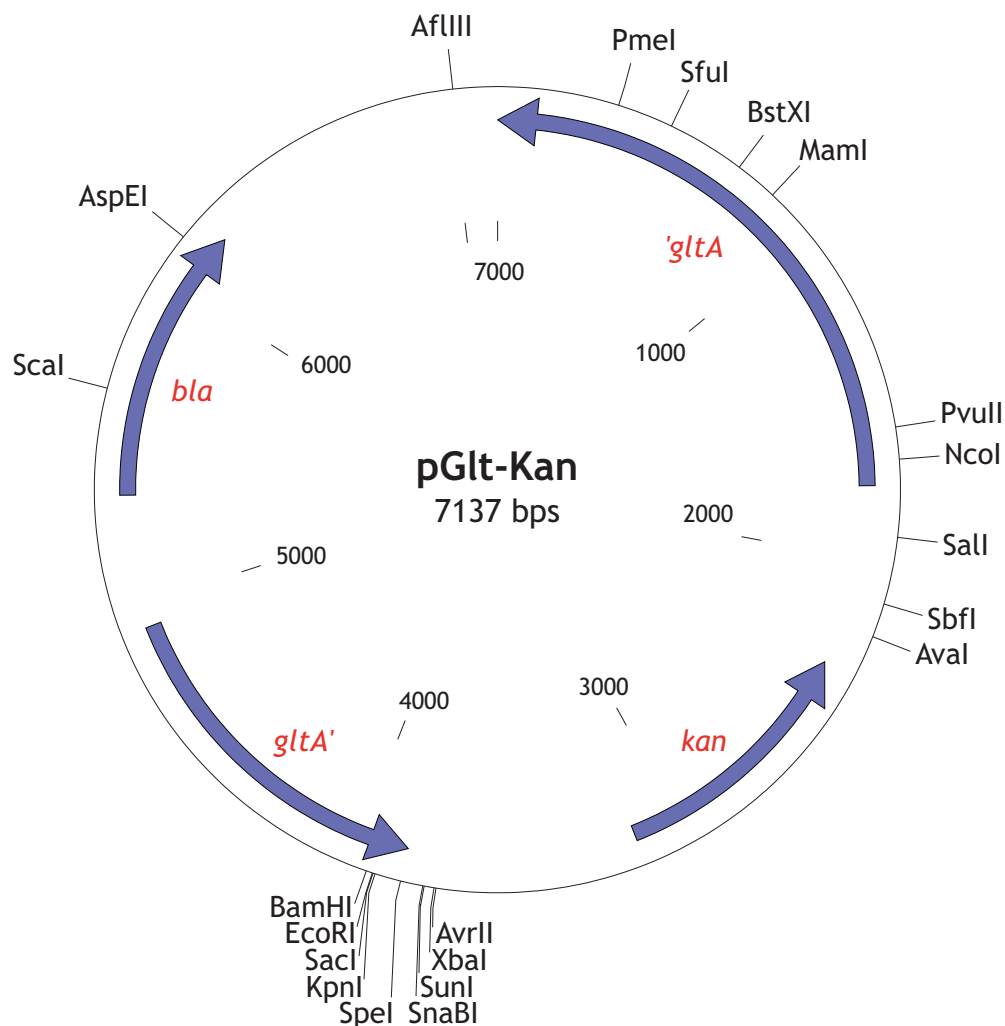
**Description:** Ectopic integration vector; cassette integrates by double recombination between plasmid and chromosomal *gltA* sequences.

**Construction:** For the vector backbone, pUC18 was digested with the PvuII, and the resulting 2364 bp fragment was self-ligated. The 5' and 3' ends of *gltA* were amplified from *B. subtilis* PY79 chromosomal DNA with *Ascl* restriction sites incorporated into the primers nearest the middle of the target gene. Recombinant PCR was then used to fuse the two portions of the genes, and the chimera was ligated into the PvuII site of the vector backbone. The *cat* gene and multiple cloning sites were amplified from pDG364 using oligonucleotides containing *Ascl* sites. The PCR products were then digested and cloned into the *Ascl* sites in the middle of the cloned *gltA* chimera.

**Use:** The plasmid is designed to integrate a cloned insert into the *B. subtilis* 168 chromosome at the *gltA* locus. The user inserts the fragment of interest into any site lying between the *gltA* fragments but outside *cat*. The plasmid is transformed into any *B. subtilis* 168 host (see below), with selection for chloramphenicol resistance. Transformants can be screened for glutamate or aspartate auxotrophy on minimal media (indicating that the resident *gltA* locus has been replaced).

**Recipient strains:** pGlt-Cm should work with any strain derived from *B. subtilis* 168.

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



**BGSC Accession:** ECE173

**Original Code:** RM34

**Reference:** Middleton, R. (unpublished).

**Sequence:** GenBank accession number [AY464559](https://www.ncbi.nlm.nih.gov/nuccore/AY464559)

**Features:**

- gltA'*-*'gltA* end fragments of the *Bacillus subtilis* 168 *gltA* (glutamate syntase, large subunit) gene
- kan* encodes kanamycin adenylyltransferase; 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)

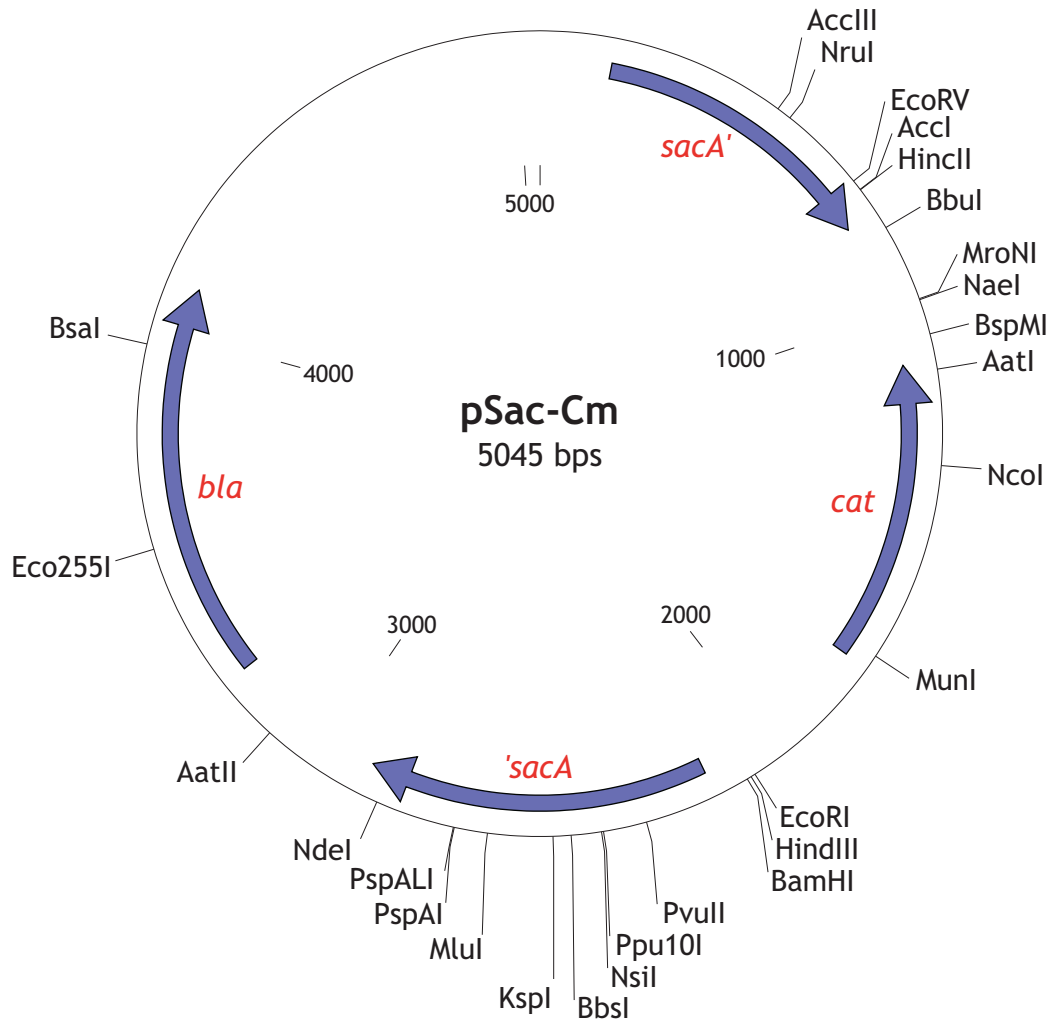
**Description:** Ectopic integration vector; cassette integrates by double recombination between plasmid and chromosomal *gltA* sequences.

**Construction:** For the vector backbone, pUC18 was digested with the PvuII, and the resulting 2364 bp fragment was self-ligated. The 5' and 3' ends of *gltA* were amplified from *B. subtilis* PY79 chromosomal DNA with *Ascl* restriction sites incorporated into the primers nearest the middle of the target gene. Recombinant PCR was then used to fuse the two portions of the genes, and the chimera was ligated into the PvuII site of the vector backbone. The *kan* gene and multiple cloning sites were amplified from pER82 using oligonucleotides containing *Ascl* sites. The PCR products were then digested and cloned into the *Ascl* sites in the middle of the cloned *gltA* chimera.

**Use:** The plasmid is designed to integrate a cloned insert into the *B. subtilis* 168 chromosome at the *gltA* locus. The user inserts the fragment of interest into any site lying between the *gltA* fragments but outside *kan*. The plasmid is transformed into any *B. subtilis* 168 host (see below), with selection for kanamycin resistance. Transformants can be screened for glutamate or aspartate auxotrophy on minimal media (indicating that the resident *gltA* locus has been replaced).

**Recipient strains:** pGlt-Kan should work with any strain derived from *B. subtilis* 168.

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



**BGSC Accession:** ECE174

**Original Code:** RM53

**Reference:** Middleton, R. (unpublished).

**Sequence:** GenBank accession number [AY464562](#)

**Features:**

- sacA'*-*'sacA* end fragments of the *Bacillus subtilis* 168 *sacA* (sucrase) 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 100 µg/ml)

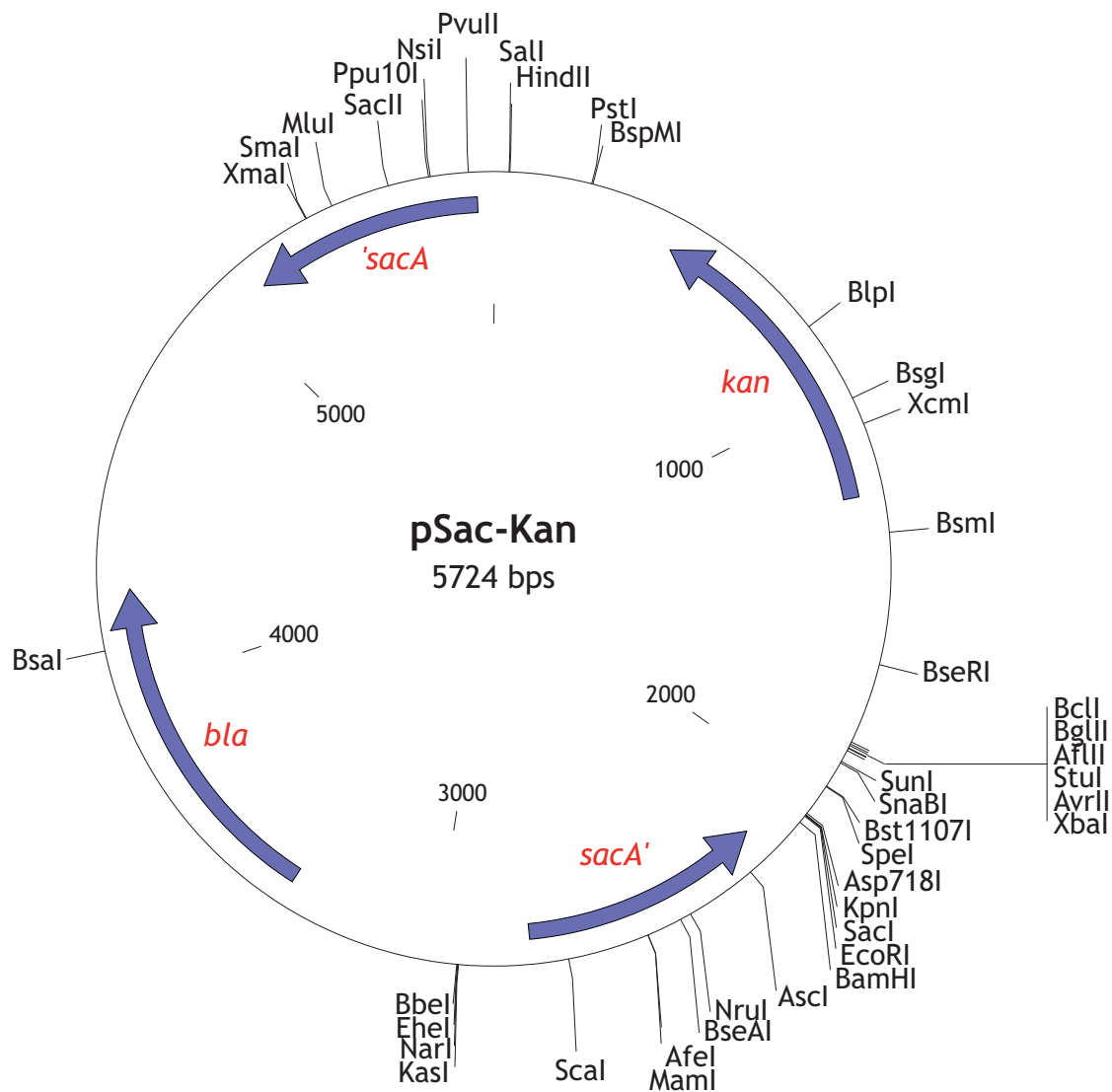
**Description:** Ectopic integration vector; cassette integrates by double recombination between plasmid and chromosomal *sacA* sequences.

**Construction:** For the vector backbone, pUC18 was digested with the PvuII, and the resulting 2364 bp fragment was self-ligated. The 5' and 3' ends of *sacA* were amplified from *B. subtilis* PY79 chromosomal DNA with *Ascl* restriction sites incorporated into the primers nearest the middle of the target gene. Recombinant PCR was then used to fuse the two portions of the genes, and the chimera was ligated into the PvuII site of the vector backbone. The *cat* gene and multiple cloning sites were amplified from pDG364 using oligonucleotides containing *Ascl* sites. The PCR products were then digested and cloned into the *Ascl* sites in the middle of the cloned *sacA* chimera.

**Use:** The plasmid is designed to integrate a cloned insert into the *B. subtilis* 168 chromosome at the *sacA* locus. The user inserts the fragment of interest into any site lying between the *sacA* fragments but outside *cat*. The plasmid is transformed into any *B. subtilis* 168 host (see below), with selection for chloramphenicol resistance. Transformants can be screened for inability to utilize sucrose as carbon source on minimal media (indicating that the resident *sacA* locus has been replaced).

**Recipient strains:** pSac-Cm should work with any strain derived from *B. subtilis* 168.

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



**BGSC Accession:** ECE175

**Original Code:** RM158

**Reference:** Middleton, R. (unpublished).

**Sequence:** GenBank accession number [AY464563](#)

**Features:**

- sacA'*-*sacA* end fragments of the *Bacillus subtilis* 168 *sacA* (sucrase) gene
- kan* encodes kanamycin acetyl transferase; selectable in either *E. coli* or *B. subtilis* (kanamycin 5 µg/ml)
- bla* encodes β-lactamase; selectable in *E. coli* only (ampicillin 100 µg/ml)

**Description:** Ectopic integration vector; cassette integrates by double recombination between plasmid and chromosomal *sacA* sequences.

**Construction:** For the vector backbone, pUC18 was digested with the PvuII, and the resulting 2364 bp fragment was self-ligated. The 5' and 3' ends of *sacA* were amplified from *B. subtilis* PY79 chromosomal DNA with AscI restriction sites incorporated into the primers nearest the middle of the target gene. Recombinant PCR was then used to fuse the two portions of the genes, and the chimera was ligated into the PvuII site of the vector backbone. The *kan* gene and multiple cloning sites were amplified from pER82 using oligonucleotides containing AscI sites. The PCR products were then digested and cloned into the AscI sites in the middle of the cloned *sacA* chimera.

**Use:** The plasmid is designed to integrate a cloned insert into the *B. subtilis* 168 chromosome at the *sacA* locus. The user inserts the fragment of interest into any site lying between the *sacA* fragments but outside *kan*. The plasmid is transformed into any *B. subtilis* 168 host (see below), with selection for kanamycin resistance. Transformants can be screened for inability to utilize sucrose as carbon source on minimal media (indicating that the resident *sacA* locus has been replaced).

**Recipient strains:** pSac-Kan should work with any strain derived from *B. subtilis* 168.

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