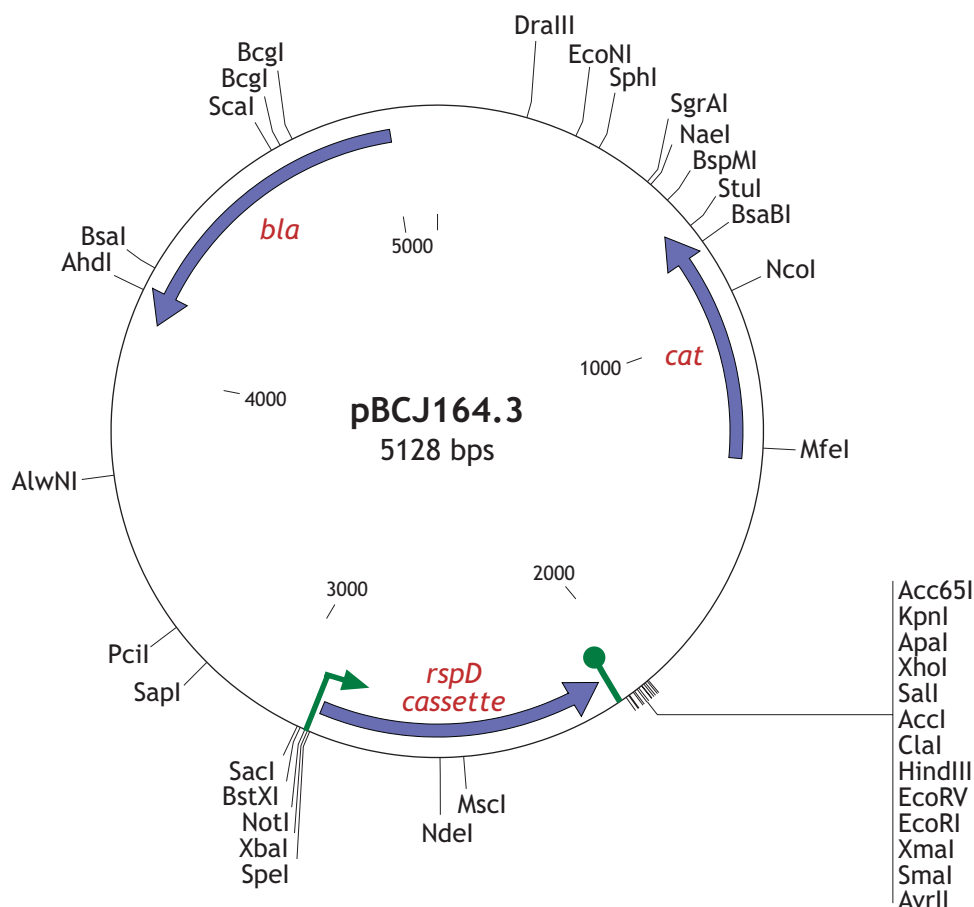


# Bacillus Genetic Stock Center

New Product Announcement

May 2004



**BGSC Accession:** ECE176

**Original Code:** TG1(pBCJ164.3)

**Reference:** Jester, B.C., J. D. Levensgood, H. Roy, M. Ibba, and K.M. Devine. 2003. Nonorthologous replacement of lysyl-tRNA synthetase prevents addition of lysine analogues to the genetic code. Proc. Natl. Acad. Sci. USA 100:14351-14356

**Sequence:** Not in database; available from BGSC at <http://www.bgsc.org/sequences/pBCJ164.htm>

**Features:**

- rspD cassette* promoter and the terminator regions of *B. subtilis* *rspD* joined by an NdeI site
- 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; facilitates a non-mutational integration at the *rspD* locus by a Campbell-type crossover event. Allows the constitutive expression of inserts cloned into the NdeI site.

**Construction:** The *B. subtilis* *rspD* promoter and terminator regions (base pairs 303442-3034772 and 3035391-303861 from the genome sequence, respectively) were amplified and tagged with BamHI and NdeI sites. The PCR products were digested with NdeI and ligated. The resulting *rspD* expression cassette was digested with BamHI and ligated into the corresponding site of pDIA5304.

**Use:** To allow the expression of inserts cloned into the NdeI site. Note: The level of expression is such that certain gene products can be deleterious. Such difficulties may be overcome by using *E. coli* TP611.

**Recipient strains:** Any transformable Rec<sup>+</sup> *B. subtilis* 168 derivative

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