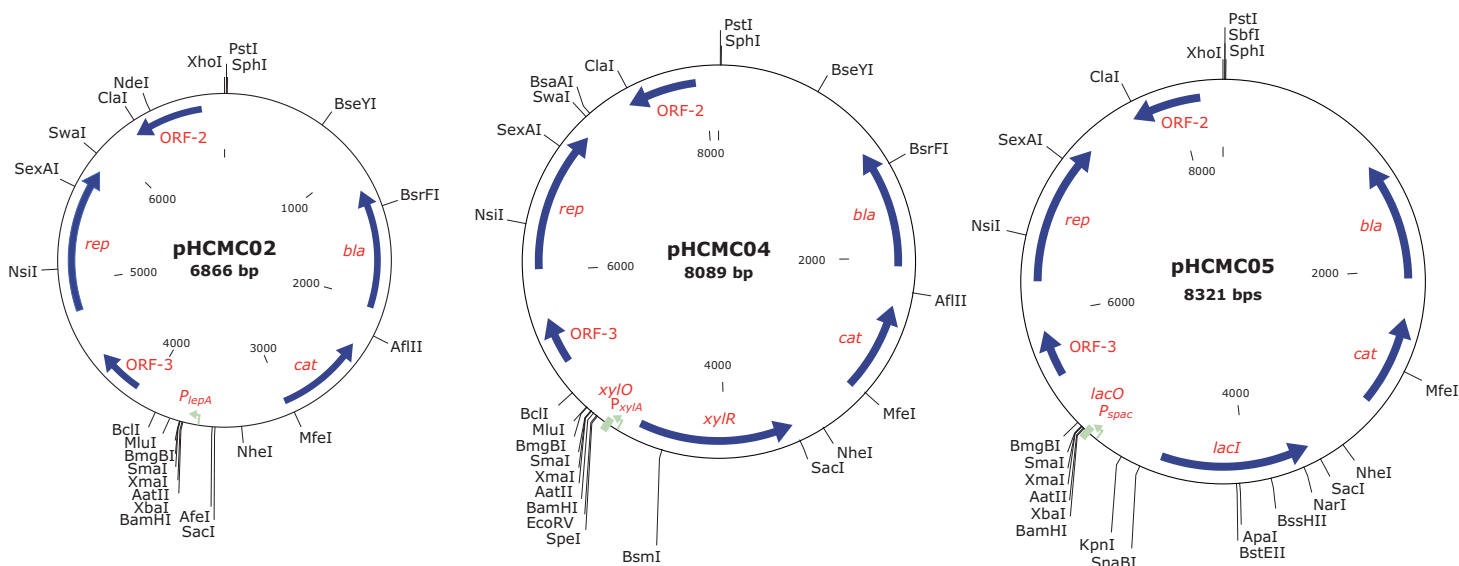


# Bacillus Genetic Stock Center

New Product Announcement

February 2006

## New Gram-Positive – *E. coli* Shuttle Expression Vectors Featuring High Structural Stability



**BGSC Accession:** ECE188, ECE189, ECE190

**Original Codes:** pHCMC02, pHCMC04, pHCMC05

**Reference:** Nguyen, H. D., Q. A. Nguyen, R. C. Ferreira, L. C. S. Ferreira, L. T. Tran, W. Schumann. 2005. Construction of plasmid-based expression vectors for *Bacillus subtilis* exhibiting full structural stability. *Plasmid* **54**:241–248.

**Sequence:** Please see: [http://www.genetik.uni-bayreuth.de/LSGenetik1/schumann\\_vectors.htm](http://www.genetik.uni-bayreuth.de/LSGenetik1/schumann_vectors.htm)

**Features:**

- rep* Replication initiation protein from theta replication plasmid pBS72 (GenBank AY102630)
- 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)
- ORF unknown function
- xyiR* represses transcription from the P<sub>xyiA</sub> promoter; xylose relieves repression
- lacI* represses transcription from the P<sub>spac</sub> promoter; IPTG relieves repression
- P<sub>lepA</sub> weakly constitutive promoter

**Description:** Production of foreign proteins in *Bacillus subtilis* has been technically difficult, in part because of the inherent structural instability of most Gram-positive plasmids carrying recombinant DNA inserts. The pHCMC series of expression vectors is unique, in that a theta-form replicating plasmid was used as a backbone, rather than the rolling circle replicating plasmids that have been more commonly used.

**Construction:** Each vector in this series was constructed on a backbone consisting of the replication regions of pBR322 (for Gram-negative replication) and pBS72 (for Gram-positives). A multiple cloning site (MCS) allows for the convenient insertion of foreign DNA. The strong *trpA* transcription terminator was inserted downstream from the MCS. A different expression cassette was inserted upstream from the MCS. For pHCMC02, the weakly constitutive *lepA* promoter was inserted. For pHCMC04, a xylose-inducible cassette was inserted. And for pHCMC05, the well-characterized IPTG-inducible P<sub>spac</sub> promoter was chosen.

**Use:** Insertion of foreign DNA into the multiple cloning site allows expression in *Bacillus subtilis* and presumably in other genetically tractable Gram-positive systems as well. Induction with pHCMC04 and pHCMC05 is accomplished with the addition of xylose (0.1-0.5%) and IPTG (0.1-0.5 mM), respectively.

**Our thanks to Wolfgang Schumann for donating pHCMC02, pHCMC04, and pHCMC05 to our collection!**