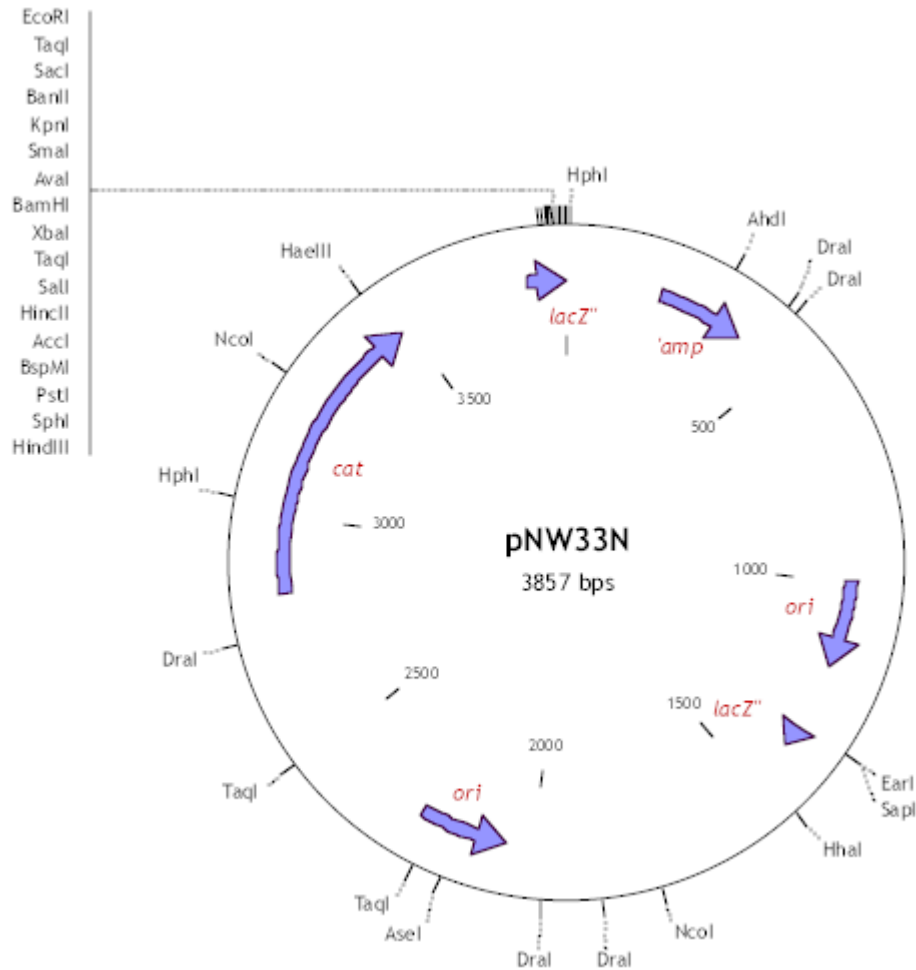


# Cloning Vector for Thermophilic *Bacillus* Strains

Thermophilic *Bacillus* vectors have had a history of cloning problems—most importantly, serious rearrangements and deletions when cloning heterologous genes (3). Plasmid pNW33N, a fifth generation vector that stably replicates in *Bacillus subtilis*, *Geobacillus stearothermophilus*, and *Escherichia coli*, is available in BGSC *E. coli* strain ECE136. It features a large multiple cloning site and encodes a chloramphenicol acetyltransferase that is expressed in both gram-positives and gram-negatives. The vector is based on cryptic plasmid replicon pBC1 (1.6 kb) isolated from *Bacillus coagulans* and *Staphylococcus aureus* plasmid pC194 (1, 2). For *E. coli*, *G. stearothermophilus*, and *B. subtilis*, use 25, 7, and 5 µg/ml of chloramphenicol.



BGSC No.	Original Code	Description	Ref.
ECE136	JM109(pNW33N)	3857 bp <i>Geobacillus-Bacillus-E. coli</i> shuttle vector in JM109; Cm <sup>R</sup>	(3)

## References:

1. De Rossi, E., P. Brigidi, M. Rossi, D. Matteuzzi, and G. Riccardi. 1991. Characterization of gram-positive broad host-range plasmids carrying a thermophilic replicon. *Res. Microbiol* **141**:389-396.
2. De Rossi, E., P. Brigidi, N. E. Welker, G. Riccardi, and D. Matteuzzi. 1994. New Shuttle Vector For Cloning in *Bacillus stearothermophilus*. *Res. Microbiol.* **145**(8):579-583.
3. Welker, N. personal communication.