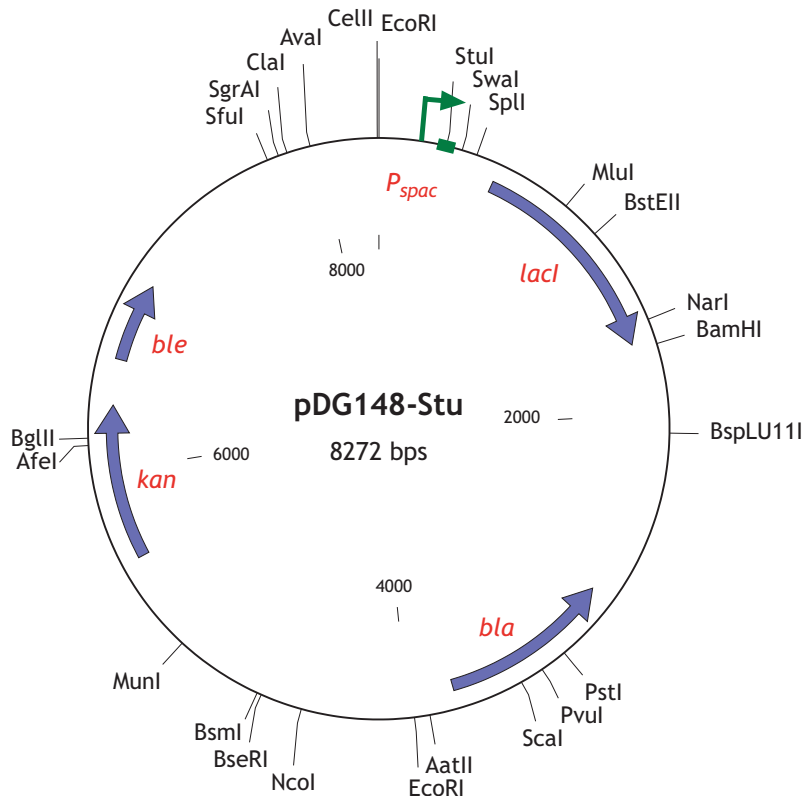


New Gram-Positive – *E. coli* Shuttle Vector Featuring Ligation-Independent Cloning and Inducible Expression



BGSC Accession: ECE145

Original Code: MC1061(pDG148-Stu)

Reference: Joseph, P., J.-R. Fantino, M.-L. Herbaud, F. Denizot. 2001. Rapid orientated cloning in a shuttle vector allowing modulated gene expression in *Bacillus subtilis*. FEMS Microbiol. Letts. **205**:91-97

Sequence: Not in database; available from BGSC at <http://www.bgsc.org/sequences/pDG148-Stu.htm>

Features:

- lacI* *lac* operon repressor from *E. coli*, engineered for expression in Gram-positive bacteria.
- P_{spac}* Hybrid promoter; regulated by the LacI repressor, inducible by IPTG.
- kan* encodes kanamycin adenyltransferase; selectable in either *E. coli* or *B. subtilis* (kanamycin or neomycin 5 µg/ml)
- ble* encodes bleomycin resistance protein (BRP); selectable in either *E. coli* or *B. subtilis* (bleomycin or phleomycin 0.5 µg/ml)
- bla* encodes β-lactamase; selectable in *E. coli* only (ampicillin 100 µg/ml)

Description: pDG148-Stu is a shuttle vector, replicating in *E. coli* from the pBR322 origin and in *Bacillus* from the pUB110 origin. It allows inducible expression of foreign inserts cloned into the *StuI* site. Oriented, ligation-independent cloning of PCR fragments is possible using the proper primers and a prepared template.

Construction: An adapter was inserted between the *HindIII* and *SphI* sites of the expression vector, pDG148.

Use: To allow oriented, ligation-independent cloning, the sequence of interest is amplified using primers with 5' overhangs. For the forward primer, the extra nucleotides are 5'-AAGGAGGAAGCAGGT-3', followed immediately by the start codon, ATG, for the amplified sequence. For the reverse primer, the extension is 5'-GACACGCACGAGGT-3'. After amplification, the PCR product is treated with T4 DNA polymerase in the presence of dATP. The vector is digested with *StuI*, then treated with T4 polymerase in the presence of dATP. The PCR product and vector now are flanked by 13 or 14 bp complementary overhangs. When they are mixed, these overhangs anneal, and the stable gapped circle is transformed directly into *E. coli*. Expression of the cloned sequence is inducible by IPTG in either *E. coli* or *B. subtilis*.

Our thanks to F. Denizot for donating pDG148-Stu to the BGSC Collection!