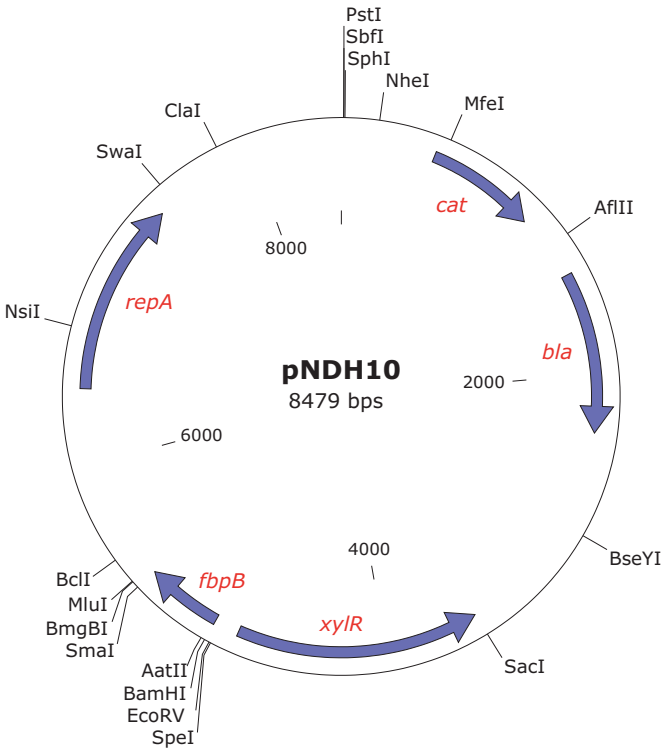
Bacillus Genetic Stock Center

New Product Announcement *July 2007*

Novel System for Immobilizing Foreign Proteins on the Cell Surface of *Bacillus subtilis*



BGSC Accession: ECE197 (see also ECE196 and 1A857) **Orignal Code:** DH5α(pNDH10)

Reference: **Nguyen, H. D., and W. Schumann.** 2006. Establishment of an experimental system allowing immobilization of proteins on the surface of *Bacillus subtilis* cells. J. Biotechnol. **122:**473-482.

Sequence: Not in database; available from BGSC at http://www.bgsc.org/sequences/pNDH10.htm

Features:

xylR	Repressor of the *B. megaterium xyl* operon; allows expression of *fbp* and fused sequences to be xylose-inducible
fbpB	C-terminal part of fibronectin binding protein B, including the sorting motif recognized by sortase A. Proteins fused to this fragment will be anchored to the cell wall of *B. subtilis* hosts that express SrtA.
repA	Initiation protein for theta-form Gram-positive plasmid replication; originally from pBS72.
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: pNDH10 is a shuttle vector, replicating at high copy number in *E. coli* in lower copy number in *Bacillus subtilis*. It features a xylose-inducible expression cassette upstream from the coding sequence of an anchoring protein that allows fused proteins to be anchored to the cell surface of a suitable host.

Construction: pNDH10 was constructed by inserting the 5' end of the fibronectin binding protein B gene (*fnbB*) of *Staphylococcus aureus* into the xylose-inducible expression vector pHCMC04 .

Use: A coding sequence inserted inframe with the plasmid *fbp* gene fragment will have xylose-inducible expression. The fusion protein will be anchored on the cell surface of *B. subtilis* strain NDH03 (=BGSC 1A857) or any other host that expresses sortase A. Plasmid pNDH09 is an integrative vector that can be used to construct such a host in the desired genetic background.

Our thanks to Wolfgang Schumann for donating pNDH09, pNDH10, B. subtilis NDH03 and to the BGSC Collection!