B. subtilis Transformations

Introduction

Bacillus subtilis transformations can be done with complete plasmids as well as linear fragments. However, the type you choose depends on your specific experiment and desired result.

1) ***Transforming B. subtilis with chromosomal DNA***
    - Use linear DNA fragments either from a genomic DNA prep, or generated in vitro by PCR with naturally competent *B. subtilis* cells
    - If integrating insert at a particular site, will need at least 500 bp of chromosomal homology blanking both sides of insert for efficient transformation
2) ***Transforming B. subtilis with integrative vector***
    - If integrating insert at a particular site on the chromosome (such as the amyE locus), you can perform the cloning in *E. coli* with an appropriate vector
    - Isolate plasmid DNA and either directly transform with miniprepped plasmid DNA or linearize via a single-site restriction enzyme digest that cuts the backbone outside the integration cassette
    - Transform linear DNA fragments into naturally competent *B. subtilis cells* , will need at least 500 bp of chromosomal homology blanking both sides of insert for efficient transformation

3) ***Transforming B. subtilis with replicative vector***
    - If transforming with a shuttle vector, perform cloning in *E. coli* and isolate plasmid DNA.
    - Can transform either naturally competent or electrocompetent *B. subtilis* hosts with the isolated circular plasmid DNA.

Protocol

For our project, we used restriction ligations to assemble our plasmids, which were then transformed in NEB5α *E. coli* cells. Backbone digests were CIP treated to prevent self-ligation. Plasmid DNA was then miniprepped, sequence-verified, and transformed into BGSC's 1A976 super-competent *B. subtilis* strain. We followed our mentor Dr. Ivan Lima's suggested protocol for maximizing *B. subtilis* transformation efficiency, copied below.

Optimized* B. subtilis *Transformation Protocol

1. Grow 1A976 *B. subtilis* culture for 5 h (until exponential stage of growth indicated by saturation; OD600 value of about 0.3-0.4):
    a. Take 2 ml from the culture and keep the rest in the fridge for future use.
2. Centrifuge at 8,000 rpm for 1m to collect the cells. Discard supernatant.

3. Add 200 ul of LB + 2% xylose and vortex. Here your are basically concentrating your cell suspension 10x.
    a. To make LB + 2% xylose solution, simply add xylose to this concentration in LB and filter sterilize it. DO NOT autoclave xylose because it will caramelize.
4. Add your plasmid (with chloramphenicol as a selective marker) and incubate 1h at 37C.
   Plate:
   - 20 ul in one LB plate (control).
   - 20 ul in one LB + chloramphenicol plate
   - 160 ul in one LB + chloramphenicol plate
   6) Seal the plates with parafilm and incubate at 37C overnight.

<u>References</u>:

Zhang X, Zhang YH. "Simple, fast and high-efficiency transformation system for directed evolution of cellulase in Bacillus subtilis." *Microb Biotechnol* 4(1): 98-105. (2011).