

Natural competent *Bacillus subtilis*

This is a protocol from openwetware.org: <http://openwetware.org/wiki/Cfrench:BacTrans2>

Generation of *Bacillus subtilis* competent cells

*N.B. This protocol assumes the use of *B. subtilis* 168 trpC2, which requires supplementation of minimal medium with tryptophan. For prototrophs this can be omitted, and for additional auxotrophies the appropriate nutrients should be added.*

1. Inoculate cells from a single fresh colony into 10 ml minimal medium (in a 250 ml conical flask).
2. Grow cells at 37°C with shaking at 200 rpm for 18 hours.
3. Subculture 1.4 ml into 10 ml prewarmed, fresh minimal medium and continue growth for 3 hours.
4. Add 11 ml starvation medium, continue growth for 2 h 45 min.
5. Either add sterile glycerol to 10% (v/v), dispense 0.3 ml aliquots and snap freeze in liquid nitrogen or continue directly with transformation.

Transformation of *B. subtilis* competent cells

For chromosomal integration of plasmid DNA by double-crossover, linearise plasmid by restriction endonuclease digestion. For genomic DNA, single-crossover or a replicating plasmid no pre-treatment is needed.

1. Thaw competent cells (if needed) at 37°C and transfer cells to a 15 ml polypropylene tube.
2. Add DNA (20 ul of plasmid digest, 10 ul of circularised plasmid or genomic DNA) and incubate at 37°C with shaking at 200 rpm for 1 hour.
3. Add 700 ul LB medium and continue growth for 1.5-2 hours depending on resistance marker for selection (e.g. 1.5 hours for erythromycin, 2 hours for chloramphenicol)
4. Plate 20-200 ul onto LB agar, grow for 18-24 hours at 37°C.

Media

Minimal salts solution (5x)

- Ammonium sulphate, 2 g; potassium hydrogen phosphate, 14.8 g; potassium dihydrogen phosphate, 5.4 g; sodium citrate, 1.9 g; magnesium sulphate heptahydrate, 0.2 g.

- Dissolve in 150 ml deionized water and adjust to pH 7.0 with hydrochloric acid/sodium hydroxide. Adjust volume to 200 ml and autoclave.

Minimal growth medium

- Per 50 ml: minimal salts solution,
 - 10 ml; glucose (50% (w/v)), 0.5 ml; casamino acids (2% (w/v)), 0.5 ml; tryptophan (10 mg/ml), 0.1 ml; iron ammonium citrate (2.2 mg/ml), 0.05 ml, deionized water, 39 ml.
 - Prepare freshly and filter sterilize.

Starvation medium

- Per 50 ml: minimal salts solution,
 - 10 ml; glucose (50% (w/v)), 0.5 ml; deionised water, 39.5 ml.
 - Filter sterilize before use.