

Development of Competence in *Bacillus subtilis* and Transformation (ISN 2.0 Protocol)

Day 0: From a single colony, inoculate 5 ml of SP medium in a tube and incubate at 37°C O/N with aeration and 150rpm

Day 1:

1. Dilute 1:50 of the O/N cultures on a 16×125 mm test tube (or equivalent) with 500µl of fresh SP medium
2. Add 1µg of DNA and incubate at 37°C with aeration, 150 rpm, 5h30min
3. Centrifuge the culture and resuspend in 1ml of LB medium (pre-heated at 37°C)
 - a. Centrifugation + Resuspension is done in an Eppendorf, but the incubation is done in the same tubes as the previous steps
4. Incubate for 1h30min at 37°C with aeration and 150rpm;
5. Plate the whole culture in desired media.
 - a. Resuspension in 50µl recommended

SP Medium Preparation

Spizizen Salts (1L)

- K₂HPO₄ 14 g
- KH₂PO₄ 6 g
- Trisodium citrate 1 g
- ddH₂O to 1 L

SP Medium (10ml)

- Spizizen salts 9.215 ml
- Glucose 50% (w/v) 400 µl
- Casein hydrolyzate 5% (w/v) 200 µl
- Tryptophan 5mg/ml 100 µl
- Ammonium ferric citrate 22mg/ml 5 µl
- Potassium glutamate 40% (w/v) 50 µl
- MgSO₄ 1M 30 µl