**B. subtilis Transformations**

Procedure for transforming *B. subtilis* bacteria.
Materials and Reagents

**LB plates containing antibiotic**

**37 C Incubators**

**HS medium:**
Spizizen’s medium supplemented with
- 0.5% glucose
- 50 μg/ml DL-tryptophane
- 50 μg/ml uracil
- 0.02% casein hydrolysate
- 0.1% yeast extract [Difco]
- 8 μg/ml arginine
- 0.4 μg/ml histidine
- 1 mM MgSO₄

**LS medium:**
Spizizen’s medium supplemented with
- 0.5% glucose
- 5 μg/ml DL-tryptophane
- 5 μg/ml uracil
- 0.01% casein hydrolysate
- 0.1% yeast extract [Difco]
- 1 mM MgSO₄
- 2.5 mM MgCl₂
- 0.5 mM CaCl₂

**10x Spizizen’s medium:**
- 2 g (NH₄)₂SO₄
- 14 g K₂HPO₄
- 6 g KH₂PO₄
- 1 g sodium citrate
- add 100 ml distilled water
- autoclave or filter sterilize with 0.25 um filter
- then add 0.1 ml 1 M MgSO₄
Transformation Protocol

Note: All materials that come into contact with bacteria must be sterile.

For transformation of *B. subtilis* we recommend the protocol of Anagnostopoulos and Spizizen (1961), slightly modified:

1. Grow *B. subtilis* overnight at 37 °C in 5 ml HS medium
2. Inoculate 20 ml LS medium with 1 ml of overnight HS culture and let shake for 3 - 4 hours at 30 °C
3. Withdraw 1 ml of his LS culture (late log/early stationary phase; OD$_{578}$), add 10 μl of 0.1 M EGTA, and incubate at RT for 5 minutes
4. Add 1 - 2 μg of plasmid DNA
5. Shake for 2 hours at 37 °C. The cells will develop antibiotic resistance during this time
6. Plate 200 ul on selective plates (5 μg/ml chloramphenicol)
7. Incubate plates at 37 C overnight.
8. Next day pick colonies and screen for clones that have your plasmid (SOP 104, SOP 105 & SOP 106).

References
