

Transformation of *B. subtilis*

Day 1 (10 min):

1. Start an overnight culture 37°C in minimal medium (MM: 10ml SMM basic salts, 125µl 40% (w/v) glucose, 100µl 2% (w/v) tryptophan, 60µl 1M Mg₂SO₄*7H₂O, 10µl 20% (w/v) casamino acids, 5µl 2.2mg/ml ferric ammonium citrate).

Day 2 (1 h):

1. Cells are diluted 1:10 in MM and grown for 3h at 37°C. Meanwhile, starvation medium (SM: 10ml SMM basic salts, 125µl 40% (w/v) glucose, 60µl 1M Mg₂SO₄*7H₂O) is prepared and prewarmed at 37°C.
2. The culture is diluted 1:2 in SM and cells are made competent with a further 2h incubation period at 37°C.
3. 400µl cell aliquots are mixed with 1µg recombinant DNA PCR and incubated at 37°C for 1 h
4. Cells are spin down, concentrated and plated on LB+Antibiotic and incubated at 37°C overnight.

Day 3

1. Colonies are tested for the integration of the recombinant DNA by PCR and sequencing.