Transformation

Preparation of competent *B. subtilis* cells and transformation with plasmid DNA – the 'Paris method'

Media

MMx5	Identical to minimal salts (x5) from Groningen method except that $MgSO_4$ is omitted.
MG	20ml MMx5; 2.5ml glucose (20% (w/v)); 0.5ml $MgCl_2$ (1M); H2O to 100ml.
MG1	50ml MG; 0.5ml casamino acids (2% (w/v)); amino acid growth factors to 50μg/ml each.
MG2	100ml MG; 0.5ml casamino acids (2% (w/v)); amino acid growth factors to 10μg/ml each.
Transformation buffer	2ml MMx5; 0.2ml $MgCl_2$ (1M); 0.25ml glucose (20% (w/v)); 0.1ml EGTA (0.1M); H_2O to 10ml.

Competent cells

1. Inoculate TBAB agar slant in a test tube (surface of the agar ~10cm^2) with a fresh culture. Incubate overnight at 30ºC (allow for sufficient oxygen supply by not tightening the screw cap).
2. Resuspend cells from the TBAB slant into 5ml of medium MG1 in a 250ml Erlenmeyer flask. Adjust the OD_{650} to ~0.7.
3. Incubate the culture for 3 hours at 37ºC in a waterbath shaker (200rev/min).
4. Dilute the culture 10-fold in 50ml of pre-warmed MG2 in a 1-1 Erlenmeyer flask.
5. Incubate for 90 minutes with vigorous shaking at 37ºC.
6. Pellet the cells by centrifugation (5000*g*, 5 minutes, 20ºC; pre-warm rotor). Save the supernatant as well; add to it glycerol to 8.5% (v/v) and glucose to 0.5%. Mix.
7. Immerse the microfuge tubes in a liquid nitrogen bath.
8. Resuspend the cell pellet gently in 4.5ml of the supernatant (plus glycerol and glucose). Mix gently.

9. Distribute competent cells in small portions in microfuge tubes (placed in liquid nitrogen) and transfer these quickly to -80ºC.

Transformation

1. Add 1-10μl of DNA solution to a 1.5ml microfuge tube.
2. Thaw quickly (37ºC, waterbath) an aliquot of frozen competent cells and dilute this 10-fold in transformation buffer. Mix gently (do not vortex).
3. Transfer 100μl of thawed cells to the DNA in the microfuge tube, mix and incubate for 20 minutes at 37ºC (without shaking).
4. Add 500μl of LB medium and incubate for 1-1½ hours at 37ºC with shaking to allow for the expression of antibiotic markers.
5. Plate on LB agar plates supplemented with appropriate antibiotics.

<u>Preparation of competent B. subtilis cells and transformation with plasmid DNA – the 'Groningen method'</u>

Media

Minimal salts (x5)	per litre: $(NH_4)_2SO_4$, 10g; K_2HPO_4, 74g; KH_2PO_4, 27g; trisodium citrate, 9.5g; $MgSO_4.7H_2O$, 1.0g; pH7.0.
Glucose	20% (w/v) solution.
Casamino acids	2% (w/v) solution.
Minimal-growth medium	per 100ml: 20ml minimal salts; 2.5ml glucose; 1ml casamino acids. If required, supplement with appropriate growth factors (amino acids and nucleotides, 20μg/ml; vitamins, 0.5μg/ml).
Starvation medium	per 100ml: 20ml minimal salts; 2.5ml glucose.

Competent cells

1. Plate the appropriate strain to check the phenotype (auxotrophic markers, antibiotic resistances etc.).
2. Inoculate 10ml of minimal growth medium in a 100ml flask with cells from a single colony.
3. Grow overnight (~16-18 hours) with agitation (waterbath shaker, 200rev/min).
4. Add 1.4ml of overnight culture to 10ml of pre-warmed fresh minimal-growth medium (plus growth factors) in a 100ml flask.

5. Grow for 3 hours at 37ºC with agitation (shaker, 200rev/min).
6. Add 11ml of starvation medium and continue growth for 2 hours at 37ºC with vigorous agitation (shaker, 300rev/min).
7. The culture is now maximally competent (for at least ½ an hour).
8. Freeze the culture for future use. Add sterile glycerol to 10% (v/v), mix and disperse in small portions (e.g. 0.5ml). Freeze quickly at -80ºC or in liquid nitrogen. The loss of competency is usually less than two-fold. Use a fresh aliquot each time.

Transformation

1. Transfer 100μl of freshly prepared competent cells to a sterile 2.5ml micro-centrifuge tube (or thaw quickly in a 37ºC waterbath an aliquot of frozen competent cells and use immediately).
2. Add 1-10μl of plasmid DNA and mix. For saturating amounts of DNA, add 0.2-0.5μg instead.
3. Incubate for 25 minutes at 37ºC in a waterbath shaker (200rev/min).
4. Add 500μl of LB medium and incubate for 1-1½ hours at 37ºC with shaking to allow for the expression of antibiotic markers.
5. Plate 0.1ml of appropriate dilutions (in LB medium) on LB agar containing selective antibiotics.
6. Incubate the plates at 37ºC.

<u>One-step Transformation Procedure</u>

1. Streak recipient strain heavily on one-half of a TBAB agar plate. Incubate for 18 hours at 37ºC.
2. Inoculate into (n + 0.5)ml of MB in a test tube (where n is the number of selective plates you will use) heavily enough so that slight turbidity is visible. Incubate with aeration for 2 hours.
3. Distribute 1ml of the competent culture into n labelled tubes. Add 0.1ml or less of sterile DNA to each tube and incubate for 1 hour.
4. Add 2.5ml of SC to each tube. Centrifuge (8000g, 10 minutes, RT) and discard supernatant.
5. Resuspend each cell pellet in 0.2ml of SC. Plate 0.1ml of undiluted suspension on selective plates.

Two-step Transformation Procedure

Preparation of competent cells

1. Streak out the strain to be made competent on an LB or TBAB agar plate as a large patch and incubate overnight at 30ºC.
2. The following morning, scrape the cell growth off the plate and use to inoculate fresh, pre-warmed SpC medium (20ml) to give an OD_{600} reading of about 0.5.
3. Incubate the culture at 37ºC with vigorous aeration and take periodic OD readings (OD_{600}) to assess cell growth.
4. When the rate of cell growth is seen to depart from exponential (i.e. no significant change in cell density over 20-30 minutes) inoculate 200ml of pre-warmed SpII medium with 2ml of stationary-phase culture and continue incubation at 37ºC with slower aeration.
5. After 90 minutes incubation, pellet the cells by centrifugation (8000*g*, 5 minutes) **at room temperature**.
6. Carefully decant the supernatant into a sterile container and **save**.
7. Gently resuspend the cell pellet in 18ml of the **saved** supernatant and add 2ml of sterile glycerol; mix gently.
8. Aliquot the competent cells (0.5ml) in sterile tubes, freeze rapidly in liquid nitrogen or a dry-ice/ethanol bath and store at -70ºC.

Transformation

1. Thaw competent cells rapidly by immersing frozen tubes in a 37ºC water bath,
2. Immediately, add one volume of SpII + EGTA to the thawed cells; mix gently.
3. In a sterile test tube add competent cells (0.2-0.5ml) to the DNA solution (<0.1ml) and incubate in a roller drum at 37ºC.
4. Dilute the transformed cells as appropriate in T Base containing 0.5% glucose and plate immediately onto selective media.

Motility Experiments

Isolating motile derivatives

1. Streak each strain carefully at one point of a 0.7% TBAB agar plate.
2. Incubate overnight at 37ºC without inverting the plates.

3. Motile cells will swarm over the surface of the plate and should be picked from as far from the site of inoculation as possible. If the strain was non-motile there will be a contained streak with a few dendritic swarms emerging from the streak. Pick from the top of one of these swarms to recover a motile derivative.