Bacterial Transformation in 96 well reaction plate

Preparation of competent cells (Inoue et al., 1990)

- Inoculate 500 ml of SOB with an overnight grown bacterial culture.
- Incubation at 19°C, shaking to an O.D.: 0,5 - 0,6.
- Place the culture for 10min on ice.
- Centrifuge the bacterial culture at 4000rpm for 10min at +4°C using cold (-20°C) 250ml centrifuge tubes.
- Resuspend each bacterial pellet first in 5ml of ice-cold TB, then add another 75ml of ice-cold TB.
- Incubate on ice for 10min.
- Centrifuge at 4000rpm for 10min at +4°C.
- Resuspend each bacterial pellet first in 5ml of ice-cold TB, then add another 15ml of ice-cold TB.
- Add 2.8ml of fresh DMSO to each.
- Mix gently.
- Keep on ice for 10min.
- Aliquot the cells into 10ml using cold, sterile “falcon” tubes and aliquote the rest into sterile cold eppendorf-tubes.
- Freeze the competent cells in liquid nitrogen.
- Store everything at -80°C.

Bacterial Transformation in 96 well reaction plate

- Melt 1 tube of 10ml frozen bacterial competent cells on ice.
- Place the 96well reaction plate containing the cloning reactions on ice-water for 10min.
- Add 100µl of competent cells to each slot (using cold tips).
- Seal the 96well reaction plate with transparent adhesive.
- Keep on ice-water for minimum 20min.
- Place immediately the 96well reaction plate on ice-water for 5min.
- Prepare a 96-well reaction plate (size 2ml) with 900µl of SOC in each well.
- Transfer the transformation in this 2ml 96well reaction plate filled with SOC.
- Cover this 2ml 96well reaction plate with gas permeable adhesive seals.
- Incubate for 1h at 37°C with shaking.
- Plate out 200µl of each transformation on appropriate antibiotic-containing LB plates.
- When plates are dry, incubate overnight (12 to 16h) at 37°C.

Composition of media and buffer:

2 litters
• SOB : 2 % (w/v) bacto tryptone 40 g
  0.5 % (w/v) yeast extract 10 g
  10 mM NaCl 1.17 g
  2.5 mM KCl 373 mg
  10 mM MgCl₂ 4 g
  10 mM MgSO₄ 4.93 g
  Adjust the pH to 7 with NaOH

• SOC : SOB + 20 mM glucose 7.2 g
  Adjust the pH to 7 with NaOH

• TB : 10 mM Pipes 3 g
  15 mM CaCl₂ 2.2 g
  250 mM KCl 18.6 g
  Adjust the pH to 6.7 avec KOH 5N
  Then add: 55 mM MnCl₂ 10.9 g
  sterilize by filtration (do not autoclave !)

Assess competency of cells
• use 5µl=50pg of puc19 (10pg/µl) to transform 100µl of competent cells. Plate 20µl of the transformation culture on LB + carbenicillin 100µg/ml and incubate at 37°C overnight.
• Estimation of the transformation efficiency:
  \[ e = \frac{\text{Nb col} \times 50}{50 \times 10^{-6}} = \text{Nb col} \times 10^6 \text{ col/µg d'ADN} \]
  Transformation efficiency of 2-5 \times 10^8 \text{ col/µg d'ADN} is currently obtained using this protocol.

Essential Points of the protocol :
• Preparation of competent cells with high transformation efficiency depend on
  o (1) growing bacteria culture at low temperature (about 19°C),
  o (2) harvesting bacterial cultures in logarithmic phase of growth,
  o (3) keeping cells on ice throughout the procedure.