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 10 min on ice
- Centrifuge the bacterial culture at 4000 rpm for 10 min at +4°C using cold (-20°C) 250 ml centrifuge tubes
- Resuspend each bacterial pellet first in 5 ml of ice-cold TB, then add another 75 ml of ice-cold TB
- Incubate on ice for 10 min
- Centrifuge at 4000 rpm for 10 min at +4°C
- Resuspend each bacterial pellet first in 5 ml of ice-cold TB, then add another 15 ml of ice-cold TB
- Add 2.8 ml of fresh DMSO to each
- Mix gently
- Keep on ice for 10 min
- Aliquot the cells into 10 ml using cold, sterile "falcon" tubes and aliquate 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 10 ml frozen bacterial competent cells on ice
- Place the 96 well reaction plate containing the cloning reactions on ice-water for 10 min
- Add 100 µl of competent cells to each slot (using cold tips)
- Seal the 96 well reaction plate with transparent adhesive
- Keep on ice-water for minimum 20 min
- Heat-shock for 15 sec. at 42°C in a water-bath
- Place immediately the 96 well reaction plate on ice-water for 5 min
- Prepare a 96 well reaction plate (size 2 ml) with 900 µl of SOC in each well
- Transfer the transformation in this 2 ml 96 well reaction plate filled with SOC
- Cover this 2 ml 96 well reaction plate with gas permeable adhesive seals
- Incubate for 1 h at 37°C with shaking
- Plate out 200 µl of each transformation on appropriate antibiotic-containing LB plates
- When plates are dry, incubate overnight (12 to 16 h) 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:
\[ e = \frac{Nb\ col \times 50}{50.10^{-6}} = Nb\ col \times 10^6\ col/\mu g\ d'ADN \]
Transformation efficiency of \(2-5 \times 10^8\ col/\mu g\ d'DNA\) 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.