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 10 min
- 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
- Heat-shock for 15 sec. at 42°C in a water-bath
- 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:

\[
e = \frac{\text{Nb col} \times 50}{50 \times 10^5} = \text{Nb col} \times 10^6 \text{ col/µg d'ADN}
\]

Transformation efficiency of \(2-5 \times 10^8 \text{ col/µg d'DNA}\) is currently obtained using this protocol.

**Essential Points of the protocol:**

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