Bacterial Transformation

Competent cells preparation (Rubidium Chloride protocol)

- Incubate colony of bacteria overnight in LB/SOC 37c shaker.
- Dilute 1:100 in YT media (16g bacto tryptone,10g bacto yeast extract, 5g NaCl, pH7.0 (with NaOH5N) – for 1L)
- Grow to OD 0.4-0.6 3-5h
- Spin cells **4C** 5000g 10min
- Remove media
- Add 100ml **TFB1**
  - Rubidium Chloride 100mM
  - Manganese Chloride 50mM
  - Potassium Acetate 30mM
  - Calcium Chloride monohydrate 10mM
  - Glycerol 15%
  - pH5.8 with acetic acid
  - filter sterilize, 4C before use
- resuspend (pipete)
- Incubate **on ice** 5min
- Spin cells **4C** 5000g 10min
- Add 5-10ml **TFB2**
  - MOPS 10mM
  - Rubidium Chloride 10mM
  - Calcium Chloride 75mM
  - Glycerol 15%
  - pH6.5 with KOH
  - Filter sterilize, 4C before use
- resuspend
- Incubate on ice 20-60min
- Freeze -80C in 1.5ml tubes (200ul)

Transformation

- Thaw competent cells on ice 15min
- Mix DNA (plasmid/ligation mix) with 100ul competent cells
- Incubate 30min on ice
- Heat shock 42C 45sec-1min
- Add 1ml LB
- Incubate 1h 37C with shaking
- Plate on LB with appropriate antibiotic