ELECTROCOMPETENT CELLS PREPARATION AND TRANSFORMATION

ADAPTED FROM THE BONNET TEAM PROTOCOL REPOSITORY

STEPS:

1. Streak the *E.coli* from glycerol stock.
2. Pre-culture.
3. Culture and preparation of electrocompetent cells.
4. Transformation test.
5. Calculate the efficiency.

MATERIALS:

To prepare 2L of culture :

- *E. coli* NEB10β (NEB, C3020K), TG1. Stored at -80°C
- pUC19 DNA (NEB N3041A): 50pg/µL in ddH2O. Stored at -20°C
- Ampicillin (Sigma): 100 mg/mL stock solution in ddH2O, filter-sterilized. Stored at -20°C
- Cam 50µg/mL (Sigma), Kana 25µg/mL(Sigma)
- SOC medium
- 2L LB medium
- 3L Pure H2O, autoclaved
- 2L of 10% (v/v) Glycerol in H2O, autoclaved (Measure 200mL of glycerol 99% in a measuring tube, add it in a 2L glass bottle, rinse the measuring tube with miliQ water, add the water in the same bottle, adjust the volume in the bottle to 2L with miliQ water)
- 2 x 250mL Measuring tubes autoclaved
- 1 x 500mL Measuring tubes autoclaved
- 4 x 500 ml centrifuge bottles (NALGENE)
- PCR tubes or 1.5mL tubes
- 1mL syringes for Eppendorf Multipipette
- 10mL, 5mL and 2mL pipettes
- 1 x 15mL tube
- High speed centrifuge (Sorvall)

- Shaking incubator at 37°C
- Electroporator (Bio-rad)
- Electroporation cuvettes (Bio-rad, 0.1 or 0.2 cm)

PROTOCOL:

Day 1:

- Streak the *E.coli* from glycerol stock (-80°C) onto a LB plate without antibiotic. Incubate the plate at 37°C overnight. The plate can be stored at 4°C for one week.

Day 2:

- Inoculate a single *E.coli* colony in 30 ml of LB medium. Grow overnight at 250 rpm and 37°C. The amount of overnight culture depends on how many liters of competent cells you are going to prepare. For 1L culture you need 4mL of overnight culture.

- Do a **negative control of contamination** with 4 antibiotics (Kam, Carb, Kana, Spec) : 2mL of inoculated culture + 2µL Antibiotic (4 different tubes). Grow overnight at 250 rpm and 37°C.

- Prepare 2YT medium or LB medium (500mL in 2L flask). Keep them at RT.

- Autoclave 3L pure H2O, 2L 10% glycerol, 3 Measuring tubes, 4 centrifuge bottles, PCR tubes or autoclaved 1.5mL tubes, PCR racks, 10mL 5mL and 2mL pipettes, few 1mL syringes for Eppendorf pipette and 1 x 15mL tube. Afterwards, keep them at 4°C.

- Book the 37°C incubator for tomorrow (4 x 2L from 9 to 13 for example).

- Book the centrifuge for tomorrow (Rotor F9 / 2500g / 4°C / during 3h).

Day 3:

Cells preparation

- Check the negative controls.

- Transfer 4 mL of overnight culture into 2-liter flask containing 1L of LB medium (1:250) without antibiotics. Homogenize.

- Do a **negative control of contamination** with 4 antibiotics (Kam, Carb, Kana, Spec): 2mL of inoculated culture + 2µL Antibiotic (4 different tubes). Grow overnight at 250 rpm and 37°C.

- Incubate at 250 rpm and 37°C until the OD_{600} is about 0.5 - 0.6 (~4h) (To measure the OD, measure the blank with 1mL LB medium).
  Note : For NEB10β, it takes about 4 hours to reach OD 0.52. You should check the OD of your culture after 3 hours and 30 minutes incubation time.

- After OD is reached, pour the culture into 4 pre-chilled 500 ml centrifuge bottles.

- Balance the weight of the bottles with the culture and chill them on ice for 30 min.

- Keep flasks to collect and decontaminate supernatants.
  Note : From here on, everything should be kept on ice, in the cold room and done as fast as possible.

- Cool down the centrifuge at 4°C.

- Spin at 2500g for 20 min at 4°C.

- Decant the sup in a "Flask –waste". **Be careful, always dry the pellet as much as possible and don't touch the flask with the bottle.**

- Use a sterilized 250mL measuring tube. Resuspend cell pellet **with a pipette and quickly, don't use vortex** in 250mL/bottle of pre-chilled pure H2O. You can pool it in 2 bottles (2 x 500mL) equilibrate and then spin at 2300g for 10 min at 4°C.

- Decant the sup in a "Flask – waste".

- Use the same sterilized 250mL measuring tube and resuspend the cell pellet as previously, in 250mL/bottle of pre-chilled pure H2O, and then spin at 2300g for 10 min at 4°C.

- Decant the sup in a "Flask – waste".

- Use a sterilized 250mL measuring tube and resuspend the cell pellet as previously, in 250 mL/bottle of pre-chilled 10% glycerol, and then spin at 2300g for 10 min at 4°C.
  Note : Take care when decanting because the cells lose adherence in 10% glycerol. Performing this right after centrifugation can reduce the loss.

- Repeat the last washing step for two times with 100mL/bottle of pre-chilled 10% glycerol.

- Carefully decant the sup and re-suspend cells in the remaining volume (about 1 ~ 2 mL) with a 2mL pipette. (If it's not possible, add 500µL glycerol 10% and re-suspend the pellet.) Transfer it in the 15mL cold tube.
  Note : This is best done by gently swirling rather than vortexing. Or use plastic pasteur pipette for gently flushing.

- Measure the OD_{600} of a 0, 5% dilution of the cell suspension. 5µL culture + 995µL H20, mix, measure. The spectrophotometer can measure only until OD_{600} = 1.0 (If the measure is too high, dilute it to1:2 dilution).
  1.0 OD_{600} = ~2.5 x 10^8 cells/mL. For High efficiency we need OD_{600} = 150-200.

- Aliquot immediately by 80µL into 1.5mL tubes, drop into a bath of liquid nitrogen, and transfer to -80°C for storage.
  For Gibson assembly we need OD_{600} = 80.

- Do a **negative control of contamination** with 4 antibiotics (Kam, Carb, Kana, Spec): Add 8mL LB medium in the 15mL Tubes with few µL of re-suspended pellet, Pour in 4 tubes (2mL/tube) + 2µL Antibiotic / tube. Grow overnight at 250 rpm and 37°C.

Day 3 or 4:

Cells transformation by electroporation and efficiency test

- Pre-warm the SOC medium at 37°C. Pre-warm the plate containing selection antibiotics at 37°C for 1 hour.

- Pre-chill cuvettes (Bio-rad, 0.1 or 0.2 cm) on ice for 5 minutes before adding the cell/DNA mixture.

- Thaw the electrocompetent cells on ice. Add a) 100 pg of pUC19 control plasmid (2µL at 50pg/L) for efficiency test (it is possible to do it with 1µL just take it into consideration when you do the efficiency calculation), or b) 5 µL of your DNA sample for experiments, or d) 1µL of Gibson Assembly product in 40 µl of the thawed competent cells, mix by gently pipetting, and transfer to the pre-chilled cuvette. Stand for 1 min.

- Perform electroporation by Bio-Rad GenePluser, Ec1 for 0.1 cm cuvette (brown) and Ec2 for 0.2 cm cuvette(green). (EC1, Bacteria = 1.8Kv and 6ms for Blanc or without cell). Flush the cuvette **immediately** with 1mL 37°C pre-warmed SOC medium and transfer to a 12mL culture tube. Recover for 1h at 37°C, 200 rpm shaking.

- Wash cuvettes with ethanol and after water two times.

Efficiency test

- Perform serial dilutions. Dilute 10µL of the culture in 990µL LB, then plate 100µL on LB Ampicillin plate (= 10^{-3})

- Dilute 10µL of $^{-3}$ in 990µL LB, then plate 100µL on LB Amp plate (= 10^{-5})
  (pUC19 = Ampi resistant)

In experiment

- Plate 100µL on a plate. Centrifuge the culture, throw 800µL, and plate the pellet resuspend in the 100µL SN.

- Incubate Overnight at 37°C.

Day 4:

- Calculate the competency in colonies.

Ex: On the 10^{-3} plate we have 27 colonies = $2,7.10^4$ UFC/mL (in our tube after electroporation)
2µL pUC19 = 100pg = $2,7.10^4$ UFC
100 ng = $2,7.10^7$ UFC
Efficiency $2,7.10^8$ UFC/µg pUC19

TROUBLESHOOTING

- **Low colony number of pUC19 transformed plate :**

1. Check the DNA quality and quantity. The OD 260:230 should be higher than 1.6 at least.
2. Check the preparation procedure of competent cells. Especially the cell concentration. The OD_{600} of 100-fold dilution of competent cells should be higher than 1.
3. Check the cuvette. Rusted or residual water on its surface ?
4. Check the voltage and time constant. The typical time constant is 4.8 to 5.1. If the time constant is lower than 4.5 ms, check your DNA preparation procedure. You should elute your DNA by pre-warmed H2O.
5. Your sample volume should be less than 5λ in 50λ transformation reaction to prevent the dilution of competent cell concentration.