Competency

Aim: To make competent E coli cells (works for DH5α and BL21).

Materials required:

1. LB miller broth
2. E coli cells
3. conical flasks
4. falcon tubes
5. Ice and ice box
6. $CaCl_2$ solution (20mM and 0.1M)
7. 80mM $MgCl_2$ solution
8. 1.5mL microcentrifuge tubes

Procedure:

Chemical method:

1. Take 10 mL LB miller broth and inoculate it with E. coli and let it grow for 12 hours. Check OD600. (Primary solution)

2. Transfer the colony in 50mL LB miller broth in 250mL (1:5 ratio) conical flask (or if possible 1:10 ratio i.e. volume of culture: volume of the conical flask)
   Note: If OD600 in step1 is ~1, add 3mL of the primary solution to the new 50mL LB miller broth.

3. Now take OD after 30-60 min to get the idea of growth and keep on checking until it reaches 0.35-0.4.

4. Transfer culture to sterile disposable ice-cold falcon tubes. Cool culture to 0-4^{0}C by incubating it on ice for 10 minutes.
   (Note: Maintaining low-temperature (0-4^{0}C) from now on is the key for getting high transformation efficiency.)

5. Centrifuge the culture at 4^{0}C at 4100rpm for 10 minutes (Since it takes time one can start with precooling in step 4 and in case it is taking more than 10 minutes just extend the time in step 4).

6. Decant the medium and resuspend the cell pellet in 30mL of ice-cold MgCl2 and CaCl2 solution (80mM MgCl2 and 20mM CaCl2) by gentle swirling. It might take time but make sure every cell has come in contact with the solution and the swirling is done in an ice box.

7. Again centrifuge at 4100rpm for 10 minutes at 4^0C.

8. Decant the media and resuspend in 2mL of ice-cold 0.1M CaCl2 for each culture.

9. Make aliquots of 100 microL each and store at -80^0C.

Transformation

Aim: To transform E coli with plasmid of interest.

Materials required:

1. competent E coli cells
2. Plasmid
3. 1.5 mL microcentrifuge tubes
4. Ice and icebox
5. LB miller broth
6. LB agar with appropriate antibiotic plate
7. micropipette and tips

Procedure:

1. Mix 30ng to 100ng of DNA/plasmid (depending on the concentration calculate the volume. Higher amount of DNA can inhibit transformation) with 100microL aliquots in 1.5mL centrifuge tubes.

2. Leave on ice for 15-30 minutes.

3. Heat shock the mixture in a water bath for 45 seconds at 42^0C.

4. Keep in ice for 5 minutes.

5. Add 1mL of LB miller broth (without antibiotics) and let it grow in the shaking incubator for 45-60 minutes (We did for 60 minutes).

6. Spread 50 microL on the appropriate antibiotic plate and place it in 37^0C static incubator.

7. For the remaining solution, centrifuge at 4000 rpm for 4 minutes and resuspend in 50 microL LB miller broth in case you want to spread all transform cells or either store the remaining solution at -80^0C.

8. Usually, waterdrop like transparent colonies start appearing after 3-4 hours but colonies appear after 12-13 hours. Don't incubate antibiotic plates for more than 24-48 hours if no growth is shown as after that contamination like fungi will start growing.

Competency and transformation

Aim: To transform E coli NEB10- beta with plasmid of interest.

Note: The protocol is the standard protocol followed for C3019I

Materials required:

1. High Efficiency Transformation Protocol using NEB 10-beta Competent E. coli (High Efficiency) (C3019H/C3019I)
2. Plasmid
3. 1.5 mL microcentrifuge tubes
4. Ice and icebox
5. LB miller broth
6. LB agar with appropriate antibiotic plate
7. micropipette and tips

Procedure:

1. Thaw a tube of NEB 10-beta Competent E. coli cells on ice until the last ice crystals disappear. Mix gently and carefully pipette 50 µl of cells into a transformation tube on ice.
2. Add 1-5 µl containing 1 pg-100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4-5 times to mix cells and DNA. Do not vortex.
3. Place the mixture on ice for 30 minutes. Do not mix.
4. Heat shock at exactly 42°C for exactly 30 seconds. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950 µl of room temperature NEB 10-beta/Stable Outgrowth Medium into the mixture.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates to 37°C.
9. Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in NEB 10-beta/Stable Outgrowth Medium.
10. Spread 50-100 µl of each dilution onto a selection plate and incubate overnight at 37°C. Alternatively, incubate at 30°C for 24-36 hours or 25°C for 48 hours.