1. Bacterial culture

For the different needs of our species have different culture methods, as follow.

1. Overnight Cultures
    1. Add 20mL autoclaved LB in a 100mL triangle bottle.
    2. Pipet 20μL of 1000X antibiotic into the LB.
    3. Select a single colony using a sterile toothpick or pipette tip.
    4. Place toothpick or pipette tip in the culture tube and stir.
    5. Remove toothpick, or leave in the bottle.
    6. Place culture tube in incubator at 37°C overnight shaking vigorously (180 RPM).
2. Plates screening
    1. Pour 20mL autoclaved LB with appropriate antibiotic into each plate.
    2. Take the appropriate amount of bacterium suspension on the plate and spread it homogeneous.
    3. Seal the plate with parafilm, place upside down and culture at constant temperature of 37° C for 12 – 14 hours.
3. Glycerol Stocks
    1. Pipet 0.5mL of glycerol into 1.5mL Eppendorf tubes
    2. Add 0.5mL of overnight culture to each tube
    3. Pipet up and down to gently mix
    4. Place the tubes in -80°C freezer
4. Making Chemically Competent E. coli Cells
    1. Inoculate 20 mL LB at 37°C while shaking（180RPM）.
    2. Shaking until OD600 is 0.4-0.6 (This step should require approximately 4-5 hours) .
    3. Pipet 1mL of glycerol into 1.5mL Eppendorf tubes.
    4. Centrifuge the subculture at 8 000 rpm at 4°C for 2 minutes.
    5. Re-suspend pellet in 1 mL of cold $CaCl_2$ (10 mM) and leave on ice for 30 minutes.
    6. Centrifuge at 8 000 rpm at 4°C for 2 minutes and re-suspend in 100μl of cold CaCl2 (10 mM, 15% glycerol solution).
    7. Then the competent cell was made and can be used to transformation or freeze at -80°C.
5. Inducible expression
    1.Shake culture bacterium solution in 1ml LB medium(contain 30 mg/L chloramphenicol) at 37° C, 200 rpm.
    2.Transfer bacterium solution (10% inoculum dose) into fresh 10ml LB culture medium, culture bacterium at 200 RPM, 37° C until OD600 = 0.6 - 0.8.
    3. Add IPTG to the solution until final concentration arrive 1mmol/L, culture at 37° C for 4 - 5 hours.