Preparation of Electroporation Competent Cell

1. Select monoclonal colony with tip, and drop it into 50ml BD tube with 10ml LB media. Set the blank control with media and tip at the mean time.
2. Culture for 14~16 hour (37℃, 220rpm).
3. The second day, transfer the bacteria solution into 1000ml LB media at the ratio of 1:100. Culture it for 2~3 hour (37℃, 220rpm), test the OD_{600} half an hour and stop culturing when the OD_{600} reach 0.3~0.4.
4. Precool the bacteria solution on ice for 30min, then distribute the solution into precooled 500ml centrifuge tube, 4℃, 2500rpm, centrifuge it for 10min.
5. Discard the supernatant, add a small amount of ddH_2O to resuspend the bacteria, and fill the tube with ddH_2O, 4℃, 2500rpm, centrifuge it for 10min.
6. Repeat step 5.
7. Discard the supernatant, add a small amount of 10% glycerol(sterilized and precooled) to resuspend the bacteria, and fill the tube with 10% glyce Electroporation rol, 4℃, 2500rpm, centrifuge it for 10min.
8. Discard the supernatant, add 5ml 10% glycerol into each tube, resuspend the bacteria and distribute the solution into 1.5ml EP tube, 300μl per tube.
9. Storage the tubes at -80℃.

Electroporation

1. Get the competent cell from -80℃ and wait for its fusion;
2. Add 1ul of plasmid into the tube;
3. Transform the liquid in the tube into a precooled electroporation cuvette;
4. Electroporate the cuvette, 2500V, 5ms.
5. Add 650ml SOC media into the cuvette immediately;
6. Plate the culture on LB plate containing corresponding antibiotics.

Knock-out

1. Primers designing;
2. Amplification the plasmid PKD3 with PCR method, run a agarose electrophoresis and purify the product with Gel Extraction Kit. PCR again with the production as templet and purify it with Gel Extraction Kit;
3. Prepare electroporation competent cell and transform the plasmid PKD46 into the cells. Plate the culture on LB plate containing ampicillin, overnight at 30℃;
4. Select successfully transformed colony, pick it into 4ml Amp^+ LB media, overnight at 30℃;

5. The second day, transfer the bacteria solution into 40ml Amp^+ LB media at the ratio of 1:100. Culture it (30℃, 180rpm) until the OD_{600} reach 0.2~0.3. Add L-arabinose to make the final concentration reach 30 mmol/L, introduce it for 1 hour in order to let the enzyme Exo, Bet and Gam express fully. Precool the bacteria solution on ice for 30min, then centrifuge it for 10min at. 4℃, 2500rpm. Discard the supernatant, wash it with 10% glycerol(sterilized and precooled) for three times, 4℃, 2500rpm, centrifuge it for 10min. Discard the supernatant, add 400μl 10% glycerol into each tube, resuspend the bacteria and distribute the solution into 1.5ml EP tube, 40μl per tube. Storage the tubes at -80℃.
7. Transform the PCR production purified in step 2 into the cell prepared in step 5. Plate the culture on LB plate containing chloramphenicol and ampicillin.
6. Pick the colony into LB media, overnight at 42℃, streak it on LB plate containing chloramphenicol, overnight at 37℃.