Competent Cell Preparation

The first method

1. Overnight cultivate glycerol bacteria, and transfer to the new no resistant medium on the second day afternoon. Measure the OD value every hour.
2. While the OD value is between 0.3-0.4, put the bacteria in the 1.5ml sterile centrifuge tube. (Full), stewing on the ice for 15-20 minutes.
3. Condition:4000rpm,2 min, centrifuge under 4℃ (Pre-cool the centrifuge to 4℃ and push the “fast cool” button)
4. Abandon supernatant, add 800μl pre cooling calcium chloride, re suspend sedimentation, bath for 30 minutes.
5. Add 200μl of calcium chloride to resuspend the precipitate to obtain competent cells, take 50μl for conversion.

The second method

1. Add 100μl pKD46-transformed E.coli BW25113 to 7ml liquid LB medium, along with 7μl ampicillin solution, shaking overnight at 30℃.
2. Take 5% of bacterium solution into 30ml no resistant liquid LB medium within 30μl ampicillin solution, cultivating at 30℃ shaker.
3. Detect OD600 each hour until it is between 0.3 and 0.4.
4. Add L-Arabinose until its concentration is 30mmol/L. Then shake it at 30℃ for 1h.
5. Take all of the bacterium solution into 1.5ml centrifuge tubes, ice bath for 10min.
6. Centrifuge the bacterium solution in the conditions of 5000rpm and 4℃ for 10min, abandoning the supernatant.
7. Suspend the sediment with pre-cooling 50% sterile glycerin till 1ml, centrifuging in the conditions of 5000rpm and 4℃ for 2min, repeat for 3 times.
8. Suspend the sediment with 300μl glycerin.