2024 NEFU_China

Preparation of electrocompetent cells

ABSTRACT

This protocol is used to prepare electrocompetent cells with high transformation efficiency.

BEFORE STARTING

All the medium and containers used in the protocol should be sterilized by high temperature autoclave.

All the steps that expose cells to air should be done in the super-clean worktable.

1.Line the E. coli strain on a solid LB medium without resistance and culture at 37℃ for 12h. Use solid LB medium with antibiotics of corresponding resistance if competent cells with plasmids are needed.

2.Pick monoclonal cells from the medium and culture them in 5 ml LB medium at 37℃ for 12h.200 rpm, 37°C, 12:00:00

Use LB medium with antibiotics of corresponding resistance if competent cells with plasmids are needed.

3.Inoculate 100 ml of LB medium with 1% volume ofE. coli culture from step 2. Use LB medium with antibiotics of corresponding resistance if competent cells with plasmids are needed.

4.Grow the cells at 37℃ shaking at 200 rpm to an OD600 of 0.55-0.6(about 1h 20min).

It should be noted that the cell density is crucial for the efficiency oftransformation. Therefore, carefully shake the culture by hands when the OD600 approaches 0.55-0.6 to avoid the decrease of transform efficiency caused by too high or too low ofthe cell density.

5.Grow the cells at 37℃ shaking at 200 rpm to an OD600 of 0.4 (about 1h 20min). It should be noted that the cell density is crucial for the efficiency of transformation. Therefore, carefully shake the culture by hands when the OD600 approaches 0.4 to avoid the decrease of transform efficiency caused by too high or too low of the cell density.

6.For all subsequent steps, keep the cells as close to 0 ℃ as possible (in an ice cuber) and chill all containers, pipette tips in ice cuber and chill all solutions in ice before adding cells. 6.Transfer the cells to two cold 50ml centrifuge bottles (45ml every bottle) and spin at 4200 rpm for 1 0 min at 4 ℃ .

7.Transfer the cells to two cold 50ml centrifuge bottles (45ml every bottle) and spin at 1000 x g for 15 min at 4℃ .

8.Carefully pour off and discard the supernatant.

9.Gently resuspend the pellet in ice-cold 10% glycerol (20ml every bottle). Centrifuge at 4 2 0 0 r p m for 20 min at 4 ℃; carefully pour off and discard the supernatant once the centrifugation is over.

10.Repeat step 9 for three times. 20ml ice pre-cooling sterilized 10% glycerol will be needed in the first two resuspension and 0.5ml glycerol for the third time.

11.Gently resuspend the pellet in ice-cold 10% glycerol (858µl every bottle). Mix well and transfer the mixture in two bottles into on bottle. Centrifuge at 1000 x g for 20 min at 4℃; carefully pour off and discard the supernatant. 12.Gently resuspend the pellet in 172 µl ice-cold GYT medium. 13.Transfer the suspension into ice-cold 1.5ml microfuge tubes (25µl every tube). 14.Freeze the suspension in -80℃ ice cuber for about 2h.