Protocol for Preparation of competent cells

·Material

Shaker

Ice Maker

Low Temperature Refrigerator

Centrifuge

LB Medium

$CaCl_2$

Glycerol

·Steps

① Pick a single colony from the newly activated E. coli plate and inoculate in a 5 ml LB liquid medium.

② Put the medium in the shaker and shake with 220rpm at 37 ℃ for 12h.

③ Draw 2ml bacterial liquid transferred to a flask containing 100ml LB liquid medium. Shake it violently at 37 ℃ for 3-4 h.

④ Draw 40ml bacterial liquid to a precooled 1.5 ml centrifuge tube and put in in ice bath for 30min.

⑤ Centrifugate the liquid at 4 ℃, 4000 rpm for 10 min. Discarded the supernatant.

⑥ Add 700μl cold 0.1M $CaCl_2$ into the tube and put it in ice bath for 30min.

⑦ Centrifugate the liquid at 4 ℃, 4000 rpm for 10 min. Discarded the supernatant.

⑧ Add 300μl cold 0.1M $CaCl_2$ and 100μl 10%glycerol into the tube and mix them well, then put it in -80 ℃ refrigerator.

·Note

① The whole process is operated under sterile condition.