

6. Transformation

Material

Competent Cell
Spreader
Plasmid (with target gene)
Ice Maker
Water Bath
Shaker
Oven
LB Liquid Medium
LB Selective Medium

Steps

- ① Take 100 μ l competent cell suspension from -80 °C refrigerator into an EP tube and put it in ice bath until it thaws.
- ② Add 1 μ l plasmid with target gene into the suspension and put it in ice bath for 30min.
- ③ Put the EP tube into 42 °C water bath for 90sec.
- ④ Transfer the tube into ice bath for 3min immediately.
- ⑤ Add 900 μ l LB liquid medium into the tube, then shake it with 300rpm at 37 °C for 1h.
- ⑥ 5krpm, 5min Centrifuge
- ⑦ Discard the 850 μ l supernatant and resuspend the remaining 150 μ l
- ⑧ Apply 150 μ l bacterium liquid in a LB selective medium plate, then flip it and put it in oven at 37°C for 16h.

Note

- ① The whole process is operated under sterile condition.
- ② Switch on the ice maker and the water bath before beginning.
- ③ The time of step ③ and ④ must be controlled precisely.