

Chemically competent cells of *E. coli*:

1. inoculate *E. coli* taken from  $-80^{\circ}\text{C}$  storage onto solid LB culture medium without antibiotics. Culture the *E. coli* in a  $37^{\circ}\text{C}$  incubator until there are small colonies on the surface of culture.
2. Take a few colonies from the plate and inoculate into liquid LB culture medium, then culture the bacteria overnight at  $37^{\circ}\text{C}$ , 200~250rpm.
3. Take 500~800 $\mu\text{L}$  bacteria liquid from the overnight culture, and then inoculate into new liquid LB culture medium. Shake the culture at  $37^{\circ}\text{C}$ , 200~250rpm for about 1.5h( when its OD reaches 0.4~0.6).
4. Pour the bacteria liquid into two 25mL centrifuge tubes, and incubate them in ice for 15~20min.
5. Put these two centrifuge tubes into a refrigerated centrifuge and spin the samples at  $4^{\circ}\text{C}$ , 6,000rpm for 10min, discard the supernatant and save the pellet.
6. Mix the pellet with 1~2mL cold 60mM glycerol- $\text{CaCl}_2$  before adding 40mL cold 60mM glycerol- $\text{CaCl}_2$ , incubate the tubes in ice for 20~30min.
7. Put these two centrifuge tubes into a refrigerated centrifuge and spin the samples at  $4^{\circ}\text{C}$ , 6,000rpm for 10min, discard the supernatant and save the pellet.
8. Repeat step 6 and 7.
9. Mix the bacteria pellet with 500 $\mu\text{L}$  cold 60mM glycerol- $\text{CaCl}_2$  and incubate the tubes in ice for 30min.
10. Split the samples with 100 $\mu\text{L}$ /tube into new EP tubes, and store the competent cells in  $-80^{\circ}\text{C}$  refrigerator.