Chemically Competent *E. coli* preparation using calcium chloride

1. Pick a single colony from a plate of *E. coli* and inoculate 3 mL of LB medium in 15 mL sterile tube. Incubate the culture overnight at 37°C, 220 rpm.
2. The next day, take 1 mL of the starter culture and inoculate 100 mL (1% v/v) of LB medium in a 500 mL Erlenmeyer flask. Grow culture for around 3 hr at 37°C, 220 rpm.
3. Transfer culture to disposable, ice-cold 50 mL polypropylene tubes. Cool culture on ice for 10 min.
4. Centrifuge culture at 4,000 $\times$ g for 10 min at 4°C.
5. Decant spent medium from the cell pellet, then invert the tubes to rest with caps on for 1 min to allow traces of medium to pool and be removed.
6. Resuspend cell pellet in 10 mL of ice-cold 0.1 M $CaCl_2$ and store on ice. Use 0.1 M $CaCl_2$, 15% glycerol solution if planning to store competent cell at -80°C.
7. Centrifuge suspension at 4,000 $\times$ g for 10 min at 4°C.
8. Decant spent medium from the cell pellet, then invert the tubes to rest with caps on for 1 min to allow traces of medium to pool and be removed.
9. Resuspend pellet in 0.2 mL of ice-cold 0.1 M $CaCl_2$ for each 50 mL of original culture. Use 0.1 M $CaCl_2$, 15% glycerol solution if planning to store competent cell at -80°C.
10. Using a chilled, sterile pipette tip, transfer 50 μL of suspended cell to pre-chilled microcentrifuge tube.
11. These preparations can be used right away, stored at 4°C for 24-48 hours, or -80°C for long-term storage. When stored at 4°C, the efficiency of transformation increases 4-6 fold during the first 12-24 hours of storage and then decreases to the original level.