This following protocol is used in Institute of Human Virology and Cancer Biology, University of Indonesia (IHVCB, UI).

Preparation of Competent Cell with Chemical Method

By following this, one can obtain up to four competent cell stocks.

1. Pick one fresh *Escherichia coli* colony, inoculate in 4 mL Luria-Bertani (LB) liquid medium. Incubate overnight at 37^{o}C, 200 rotations per minute (rpm), along with control (by inoculating *E. coli* in LB liquid medium with ampicillin, the ratio is 1:1000).
2. Inoculate 100 uL culture into 20 mL fresh LB liquid medium (200-fold dilution) into 50 mL Falcon tube. Incubate at 37^{o}C, 200 rpm for 2-3 hours until $OD_{590} \leq 0.4$ with cell concentration approximately 10^8 cells/mL (log phase of the bacteria).
3. Incubate in ice for approximately 30-60 minutes (at this point, all materials and equipment should be chilled including Falcon tubes, solutions, microtubes, and pipette tips).
4. Centrifuge at 3500 rpm, 4^{o}C, for 10 minutes without break.
5. Discard supernatant, add 1/5 volume (4 mL) 100 mM cold $MgCl_2$ and dissolve properly. Incubate in ice for approximately 15-20 minutes.
6. Centrifuge at 3500 rpm, 4^{o}C, for 10 minutes without break.
7. Discard supernatant, add 1/50 culture volume (400 uL)100 mM cold $CaCl_2$ and dissolve properly. Incubate in ice for one hour.
8. Centrifuge at 3500 rpm, 4^{o}C, for 10 minutes without break.
9. Discard supernatant, add 1/100 culture volume (200 uL) 100 mM cold CaCl2 and dissolve properly.
10. Aliquot 50 uL into new prechilled microtubes and the cells can immediately be transformed, or add ¼ volume 75% sterile glycerol for storage at -80^{o}C (competent cells lasted for one month).

Cell Transformation

1. Add 1 ng of DNA for each 50 uL competent cell stock, incubate in ice for one hour. In the meantime, thaw super optimal broth with catabolite repression (SOC) medium from freezer at room temperature.
2. Perform heat shock in water bath at 38^{o}C for 90 seconds, immediately transfer back the tubes into ice for 60 seconds.

3. Add 200 uL SOC medium into one tube at room temperature. Incubate at 37^{o}C, 200 rpm for one hour.
4. Spread 50 uL of the medium into plate agar with appropriate antibiotic for selection. Incubate the plate upside down at 37^{o}C overnight.
5. For colony selection, one may perform polymerase chain reaction (PCR) colony.