How to prepare the *Escherichia coli* competent cells for electroporation

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Note that (most of) *E. coli* auxotroph expression host strains from Addgene and RIKEN Bioresource Center do NOT carry any antibiotic resistance marker (see Table 1 in our website <https://fesworld.jp/EcoliStrains.html>). Therefore one should carefully avoid possible contamination of the *E. coli* cells when use.

1. Streak the *E. coli* auxotroph cells from a glycerol stock onto a Luria-Bertani (LB) plate, and incubate this plate at 37 °C (overnight, no antibiotics).
2. Pick a single colony from the plate and put it into 5-ml LB medium (in a small tube). Incubate the resulting tube(s) using a lab shaker at 250 rpm and at 37 °C (overnight).
3. Inoculate 50 μl of the overnight culture into 5-ml LB medium (in a small test tube), and let the cells grow using a shaker at 250 rpm and at 37 °C for 4 h.
4. Centrifuge the resulting tube at 5,000 rpm at 4 °C for 6 min, and then discard the supernatant.
5. Re-suspend the cell pellet with 1-ml cold ddH_2O (sterilized distilled water), and transfer it into a 1.5-ml (Eppendorf) tube.
6. Centrifuge the Eppendorf tube at 5,000 rpm at 4 °C for 2 min, and discard the supernatant.
7. Re-suspend the cell pellet in 50-μl cold ddH_2O (sterilized distilled water).
8. Repeat the steps 6-7 once more.*
9. The resultant competent cells suspended in 50-μl cold ddH_2O are ready for **electroporation**.

*For storage of the competent cells as a frozen glycerol stock at -80 °C, one needs to suspend the cell pellet in **50 μl of 10% glycerol (in ddH_2O) at step 8**. The resulting competent cells kept as a frozen glycerol stock at -80 °C can also be used for electroporation.