

## Transformation of chemically competent *E.coli*

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Transformation of chemically competent *E.coli* is a routine technology used in many labs. We use protocols presented here for chemically competent DH5 $\alpha$  cells (subcloning efficiency) from Invitrogen. During past years we found that usage of these relatively cheap cells is more efficient than preparation of competent cells in the lab. There is only one disadvantage of this product. The cells are supplied in 1 ml portions, what is usually too much for one experiment, since we use 100 $\mu$ l aliquots for every transformation. Therefore, for transformation with ligation reaction (long protocol) we use fresh aliquots only. The rests of thawed cell suspension may be used for transformation with a plasmid, using short protocol.

### Long protocol

1. Thaw the vial with *E.coli* on ice.
2. During this time pre-chill desired number of eppendorf tubes (1.5ml).
3. Aliquot bacterial suspension in pre-chilled tubes (100  $\mu$ l per tube).
4. Label and freeze remaining bacterial suspension.
5. To the aliquots add 1 to 5  $\mu$ l of your ligation mix.
6. Incubate 30 min on ice.
7. During this time pre-heat water bath to 42°C (don't use heating block!)
8. Incubate samples at 42°C in the water bath for exactly 1 min.
9. Immediately transfer samples on ice, leave for 2-3 minutes.
10. Add 400 $\mu$ l of SOC medium (Invitrogen) to each sample.
11. Incubate at 37°C for 45-60 minutes with shaking at 100 rpm.
12. Plate 100  $\mu$ l of bacterial suspension on agar plate.
13. Centrifuge remaining sample for 5 min at 6000 rpm.
14. Aspirate 300 $\mu$ l supernatant.
15. Resuspend bacterial pellet in the remaining medium.
16. Plate everything on agar plate.
17. Incubate plates at 37°C for 18 h.

### Short protocol

1. Thaw already used (from the long protocol) or fresh vial with *E.coli* on ice.
2. During this time pre-chill eppendorf tubes (1.5ml).
3. Aliquot bacterial suspension in pre-chilled tubes (100  $\mu$ l per tube).
4. To the aliquots add 1  $\mu$ l of your plasmid solution (10-100ng plasmid).
5. Incubate 30 min on ice.
6. During this time pre-heat water bath to 42°C (don't use heating block!).
7. Incubate samples at 42°C in the water bath for exactly 1 min.
8. Immediately transfer samples on ice, leave for 2-3 minutes.
9. Plate 10  $\mu$ l of bacterial suspension on agar plate.
10. Transfer some bacteria to another plate using a loop.
11. Incubate plates at 37°C for 18 h.