

Transformation of *E. coli*

The following protocol is suitable for chemically competent *E. coli*.

1. Thaw an aliquot of competent bacteria on ice for 20 min.
 - Use 100 μL of competent bacteria per DNA to be transformed.
 - Once bacteria are thawed, mix gently on ice by pipetting up and down slowly.
2. Into an eppi tube, place the DNA that is to be transformed; chill on ice for 5 min prior to adding the competent bacteria (step 3).
 - If you are transforming a ligation reaction, inactivate the ligase at 65°C for 20 min prior to transformation.
3. Add 100 μL of thawed competent bacteria to the eppi tube containing the DNA.
4. Incubate on ice 20 min.
5. Heat shock the mixture by incubating at 42°C for 2 min.
6. Immediately return to ice and incubate ≥ 2 min.
7. Spread the bacteria on LB agar medium supplemented with the appropriate antibiotic (e.g., ampicillin).
8. Incubate at 37°C for 12 – 16 hours.