Transformation of competent E. coli

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ABSTRACT

Marburg iGEM 2021 Team's Competent E. coli transformation protocol

PROTOCOL CITATION

yasoo 2021. Transformation of competent E. coli. **protocols.io** https://protocols.io/view/transformation-of-competent-e-coli-bu5tny6n

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MATERIALS TEXT

Chemically competent E. coli
~100 Plasmid DNA
LB/SOC Medium
Antibiotic containing Agar Plate (pre-heat for higher efficiency)

BEFORE STARTING

Prepare a container with ice
Check if there are antibiotic plates ready, otherwise new plates need to be cast
Heat up the heat block for the heat shock

1 Briefly thaw chemically competent E. coli cells on ice. 3m

2 Add ~ **100 ng** of plasmid-DNA to the cells. Alternatively just use **1 µl or 2 µl** for retransformations and **5 µl** for freshly cloned constructs (Golden Gate reactions).

3 Incubate on ice for **00:30:00** (can be skipped for retransformations). 30m

4 Heat shock the cells at **42 °C** for **00:00:45** (30 seconds for NEB® Turbo cells). 45s

5 Recover on ice for **00:05:00** , then add **500 µl** of SOC Medium **Contributed by users** to the cells. 5m

Alternatively use LB medium, but SOC is better for the cells.

6 Incubate at **37 °C** for 1 hour (if construct confers amp resistance) or 2 hours (if construct confers kan, cam, tet or spec resistance). Use the incubation time to prepare the plates and pre-warm them in an incubator. 2h

7 Plate **100 µl** on pre-warmed agar plates containing the corresponding antibiotic.

8 Optionally you can centrifuge for **00:05:00** at **3500 x g** , resuspend the pellet in the remaining ~ **100 µl** and plate it. 5m