

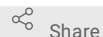


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Transformation of competent E. coli

yasoo^{1,2}¹Philipps-Universität Marburg; ²OpenPlast iGEM Team

1 Works for me



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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 ^{2h} spec resistance). Use the incubation time to prepare the plates and pre-warm them in an incubator.
- 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 ^{5m} and plate it.