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Transformation

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

This protocol is designed to transform plasmids from distribution kits of iGEM 2017, 2018, 2019 as well as those synthesized by Mission Biotech with yT&A backbone into E.coli DH5-α competent cells. Chloramphenicol is used for selection in transformation when the plasmids are retrieved from iGEM distribution kits, while ampicillins is utilized when the plasmid are derived from Mission Biotech.

ATTACHMENTS

Transformation.pdf

PROTOCOL CITATION

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ATTACHMENTS

Transformation.pdf

GUIDELINES

The ratio between competent cells and plasmid is 10 : 1. We will apportion 20 µL of competent cells for this protocol.

SAFETY WARNINGS

Make sure that all the steps should be operated in laminar flow (except heat shock).

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BEFORE STARTING

Follow the "preparation" section of protocol.

Preparation

Sterilize styrofoam box with alcohol.

1

2 Turn on the UV light of the laminar flow for **00:10:00** to **00:15:00** in order to sterilize it.

3 Add ice into the styrofoam box and sterilize it again.

4 Place the styrofoam into the laminar flow.

5 Set the dry bath incubator at **42 °C**

Protocol

6 Apportion 20uL of E-coli DH5α into an eppendorf.

> From step 6 to 8, perform them on ice and in the laminar flow.

7 Add 2uL of target plasmid into the eppendorf and vortex

8 Ice the eppendorf for 3-5 min.

9 Heat shock the eppendorf for 1 min at 42°C in a dry bath incubator.

10 Ice the eppendorf for 3-5 min.

11 Add 100uL of antibiotic-free LB media into the eppendorf.

12 Incubate the eppendorf in **37 °C** , shaking for **01:00:00**

13 Spread the transformed bacteria on agar plate containing antibiotics.

14 Incubate the agar plate for **Overnight (16-18 hours)**