

Transformation

Introduction

http://parts.igem.org/Help:Transformation_Protocol

Estimated time: 3 hours (plus 14-18 hour incubation)

Materials

- › Resuspended DNA (*Resuspend well in 10ul dH2O, pipette up and down several times, let sit for a few minutes*)
- › Competent cells (http://parts.igem.org/Help:Protocols/Competent_Cells) (*50ul per transformation*)
- › Ice (in ice bucket/container)
- › 2ml tube (*1 per a transformation*)
- › 42°C water bath
- › SOC media (*check for contamination!*)
- › Petri dishes with LB agar and appropriate antibiotic (*2 per transformation*)
- › glass beads or spreader
- › 37°C incubator
- › 10pg/ul RFP Control (pSB1A3 w/ BBa_J04450)
- ›

Procedure

1. Start thawing the competent cells on ice.
2. Add 50 µL of thawed competent cells into pre-chilled 2ml tube, and another 50µL into a 2ml tube, labelled for your control.
3. Add 1 - 2 µL of the resuspended DNA to the 2ml tube. Pipet up and down a few times, gently. Make sure to keep the competent cells on ice.
4. Add 1 µL of the RFP Control to your control transformation.
5. Close tubes and incubate the cells on ice for 30 minutes.
6. Heat shock the cells by immersion in a pre-heated water bath at 42°C for 60 seconds.
7. Incubate the cells on ice for 5 minutes.
8. Add 200 µl of SOC media (make sure that the broth does not contain antibiotics and is not contaminated) to each transformation
9. Incubate the cells at 37°C for 1 hour while the tubes are **rotating** or **shaking**.
10. Label two petri dishes with LB agar and the appropriate antibiotic(s) with the part number, plasmid backbone, and antibiotic resistance. Plate 20 µl and 200 µl of the transformation onto the dishes, and spread. This helps ensure that you will be able to pick out a single colony.

11. For the control, label two petri dishes with LB agar (AMP). Plate 20 μ l and 200 μ l of the transformation onto the dishes, and spread.
12. Incubate the plates at 37°C for 12-14 hours, making sure the agar side of the plate is up. If incubated for too long the antibiotics start to break down and un-transformed cells will begin to grow. This is especially true for ampicillin - because the resistance enzyme is excreted by the bacteria, and inactivates the antibiotic outside of the bacteria.
13. You can pick a single colony, make a glycerol stock, grow up a cell culture and miniprep (<http://parts.igem.org/Help:Protocols/Miniprep>).
14. Count the colonies on the 20 μ l control plate and calculate your competent cell efficiency.
- 15.