Help:Protocols/Transformation

Transformation Protocol

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

Transformations are essential to using the distribution kits sent out by the Registry. They can also be one of the more fickle laboratory techniques. We recommend the following protocol as it is the same one used at iGEM HQ.

We have tested transformations of the distribution kit with this protocol and have found that it is the best protocol to use with Registry parts and ensures the highest efficiency for the transformation. This protocol may be particularly useful if you are finding that your transformations are not working, or yielding few colonies.

At iGEM HQ we make our own stocks of NEB 10beta competent cells. Competent cells purchased from vendors will have better efficiency.

Transforming Your Part from iGEM Videos.

Materials

- Resuspended DNA (*Resuspend well in 10ul dH20, pipette up and down several times, let sit for a few minutes*)
- Competent cells (*50ul per transformation*)
- Ice (in ice bucket/container)
- 2ml tube (*1 per a transformation*')
- 42ºC water bath
- SOC media (*check for contamination!*)
- Petri dishes with LB agar and appropriate antibiotic (*2 per transformation*)
- glass beads or spreader

- 37ºC 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 42ºC 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 37ºC for 2 hours while the tubes are rotating or shaking. **Important:** *2 hour recovery time helps in transformation efficiency, especially for plasmid backbones with antibiotic resistance other than ampicillin*.
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 μl and 200 μl of the transformation onto the dishes, and spread.
12. Incubate the plates at 37ºC 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.
14. Count the colonies on the 20 µl control plate and calculate your competent cell efficiency.