Retreving DNA from the iGEM distribution kit

Introduction

Adapted by Jacob Mejlsted from iGEM's protocol

Materials

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- Resuspended DNA to be transformed
- 10pg/µl Positive transformation control DNA (e.g. pSB1C3 w/ BBa_J04450, RFP on high-copy chloramphenicol resistant plasmid. Located in the Competent Cell Test Kit.)
- Competent Cells (50µl per sample)
- 1.5mL Microtubes
- SOC Media (950µL per sample)
- Petri plates w/ LB agar and antibiotic (2 per sample)

Equipment

- Floating Foam Tube Rack
- Ice & ice bucket
- Lab Timer
- 42°C water bath
- 37°C incubator
- Sterile spreader or glass beads
- Microcentrifuge

Procedure

Method: Day 1

1. Resuspend DNA in selected wells in the Distribution Kit with 10µl dH20. Pipet up and down several times, let sit for a few minutes. Resuspension will be red from cresol red dye.

2. Label 1.5ml tubes with part name or well location. Fill lab ice bucket with ice, and pre-chill 1.5ml tubes (one tube for each transformation, including your control) in a floating foam tube rack.

3. **Thaw competent cells on ice:** This may take 10-15min for a 260µl stock. Dispose of unused competent cells. Do not refreeze unused thawed cells, as it will drastically reduce transformation efficiency.

4. **Pipette 50µl of competent cells into 1.5ml tube:** 50µl in a 1.5ml tube per transformation. Tubes should be labeled, pre-chilled, and in a floating tube rack for support. Keep all tubes on ice. Don't forget a 1.5ml tube for your control.

5. **Pipette 1µl of resuspended DNA into 1.5ml tube:** Pipette from well into appropriately labeled tube. Gently pipette up and down a few times. Keep all tubes on ice.

6. **Pipette 1µl of control DNA into 2ml tube:** Pipette 1µl of 10pg/µl control into your control transformation. Gently pipette up and down a few times. Keep all tubes on ice.

7. **Close 1.5ml tubes, incubate on ice for 30min:** Tubes may be gently agitated/flicked to mix solution, but return to ice immediately.

8. **Heat shock tubes at 42°C for 45 sec:** 1.5ml tubes should be in a floating foam tube rack. Place in water bath to ensure the bottoms of the tubes are submerged. Timing is critical.

9. **Incubate on ice for 5min:** Return transformation tubes to ice bucket.

10. **Pipette 950µl SOC media to each transformation:** SOC should be stored at 4°C, but can be warmed to room temperature before use. Check for contamination.

11. **Incubate at 37°C for 1 hours, shaking at 200-300rpm**

12. **Pipette 100µL of each transformation onto petri plates** Spread with sterilized spreader or glass beads immediately. This helps ensure that you will be able to pick out a single colony.

13. **Incubate transformations overnight (14-18hr) at 37°C:** Incubate the plates upside down (agar side up). If incubated for too long, colonies may overgrow and the antibiotics may start to break down; un-transformed cells will begin to grow.

Method: Day 2

14. **Pick single colonies:** Pick single colonies from transformations: do a colony PCR to verify part size, make glycerol stocks, grow up cell cultures and miniprep.

15. **Count colonies for control transformation:** Count colonies on the 100µl control plate and calculate your competent cell efficiency. Competent cells should have an efficiency of 1.5x10^8 to 6x10^8 cfu/µg DNA.