Transformation Protocol

Introduction

When transforming competent cells, both timing and temperature are very important. Use a lab timer, follow the incubation temperatures closely, and keep materials on ice when required.

Materials

- Competent cells
- 2mL tubes
- Resuspended DNA
- Agar plates

Procedure

1. Thaw competent cells on ice.

   This may take 10-15 minutes for a 260µL stock. Dispose of unused competent cells. Do not refreeze unused thawed cells, as it will drastically reduce transformation efficiency.

2. Pipette 50µL of competent cells into 2mL tube.

   50µL in a 2mL 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 2mL tube for your control.**

3. Pipette 1µL of resuspended DNA into 2mL tube.

   Pipette from well into the appropriately labeled tube. Gently pipette up and down a few times. Keep all tubes on ice.

4. Pipette 1µL of control DNA into 2mL tube.

   Pipette 1µLof 10pg/µL control into your control transformation. Gently pipette up and down a few times. Keep all tubes on ice.

5. Close 2mL tubes, incubate on ice for 30 minutes.

   Tubes may be gently agitated/flicked to mix solution, but return to ice immediately.

6. Heat shock tubes at 42°C for 1 minute.

   2mL 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.

7. Incubate on ice for 5 minutes.

   Return transformation tubes to ice bucket.

8. Pipette 200µL SOC media to each transformation.

   SOC should be stored at 4°C, but can be warmed to room temperature before use. Check for contamination.

9. Incubate at 37°C for 2 hours, shaker or rotor recommended.

10. Pipette each transformation on two petri plates for a 20μL and 200μL plating.

    Pipette 20μL and 200μL of the transformation onto appropriately labeled plates. Spread with sterilized spreader or glass beads immediately. This helps ensure that you will be able to pick out a single colony.

11. Incubate transformations overnight (14-18 hours) at 37°C.

    Incubate the plates upside down (agar side facing up). If incubated for too long, colonies may overgrow and the antibiotics may start to break down; un-transformed cells will begin to grow.

12. 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.

13. Count colonies for control transformation.

    Count colonies on the 20μL control plate and calculate your competent cell efficiency. Competent cells should have an efficiency of $1.5*10^8$ to $6*10^8$ cfu/μg DNA.