Single Tube Transformation Protocol

Before You Start

Estimated bench time: 1 hour
Estimated total time: 2 hours (plus 14-18 hour incubation)

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.

- **Read through the entire protocol before starting!**

Materials

- Resuspended DNA to be transformed
- 10pg/μl Positive transformation control DNA (e.g. pSB1C3 w/ BBa_J04450, RFP on high-copy chloramphenicol resistant plasmid.
- 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
- Pipettes and Tips (10μl, 20μl, 200μl recommended)
- Microcentrifuge

Method

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. **Spin down cells at 6800g for 3mins and discard 800μL of the supernatant. Resuspend the cells in the remaining 100μL, and pipette each transformation onto petri plates** Spread with sterilized spreader or glass beads immediately. This increases the chance of getting colonies from lower concentration DNA samples.
14. **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.
15. **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.
16. **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.