Single Tube Transformation Protocol

Aim

To transform competent bacteria with plasmids.

Material

- Resuspended DNA to be transformed
- 10 pg/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 (50 µl per sample)
- 1.5 ml microtubes
- SOC media (950 µl per sample)
- Petri plates w/ LB agar and antibiotic (2 per sample)

Equipment

- Floating foam tube rack
- Ice & ice bucket
- Lab timer
- Water bath at 42°C
- Incubator at 37°C
- Sterile spreader or glass beads
- Pipettes and tips (10 µl, 20 µl, 200 µl recommended)
- Microcentrifuge

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Protocol

1. Resuspend DNA in selected wells in the Distribution Kit with 10 µl dH_2O. Pipet up and down several times, let it sit for a few minutes. Resuspension will be red from cresol red dye.
Selected wells of Plate …. :

2. Label 1.5 ml tubes with part name or well location. Fill lab ice bucket with ice, and pre-chill 1.5 ml tubes (one tube for each transformation, including your control) in a floating foam tube rack.
3. Thaw competent cells on ice: This may take 10-15 min for a 260 µl stock. Dispose of unused competent cells. Do not refreeze unused thawed cells, as it will drastically reduce transformation efficiency.
4. Pipette 50 µl of competent cells into 1.5 ml tube: 50 µl in a 1.5 ml 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 1.5 ml tube for your control.
5. Pipette 1 µl of resuspended DNA into 1.5 ml tube: Pipette from well into appropriately labeled tube. Gently pipette up and down a few times. Keep all tubes on ice.
6. Pipette 1 µl of control DNA into 2 ml tube: Pipette 1 µl of 10 pg/µl control into your control transformation. Gently pipette up and down a few times. Keep all tubes on ice.
7. Close 1.5 ml tubes, incubate on ice for 30 min: Tubes may be gently agitated/flicked to mix solution, but return to ice immediately.
8. Heat shock tubes at 42°C for 45 s: 1.5 ml 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 5 min: Return transformation tubes to ice bucket.
10. Pipette 950 µl SOC media to each transformation: SOC should be stored at 4°C, but can be warmed to room temperature before use. Check for contamination.

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11. Incubate at 37°C for 1 h, shaking at 180 rpm.
12. Pipette 100 µl 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 6800 g for 3 min and discard 800 µl of the supernatant. Resuspend the cells in the remaining 100 µl, 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 (14-18 hr) 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.