Date: 20170907

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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

- Ligated DNA to be transformed: - pET32a E1-1, pET32a E1-2, pET32a E2, pET32a E3, pET32a E4
- Competent Cells (50 µl per sample) DH5α
- 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
- 42°C water bath
- 37°C incubator
- Sterile spreader or glass beads
- Pipettes and Tips (10 µl, 20 µl, 200 µl recommended)
- Microcentrifuge

Method

1. 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.
2. **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.
3. **Pipette 50 µl of competent cells into 1.5ml 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.
4. **Pipette 10 µl of ligated 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.
5. **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.
6. **Heat shock tubes at 42°C for 45 sec:** 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.
7. **Incubate on ice for 5 min:** Return transformation tubes to ice bucket.

8. **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.
9. **Incubate at 37°C for 1 hours, shaking at 180 rpm**
10. **Pipette 200 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.
11. **Spin down cells at 6 800 x g for 3mins and discard 550 µl of the supernatant. Resuspend the cells in the remaining 200 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.
12. **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.
13. **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.