Date: 2017/08/23
Operators: Alexis

Solubilisation of Eurofins Plasmid E4 gene: pEXA258-E4

Equipment:

- $TE_{0.1}$ 10X
- Pipettes p10, p20, p200, p1000 and associated cones
- Electric propipet and plastic 5 ml or 20 ml or 50 ml pipettes
- Microcentrifuge Eppendorf Tubes (1.50 ml)
- Sterile Water
- Bunsen Burner
- Lyophilized pEXA258-E4

Protocole

1. Dilute $TE_{0.1}$ 10X, 10 times to obtain $TE_{0.1}$ 1X
2. Centrifuge lyophylized DNA (3.6 µg) at 14 800 rpm, for 2 minutes
3. Add 360 µl of $TE_{0.1}$ 1X
4. Vortex and centrifuge again at 14 800 rpm, for 2 minutes
5. Measure concentration with UV5 spectrophotometer machine:

Gently mix the solution between before each measurement

Sample of: pEXA258-E4 Concentrations (ng/µl)	Average concentration (ng/µl) of: pEXA258-E4
9.32	8.84
8.58	
8.62	

Prepare a solution of 8.84 pg/µl, by diluting the solution of 8.84 ng/µl
Mix:

$TE_{0.1}$ 1X	1 ml
pEXA258-E4 8.84 ng/µl	1 µl

Store at -20°C

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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

- DNA to be transformed : pEX-A258 E4 (8.84 pg/µl)
- Competent Cells (50 µl per sample) of DH5α (max efficiency)
- 1.5 ml Microtubes
- SOC Media (950 µl per sample)
- Petri plates w/ LB agar and antibiotic CARB (2 per sample)

Operators : Ersin, Diane

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. **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.
2. **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.
3. **Pipette 1µl = 8.84 pg/µl of DNA pET43.1a E4 (8.84 pg/µl) and add to a DH5α 1.5 ml tube:** Pipette from tube into appropriately labeled tube. Gently mix a few times. Keep all tubes on ice.
4. **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.
5. **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.
6. **Incubate on ice for 5 min:** Return transformation tubes to ice bucket.
7. **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.
8. **Incubate at 37°C for 1 hours, shaking at 200-300 rpm**
9. **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.
10. **Spin down cells at 6 800 x g for 3mins and discard 600 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.

11. **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.