Heat shock + Electroporation protocol for *S. epidermidis*

The following protocol is adapted from the publications specified below:

Lee, Jean YH, et al. "Mining the Methylome Reveals Extensive Diversity in Staphylococcus epidermidis Restriction Modification."*Mbio*10.6 (2019).

Chen, Y. Erin, et al. "Decoding commensal-host communication through genetic engineering of Staphylococcus epidermidis."*bioRxiv*(2019): 664656.

Materials :

- B media (BM) (1% BactoTM Proteose Peptone No.3, 0.5% yeast extract, 0.5% NaCl, 0.1% K2HPO4, 0.1% glucose)
- Tryptic Soy Broth (TSB)
- TSB + 0.5M sucrose (filter sterilized)
- TSB + erythromycin agar plates
- TSB agar plates
- Ice cold 10% (weight/volume) glycerol
- Ice cold 10% (weight/volume) glycerol + 0.5M sucrose (filter sterilized)
- Ice cold water
- 2mm gap width electroporation cuvettes
- 0.25µM dialysis filters
- epiFlex construct
- sterile dH20
- Water bath

Methods

Electrocompetent *S. epidermidis*

- Grow S. epidermidis to early stationary phase cultures (8 h) in 10 ml of BM at 37°C shaking at 250 rpm.
- Add culture to 90 ml of fresh, prewarmed BM.
- Reincubate cultures to an OD600 between 0.8 - 0.9.
- Chill in an ice slurry for 10 min.
- Harvest cells at 3,900xg for 5 min at 4ºC
- Discard supernatant
- Resuspend in 100ml ice cold autoclaved water
- Repeat centrifugations and resuspend with 50mL, 20mL, 10mL autoclaved water successively
- Centrifuge once more and then resuspend with 250µL ice-cold 10% glycerol, make 50µL aliquots and store at -80°C

Electroporation of *S. epidermidis*

- Add 5µg to dialysis filter and dialyze DNA on sterile nuclease free ultrapure water for 30min to 1hour
- Thaw cells on ice for 5 minutes on ice and then 5 minutes at room temperature.
- Centrifuge at 5000xg for 1 minute and resuspend in 50µl of ice cold 10% (weight/volume) glycerol + 0.5M sucrose.

- Add DNA to cells, flick to mix then heatshock at 56°C for 2 minutes
- Transfer mixture to electroporation cuvette and pulse in electroporator
- Pulse settings: 2.5 kV, 100 Ω, 25 µF at room temperature.
- Transfer cell/DNA mixture to 1mL prewarmed TSB+ 0.5M sucrose and leave to recover for 2 hours at 37°C shaking at 250 rpm.
- Plate cells on selective plates and non-selective plates as a control.
- Leave to incubate at 37°C.