Electroporation of *R. tropici* and *S. meliloti (Isaacs Lab)*

1. Grow up rhizobia cells in LBmin at 30˚C to OD = 0.7 (~2 days)
2. Place cells on ice
3. For each sample, spin down 1ml culture in 1.5mL eppendorf tube ~10000 rpm for 30s. Discard supernatant and wash with 1ml ice cold qH20. (milliQ water)
4. Repeat step 3.
5. After second wash, resuspend cells in 50ul qH20.
6. Add 50 ng plasmid DNA
7. Transfer (gently) to 1mm electrocuvette (prechilled)
8. Electroporate (1800V) - wipe down sides of cuvette and make sure it's completely dry

On the machine: Preset protocols > Bacteria/Yeast > *E. coli*

Test pulse ( Ω button), then pulse. Time constants should be close to 4.8ms.

9. Transfer to 1mL TSB media (in culture tube).
10. Recover for 4hrs at 30˚C .
11. Plate 100ul on selective agar plates

Electroporation of *R. tropici* and *S. meliloti (Dellaporta Lab)*

*Preparation of Electrocompetent Cells*

1. Grow up 3ml starter culture of rhizobia cells at 30˚C in TSB until OD is between 0.6 and 1.0. (2-3 days)
2. Inoculate 500ml TSB with the starter culture and incubate at 30˚C until OD is between 0.6 and 0.8 (~16-24hrs)
3. Place cells on ice (30min or overnight)
4. Spin cells at 3000rpm for 20min in 2x large bottles (250ml in each) in Beckman centrifuge at 4˚C
5. Decant supernatant, resuspend pellet in the small amount of LB remaining by vortexing
6. Add 250ml of ice cold water to each bottle, mix by swirling.
7. Spin at 3000rpm in Beckman centrifuge at 4˚C for 20min.
8. Decant supernatant, resuspend pellet in the small amount of water remaining by swirling.
9. Add 50ml of ice cold water to each bottle, mix by swirling, and transfer into the same large bottle.
10. Spin at 3000rpm in Beckman centrifuge at 4˚C for 20min.
11. Decant supernatant, resuspend pellet in the small amount of water remaining by swirling.
12. Add 100ml ice cold 10% glycerol, mix by swirling.
13. Spin at 3000rpm in Beckman centrifuge at 4˚C for 20min.
14. Decant supernatant, resuspend pellet in the small amount of glycerol remaining by swirling.
15. Top up with ice cold 10% glycerol to 1.5ml.
16. Aliquot 20µl portions into 500µl microtubes, and place in dry ice-ethanol to quick freeze.
17. Store at -80˚C.

*Electroporation*

1. Thaw electrocompetent rhizobia cells on ice
2. Add 50 ng plasmid DNA, tap gently to mix.
3. Transfer (gently) to 2mm electrocuvette (prechilled)
4. Electroporate at 2.5kV, 250µFd, 200 Ω - wipe down sides of cuvette and make sure it's completely dry

On the machine: Preset protocols > Bacteria/Yeast > *E. coli*
Test pulse (Ω button), then pulse. Time constants should be close to 6.0ms.
5. Transfer to 1mL TSB media (in culture tube).
6. Recover for 4 hrs.
7. Plate 100ul on selective agar plates