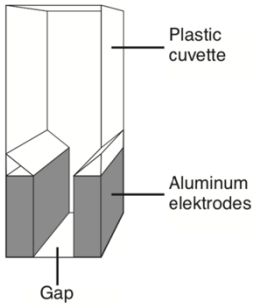
Updated on 03/12/2021

Agrobacterium Transformation

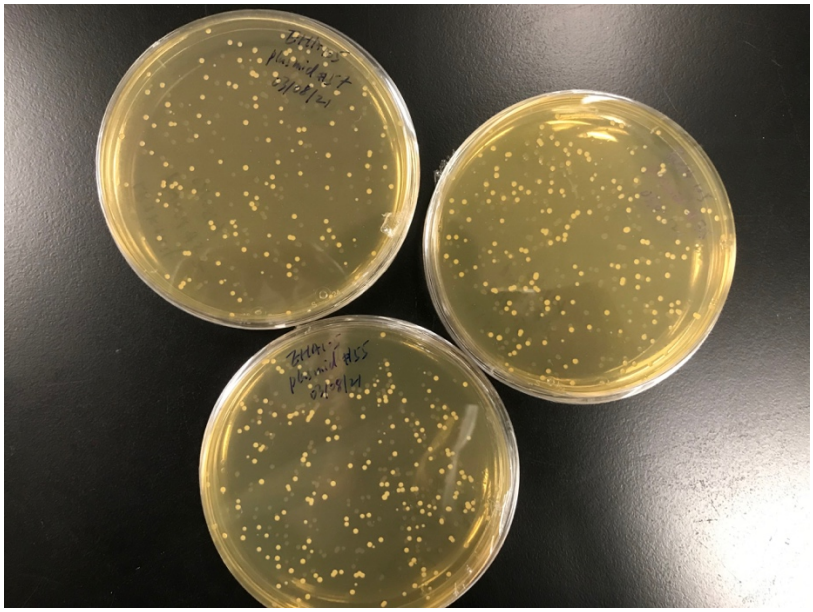
- Before started
  - Prepare cuvette
    - The cap size of the cuvette in the Soltis lab is 0.1 cm
    - Wash with deionized water
    - Put inside a baker with 70% EtOH
    - Dry inside the hood; put on top of a piece of plastic wrap
    - Wrap it and put inside 4 C (to improve transformation efficiency)
  - Pre-warm recover SOB and LB plates at 28 C



- Get the electrocompetent cell from the -80; put on ice (thaw for 10 min)
- Add 1 vl plasmid (~100 ng) into 100 vl agrobacterium; mix well, and put on ice
- Operate the electroporator
  - The one in the Soltis lab: Eppendorf 2510
  - Transfer the mixture to the cuvette (no bubbles! By tapping down 2X)
  - Wipe the cuvette
  - Set the voltage
    - 0.1 cm gap size: 1.8 kV
    - 0.2 cm gap size: 2.5 kV
  - Start the charging/discharging procedure
    - Pressing the PULSE key twice
  - Removing the sample

- Recovery
  - Immediately add 1 ml SOB into the cuvette and pipette up and down to mix
  - Add the mixture to the 1. 5 ml centrifuge tube
- Shake in 28 C for 1-2 h
  - Put the tube inside a flask
- Use 100-200 vl culture (~1/10 of the total volume) and spread on a LB plate with appropriate antibiotics (e.g., LB+K+Rif for strain EHA105) using glass beads
  - Wrap the plate; upside down; 28 C
  - it will take ~ 2 days

Updated on 03/12/2021



20210310_transformed.agro_plasmid55-57-75