Electrocompetent Agrobacterium

1. Grow seed culture to saturation (36-48 hours), you will need ~5mL
2. Inoculate 1 L of YEP media (20g peptone, 10 g yeast / 1L) 1:200 with saturated agro. Alternatively inoculate 500ml YEP 1:100. Grow culture to an OD600 of 1.5 (24 hours or so). Proceed on ice.
3. Spin cells at 5000 rpm for 15 minutes, resuspend (wash) in 1 volume cold, sterile H_2O
4. Spin cells at 5000 rpm for 15 minutes, resuspend (wash) in 0.5 volume cold, sterile H_2O
5. Spin cells at 5000 rpm for 15 minutes, resuspend (wash) in 0.02 volume cold, sterile **10% (v/v) glycerol**.
6. Spin cells at **6000** rpm for 15 minutes, resuspend (wash) in 0.001 volume cold, sterile **10% (v/v) glycerol**. Pipette the glycerol onto cells and gently stir until well mixed. The cells will be viscous. Final volume will be 0.003X original culture vol.
7. Dispense cells in ~80µl aliquots into screw top tubes—sample is viscous and challenging to pipette accurately, using a P1000 makes pipetting quicker and less difficult. Place tubes in freezer box and freeze quickly by adding liquid nitrogen.
8. Store at –80°C. Thaw on ice before use.

Electroporation with gene pulser:

1. Add a small amount of plasmid DNA (<100ng) to 40-80µl of competent cells in a tube on ice. Stir gently.
2. Pipette Agro cells with DNA into a chilled 0.2 cm electroporation cuvette. Shake cells to bottom of cell
3. Pulse cells at 2.5kV.
4. Add 1 ml YEP media to cuvette, mix, and immediately transfer cells into sterile test tubes.
5. Allow cells to shake at 28°C for 2-4 hours.
6. Plate 10 µl and 100µl on selective media.