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Electrocompetent Agrobacterium preparation

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1 Works for me dx.doi.org/10.17504/protocols.io.pdsdi6e

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ABSTRACT

Preparing electrocompetent Agrobacterium - used for the GV301 strain that we currently have in stock in Salt Lab

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). If you dont have YEP, just use normal LB media. Proceed on ice.

3 Spin cells at 5000 rpm for 15 minutes, resuspend (wash) in 1 volume cold,sterile H2O

4 Spin cells at 5000 rpm for 15 minutes, resuspend (wash) in 0.5 volume cold,sterile H2O

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.