Making electrocompetent Agrobacterium tumefaciens

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ABSTRACT

This protocol yields about 5 ml of electrocompetent *Agrobacterium tumefaciens* cells, aliquotted into 80 ul you get 50-60 tubes out of it.

Rifampicin is dark red and the colour will change over time, it does however NOT interfere with OD600 measurements, so you can use LB to blank.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

Things you need to prepare

1

- LB medium (for 1 litre combine 10 g Tryptone, 5 g Yeast Extrad and 10 g NaCl, adjust pH to 7.0 and autoclave)
- 10% glycerol solution (ice cold)
- H2O (ice cold)
- liquid nitrogen
- 50 - 60 x 1.5 ml tubes (pre-chill in fridge)
- 10 ml pipette tips
- 8 x 50 ml tubes (pre-chill in fridge)
- Your favourite strain of *Agrobacterium tumefaciens* grown on a plate

Depending on your strain of Agrobacterium you'll need the following antibiotics:

- **AGL1**: rifampicin 100 ng/ul
- **LBA 4404**: rifampicin 100 ng/ul, streptomycin 100 ng/ul --> grows slow and clumps
- **GV3101::pMP90**: rifampicin 100 ng/ul, gentamicin 25 ng/ul --> grows fastest of the three

Day 1

2 Inoculate **3 ml LB** (containing the appropriate antibiotics) with **1 colony of *Agrobacterium tumefaciens*** in the morning and incubate at **28°C at 250 rpm** for the rest of the day.

28 °C
3 ml LB
05:00:00

3 In the evening inoculate **3 x 150 ml LB** (containing the appropriate antibiotics) with **1 ml each of the culture** grown throuout the day.

28 °C
450 ml LB

4 Incubate at **28°C at 250 rpm** until **OD600 reaches 0.8 - 1.0** (This usually takes around 30 - 40 hours).

28 °C

30:00:00

Day 3

5 Check OD600, and combine all 3 cultures once **OD600 reaches 0.8-1.0**

6 Chill culture by **putting it on ice for 15 minutes and swirling it regularly.**

From here on everything needs to happen on ice or at 4°C!

00:15:00

4 °C

7 Distribute culture into **8 x 50 ml tubes** and pellet by centrifugation at **3000 - 4000 x g for 20-30 minutes at 4°C.**

00:30:00

4 °C

8 Discard supernatant and resuspend each pellet in **10 ml ice cold H2O.**

10 ml H2O

9 Adjust volume to **50 ml each with ice cold H2O.**

10 Pellet cells by centrifugation at **3000 - 4000 x g for 20-30 minutes at 4°C.**

00:30:00

4 °C

11 Discard supernatant and resuspend each pellet in **10 ml ice cold H2O.**

10 ml H2O

12 Combine solutions into **4 x 50 ml** tubes and adjust to **50 ml with ice cold H2O.**

13 Pellet cells by centrifugation at **3000 - 4000 x g for 20-30 minutes at 4°C.**

00:30:00

4 °C

14 Discard supernatant and resuspend each pellet in **10 ml ice cold H2O.**

10 ml H2O

15 Combine solutions into **2 x 50 ml** tubes and adjust to **50 ml with ice cold H2O.**

16 Pellet cells by centrifugation at **3000 - 4000 x g for 20-30 minutes at 4°C.**

00:30:00

4 °C

17 Discard supernatant and resuspend each pellet in **10 ml ice cold H2O.**

10 ml H2O

18 Combine solutions into **1 x 50 ml** tubes and adjust to **50 ml with ice cold H2O.**

19 Pellet cells by centrifugation at **3000 - 4000 x g for 20-30 minutes at 4°C.** (Use a counterbalence tube)

00:30:00

4 °C

20 Discard supernatant and resuspend pellet in **4.5 ml ice-cold 10% glycerol.**

4.5 ml 10% glycerol

21 Dispense **80 ul aliquots** into pre-chilled 1.5 ml tubes and flash freeze in liquid nitrogen.

22 Store aliquots at -80°C until use.