Electroporation of Agrobacterium

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ABSTRACT

A quick guide on how to electroporate your plasmid of interest into electrocompetent *Agrobacterium tumefaciens* cells.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

Preparation

1 **Media**:

- **YM plates** (For 1 litre: 10 g Mannitol, 0.4 g Yeast Extract, 0.5 g K_2HPO_4, 0.1 g NaCl, 0.2 g $MgSO_4$ $7H_2O$, 15 g Agar) --> autoclave and add appropriate antibiotics before making plates.
- **SOB** (For 1 litre: 0.5 g NaCl, 20 g Tryptone, 5 g Yeast Extract) --> autoclave
- **2M Glucose solution** (For 50 ml: 18 g Glucose) --> filter sterilize
- **SOC** (For 100 ml: 99 ml SOB, 1 ml 2M glucose solution)

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

Preparation of Electroporation mixture

2 Get aliquotted electrocompetent Agrobacteria from -80°C freezer

PROTOCOL

Making electrocompetent Agrobacterium tumefaciens
by Johannes Wolfram Debler, Curtin University

PREVIEW RUN

2.1

- 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

2.2 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

2.3 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

2.4 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

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

2.6 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

2.7 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

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

10 ml H2O

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

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

00:30:00
4 °C

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

10 ml H2O

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

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

00:30:00

4 °C

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

10 ml H2O

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

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

00:30:00

4 °C

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

10 ml H2O

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

2.19 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

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

4.5 ml 10% glycerol

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

2.22 Store aliquots at -80°C until use.

3 add **0.1 - 0.5 µl plasmid** to **80 µl Agro aliquot**

4 mix gently and transfer into 1 mm electroporation cuvette

Electroporation

5 select Agrobacterium program on electroporator (check manufacturers handbook) and deliver pulse

Regenerate electroporated cells

6 flush cuvette with **500 µl SOC** medium and transfer back to 1.5 ml tube

7 incubate at **28°C at 280 rpm for 3-4 hours**

Plating of electroporated cells

8 transfer **20 µl** into new 1.5 ml tube

9 add **500 µl SOC**

10 plate **20 µl of SOC diluted Agro** onto YM plates containing the correct antibiotics for your strain of Agrobacterium plus an antibiotic to select for your plasmid.

11 incubate at **28°C for 2-3 days** until colonies become visible