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Protocol status: Working
 We use this protocol and it's working

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🌐 Agro Preparation for *Mimulus in Planta* Transformation

📁 In 1 collection

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Mimulus

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ABSTRACT

This protocol is part of a [collection for *Mimulus in planta* transformation](#).

ATTACHMENTS

[Mimulus in planta transformation.pdf](#)

SAFETY WARNINGS

⚠ See SDS (Safety Data Sheet) for warnings and hazards.

Keywords: Agro Preparation,
Mimulus, Stable
Transformation, Plants

1 Inoculate 4 agro colonies into separate 5 mL tubes of LB+Kan+Gent+Rif.

1.1 Shake overnight at 28 °C - 30 °C .

2 Colony PCR from the 5 mL culture to check that it has your insert.

3 Inoculate correct agro colony into a flask of 300 mL LB+Kan+Gent+Rif, and cover with foil.

3.1 Shake for 12:00:00 to 16:00:00 at 28 °C - 30 °C .

Note

If left longer, the bacteria will begin to die.

4 Make glycerol stocks by adding 1 mL of agro to glycerol stock tube.

4.1 Place in liquid nitrogen then store in -80 °C freezer.

5 Transfer contents of flask into 500 mL centrifuge bottles and label them.

6 Balance the bottles (use the scale and spray bottle of water to balance).

7 Centrifuge to pellet the Agro.

SPEED = 6000 x g

TIME = 00:15:00

TEMP = 4 °C

8 While centrifuging, make the resuspension solution:

300 mL total for each construct

5% sucrose = 15 g sucrose

[M] 0.1 Molarity (M) acetosyringone = 2 mL/L = 600 µL

8.1 Make acetosyringone fresh by dissolving 0.0196 g /mL in methanol.

8.2 Dissolve sucrose.

8.3 Add acetosyringone.

8.4

Add dH₂O to bring total volume to 300 mL , and mix.

- 9 After centrifuging, pour off liquid into the original flasks the Agro was grown in, and bleach the flasks.
- 10 Resuspend the Agro pellet in 300 mL resuspension solution by adding the solution to the centrifuge bottle and shaking.
- 11 Pour into spray bottle for infiltration.
- 12 Add 300 μ L Silwett to the Agro and shake to mix.