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Agro Preparation for *Mimulus in Planta* Transformation

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Mimulus

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ABSTRACT

This protocol is part of a collection for *Mimulus in planta* transformation.

EXTERNAL LINK

http://mimubase.org/FTP/Protocols/Stable_Transformation/Mimulus%20in%20planta%20transformation.pdf

Mimulus in planta transformation.pdf

SAFETY WARNINGS

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

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**.

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

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 dH_2O 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 µl Silwett** to the Agro and shake to mix.