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## 🌐 Agro Preparation for *Mimulus in Planta* Transformation

📁 In 1 collection

DOI

<https://dx.doi.org/10.17504/protocols.io.3rqgm5w>



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Mimulus



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## Manuscript citation:

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**We use this protocol and it's working**

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## Abstract

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

## Attachments



[Mimulus in planta tr...](#)

39KB

## 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 .

**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  $\mu$ L
- 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<sub>2</sub>O 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  $\mu$ L Silwett to the Agro and shake to mix.