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Agrobacterium-Mediated Transient Expression in Nicotiana benthamiana Leaves

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Maryam Rahmati Ishka[1]

[1]Boyce Thompson Institute (BTI)

Maryam Rahmati Ishka
Boyce Thompson Institute (BTI)

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We use this protocol and it's working

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Disclaimer

n/a

Abstract

This procedure outlines the Agrobacterium infiltration of tobacco leaves for the transient expression of fluorescently labeled proteins.

Image Attribution

n/a

Guidelines

n/a

Materials

Infiltration medium: 10 mM $MgCl_2$, 10 mM MES (pH 5.6). Autoclave for 20 min. Add acetosyringone immediately before using it MgCl2 **Applied Biosystems (ThermoFisher Scientific)**
20X MES Buffer **Thermo Fisher Scientific Catalog #**NP0002
Acetosyringone **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**2478-38-8
1g Rifampicin USP Grade **G-Biosciences Catalog #**RC-191 to a final concentration of 200 µM.

Safety warnings

n/a

Ethics statement

n/a

Before start

n/a

Transform constructs into Agrobacterium tumefaciens strains that are compatible with vectors. Strain GV3101 is typically used (GV2260 can also be used). Make glycerol stock of.

1 Refresh bacteria containing the construct of interest from glycerol stock on agar YEP (or any other bacterial media such as LB) medium with an appropriate antibiotic**.** Incubate at 28°C for two days. If *A. tumefaciens* GV3101 is used add **rifampicin**.

2 Inoculate **5 mL** of liquid YEP medium with appropriate **antibiotic** in a test tube. Grow at 28°C for **24 hours**
with shaking at 250-300 rpm. Be sure to grow bacterial strain with **p19** as a silencing inhibitor as well!

3 Measure the OD_{600} using 100 µL of bacterial culture.

4 Calculate the culture volume needed for an OD_{600} of 0.2 - 0.5 per construct for a 4 mL total volume of Infiltration medium. 4 mL are used for test spot infiltration. The specific OD_{600} to use depends on how
well expressed your construct is in *N. benth*, the general range is 0.1 – 0.5. Use an OD_{600} of 0.3 for P19.
(I use 0.3 for all constructs and it works well).

5 Pipette the appropriate volume of each strain together in 2 mL tubes.

6 Centrifuge combined bacteria at 8000×*g* for 2 min at room temperature. Carefully remove all supernatant.

7 Add 200 mM **acetosyringone** to the **Infiltration medium** at a 1:1000 ratio.
Note: acetosyringone is light-sensitive, keep out of light until ready for use.

8 Add 2 mL of infiltration medium with **acetosyringone** to each pellet, and resuspend. Leave in the dark at room temperature for 1–2 hrs (or even longer but not overnight).

9 Infiltrate 4-week-old N. benthamiana (pre-flowering) leaf using 1 mL syringe.

10 Grow plants for 2-3 days after infiltration (up to 5 days, depending on expression level of constructs).

11 Punch **1 cm** leaf discs from leaves and observe under the microscope to visualize the fluorescence.

Protocol references

n/a