Infiltration of *Nicotiana benthamiana leaves* with Agrobacterium for transient transformation

Simplified version. Based on Sparkes, Imogen A, John Runions, Anne Kearns, and Chris Hawes. "Rapid, Transient Expression of Fluorescent Fusion Proteins in Tobacco Plants and Generation of Stably Transformed Plants." Nature Protocols 1, no. 4 (November 2006): 2019–25. https://doi.org/10.1038/nprot.2006.286.

Notes

- *Agrobacterium tumefaciens* strain GV3101 is grown in LB with selection agents (rifampicin, gentamicin, and those corresponding to the selection genes in the plasmid) at 28ºC, 200 r.p.m. aprox. 16h.
- Plants should be 3-6 weeks old, growing at 25 °C, 16 hr light, 8 hr dark.
- The infiltration medium should be prepared just before the infiltration.

1. Preparation of the infiltration medium

- 0,050 g D-Glucose
- 1 mL MES [2-(N-morpholino)ethanesulfonic acid, 10x stock]
- 1 mL $Na_3PO_4.12H_2O$ (stock a 10x)
- 25 uL acetoseringone (stock at 200 mM)
- Add dH_2O to the final volume of 10 mL.

2. Agrobacterium preparation

- Centrifuge the Agrobacterium culture 5-10 min at 4000 rpm.
- Remove the supernatant inverting the tube.
- Add 1 mL of infiltration medium and resuspend the pellet (agitate or use the vortex).
- Make a 1:10 dilution for a plastic cuvette: 100 uL of Agrobacterium to 900 uL of infiltration solution. Measure optical density at 600 nm.
    - Dilute the Agrobacterium suspension in the infiltration medium to OD=0.1, in a final volume of 1 mL (1000 uL). Use the obtained absorbance value in the formula

Absi x Vi = Absf x Vf

(Absi x 10) x Vi = 0.1 x 1000

- Prepare the dilution in 2 mL tubes.

3. Infiltration

- Choose the youngest fully expanded leaves.

- Fill the syringe (without the needle) with circa 0.5 mL of Agrobacterium.
- Press gently on the abaxial page (bottom), outside the midrib area and slowly press the plunger until a dark-green infiltrated area appears, or the entire leaf area. Repeat on another part of the same leaf or change to other leaf.
- Mark the infiltrated area or leaf.

➢ Leaf sections with circa 0.5 cm are examined by confocal microscopy for detection of the reporter protein, 2 to 4 days after infiltration.

Vídeo https://www.youtube.com/watch?v=GHc7PU_jG2M

Stock solutions

(10x Stock): 500 mM MES [4,88 g of 2-(N-morpholino)ethanesulfonic acid in 50 mL of dH_2O]. Keep at 4ºC.

(10x Stock): 20 mM $Na_3PO_4.12H_2O$ (0,38018 g em 50 mL) solvente dH_2O. Manter a 4ºC.

200 mM acetoseringona (3'-5'-dimethoxyl-4'-hydroxyacetophenone) (0,0392 g em 1 mL) solvente DMSO. Distribuir em doses de 25 µL e manter a -20 °C.