Infiltration of *Nicotiana benthamiana* Protocol for Transient Expression via *Agrobacterium*

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[Abstract] Transient expression in tobacco plant (*Nicotiana benthamiana*) is used to determine the subcellular location of a protein of interest when tagged with a reporter such as green fluorescent protein (GFP), or to mass produce proteins without making transgenic plants. The root tumor bacteria, *Agrobacteria*, are used to introduce the target gene expression cassette into benthamiana mesophyll cells.

**Materials and Reagents**

1. *Agrobacterium* strain hosting a plant expression construct (usually driven by *Cauliflower mosaic virus* 35S promoter)
2. Healthy *Nicotiana benthamiana* (*N. benthamiana*) plants 2-4 weeks
3. MES
4. MgCl₂ stock
5. Antibiotics
6. Acetosyringone
7. LB media with appropriate antibiotics (see Recipes)
8. Acetosyringone stock (see Recipes)
9. MES-K (see Recipes)
10. Resuspension solution (see Recipes)
11. Acetosyringone datasheet (Sigma-Aldrich) (see Recipes)

**Equipment**

1. Centrifuge for 50 ml tubes
2. Spectrometer
3. Syringe
4. UV lamp (optional)
5. Fluorescence microscope (optional)
6. Confocal laser scanning microscope (optional)
Procedure

1. Inoculate one single colony of *Agrobacterium* in 5 ml LB with appropriate antibiotics. Grow overnight at 28-30 °C.
   \textit{Note: I usually use 100 μg/ml gentamicin (maintain the virulence of Agrobacterium strain GV3101) and 50 μg/ml spectinomycin (selective marker for shuttle vector) for most of the shuttle vectors.}

2. Use 1 ml of the overnight culture to inoculate 25 ml LB (with same antibiotics, plus 20 μM acetosyringone added after autoclaving and immediately before use) and grow overnight.

3. Measure the $A_{600}$ of overnight culture.

4. Precipitate the bacteria (5,000 x g, 15 min), resuspend the pellet in Resuspension Solution. The final $A_{600}$ should be adjusted to 0.4.

5. Leave on the bench (room temperature) for 2-3 h (or overnight) before infiltration.

6. Perform the infiltration with 5 ml syringe. Simple press the syringe (no needle) on the underside of the leaf (\textit{Note: Avoid cotyledons}), and exert a counter-pressure with finger on the other side. Successful infiltration is often observed as a spreading “wetting” area in the leaf.

7. (Optional) Check the GFP fluorescence by a portable long-wavelength UV lamp 2-5 days after infiltration. This only applies to strong expression of GFP signal (as green from red background).

8. Observe the fluorescence labeled protein under a fluorescent microscope or confocal laser scanning microscope. Or harvest leaves for protein purification.

Recipes

1. LB media with appropriate antibiotics
   Usually two antibiotics used: one to maintain *Agrobacteria* virulence, one for the shuttle vector

2. Acetosyringone stock
   100 mM in ethanol, stored at -20 °C

3. MES-K (0.5 M) (pH 5.6)
   First make 0.5 M MES, adjust pH with KOH to 5.6

4. Resuspension solution
   10 mM MgCl$_2$
   10 mM MES-K (pH 5.6)
   Autoclave 15 min
   100 μM acetosyringone (\textit{note: Added after autoclaving and immediately before using})
5. Acetosyringone datasheet

Synonyms 3', 5'-Dimethoxy-4'-hydroxyacetophenone
Synonyms Acetosyringone
4'-Hydroxy-3', 5'-dimethoxyacetophenone
Molecular Formula C_{10}H_{12}O_{4}
Molecular Weight 196.20
CAS Number 2478-38-8
Beilstein Registry Number 1966119
EG/EC Number 2196105
MDL number MFCD00008748

References