Agrobacterium infiltration of *Nicotiana benthamiana* for transient protein expression – Catherine Espinoza Patharkar

Transient expression in the wild 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. A domesticated version of the crown gall forming bacteria, *Agrobacterium tumefaciens*, is used to introduce the target gene expression cassette into *N. benthamiana* leaves.

Materials

- *Agrobacterium* strain hosting a plant expression construct (usually driven by Cauliflower mosaic virus 35S promoter)
- Healthy non-flowering tobacco (*Nicotiana benthamiana*) plants (3-4 weeks old)
- MES/KOH (pH 5.6)
- $MgCl_2$
- Acetosyringone
- LB media with appropriate antibiotics

The time frame for the protocol described here is as follows:

- Day 1: *Agrobacterium* transformation with binary vectors.
- Day 3: Start *Agrobacterium* culture (including Agrobacterium containing p19 strain). *p19 is a suppressor of gene silencing from the tomato bushy stunt virus and must be coinfiltrated for proper gene expression.* Water tobacco plants.
- Day 4: Infiltration of transformed *Agrobacteria* in *N. benthamiana* leaves
- Day 6-10: Imaging of results.

Agrobacterium-Mediated Infiltration of *N. benthamiana*

This method was modified from Voinnet et al. (2003).

1. Prepare the Activation buffer

Reagent stock concentration	10 ml	Final concentration
1 M MES/KOH (pH 5.6)	100 µl	10 mM
10 mM $MgCl_2$	10 ml	10 mM
150 mM Acetosyringone (3′,5′-Dimethoxy-4′-hydroxyacetophenone) in DMSO, stored at −20°C	10 µl	150 µM

2. Measure the OD_{600} of the overnight cultures.
3. *N. benthamiana* will be infiltrated with a solution containing OD_{600}=0.3 of each experimental Agro containing construct and OD_{600}=0.1 of p19. Calculate the volume of cultures ($V_{construct}$; V_{p19}) needed according to the formulas:

$V_{construct} = V_{final} \times 0.3/OD_{600}$; $V_{p19} = V_{final} \times 0.1/OD_{600}$. One mL of infiltrate is often enough to complete a small experiment. Plan your final volume accordingly.

4. Mix calculated volumes of construct(s) and p19 cultures into a 1.5 ml Eppendorf tube.
5. Immediately centrifuge the mixtures at maximum speed for 30 sec at room temperature.
6. Discard the supernatant and resuspend the mixtures in V_{final} of activation buffer.
7. Incubate the mixtures for at least 30 min at room temperature in dark.
8. Infiltrate the mixtures using a 1-mL syringe without a needle into the abaxial (bottom) side of *N. benthamiana* leaves. Trace the infiltrated area with a marker. Incubate the plants in the greenhouse for 2-6 d, depending on the level of protein expression. Two days of expression is often maximal.

A video protocol can be viewed at: https://rahulpatharkar.000webhostapp.com/?p=313