

Transient expression assay in *N. benthamiana* leaves for intracellular localization study

Introduction

Transient expression assay using *N. benthamiana* is an easy and simple way to examine intracellular localization of fluorescently-tagged proteins. Subcellular localization of proteins of one's interest can be observed within four days if you already have agrobacterium with the constructs to transform Arabidopsis or other plants. If you have several constructs for tagged proteins by different fluorescent proteins, they can be coexpressed and observed in the same cell by inoculating mixed agrobacteria. This method would be extremely useful when you want to compare localization of your proteins with established organelle markers, or when you want to look for some effects of your proteins that are toxic and stable transformants are not recovered. Although the exact localization should be confirmed in stable transgenic plants or by immuno-staining, this method would still be a powerful tool to advance the research.

Before starting the experiment

Use 3~4-week old *N. benthamiana* plants.

Spread transformed agrobacteria on Yeb media plates and incubate for 1~2 days.

Spread P19 on the Yeb plate, which is used to avoid gene silencing.

(Voinnet, Rivas, Mestre, & Baulcombe, 2003)

Voinnet, O., Rivas, S., Mestre, P., & Baulcombe, D. (2003). An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. *The Plant journal : for cell and molecular biology*, 33(5), 949-56.

Materials:

Infiltration medium

10 mM MES

10 mM MgCl₂

0.15 mM acetosyringone

Yeb medium (final 500ml)

2.5 g Beef-extract

0.5 g Yeast-extract

2.5 g sucrose

0.245 g MgSO₄ 7H₂O

Day 1; Cell culture

1. Inoculate the agrobacterium grown on the Yeb plate to 5 ml of liquid Yeb medium, which contains proper antibiotics, and culture at 30 °C.

Day 2; Infiltration

1. Centrifuge to collect cells at 4,500 rpm for 10 min
2. Discard the supernatant, add 1ml infiltration media, and resuspend by pipetting (without vortexing)
3. Measure the OD₆₀₀ of the cells using spectrophotometer
4. Add infiltration media to adjust OD₆₀₀ = 1 and stand it at RT for 3 hours.
5. Mix the same volume of agrobacterium that you want to infiltrate and P19 in a 1.5 ml micro centrifugation tube. If you want to express more than two genes together, the same amount of P19 as the sum of agrobacteria cultures should be added. Add the infiltration medium up to 1ml.
(In our lab, we usually adjust the final OD₆₀₀ of FLS2 at 0.02 by mixing 20 µl of FLS2, 20 µl of P19, and 960 µl of infiltration medium)
6. Infiltrate the mix from the abaxial side of leaves of *N.benthamiana* using 1ml syringe.



Figure 1. How to infiltrate.

Day 4; Observation of the expressed proteins under microscopes

1. Excise the inoculated region of the leaves into about 3 mm x 3 mm sections.
2. Mount the sample in water and put a cover slip on the sample carefully to avoid bubbles.

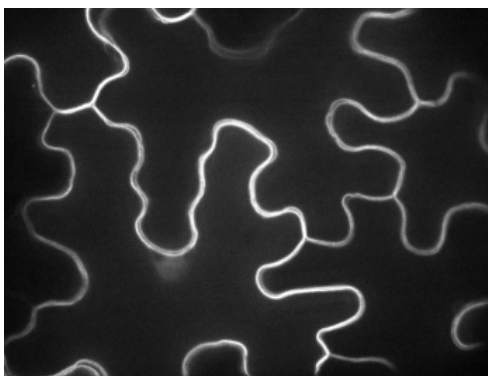


Figure 2. The localization of FLS2 (FLAGELLIN SENSING 2) on the plasma membrane.