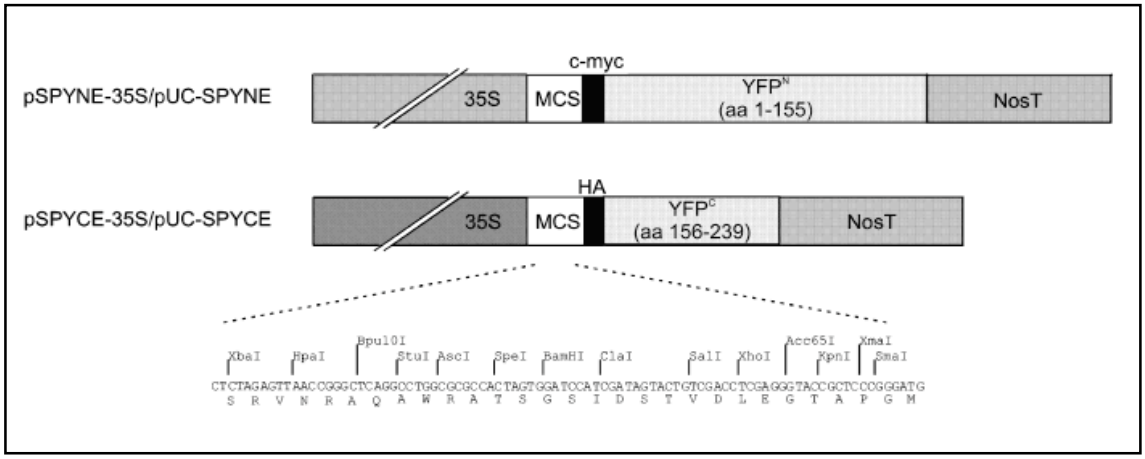
## Bimolecular Fluorescence Complementation (BiFC) in living plant cells

To confirm protein-protein interaction *in vivo*, biochemical works using GST pull-down and co-immunoprecipitation are generally used. Recently, however, some new experimental techniques using fluorescent proteins for this purpose have been developed, such as BiFC (bimolecular fluorescence complementation), and FRET/BRET (fluorescence/bioluminescence resonance energy transfer) [See review for details: Bhat et al , Plant Methods, 2006]. Of these new methods, BiFC is the simplest and easiest to introduce into the lab because a normal fluorescence microscope is sufficient, whereas other methods require more specialized equipment. These new approaches are valuable, as they allow researchers to observe *what* and *where* interactions occur at the subcellular level of living plant cells at the same time.

**Vectors:** Choose either vector set depending on which transformation method for transient expression you will use.




**Walter et al., Plant J., 40, 428-438 (2004)**

A. pSPYNE-35S, pSPYCE-35S (plant expression binary vectors, for agro-infiltration)
B. pUC-SPYNE, pUC-SPYCE (pUC-based high copy plasmid vectors, for PEG-mediated protoplast transformation)

**Notes:**

- Gateway™ (Invitrogen) compatible destination vectors for all are also available.
- For Agro-infiltration, co-transformation of 35S::p19 with your constructs makes enhanced expression. p19 is RNA silencing repressor protein from *Tomato bushy stunt virus* (Voinnet et al., Plant J., 2003). 35S::p19 can be requested from Dr.

David baulcombe (John Innes center, UK).

- All vector plasmids can be requested directly from Dr. Klaus Harter (Tubingen University, Germany: klaus.harter@zmbp.uni-tuebingen.de).

## Procedure:

- Make BiFC constructs with your favorite genes.
- Co-transform plasmid set (SPYNE and SPYCE) by either transient expression assay method A or B (see below).
- View the image of the living plant cells with YFP-derived fluorescence using fluorescence microscope.

## Transient expression assay methods:

### A. Agro-infiltration for *Nicotiana benthamiana* (modified protocol of David Baulcombe lab)

1) Inoculate your agro into 5 ml LB to make pre-culture (or directly inoculate into 50 ml LB) by using glycerol stock or picking up the colony from the petri-dish. Grow it at 28$^{o}$C, O/N. Use:

-5 ml (or 50 ml) L-broth + antibiotics (Kanamycin, Rifampicin, etc…)

If you'd like to use pre-culture for 50ml inoculation, use 1ml of pre-culture. For 50 ml culture, add the following ingredients:

-10 µl 100mM Acetosyringone (20 µM final): Stock must be made into DMSO

-500µl 1M MES-KOH, pH5.7 (10 mM final) [optional, filter-sterilized]

2) Precipitate the bacteria by centrifuge (5000g, 15-20 min, RT) and resuspend the pellet in 5ml of Agro-infiltration buffer containing:

-10 mM MgCl2

-10 mM MES-KOH, pH 5.7

-150 -200µM Acetosyringone (freshly adding just before using)

3) Check OD600 by making 1:10 dilution into another eppendorf tube and adjust agro's OD to 0.5 (1 agro/injection), 1.0 (2 agros/injection), 1.5 (3 agros/injection) using Agro-infiltration buffer.

4) Leave it on the bench for 2 to 4 hours before agro-infiltration (or overnight).

5) Mix them up (final OD of each agro is 0.5) and perform the infiltration with a 1-2 ml syringe. Simply press the syringe (no needle) on the underside of the leaf, and exert a counter-pressure with your finger on the other side. Infiltrate the maximum number of leaves, avoiding cotyledons. Use top leaves if you expect high expression of transgene.

6) 2-5 days after infiltration (5 days is best), harvest the samples or perform your experiment.

**B. PEG-mediated transformation for *Arabidopsis* mesophyll protoplasts**

We strongly recommend using Jen Sheen's lab's protocol for this work because they established this experiment very well. You can download the protocol file from their web site (registration required). They also have an excellent movie featuring the details of all procedures.

## References:


Bhat, R.A., Lahaye, T. and Panstruga, R. (2006) The visible touch: in planta visualization of protein-protein interactions by fluorophore-based methods, Plant Methods, 2, 12

Walter, M., Chaban, C., Schutze, K., Batistic, O., Weckermann, K., Nake, C., Blazevic, D., Grefen, C., Schumacher, K., Oecking, C., Harter, K., and Kudla, J. (2004) Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation, Plant J., 40, 428-438

Jen Shhen lab's web site: http://genetics.mgh.harvard.edu/sheenweb/main_page.html

David Baulcombe lab's web site: http://www.ayeaye.tsl.ac.uk/index.php?option=com_content&task=view&id=42&Itemid=226