Transient gene expression assay (Yuan Lab)

1. Strike out agrobacterium containing construct of interest onto a Kan+Rif+Gent plate (or other antibiotic+Rif+Gent). Incubate at 28 °C for 2 days.
2. Inoculate 20ml of LB broth with Kan+Rif+Gent (or other antibiotic+Rif+Gent) in a 50ml Falcon tube by scraping agro from plate with a pipet tip and ejecting it into the liquid.
3. Shake overnight at 28 °C (16-20 hr).
4. Spin down agro for 10 minutes at 6,000g. Remove supernatant.
5. Completely resuspend pellet in 40mL dH_20 by shaking (DO NOT USE PIPET).
6. Using a 1cc syringe with no needle, gently inject the abaxial surfaces of young leaves with the resuspended agro solution.
7. In 4-6 days, fluorescence/pigment should become visible. Examine leaves under confocal microscopy to visualize protein localization.

Antibiotic	**Powder storage**	**Stock solution**	**Final concentration**	**µL stock/ mL media**	**Resistance**
Kan	RT	50 mg/ml in water	50 µg/ml	1	Your plasmid
Gent	4°C	50 mg/ml in water	50 µg/ml	1	Ti plasmid marker (agro)
Rif	-20ºC	12.5 mg/ml in methanol	25 µg/ml	2	Agro chromosomal marker

NOTES:

Agrobacteria are very sensitive to temperature. The entire process should take place at 28°C or cooler.

Plant infiltration works best when the stomata of leaves are open; cool, sunny mornings are ideal!

Both *M. parishii* and *M. lewisii* works well with this protocol.

Co-infiltration (2 constructs) works well; simply combine 20mL of resuspended agro from each desired construct and invert gently to mix. Remember to use different fluorescence tags for this process.