Transient Agroinfiltration of tobacco leaves; works better with cotyledons of about 1 week old plants

- Grow agrobacteria containing your construct in 2ml LB supplemented with appropriate antibiotics (50 μg of kanamycin and 50 μg of rifampin per ml) at 28 C 1-2 days.
- Inoculate 0.250 ml in 25 ml LB supplemented with 10 mM MES pH 5.6, 20-100 μM acetosyringone as well as the antibiotics and grow at 28 C to an OD600 of 0.8
- Pellet cells (not more than 3000g) and resuspended in MMA (10 mM MgCl2, 10 mM MES [pH 5.6], 100 μM acetosyringone) to a final optical density at 600 nm (OD600) of 0.5 to 1.5 (optimal 0.8). KEEP at room temperature for 1 – 3 hours
- Note: When two agrobacteria are to be used for co-transformation make sure OD600 of each is Approximaately 0.5
- Infiltrate 100 μL per spot in a fully expanded leaf using needless syringe.
- Keep plants in dim light or dark at 23 C under high humidity for 1-2 days.
- Apply your treatment if you wish to the infiltrated area and incubate again.
-
- Assay for the expression of your gene reporter.

MMA

10 mM MgCl2,

10 mM MES [pH 5.6],

100 μM acetosyringone

Acetosyringone

FW 196.2

References.

Journal of Virology, July 2001, p. 6440-6449, Vol. 75, No. 14

Planta. 2003 Mar;216(5):824-33. Epub 2002 Nov 02.

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The promoter of a rice glycine-rich protein gene, *Osgrp-2*, confers vascular-specific expression in transgenic plants

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Agroinfiltration

Agroinfiltration was assayed essentially as described by Yang et al. (*2000*). *Agrobacterium tumefaciens* strain LBA 4404 containing each individual construct was incubated in 2 ml LB medium supplemented with appropriate antibiotics (kanamycin and streptomycin) and then inoculated (1%) into 25 ml LB with 10 mM Mes, 20 μM acetosyringone as well as the antibiotics. The culture was grown overnight to log phase (OD6000.8) at 28 °C, centrifuged and resuspended in MMA solution (10 mM Mes, 10 mM MgCl2, 100 μM acetosyringone) to a final OD600 of 1.5. One hundred microliters of the bacterial suspension per spot was infiltrated into intercellular spaces of near fully expanded leaves using a 1-ml plastic syringe. Typically, up to ten infiltrating spots separated by veins could be arranged in a single leaf of tobacco (*N. tabacum* cv. Xanthi nc). After agroinfiltration, the treated plants were maintained in the greenhouse for 2-6 days before GUS assay of the infiltrated areas.

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Role of the 3'-Untranslated Regions of Alfalfa Mosaic Virus RNAs in the Formation of a Transiently Expressed Replicase in Plants and in the Assembly of Virions

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***Agrobacterium*-mediated transient expression.** All pMOG constructs were transformed to *Agrobacterium tumefaciens* strain LBA 4404 by electroporation. pMOG800 was generally used as a negative control. Cultures of 5 ml were grown for 48 h at 28°C in LC medium containing 50 μg of kanamycin per ml and 50 μg of rifampin per ml. Of this culture, 1 ml was grown overnight at 28°C in 100 ml of minimal A medium [46 mM K2HPO4, 33 mM KH2PO4, 7.5 mM (NH4)2SO4, 1.5 mM C6H5Na3O7 · 2H2O, 1 mM MgSO4, 0.2% glucose, 100 μM CaCl2], containing 10 mM *N*-morpholinoethanesulfonic acid (MES) (pH 5.6), 40 μM 3', 5'-dimethoxy-4'-hydroxyacetophenone (acetosyringone), 50 μg of kanamycin per ml, and 50 μg of rifampin per ml. Subsequently, the cells were pelleted and resuspended in MMA (10 mM MgCl2, 10 mM MES [pH 5.6], 200 μM acetosyringone) to a final optical density at 600 nm (OD600) of 0.5 to 1.0. When two *A. tumefaciens* strains were coinfiltrated, the OD600 of each strain was at least 0.5 and the OD600 of the culture was between 1.0 and 1.5. The cells were kept at 23°C for 1 to 3 h prior to infiltration.

N. benthamiana leaves were infiltrated using a syringe without a needle. The plants were kept humid at 23°C under mild light conditions (1,000 lux). One day after infiltration, the plants were transferred to the greenhouse. If necessary, leaves were inoculated with P12 virus 2 days after infiltration, as described previously (47). P12 virus consists of virions containing RNA 3 and virions containing RNA 4. The plants were kept in the greenhouse. Plants were inoculated with wild-type AMV as described previously (60).

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pGD vectors: versatile tools for the expression of green and red fluorescent protein fusions in agroinfiltrated plant leaves

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For 'agroinfiltration', suspensions of transformed C58C1 bacteria were adjusted to an OD600 of 0.6 in MES buffer (10 mm MgCl2, 10 mm MES, pH 5.6), and acetosyringone was added to a final concentration of 150 μm. Bacterial suspensions were then maintained at room temperature for 2-3 h. For co-infiltration of different *Agrobacterium* transformants, equal volumes of each culture suspension were mixed prior to infiltration. Infiltrations were conducted by gently appressing a 1-ml disposable syringe to the abaxial surface of fully expanded leaves that were approximately 2.5 cm wide at the midleaf and slowly depressing the plunger. A sufficient amount of bacterial suspension was used to completely infiltrate the leaves and give a water-soaked appearance. This typically required 1-4 infiltration sites per leaf. Following agroinfiltration, plants were maintained in the laboratory under continuous fluorescent lighting for at least 24 h. Plants kept for longer periods were maintained in a growth chamber at 22°C with a 16 h/8 h. light/dark photoperiod. Leaves were examined by microscopy between 40 h and 90 h post-infiltration.