# Functional transient genetic transformation of *Arabidopsis* leaves by biolistic bombardment

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**Transient gene expression is an indispensable tool for studying functions of gene products. In the case of plants, transient introduction of genes by *Agrobacterium* infiltration is a method of choice for many species. However, this technique does not work efficiently in *Arabidopsis* leaf tissue, the most widely used model system for basic plant biology research. Here we present an optimized protocol for biolistic delivery of plasmid DNA into the epidermis of *Arabidopsis* leaves, which can be easily performed using the Bio-Rad Helios gene gun system. This protocol yields efficient and reproducible transient expression of diverse genes and is exemplified here for use in a functional assay of a transcription repressor and for the subcellular localization and cell-to-cell movement of plant viral movement protein. This protocol is suitable for studies of biological function and subcellular localization of the gene product of interest directly *in planta* by utilizing different types of activity-based assays. Using this procedure, the data are obtained after 2–4 d of work.**

## INTRODUCTION

Transient gene expression in a model system provides invaluable insights into many protein functions, such as subcellular localization and trafficking, protein–protein interactions, protein stability and degradation and protein activity (e.g., transcriptional activation), as well as into cellular processes induced by the transcription of the expressed gene, e.g., RNA silencing. *Arabidopsis thaliana* is the most widely used model organism for plant biology research due to the availability of its complete genome sequence and wealth of genetic genomic tools and resources, which include extensive collections of T-DNA insertion mutants. At present, the best method for *Arabidopsis* genetic transformation is floral dipping[1–3]. This technique has been adopted by virtually all research groups working with genetically modified *Arabidopsis* plants. Floral dip, however, is designed for the production of transgenic plants, and it is not usable for most transient transformation applications. Stable gene expression in transgenic plants, although indispensible for many studies, suffers from a number of disadvantages in comparison with transient expression. For example, production of stably transformed plants requires a significant time investment, and, even when the transgenic plant line is generated, the stably integrated transgene is often silenced transcriptionally and/or post-transcriptionally. Moreover, stable expression of the transgene may produce deleterious effects, and position effects on the expression of transgenes can further complicate the evaluation of the experimental data[4]. Finally, the absence of an efficient and reliable method for transient protein expression has been a key impediment to analyzing how the wealth of available mutations in *Arabidopsis* may affect protein localization and function. Thus, there is a need to develop an efficient, reproducible and relatively simple methodology for transient genetic transformation of *Arabidopsis* tissues. As many studies of proteins of interest are initiated using leaves, which represent the *Arabidopsis* organs that are the largest and easiest to harvest, manipulate and observe, it is preferable that the transformation technology is adapted for use with leaf tissues.

There are three types of basic approaches for transient gene expression in *Arabidopsis*: polyethylene glycol (PEG)-mediated transformation of protoplasts, infiltration with *Agrobacterium tumefaciens* (agroinfiltration) and biolistic bombardment. PEG-mediated transfection of *Arabidopsis* mesophyll protoplasts is efficient[5,6], but it is time consuming and can be used only for studies in isolated protoplasts, which are in a totally different context from the plant tissues. For example, the subcellular organization of the cytoskeleton and ER are altered by the removal of the cell wall to prepare protoplasts, and the trafficking of signals and macromolecules between cells cannot be analyzed using this approach. Transient gene expression by agroinfiltration is the favored technique in several plant species, e.g., *Nicotiana benthamiana*, representing a relatively noninvasive and cost-effective method that allows for fine-tuning of the expression levels by altering the concentration of the infiltrated *Agrobacterium* cell suspension[7,8]. One notable example of agroinfiltration of *Arabidopsis*[9] is its use for induction of gene silencing[10,11]. However, the best substrate for the *Agrobacterium*-mediated transient gene expression in *Arabidopsis* is its root[12,13], whereas agroinfiltration of leaves generally yields low and highly variable expression, and the efficiency in several *Arabidopsis* ecotypes is extremely low or negligible[14]. For example, using this procedure[14] to express a yellow-fluorescent protein (YFP)-tagged cell-to-cell movement protein (MP) of *Tobacco mosaic virus* (TMV), we obtained very low transient expression in leaves of *Arabidopsis* ecotype Col-0: examination of 20 infiltrated leaves led to the identification of only a single epidermal cell expressing MP-YFP (S.G.L., unpublished data). In contrast, the same construct was expressed efficiently using the transformation technique reported here.

Microparticle bombardment is another popular technique for transient gene expression, used in a variety of plant species[15]. Whereas a procedure for *Arabidopsis* bombardment has been reported[16], its efficiency and reproducibility is very low as compared with transient expression by biolistic bombardment of other

species, such as *N. benthamiana* and tobacco (*N. tabacum*) (e.g., see ref. 17). Hence, many in the plant research community are often forced to use these latter species for transient expression and studies of *Arabidopsis* genes and gene products. Such heterologous systems, although useful, fall short of a true *in vivo* approach. Furthermore, they also do not allow to take advantage of the wide-range of *Arabidopsis*-based research resources, such as T-DNA insertion mutants, ethane methyl sulfonate-mutagenized lines or RNAi-silenced lines. An efficient transfection system to analyze transiently expressed gene products in well-characterized *Arabidopsis* mutant and RNAi-silenced backgrounds, which is difficult to do in other plant systems, will provide the means to develop critically needed functional assays in this key model plant.

Here we describe a protocol for the efficient delivery of plasmid DNA into the epidermis of *Arabidopsis* leaves by microparticle bombardment[18], which can be achieved easily using the Bio-Rad Helios gene gun system. Our technique is characterized by its high efficiency, reproducibility and suitability for the robust transient expression of a variety of functional *Arabidopsis* proteins with diverse biological activities and subcellular localization specificities. Furthermore, this procedure also allows for the coexpression of numerous proteins, tagged and/or untagged, by bombardment with multigene expression constructs, such as the RSC/SAT vector series, which is suitable for simultaneous expression of up to seven different proteins from the same plasmid[19,20]. Although suitable for virtually any application requiring transient gene expression in *Arabidopsis* tissues, our protocol is especially useful for transient transformation and analyses of specific transgenic and/or mutant *Arabidopsis* lines, experiments requiring rapid and efficient transformation of *Arabidopsis* leaf tissues and experiments that aim to avoid *Agrobacterium*-mediated transformation due to potentially unpredictable effects of bacterial effector proteins known to be exported into the plant cells together with the transforming T-DNA[21,22]. Furthermore, the more standard high-pressure gene gun (e.g., the Biolistic PDS-1000-He Particle Delivery System), with its fixed pressure system, produces extensive damage in *Arabidopsis* leaf tissue, making it inefficient and unsuitable for transient expression assays. In contrast, the ability to vary the pressure to much lower levels using the Helios gene gun system allows one to minimize the damage to *Arabidopsis* leaf cells. We have successfully used this method to study transcriptional repressor activity of *Arabidopsis* SWIRM-domain polyamine oxidase protein 1/lysine demethylase-like protein 1 (SWP1/LDL1) in a transgenic *Arabidopsis* plant line[18]. As any transient gene delivery, the procedure may result in certain, although very limited in our experience, cell damage. It is also relatively expensive, requiring the use of gold microparticles and a costly gene gun system. Nevertheless, it is the only technique for obtaining efficient transient expression of proteins in *Arabidopsis* leaves, thereby making it an essential tool for rapidly examining the subcellular localization and activities of proteins in wild-type or mutant *Arabidopsis* backgrounds. This method can be also applied to induce RNA silencing in *Arabidopsis* leaves by introducing antisense or inverted-repeat sequence of the target gene. On the other hand, although the number of the transformed cells are more than suitable for visualization of the expressed proteins and protein/RNAi activity assays, they are most likely to be insufficient for the purification of large amounts of the expressed protein from the transformed tissues.

### Experimental design

**Choice of gene gun system.** The hand-held Bio-Rad Helios Gene Gun is the system of choice. The ability to vary the pressure for bombardment down to levels lower than those obtained with the standard high-pressure fixed gene gun setups, such as the Biolistic PDS-1000-He Particle Delivery System, so as to avoid extensive damage of the *Arabidopsis* leaves, is critical.

**Choice of plasmid DNA.** Any plasmid with any protein coding sequence under the control of promoter/terminator system active in *Arabidopsis* cells (e.g., the *Cauliflower mosaic virus* 35S promoter/terminator system) can be used. Simultaneous expression of multiple genes can be achieved by using the RCS/SAT vector system[19,20]. A high concentration of plasmid DNA is needed for efficient coating of microparticles, hence high copy number plasmids represent a more convenient choice. To obtain high efficiency of transformation, the plasmid DNA must be purified using common commercial kits rather than self-prepared reagents.

**Plant and growth conditions.** Six- to eight-week-old *Arabidopsis* plants grown under short day (8-h light at 23 °C/16-h dark at 20 °C) are used. Light and humidity must be properly controlled to obtain healthy plants. The leaves from the plants maintained under these conditions have the optimal size for the bombardment (microparticles are delivered to a 10- to 12-mm-diameter area). If a specific experiment requires expression leaves from plants at different developmental stages, the gene gun pressure should be determined empirically.

**Design of controls.** An internal positive control for overall expression efficiency (i.e., to ascertain the quality of cartridge loaded with DNA-coated microparticles and efficiency of the bombardment) can be provided by coexpressing the tested gene with a control gene. For example, plasmid DNA coding for a GFP-tagged tested protein can be mixed with plasmid DNA coding for a free DsRed2 and used to coat microparticles. GFP and DsRed2 can be visualized easily using the appropriate sets of filters. In this experimental design, the expression levels of the tested GFP fusion can be compared directly with expression levels of DsRed2 in different tissues or mutant backgrounds.

## MATERIALS

### REAGENTS

- *Arabidopsis thaliana* plants, 6- to 8-weeks old
- Plasmid DNA for expression of the gene of interest (at >0.5 μg μl$^{-1}$ in $H_2O$, up to 50 μg total), purified using common commercial kits
- Gold microparticles, 0.6 or 1.0 μm diameter (Bio-Rad Laboratories, cat. no. 165-2263 and 165-2262, respectively)
- Spermidine (Sigma, cat. no. S0266-1G)
- $CaCl_2$ (Sigma, cat. no. C2661-500G)
- Absolute ethanol (Pharmco, cat. no. 111ACS200) ! CAUTION Absolute ethanol is flammable. ▲ CRITICAL The bottle has to be opened freshly for each experiment, as ethanol that has absorbed moisture from air tends to give poor results.
- Tefzel tubing (Bio-Rad, cat. no. 165-2441)

- Polyvinylpyrolidone (PVP), MW 360,000 (included in the Tefzel tubing kit provided by Bio-Rad)
- 5-ml syringe (without needle)
- 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid, cyclohexylammonium salt (X-Gluc) (Fermentas, cat. no. R0852)
- $NaH_2PO_4$ (Sigma, cat. no. S8282-500G)
- $Na_2HPO_4$ (Sigma, cat. no. S7907-500G)
- Ethylenediaminetetraacetic acid (EDTA), disodium salt (Sigma, cat. no. E5134)
- *N*, *N*′-Dimethyl formamide (Sigma, cat. no. D4551-250ML) ! CAUTION This solvent should be handled under chemical hood.
- Double-distilled water or water with equivalent quality prepared by Millipore MiliQ system ($ddH_2O$)

**EQUIPMENT**

- Ultrasonic cleaner (e.g., Misonix ultrasonic cleaner, frequency 40 Hz)
- Vortex mixer with adjustable speed
- Bio-Rad Helios cartridge preparatory station (Bio-Rad, cat. no. 165-2420)
- Tubing cutter (Bio-Rad, cat. no. 165-2422)
- Bio-Rad Helios gene gun (Bio-Rad, cat. no. 165-2432)
- Tank with helium gas ! CAUTION Gas under pressure should be handled with care according to the institutional regulations.
- Helium gas regulator (Bio-Rad, cat. no. 165-2413)
- Tank with dry $N_2$ gas
- Window screen mesh, cut into 10 cm × 10 cm squares
- Flat Styrofoam surface (e.g., a lid of a Styrofoam box)
- Epifluorescence or confocal microscope
- 37 °C air incubator
- *Arabidopsis* growth chamber

**REAGENT SETUP**

**Spermidine solution** Prepare stock solution of 3.0 M spermidine in $ddH_2O$. This solution can be stored at −20 °C for at least 1 year. Prepare working solution (50 mM in $ddH_2O$) from stock solution just before use.

**PVP-ethanol solution** Prepare stock solution of PVP in absolute ethanol (20 mg $ml^{-1}$). This solution can be stored at −20 °C for at least 1 year. Prepare working solution (50 µl of stock solution in 20 ml absolute ethanol) just before use.

**$CaCl_2$ solution** Prepare 1 M $CaCl_2$ in $ddH_2O$.

**β-Glucuronidase assay buffer** Prepare 50 mM phosphate buffer (add 9.75 ml of 0.2 M $NaH_2PO_4$ and 15.25 ml of 0.2 M $Na_2HPO_4$ for a total of 100 ml buffer), 15 mM EDTA, 0.01% (vol/vol) Tween-20, pH 7.0. If necessary, adjust pH by adding KOH. This solution can be stored at 4 °C for at least 1 month.

**GUS assay substrate** Just before use, dissolve 5–10 mg of X-Gluc in 0.1 ml of *N*, *N*′-dimethyl formamide and add 10 ml of β-glucuronidase (GUS) assay buffer.

**EQUIPMENT SETUP**

**Helios gene gun system and tubing prep station** Set up and used according to the manufacturer's instructions.

## PROCEDURE

### Plant growth ● TIMING 6–8 weeks

**1|** Grow 1–2 *Arabidopsis* plants on Pro-Mix BX in a pot (10 cm× 10 cm × 10 cm) in an environment-controlled chamber with a short photoperiod (8-h light at 23 °C/16 h dark at 20 °C) under light (130–150 µE $m^{-2}$ $s^{-1}$), and 40–65% relative humidity for 6–8 weeks[23]. Occasionally, apply commercially available fertilizer according to the instructions[23]. Leaves with the size larger than 15 mm width and 35 mm length (the length measurement includes the petiole) are best suitable for the experiments (**Fig. 1a**).

▲ CRITICAL STEP Ensure that the plants are healthy and well maintained. Leaves harvested from plants grown under inappropriate conditions yield poor transformation efficiency. The short photoperiod is required to obtain larger leaves; thus, growing plants under long photoperiod conditions will result in much smaller leaves, which are less convenient for the experiments.

### DNA precipitation onto the surface of gold microparticles ● TIMING Day 1, 20 min

**2|** Weigh 12 mg of gold microparticles and transfer into a 1.5-ml microcentrifuge tube. Add 100 µl of 0.05 M spermidine. Sonicate the mixture for 10 s, then vortex the tube vigorously for 10 s to disperse gold particles.

? TROUBLESHOOTING

**3|** Add 25–50 µg of plasmid DNA, in a <100 µl volume (ideally 50 µl), to the gold microparticle suspension. Sonicate the tube for 10 s, then vortex vigorously for 5 s at full speed.

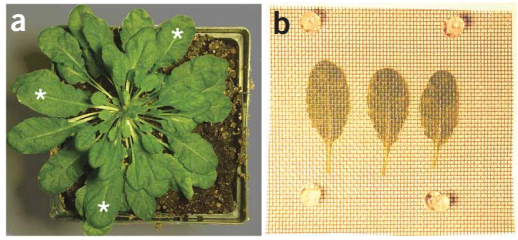
▲ CRITICAL STEP Dispersing the gold microparticles by sonication and vortexing is required for uniform DNA coating of the particle surface.

? TROUBLESHOOTING

**4|** Lower the speed of vortex. Open the tube, while continuing to mix the gold microparticle suspension (you must be able to vortex with the lid open without spilling the suspension from the tube), and add 100 µl of 1.0 M $CaCl_2$ slowly, dropwise, with ~5 s between each drop.

▲ CRITICAL STEP The 1.0 M $CaCl_2$ solution must be added slowly, constantly mixing, to precipitate DNA evenly on the surface of the gold particles.

? TROUBLESHOOTING




An *Arabidopsis* plant ready for bombardment

*Arabidopsis* leaves immobilized in preparation for bombardment

**Figure 1** | *Arabidopsis* plant and leaves suitable for biolistic bombardment. (**a**) A 7-week-old plant of suitable age and leaf size grown as described in Step 1. The leaves indicated with asterisks were removed and processed for bombardment with TMV MP-YFP for the transient expression and cell-to-cell movement experiments in **Figure 3**. (**b**) Leaves immobilized for bombardment. The harvested leaves were flattened and placed abaxial side-up under a piece of window screen mesh as described in Step 14. The microparticles are delivered by aiming the gun at the center of the midrib of each of the immobilized leaves.

**5|** Allow the suspension to settle at room temperature (i.e., approximately 23–25 °C) for ~10 min. Meanwhile, connect the Tefzel tubing to $N_2$ airflow and dry the inner wall of the tubing for >5 min.

**6|** Centrifuge at 8,000*g* at 25 °C for 30 s in a microcentrifuge to collect the gold microparticles.

**7|** Remove the supernatant without disturbing the pellet. Add 1 ml of absolute ethanol to the gold microparticles, resuspend and centrifuge at 8,000*g* at 25 °C for 30 s.
**? TROUBLESHOOTING**

**8|** Repeat Step 7 twice and remove the supernatant completely. Resuspend the gold microparticles in 0.5 ml of PVP-ethanol solution and transfer the microparticle suspension to a 15-ml conical tube. Wash the microcentrifuge tube with 0.5 ml of PVP-ethanol solution to collect the remainder of the microparticles and add them to the conical tube. Adjust the total volume of microparticle suspension in the conical tube by adding PVP-ethanol to 3.0 ml. Sonicate the resulting mixture for 10 s to disperse the gold microparticles before proceeding to Step 9.
▲ **CRITICAL STEP** Sonicating at this step is required for efficient particle loading into the Tefzel tubing in Step 9.
**? TROUBLESHOOTING**

**Cartridge preparation with tubing prep station ● TIMING Day 1, 30 min**
**9|** Close the $N_2$ flow after drying the Tefzel tubing. Remove the tubing from the tubing prep station, and load it with the DNA-coated gold microparticle suspension using a 5-ml syringe connected to the Tefzel tubing through a short segment of flexible Tygon tubing.
▲ **CRITICAL STEP** Minimize the handling time for Steps 7–9.
**? TROUBLESHOOTING**

**10|** Place the Tefzel tubing horizontally in the tubing prep station immediately after loading. Allow the Tefzel tubing with gold microparticle suspension to lie for 5 min for 1-μm gold or for 15 min for 0.6-μm gold to settle the microparticles on the inner surface of the Tefzel tubing.
**? TROUBLESHOOTING**

**11|** Remove ethanol from the tubing, using the 5-ml syringe. After ethanol removal, the gold microparticles must remain on the inner surface of the Tefzel tubing. Turn the tubing 180°, wait for 5 s, then rotate the tubing at a speed of 60 r.p.m. for 30 s. Open the $N_2$ flow for 10 min to dry the tubing.
*Note*: The manufacturer recommends to keep rotating the tube while drying the inner surface of the tubing. However, our protocol simplifies the procedure without reducing transformation efficiency as compared with the manufacturer's protocol.

**12|** Cut the microparticle-loaded Tefzel tubing into 1-cm-long segments (cartridges) using the Tubing Cutter supplied with the tubing prep station. A 70-cm-long Tefzel tubing loaded with gold microparticle prepared using this protocol should yield ~50 cartridges. The cartridges can be kept at −20 °C in a vial containing drying agent, such as silica gel. We place several pieces of Drierite in the scintillation vial with a cotton ball to secure Drierite to the bottom of the container, place the prepared cartridges on top of the cotton ball and tightly close the vial with its lid.
■ **PAUSE POINT** The cartridges can be stored for several months in dry environment at −20 °C.

**Microbombardment ● TIMING Day 1, 10 min**
**13|** Select well-expanded leaves (larger than 15 mm × 35 mm, see above) from 4- to 6-week-old plants, before flowering.

**14|** Remove the selected leaves with a sharp razor blade and immediately place them with the abaxial sides facing up onto a flat Styrofoam surface. The abaxial side of the leaf represents a better substrate for bombardment due to its lower trichome density and thinner cuticle. Cover the leaves with a piece of window screen mesh and secure the mesh with pushpins to the Styrofoam surface (**Fig. 1b**). Maintaining leaves flat using the window screen mesh increases the efficiency of the particle delivery and minimizes the damage to the tissue during the bombardment.

**15|** Load the cartridge into the gun and shoot at a pressure of 80–110 psi for 1-μm gold and 140–160 psi for 0.6-μm gold. (Note that this pressure has been adapted for *Arabidopsis* leaves, and it may be too low for other plant species, such as tobacco or *N. benthamiana*, thus resulting in low transformation efficiency). Hold the tip of the barrel liner of the gene gun as close as possible to the leaf tissue and aim to the center of the midrib of the leaf. Note that this procedure is aimed at transformation of the epidermal cell layers of the leaf. We usually use one cartridge per leaf, as each leaf has enough surface area only for one shot that spreads microparticles over an area of 10–12 mm in diameter.
**? TROUBLESHOOTING**

**16|** Remove the window screen mesh, remove the leaves from the Styrofoam surface and place them into Petri dish (1–2 leaves per 90-mm dish) over three layers of wet Whatman filter paper, seal the Petri dish with Parafilm and leave it at room temperature for 16–48 h to allow expression of the delivered transgene, followed by epifluorescence/confocal microscopy observation (see Step 17) or analyses of protein activity, such as GUS assay (see Step 18).
▲ **CRITICAL STEP** The time period between the bombardment and the microscopy/activity assay should be determined empirically. For example, for imaging GFP and its different spectral variants, 24–36 h is usually sufficient, whereas for imaging DsRed2, expression/protein maturation time of 48 h may be required. Thus, for simultaneous detection of coexpressed GFP-based fluorescent tags and DsRed2, observation after 48 h is recommended.

**Observations of the expressed proteins/biological assay of the expressed gene products ● TIMING Days 2–4**
**17|** For observation by microscopy, the bombarded area is excised from the leaf tissue, avoiding the major veins. The tissue is mounted on a slide glass in a drop of water and covered by a cover glass. The mounted tissue is observed immediately to avoid excessive stress due to excising and mounting. The fluorescence signal derived from the expression of tested protein will be visible in the bombarded area after 24–48 h. For internal control for expression efficiency, free fluorescent protein with nonoverlapping spectral properties can be coexpressed with the fluorescently tagged tested protein (e.g., free DsRed2 and GFP fusion, respectively).

**18|** For the GUS assay, the bombarded leaf is submerged in the GUS assay substrate and incubated in a 37 °C air incubator until the dark blue color of the GUS product appears (typically, overnight). The reaction is stopped by placing the leaf into absolute ethanol. Continue to incubate the leaf in absolute ethanol to extract chlorophyll until the leaf loses its green color and becomes white.

● **TIMING**
Step 1, plant germination and growth: 6–8 weeks
Steps 2–16, gold particle DNA-coating, cartridge preparations and transgene delivery: 1 h (day 1)
Steps 17 and 18, epifluorescence/confocal microscopy and GUS assay (or any other biological assay for the protein of interest): 1–16 h (days 2–4)

**? TROUBLESHOOTING**
Troubleshooting advice can be found in **Table 1**.

**TABLE 1 |** Troubleshooting table.

| Steps | Problem | Possible reason | Solution |
|---|---|---|---|
| 2 | Low expression level with sufficient amount of micro-particles loaded into the cartridge | Poor quality of DNA-coated microparticles | Use DNA solution with higher concentration |
| 3 | | | Sonicate the microparticle suspension to disperse the particles more evenly |
| 4 | | | Add 1 M $CaCl_2$ more gradually, taking longer time to do it |
| 7–9 | Low expression level with insufficient amount of micro-particles loaded into the cartridge | Poor loading efficiency | Use fresh, unopened ethanol bottle. Ethanol that has been opened and has absorbed moisture gives poor loading efficiency |
| | | | Sonicate the DNA-coated microparticle suspension to disperse the particles more evenly |
| 10 | | | The Tefzel tubing must remain stationary after the microparticle loading, precisely as described in this step |
| 15 | Low expression level with well-prepared cartridges | Inappropriate bombardment conditions | Use the pressure values indicated in this step. A pressure that is too low will produce poor transformation, whereas a pressure that is too high will damage the cells |

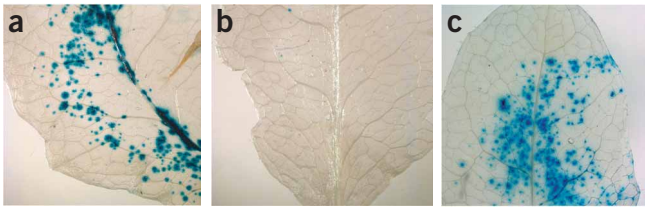



**Figure 2** | Transient gene expression to assay transcriptional repressor activity of *Arabidopsis* SWIRM-domain polyamine oxidase protein 1/lysine demethylase-like protein 1 (SWP1/LDL1) in a transgenic *Arabidopsis* plant line. SWP1 was fused to a chimeric transcriptional activator containing the yeast Gal4 DNA-binding domain (mGal4, modified for optimal activity in *Arabidopsis*[24]) fused to the VP16 transcriptional activator from *Herpes simplex* virus and transiently expressed from a CaMV 35S promoter by bombardment of its encoding construct into transgenic *Arabidopsis* plants that carry a *GUS* reporter transgene driven by a mGal4-VP16-inducible *Gal4-UAS* promoter (obtained from Dr J. Haseloff). For detailed experimental design, see ref. 18. (**a**) mGal4-VP16 induces expression of the GUS reporter. (**b**) Inhibition of GUS expression by transcriptional fusion of SWP1 to mGal4-VP16. (**c**) Coexpression of free SWP1 and mGal4-VP16 does not inhibit the mGal4-VP16-induced GUS expression.

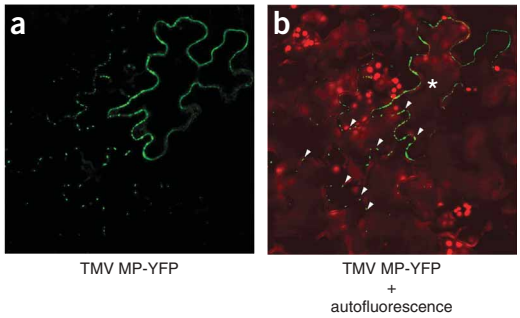



**Figure 3** | Transient gene expression to assay TMV MP movement in *Arabidopsis* leaf epidermis. *TMV MP-YFP* driven by a CaMV 35S promoter was bombarded into the *Arabidopsis* leaf epidermis. After 48 h, TMV MP-YFP was visualized by laser scanning confocal microscopy directly in the bombarded leaf. (**a**) TMV MP-YFP localization and (**b**) YFP signal is indicated in green, and plastid autofluorescence is indicated in red. The image is a single confocal section. Note that TMV MP-YFP shows a specific cell wall-associated punctate localization diagnostic of plasmodesmata[25–28] (some of these TMV MP-YFP-labeled plasmodesmata are indicated by arrowheads). Moreover, the transiently expressed TMV MP-YFP is functional because it moves from the initially transformed cell (marked with asterisk) to the adjacent cells. It is important to note that although gold microparticles are delivered into numerous cells in the bombarded area they are seldom found in multiple adjacent cells: the clusters of the several cells observed after 48 h are due to the cell-to-cell movement of TMV MP-YFP from the initially transformed cells. This allows for monitoring cell-to-cell movement easily as the initially transformed cell is characterized by higher fluorescence intensity.

## ANTICIPATED RESULTS

A good transient expression system is defined as one that is highly reproducible, gives high transformation efficiency (i.e., a number of transformed cells sufficient for statistically significant analyses) without producing significant detrimental effects or artifacts by the transformation procedure itself, and that is broadly applicable to different types of functional assays. Generally, the overall efficiency of the transfection by biolistic bombardment depends on the quality of the DNA-coated microparticles and the efficiency of their delivery into the cells while minimizing cell damage. This protocol is optimized for delivery and transient expression of genes in the leaf epidermis of *Arabidopsis*, and it routinely results in transformation of up to 40 cells per typical microscope field at $\times$10 magnification.

We have used this protocol for two different types of experiments as presented in **Figures 2** and **3**: a transcriptional repression analysis based on *in situ* GUS activity assay (see **Fig. 2** and ref. 18) and visualization of the subcellular localization and cell-to-cell movement of the *Tobacco mosaic virus* 30-kDa movement protein tagged with YFP (TMV MP-YFP; **Fig. 3**). Note that the data in **Figure 2** were obtained using a specific transgenic *Arabidopsis* line[18], and they would have been impossible without a technique for efficient transient transformation of *Arabidopsis* leaves. Our protocol, therefore, is applicable to very diverse types of proteins and experimental designs. We expect that this protocol can be readily adopted for other types of functional assays both in wild-type and mutant *Arabidopsis* plants.



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