



Cold Spring Harbor Protocols

FAST Technique for *Agrobacterium*-Mediated Transient Gene Expression in Seedlings of *Arabidopsis* and Other Plant Species

Jian-Feng Li and Andreas Nebenführ

Cold Spring Harb Protoc 2010; doi: 10.1101/pdb.prot5428

Email Alerting Service

Receive free email alerts when new articles cite this article - [click here](#).

Subject Categories

Browse articles on similar topics from *Cold Spring Harbor Protocols*.

To subscribe to *Cold Spring Harbor Protocols* go to:
<http://cshprotocols.cshlp.org/subscriptions>

Protocol

FAST Technique for *Agrobacterium*-Mediated Transient Gene Expression in Seedlings of *Arabidopsis* and Other Plant SpeciesJian-Feng Li¹ and Andreas Nebenführ²

Department of Biochemistry and Cellular and Molecular Biology, University of Tennessee, Knoxville, TN 37996, USA

INTRODUCTION

Genome sequencing has identified a massive number of uncharacterized genes in *Arabidopsis thaliana* and several other plant species. To decipher these unknown gene functions, several transient expression assays have been developed as rapid and convenient alternatives to the lengthy creation of transgenic plants. As one of these transient assays, *Agrobacterium*-mediated transformation harnesses the natural capability of *Agrobacterium* to transfer foreign DNA into plant cells with intact cell walls. However, pioneering applications of *Agrobacterium*-based transient transformation to *Arabidopsis* have led to rather limited success with great variability. In this protocol, we describe a Fast *Agrobacterium*-mediated Seedling Transformation (FAST) technique for transient gene expression analysis in *Arabidopsis* and other dicot or monocot species. This technique makes use of the cocultivation of young plant seedlings with *Agrobacterium* in the presence of the surfactant Silwet L-77. The young seedlings can be grown easily and were found to be more susceptible to *Agrobacterium* transformation compared with adult plants. The surfactant facilitates transformation of plant cells, thus replacing wounding or a device-dependent vacuum step during plant transformation. This protocol provides a quick, efficient, and economical assay for gene function in intact plants with minimal manual handling and without the need for a dedicated device.

RELATED INFORMATION



This protocol was modified from the method by Li et al. (2009); <http://www.plantmethods.com/content/5/1/6>.

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <I>, and recipes for reagents marked with <R>.

Reagents

Agrobacterium tumefaciens GV3101::pMP90 (or other suitable strain) containing binary plasmid for expression of gene of interest

All solutions containing *Agrobacterium* and all equipment contacting *Agrobacterium* cultures must be sterilized appropriately.

Antibiotics, including 50 µg/mL gentamycin for *Agrobacterium* and the following choices, depending on the binary plasmid:

¹Present address: Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, USA

²Corresponding author (nebenfuehr@utk.edu).

Cite as: Cold Spring Harb Protoc; 2010; doi:10.1101/pdb.prot5428

www.cshprotocols.org

<I>50 µg/mL kanamycin
 100 µg/mL spectinomycin
 <R>Cocultivation medium
 <R>LB (Luria-Bertani) liquid medium
 <R>MS plates (0.5X) (optional; see Step 14)
 <I>500 µg/mL carbenicillin can be added to increase seedling survival.
 Seedlings of interest
Possibilities include 40 4-d-old Arabidopsis (or tobacco) seedlings or 15 4-d-old tomato or 5-d-old rice (or switchgrass) seedlings. These numbers are for cocultivation in a 100 × 20-mm Petri dish. For cocultivation vessels with smaller volumes, reduce the number of seedlings accordingly (see Step 10).
 <I>Surface sterilization solution (1% bleach; i.e., 0.05% sodium hypochlorite)
 <R>Washing solution for *Agrobacterium*
 <R>YEB medium

Equipment

Aluminum foil
 Centrifuges and appropriate tubes
 Forceps (optional; see Step 10)
 Incubator preset to 28°C
 Micropipettor and tips
 Petri dish (100 × 20 mm)
Alternatively, 24-well plates or 96-well plates can be used (see Step 10).
 Pipettes
 Plant growth chamber
 Roller drum or shaker for bacterial culture
 Spatula (sterile)
 Spectrophotometer

METHOD

Choose Procedure A (Steps 1-6) or Procedure B (Steps 7-8), then continue with Step 9. Procedure A offers better control of the growth stage of the bacteria, whereas Procedure B is simple and fast.
 Plant seedlings should have been germinated several days in advance, depending on the species. For *Arabidopsis*, the optimal time for cocultivation is 4 d after germination.

Procedure A

1. One day before cocultivation, inoculate a single colony or frozen glycerol stock of *A. tumefaciens* GV3101::pMP90 (Koncz and Schell 1986) into 2 mL of LB medium with appropriate antibiotics (50 µg/mL gentamycin for *Agrobacterium*, plus antibiotic for binary plasmid). Grow at 28°C for 18-24 h.
2. On the day of cocultivation, dilute the saturated *Agrobacterium* culture into 10 mL of fresh YEB medium without antibiotics to OD₆₀₀ = 0.3. Shake vigorously at 28°C until the OD₆₀₀ reading reaches >1.5.
This step generally takes ~10-12 h.
See Troubleshooting.
3. Pellet the *Agrobacterium* cells by centrifugation at 6000g for 5 min and discard the YEB medium.
4. Resuspend the *Agrobacterium* cells in 10 mL of washing solution by pipetting.
5. Pellet the *Agrobacterium* cells again by centrifugation at 6000g for 5 min and discard the supernatant.
6. Resuspend the *Agrobacterium* cells in 1 mL of washing solution by pipetting. Continue with Step 9.

Procedure B

7. Two days before cocultivation, inoculate *A. tumefaciens* GV3101::pMP90 (Koncz and Schell 1986) on YEB plates with appropriate antibiotics (50 µg/mL gentamycin for *Agrobacterium*, plus antibiotic for binary plasmid), and grow at 28°C for ~48 h.
8. On the day of cocultivation, scrape the bacterial colonies from the plate with a sterile spatula and resuspend directly in washing solution.
A bacterial colony volume of ~0.5 mL is typically sufficient for several small-scale cocultivation experiments.

Common Procedure

9. Dilute 100 µL of *Agrobacterium* cell suspension 10 times with washing solution, measure OD₆₀₀ and calculate the OD₆₀₀ of the original cell suspension.
10. To a clean 100 × 20-mm Petri dish, add 20 mL of cocultivation medium and transfer 40 4-d-old *Arabidopsis* (or tobacco) seedlings or 15 4-d-old tomato or 5-d-old rice (or switchgrass) seedlings into the medium.
Take care not to mechanically damage the seedlings. For example, lift the seedlings with forceps inserted under the cotyledons; it is also possible to pick them up by the root.
Alternatively, different cocultivation vessels can be used with smaller volumes, e.g., 24-well plates with 0.5 to 1 mL per well or 96-well plates with 100 µL per well. The number of seedlings should then be reduced accordingly.
11. Add *Agrobacterium* cell suspension to the cocultivation medium to a final density of OD₆₀₀ = 0.5 and mix well by gentle shaking.
Calculate the volume of Agrobacterium suspension added to the cocultivation medium based on its starting OD₆₀₀. For example, if the OD₆₀₀ of Agrobacterium suspension calculated in Step 9 is 12, add $20 \times 0.5/12 = 0.83$ mL of Agrobacterium suspension to the cocultivation medium.
12. Wrap the Petri dish with aluminum foil and incubate in a plant growth chamber for 36-40 h for *Arabidopsis* seedlings, 60 h for tobacco (or tomato) seedlings or 6 d for rice (or switchgrass) seedlings before subsequent analysis. Incubate at the usual growth temperature for the species used (e.g., 22°C for *Arabidopsis*) in this and the following steps.
Shorter cocultivation periods may be possible; however, this usually comes at the expense of transformation efficiency. If only relatively few transformed cells are required, e.g., to test for expression of a fluorescently tagged construct, as little as 6 h of cocultivation may be sufficient for Arabidopsis.
See Troubleshooting.
13. After the cocultivation period, remove cocultivation solution and replace with surface sterilization solution to remove epiphytic bacteria. Incubate for 10 min, then wash three times with H₂O.
14. (Optional) After surface sterilization, place seedlings on 0.5X MS plates and cultivate for up to 1 wk prior to analysis.
In this case, addition of 500 µg/mL carbenicillin to remove remaining Agrobacterium cells can increase seedling survival.

TROUBLESHOOTING

Problem: *Agrobacterium* density does not reach OD₆₀₀ >1.5 within 12 h.

[Step 2]

Solution: Start with freshly transformed *Agrobacterium* cells in Step 1, if possible. Also, consider shaking the cells more vigorously.

Problem: Low transformation efficiency is achieved.

[Step 12]

Solution: Consider the following:

1. If low transformation efficiency is accompanied by severe necrosis of plant seedlings, consider reducing the bacterial density and/or Silwet L-77 concentration during cocultivation.
2. If no necrosis occurs, consider increasing the density of bacteria or extending the cocultivation period. For details, see Li et al (2009).

Problem: Transformation efficiency is variable from seedling to seedling.

[Step 12]

Solution: Variation of transformation efficiency is presumably due to an uneven distribution of *Agrobacterium* cells during cocultivation. Increasing the number of seedlings in an assay may allow an overall constant output to be obtained. If possible, gentle shaking of the cocultivation medium during cocultivation may help to reduce the variation of transformation efficiency between seedlings.

DISCUSSION

The FAST technique is a simple method to quickly assess gene function in *Arabidopsis* and other plant species. It results in a high percentage of epidermal cells as well as mesophyll cells transiently expressing a gene of interest. Among other uses, it can be employed to identify subcellular localization of fusion constructs, or to detect protein-protein interactions in vivo with bimolecular fluorescence complementation (BiFC) or Förster resonance energy transfer (FRET) (Li et al. 2009). The high transformation efficiency can also be exploited for biochemical analyses. For example, we were able to detect an expressed protein of interest by Western blot in total protein extracts from as few as eight seedlings (Li et al. 2009). The protocol can also be adapted for 96-well plates and thus can be employed for high-throughput screening approaches (Li et al. 2009).

Compared to other protocols (e.g., Marion et al. 2008), FAST offers the advantages of involving minimal hands-on manipulation and not requiring any specialized equipment. A high level of variation of transformation efficiency from seedling to seedling can easily be compensated for by an increase in the number of seedlings included during cocultivation. However, transient expression occurs primarily in cotyledons, less frequently in hypocotyls, and never in roots. If cell-type specific expression in these other tissues is required, another method of transient expression (e.g., particle bombardment) or seedling transformation with *Agrobacterium rhizogenes* (Campanoni et al. 2007) should be used.

ACKNOWLEDGMENTS

Work in the Nebenführ lab is supported by the National Science Foundation (grant MCB-0822111).

REFERENCES

- Campanoni P, Sutter JU, Davis CS, Littlejohn GR, Blatt MR. 2007. A generalized method for transfecting root epidermis uncovers endosomal dynamics in *Arabidopsis* root hairs. *Plant J* **51**: 322–330.
- Koncz C, Schell J. 1986. The promoter of T_L-DNA gene 5 controls the tissue-specific expression of chimeric genes carried by a novel type of *Agrobacterium* binary vector. *Mol Gen Genet* **204**: 383–396.
- Li JF, Park E, von Arnim AG, Nebenführ A. 2009. The FAST technique: A simplified *Agrobacterium*-based transformation method for transient gene expression analysis in seedlings of *Arabidopsis* and other plant species. *Plant Methods* **5**: 6.
- Marion J, Bach L, Bellec Y, Meyer C, Gissot L, Faure JD. 2008. Systematic analysis of protein subcellular localization and interaction using high-throughput transient transformation of *Arabidopsis* seedlings. *Plant J* **56**: 169–179.