Please cite this article as: Naoko *et. al.*, (2016). Measurement of Uptake and Root-to-Shoot Distribution of Sulfate in *Arabidopsis* Seedlings, *Bio-protocol* 6 (1): e1700. DOI: 10.21769/BioProtoc.1700.

bio-protocol http://www.bio-protocol.org/e1700 Vol 6, Iss 1, Jan 05, 2016

Measurement of Uptake and Root-to-Shoot Distribution of Sulfate in *Arabidopsis* Seedlings

Naoko Yoshimoto[1, 2*], Tatsuhiko Kataoka[2], Akiko Maruyama-Nakashita[2, 3] and Hideki Takahashi[2, 4*]

[1]Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan; [2]RIKEN Plant Science Center, Japan; [3]Graduate School of Agricultural Science, Kyushu University, Kyushu, Japan; [4]Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, USA

[*]For correspondence: naokoy@faculty.chiba-u.jp; htakaha@cns.msu.edu

[Abstract] Sulfur is an essential macronutrient required for growth and development of plants. Plants take up sulfate from the soil environment through the function of plasma membrane-bound sulfate transporters expressed at the root surface cell layers. Plants then utilize the incorporated sulfate as the main sulfur source to synthesize sulfur-containing compounds such as cysteine and methionine. Measurement of root sulfate uptake capacity is essential for analyzing mutants showing altered levels of sulfate transporters and/or sulfur metabolic enzymes as a result of genetic modification or due to the effect of intrinsic or environmental factors modulating their gene expression. The method described in this protocol allows quantitative investigation of sulfate uptake rates and root-to-shoot sulfate distribution in *Arabidopsis* seedlings using [^{35}S] sulfate as a radioactive tracer. The method is designed for parallel comparisons of multiple *Arabidopsis* accessions, mutants or transgenic lines at the seedling stage.

Materials and Reagents

1. Nylon mesh (300 μm mesh opening) (NBC Meshtec, model: NMG 58 or similar types)
2. 9 cm x 9 cm square Petri dishes (Simport, catalog number: D210-16)
3. Plastic frame [exterior dimension, 10 cm (W) x 12 cm (H) x 1.5 cm (D); window size, 7.5 cm (W) x 9 cm (H)]]
4. Plastic container [13 cm (W) x 13 cm (H) x 3 cm (D)]-should be transparent and with no color
5. Double-sided adhesive tape (NICHIBAN, model: NW-10 or similar types)
6. Single-sided adhesive tape (Shamrock Scientific Specialty Systems, model: ST-12-1 or similar types)
7. Paper towel (NIPPON PAPER INDUSTRIES CO., catalog number: 37016 or similar types)
8. Scintillation vials (Sigma-Aldrich, catalog number: Z190527 or similar types)
9. Seeds of *Arabidopsis* (*Arabidopsis thaliana*)

Please cite this article as: Naoko *et. al.*, (2016). Measurement of Uptake and Root-to-Shoot Distribution of Sulfate in *Arabidopsis* Seedlings, *Bio-protocol* 6 (1): e1700. DOI: 10.21769/BioProtoc.1700.

10. Sterile deionized water
11. Agar (Wako Pure Chemical Industries, Siyaku, catalog number: 016-11875 or similar types suitable for plant growth)
12. Sterile agar medium for growth of *Arabidopsis* seedlings [General nutrient medium can be used. Nutrient source and concentrations may be modified depending on research purposes, *e.g.,* sulfate concentrations to be adjusted to 1,500 μM or 15 μM sulfate (Maruyama-Nakashita *et al*., 2015)]
13. Sterile liquid medium for labeling and post-labeling incubation (Nutrient source and concentrations to be adjusted the same as the agar medium mentioned above)
14. $Na_2{}^{35}SO_4$ aqueous solution, 10 mCi/ml (370 MBq/ml) (American Radiolabeled Chemicals, catalog number: ARS0105)
15. Hydrochloric acid (HCl) (Sigma-Aldrich, catalog number: H1758)
16. Ultima Gold™ scintillation cocktail (PerkinElmer, catalog number: 6013321)

Equipment

1. Growth chamber (NKsystem, model: LPH-241SP or similar types. Set the growth conditions appropriately depending on research purposes, *e.g.,* 22 °C, 16 h light/8 h dark cycles)
2. Autoclave (TOMY SEIKO CO., model: LSX-500 or similar types)
3. Mechanical pipettes (Gilson, model: P-200 and P-1000) and tips
4. Analytical balance (readability, 0.1 mg) (Cole-Parmer Instrument Company, Mettler Toledo, model: MS204TS or similar types)
5. Shaker (either reciprocating or rotary shaker) (TAITEC CORPORATION., model: NR-10 or similar types)
6. Liquid scintillation counter (Hitachi Aloka Medical, model: AccuFLEX LSC-7400 or similar types)
7. Utility knife (KOKUYO, model: HA-S200YR or similar types)
8. Stainless steel Forceps (Sigma-Aldrich, catalog number: Z168696 or similar types)
9. Surgical scissors (stainless steel dissecting scissors) (Sigma-Aldrich, catalog number: Z265977 or similar types)

Procedure

A. Preparation of mesh-embedded agar medium

1. Cut the nylon mesh (300 μm opening) into 1 cm x 8.7 cm strips. Wrap the strips of the nylon mesh tightly in aluminum foil, autoclave them and let them dry. Keep the nylon mesh in aluminum foil flattened during autoclaving and drying processes.
2. Prepare agar medium, on which you grow *Arabidopsis* seedlings, in 9 cm x 9 cm square Petri dishes. The agar concentrations should be 0.8% or higher to keep the

Please cite this article as: Naoko *et. al.*, (2016). Measurement of Uptake and Root-to-Shoot Distribution of Sulfate in *Arabidopsis* Seedlings, *Bio-protocol* 6 (1): e1700. DOI: 10.21769/BioProtoc.1700.

roots remain on the surface of the medium.

3. Embed the autoclaved nylon mesh vertically into a solidified agar medium. Insert the longer side of the mesh vertically against the surface of the agar medium at the position approximately 2 cm apart from one side of the Petri dish (Figure 1A). Approximately one-half area of the mesh should be embedded in the agar medium. As a result, each agar medium is partitioned into 2 cm x 9 cm and 7 cm x 9 cm areas by a nylon mesh (Figure 1B).
4. Fill in the gap between the upper side of the nylon mesh and the agar medium in the 2 cm x 9 cm area, by pouring trace amount of melted agar medium using a mechanical pipette (Figures 1C-D). Solidify the agar medium at room temperature.

B. Vertical plate culture of *Arabidopsis* seedlings

1. Sterilize the surface of *Arabidopsis* seeds following a standard protocol (Li, 2011) or similar methods described elsewhere. Immerse the seeds in sterile deionized water at 4 °C under dark for 3 d or more to enhance germination.
2. Place the surface sterilized seeds on the mesh-embedded agar medium described above, approximately 2 mm apart from the nylon mesh in the 2 cm x 9 cm area. Do not sow seeds close to each side of the Petri dish (Figure 1E).
3. Set the agar plates vertically in a growth chamber and grow *Arabidopsis* seedlings under a controlled condition. The roots of *Arabidopsis* will penetrate through the nylon mesh, and elongate on the surface of the agar medium (Figure 1F). If the roots are not attached onto the surface of the medium after penetrating through the nylon mesh, dab those roots gently onto the surface of the medium using a sterile pipette tip or forceps.
4. Following an appropriate period of incubation in a growth chamber (*e.g.,* 10-15 d, which is generally an appropriate period to obtain the seedlings in size suitable for the analysis, for wild-type plants when grown on standard nutrient medium), you will obtain a strip of nylon mesh holding *Arabidopsis* seedlings to be used for the ^{35}S-labeling experiment.

Please cite this article as: Naoko *et. al.*, (2016). Measurement of Uptake and Root-to-Shoot Distribution of Sulfate in *Arabidopsis* Seedlings, *Bio-protocol* 6 (1): e1700. DOI: 10.21769/BioProtoc.1700.

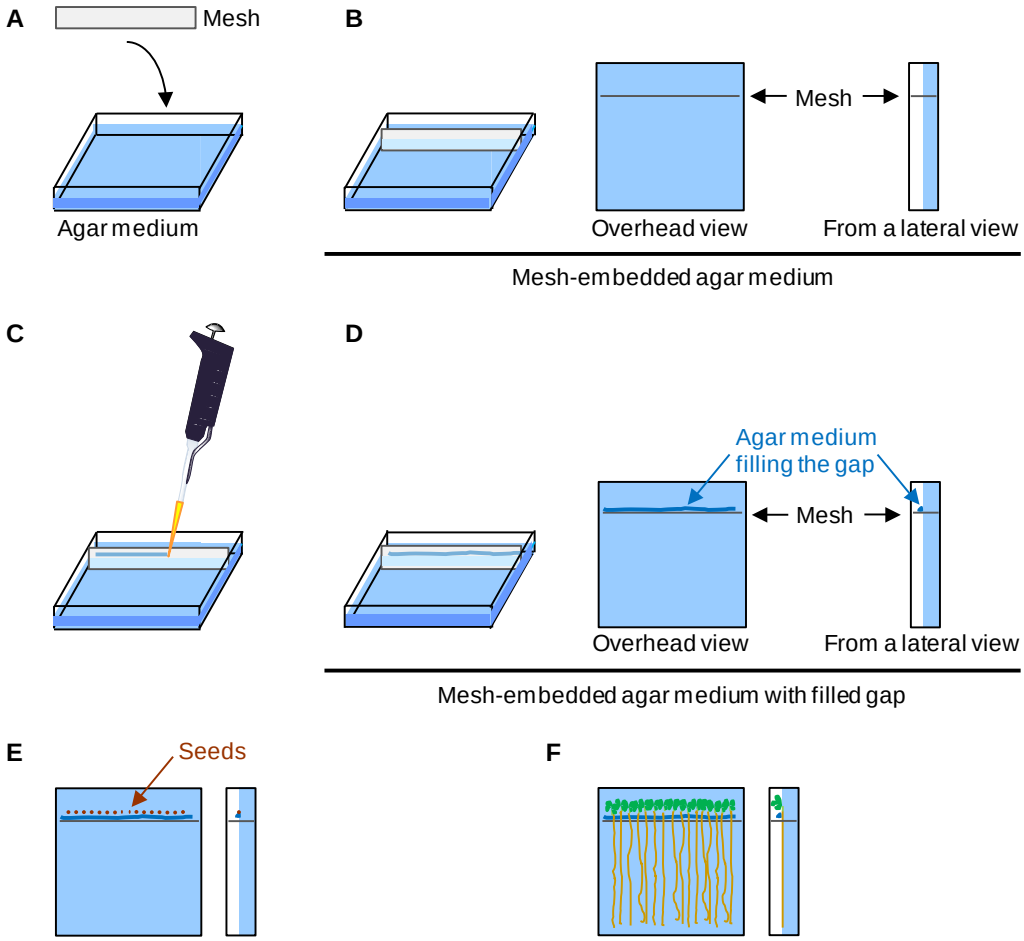

Figure 1. Schematic illustration of mesh-embedded agar medium for vertical plate culture of *Arabidopsis* seedlings. A. Inserting an autoclaved mesh into an agar medium. B. Mesh-embedded agar medium. C. Filling in the gap between the upper side of the mesh and the agar medium by pouring trace amount of melted agar medium using a pipette. D. Mesh-embedded agar medium with filled gap. E. Seeding of *Arabidopsis*. F. *Arabidopsis* seedlings grown through the mesh. (Yoshimoto *et al.*, 2007)

C. Preparation for ^{35}S-labeling

1. Make a plastic frame to place the nylon mesh holding the *Arabidopsis* seedlings. Cut a rectangular window [7.5 cm (W) x 9 cm (H)] on the lid of a plastic tip box [10 cm (W) x 12 cm (H) x 1.5 cm (D)] using a utility knife. To support the nylon mesh in the following step (step C4), put double-sided adhesive tapes on both sides of the plastic frame (Figure 2A).
2. Pour 50 ml of liquid medium into a 9 cm x 9 cm square Petri dish, and place it under a plastic frame (Figure 2A).
3. Pull the nylon mesh, which holds the *Arabidopsis* seedlings, from the agar medium using forceps (Figure 2A). Handle them carefully not to damage the roots.
4. Carefully place two edges of the nylon mesh onto the double-sided adhesive tapes

Please cite this article as: Naoko *et. al.*, (2016). Measurement of Uptake and Root-to-Shoot Distribution of Sulfate in *Arabidopsis* Seedlings, *Bio-protocol* 6 (1): e1700. DOI: 10.21769/BioProtoc.1700.

attached to the plastic frame. Then, fix the two edges of the nylon mesh to the plastic frame using single-sided adhesive tapes (Figure 2B). Usually, 4-6 strips of nylon mesh can be placed in parallel on one plastic frame (Figure 2C). After placing all the meshes on the frame, securely fix them to the frame using single-sided adhesive tapes (Figure 2D). Submerge the roots elongated under the nylon mesh into the liquid medium using forceps. This plastic frame device allows you to create approximately 1 cm of space above the surface of the liquid medium underneath the nylon mesh. Finally, place a transparent plastic container [13 cm (W) x 13 cm (H) x 3 cm (D)] bottom side up to cover the plastic frame holding the plants (Figure 2E). This prevents desiccation of aerial parts of plants. Incubate plants for > 10 min.

5. Preparation of ^{35}S-labeling medium: Add [^{35}S] sodium sulfate ($Na_2{}^{35}SO_4$) aqueous solution to the liquid medium. Usually, the appropriate amount of $Na_2{}^{35}SO_4$ to be added is 0.5-5% of the total sulfate contained in the liquid medium.

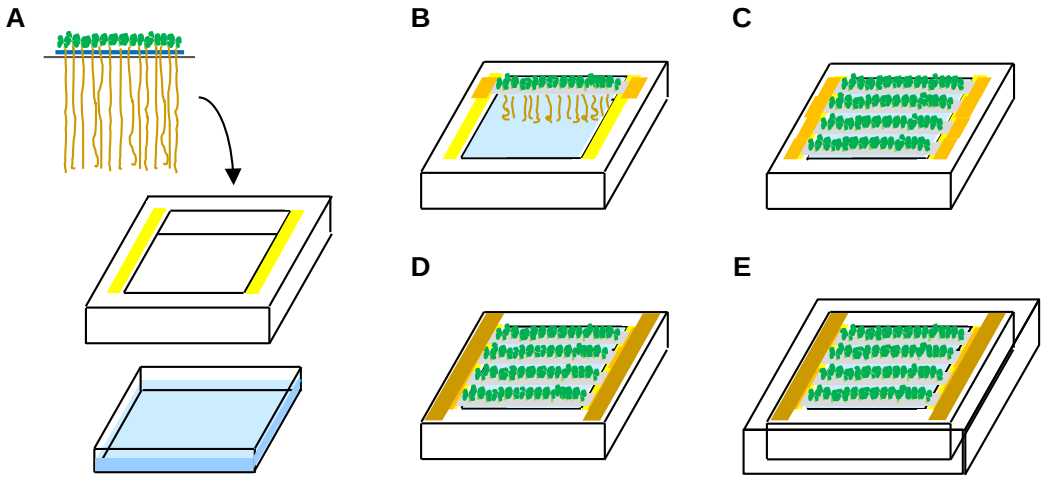

Figure 2. Schematic illustration of a plastic frame device holding nylon mesh and *Arabidopsis* seedlings. A. Organization of a nylon mesh holding *Arabidopsis* seedlings, a plastic frame with double-sided adhesive tapes (yellow), and a square Petri dish containing the liquid medium. B. Plastic frame holding the nylon mesh and *Arabidopsis* seedlings. A square Petri dish is placed underneath the plastic frame. Two edges of the nylon mesh are placed onto the double-sided adhesive tapes attached to the plastic frame, and fixed with single-sided adhesive tapes (orange). Roots of *Arabidopsis* are submerged in the liquid medium. C. Plastic frame holding four strips of nylon mesh with *Arabidopsis* seedlings. D. The final setting of the plastic frame device ready for the ^{35}S-labeling experiment. Four strips of nylon mesh are securely fixed to the plastic frame using single-sided adhesive tapes (brown). E. The final setting of the plastic frame device ready for the ^{35}S-labeling experiment covered by a transparent plastic container which prevents desiccation of aerial parts of plants. (Yoshimoto *et al.*, 2007)

Please cite this article as: Naoko *et. al.*, (2016). Measurement of Uptake and Root-to-Shoot Distribution of Sulfate in *Arabidopsis* Seedlings, *Bio-protocol* 6 (1): e1700. DOI: 10.21769/BioProtoc.1700.

D. ^{35}S-labeling of roots

1. To start ^{35}S-labeling, remove the transparent plastic container [13 cm (W) x 13 cm (H) x 3 cm (D)] that was covering the plants, and transfer the whole plastic frame device including the mesh and seedlings on top of a 9 cm × 9 cm square Petri dish which contains 50 ml of ^{35}S-labeling medium. Submerge the roots into the ^{35}S-labeling medium using forceps. Place a transparent plastic container [13 cm (W) x 13 cm (H) x 3 cm (D)] back to the position to cover the plastic frame device and plants to prevent desiccation of aerial parts of plants during incubation. Then, incubate the plants for 10-60 min depending on the objectives of the experiment.
2. To stop ^{35}S-labeling, rinse the roots of *Arabidopsis* twice in 60 ml of non-labeled liquid medium in 9 cm x 9 cm square Petri dishes.
3. To wash out the radioactivities remaining in the apoplastic space of roots, submerge the roots in 75 ml of non-labeled liquid medium. Place a transparent plastic box [13 cm (W) x 13 cm (H) x 3 cm (D)] over the plastic frame device and plants to prevent desiccation of aerial parts of plants during incubation. Incubate the plants for 60 min.
4. Harvest plant tissues from step D2 or D3. First remove the nylon mesh and the plants from the plastic frame, then cut the roots beneath the nylon mesh using surgical scissors, and detach the roots and the aerial parts of plants separately from the mesh. Remove the liquids remaining on the root surface by using a paper towel. Weigh the fresh weight of plant tissues using an analytical balance.

E. Determination of incorporated radioactivity

1. Place the harvested plant tissues (< 200 mg FW) in a liquid scintillation vial.
2. In separate vials, make standard samples for calibration of radioactivity, using aliquots of the ^{35}S-labeling medium from step C5 (*e.g.,* 0, 1, 3, 10, 30, and 100 µl).
3. Add 1 ml of 100 mM hydrochloric acid and gently shake the vials overnight at room temperature in the dark using a shaker, to digest the plant tissue.
4. Add 2 ml of Ultima Gold™ scintillation cocktail and mix thoroughly.
5. Measure the radioactivity using a liquid scintillation counter.
6. Generate a calibration curve by plotting the amount of sulfate versus the radioactivity of the standard samples. Calculate the amount of ^{35}S-sulfate in plant tissues using the calibration curve. Calculate the sulfate uptake rates based on the amount of ^{35}S-sulfate incorporated into plant tissues, divided by the fresh weight of plant tissues (step D4) and the period of time for incubation in the ^{35}S-labeling medium (step D1). The root-to-shoot sulfate distribution in plants can be estimated based on the amount of radioactivity separately detected in roots and shoots.

Please cite this article as: Naoko *et. al.*, (2016). Measurement of Uptake and Root-to-Shoot Distribution of Sulfate in *Arabidopsis* Seedlings, *Bio-protocol* 6 (1): e1700. DOI: 10.21769/BioProtoc.1700.

Notes

The composition of agar and liquid media, the concentration of [^{35}S] sodium sulfate in the ^{35}S-labeling medium, and the labeling period, may vary according to the research objectives (*e.g.*, measurement of high- or low-affinity sulfate uptake rate). This protocol is adapted from Kataoka *et al*. (2004a), Kataoka *et al*. (2004b), Maruyama-Nakashita *et al*. (2004), Maruyama-Nakashita *et al*. (2006), Maruyama-Nakashita *et al*. (2015) and Yoshimoto *et al*. (2007).

Acknowledgments

N.Y. receives grant support of JSPS KAKENHI Grant Number 26460118. A.M.N. receives grant support of JSPS KAKENHI Grant Number 24380040 and 15KT0028. H.T. receives grant support from the National Science Foundation (MCB-1244300) and AgBioResearch.

References

1. Kataoka, T., Hayashi, N., Yamaya, T. and Takahashi, H. (2004a). Root-to-shoot transport of sulfate in *Arabidopsis*. Evidence for the role of SULTR3;5 as a component of low-affinity sulfate transport system in the root vasculature. *Plant Physiol* 136(4): 4198-4204.
2. Kataoka, T., Watanabe-Takahashi, A., Hayashi, N., Ohnishi, M., Mimura, T., Buchner, P., Hawkesford, M. J., Yamaya, T. and Takahashi, H. (2004b). Vacuolar sulfate transporters are essential determinants controlling internal distribution of sulfate in *Arabidopsis*. *Plant Cell* 16(10): 2693-2704.
3. Li, X. (2011). *Arabidopsis* growing protocol-A general guide. *Bio-protocol* Bio101: e126.
4. Maruyama-Nakashita, A., Nakamura, Y., Yamaya, T. and Takahashi, H. (2004). A novel regulatory pathway of sulfate uptake in *Arabidopsis* roots: implication of CRE1/WOL/AHK4-mediated cytokinin-dependent regulation. *Plant J* 38(5): 779-789.
5. Maruyama-Nakashita, A., Nakamura, Y., Tohge, T., Saito, K. and Takahashi, H. (2006). *Arabidopsis* SLIM1 is a central transcriptional regulator of plant sulfur response and metabolism. *Plant Cell* 18(11): 3235-3251.
6. Maruyama-Nakashita, A., Watanabe-Takahashi, A., Inoue, E., Yamaya, T., Saito, K. and Takahashi, H. (2015). Sulfur-responsive elements in the 3'-nontranscribed intergenic region are essential for the induction of SULFATE TRANSPORTER 2;1 gene expression in *Arabidopsis* roots under sulfur deficiency. *Plant Cell* 27(4): 1279-1296.

Please cite this article as: Naoko *et. al.*, (2016). Measurement of Uptake and Root-to-Shoot Distribution of Sulfate in *Arabidopsis* Seedlings, *Bio-protocol* 6 (1): e1700. DOI: 10.21769/BioProtoc.1700.

7. Yoshimoto, N., Inoue, E., Watanabe-Takahashi, A., Saito, K. and Takahashi, H. (2007). Posttranscriptional regulation of high-affinity sulfate transporters in *Arabidopsis* by sulfur nutrition. *Plant Physiol* 145(2): 378-388.