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# Translating Ribosome Affinity Purification (TRAP) to Investigate Arabidopsis Thaliana Root Development at a Cell Type-Specific Scale

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| **Corresponding Author:** | Martha Thellmann, M.Sc.<br>Universitat Zurich<br>Zurich, Zurich SWITZERLAND |
| **Corresponding Author's Institution:** | Universitat Zurich |
| **Corresponding Author E-Mail:** | martha.thellmann@botinst.uzh.ch |
| **Order of Authors:** | Martha Thellmann, M.Sc. |
| | Tonni Grube Andersen |
| | Joop EM Vermeer |
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**TITLE:**

# Translating Ribosome Affinity Purification (TRAP) to Investigate *Arabidopsis Thaliana* Root Development at a Cell Type-Specific Scale

**AUTHORS AND AFFILIATIONS:**

Martha Thellmann[1], Tonni G. Andersen[2], Joop E. M. Vermeer[1,3]

[1]Department of Plant and Microbial Biology, University of Zurich, Zurich, Switzerland
[2]Biophore, UNIL, Lausanne, Switzerland
[3]Laboratory of Cell and Molecular Biology, Institute of Biology, University of Neuchâtel, Neuchâtel, Switzerland

**Corresponding Author:**

Joop E. M. Vermeer (joop.vermeer@unine.ch)

**Email Addresses of Co-authors:**

Martha Thellmann (Martha.thellmann@botinst.uzh.ch)
Tonni G. Andersen (tandersen@mpipz.mpg.de)

**KEYWORDS:**

arabidopsis, TRAP, TRAP-seq, translatome profiling, cell type-specific RNA-seq, lateral root formation, endodermis differentiation

**SUMMARY:**

Translating ribosome affinity purification offers the possibility to dissect developmental programs with minimal processing of organs and tissues. The protocol yields high-quality RNA from cells targeted with a green fluorescent protein-labeled ribosomal subunit. Downstream analysis tools, such as qRT-PCR or RNA-seq, reveal tissue and cell type-specific expression profiles.

**ABSTRACT:**

In this article, we provide hands-on instructions to obtain translatome data from different *Arabidopsis thaliana* root cell types via the translating ribosome affinity purification (TRAP) method and consecutive optimized low-input library preparation. As starting material, we use plant lines that express the GFP-tagged ribosomal protein RPL18 in a cell type-specific manner using adequate promoters. Prior to immunopurification and RNA extraction, the tissue is snap frozen, which preserves tissue integrity and simultaneously allows the execution of time series studies with high temporal resolution. Notably, cell wall (CW) structures remain intact, which is a major drawback in comparison with alternative procedures such as fluorescence-activated cell sorting (FACS)-based approaches, which rely on tissue protoplasting to isolate distinct cell populations. Additionally, unlike laser capture microdissection-based techniques, no tissue fixation is necessary, which allows high-quality RNA to be obtained. However, sampling from subpopulations of cells and only isolating polysome-associated RNA severely limits RNA yields. Therefore, it is necessary to apply very sensitive library preparation methods for successful data

acquisition by RNA-seq. TRAP offers an ideal tool for plant research, because many developmental processes involve CW-related and mechanical signaling pathways. The use of promoters to target specific cell populations bridges the gap between organ and single-cell level studies that suffer from little resolution or very high costs. Here we apply TRAP to study cell-cell communication in lateral root formation.

**INTRODUCTION:**

Driven by the increasing application of next-generation sequencing techniques, spatial resolution in developmental biology could be augmented. Contemporary studies aim at dissecting tissues down to specialized cell types or single-cell level[1–4]. To this end, a plethora of different methods has been devised over the last 50 years (see **Figure 1A**)[5–15].

Many tools in plant science have been adaptations of techniques that were pioneered in animal research. This is not the case for the method we are introducing in detail in this article. In 2005, equipped with a strong background in protein translation, Zanetti et. al. set out to engineer ribosomal proteins for subsequent affinity purification[16]. Thus, they could avoid time-consuming and labor-intensive polysome profiling, which is based on ultracentrifugation with a sucrose gradient and has been used to assess translating ribosomes since the 1960s[17,18]. The method has since been referred to as translational ribosome affinity purification (TRAP)[16]. After successful translatome studies in plants, Heiman et al. adapted TRAP for animals[19] and others extended its application to yeast[20], *Drosophila*[21], *Xenopus*[22], and zebrafish[23,24].

Although genetic modification of the model system is a prerequisite for TRAP, which limits its application to species amenable to genetic transformation, one can simultaneously harness this drawback to target subsets of cells that are of special interest and otherwise extremely difficult to isolate from the intact tissue/organ[25], such as highly branched dendritic cells in a mouse brain or fungal hyphae in infected plant tissue. In plants, all cells are held in place via cell walls (CWs) that form the basis of the hydrostatic skeleton[26]. To free a plant cell from this matrix, scientists have either to physically cut the cell out of its surrounding tissue through laser capture microdissection (LCM)[27] or to perform enzymatic digestion of the CWs[28]. In protoplasts, the population of interest is fluorescently labeled and can be separated via fluorescence-activated cell sorting (FACS)[7]. LCM usually requires a sample to be fixed and embedded in wax, which ultimately deteriorates the quality of the isolated RNA[29]. FACS-based methods yield high-quality RNA, but the process of protoplasting itself introduces differences in gene expression[30], and tissues with modified and thick secondary CWs are notoriously difficult to treat. Moreover, many developmental processes in plants are assumed to rely on mechanically transmitted signals. Therefore, the integrity of the CW is of paramount importance[31]. Two methods that use a shortcut to circumvent cell isolation by operating on the level of the nuclei, are fluorescence-activated nuclear sorting (FANS) and isolation of nuclei tagged in specific cell types (INTACT). Like TRAP, they use cell type-specific promoters to mark nuclei that subsequently get enriched via sorting or pull down assays, respectively[8,15]. A major challenge for all these approaches is getting sufficient RNA from subsets of cells in a tissue. As TRAP samples only a fraction of the cellular RNAs, sample collection is a considerably difficult. Therefore, especially sensitive library preparation protocols are needed to produce high-quality data from low input amounts.

Since its establishment, TRAP has been either used in combination with DNA microarrays or, as sequencing costs dropped significantly, RNA-seq[10,32,33]. As reviewed in Sablok et al.[34], many research questions have been elucidated using this technique. We are convinced that more reports will follow in the coming years, because the technique is very versatile when using different promoters to target specific cell types, maybe eventually even in an inducible way, and may be combined with probing the plant's reaction to many biotic and abiotic stress factors. Additionally, where stable transgenic lines are not available, hairy root expression systems have also been successfully used to perform TRAP in tomato and *Medicago*[35,36].

The goal of this article is to provide a detailed description of TRAP, to highlight critical steps in the technique, and to provide guidance for a possible library preparation method.

A generic TRAP experiment will essentially consist of the following steps (see also **Figure 1B**): (1) preparation of plant material including cloning of ribosome-tagging construct, transgenic line production, and selection, growing and bulking up of seeds, sterilization, and plating, as well as stress application/treatment (optional) and tissue harvesting, (2) immunopurification including tissue homogenization and clearing of the crude extract, bead wash and immunopurification, and washing steps, (3) RNA extraction and quality assessment, and (4) library preparation.

The *Arabidopsis* root has been a model system to study plant development[37,38]. Here, the application of TRAP is showcased in the context of plant lateral root development. In plants, the buildup of the entire root system is very important for the survival of the organism[39]. In *Arabidopsis*, lateral roots originate from pericycle tissue that resides next to xylem vessels and is therefore termed the xylem pole pericycle (XPP; see **Figure 2C**)[40]. Some XPP cells located deep inside the root acquire a founder cell identity and, upon a local hormonal trigger, start to proliferate by swelling and dividing anticlinally[41]. However, due to the presence of a rigid CW matrix, this process exerts mechanical stress on the surrounding tissues. The overlying endodermis is particularly affected, because it is in the way of the lateral root growth axis[42–44]. In fact, the newly forming primordium will have to grow through the overlying endodermis cells (**Figure 2C2**), whereas cortex and epidermal cells are just pushed aside for the primordium to finally emerge[45,46]. Recent work in our lab has shown that the endodermis actively contributes to proliferation in the pericycle. Targeted blocking of endodermal hormonal signaling is sufficient to inhibit even the very first division in the XPP cells[47]. Hence, pericycle-endodermis communication constitutes a very early checkpoint for lateral root development in *Arabidopsis*. However it is not known how this signal is conveyed. To unravel this mystery, we chose the TRAP-seq approach to target XPP and endodermal cells. To enrich for cells in the lateral root program, we mimicked a hormonal trigger by exogenously applying an analog (1-naphthaleneacetic acid, NAA) of auxin[48], a plant hormone involved in cell elongation, which at the same time allowed us to temporally examine the initial phase of lateral root formation.

**PROTOCOL:**

**1. Cloning of transgene, transgenic line production, and selection**

1.1. Clone the promoter of choice in the appropriate entry vector. Use a recombination-based cloning method (**Table of Materials**) and recombine the promoters in pDONRP4-P1r. Clone RPL18 (with affinity tag or fluorescent protein of choice) using recombination-based cloning in pDONRP1-P2[49].

1.2. Combine the entry vector containing *RPL18* with the promoter-containing entry vector in a two fragment recombination reaction into the appropriate destination vector with the FAST-red selection cassette[50] to facilitate direct selection of transgenic seeds.

1.3. Verify the recombined vector by sequencing and transform it into suitable, competent agrobacteria. Flower dip *Arabidopsis* plants and after 3−4 weeks harvest and select T1 seeds[51].

1.4. Use microscopy to identify well-expressing lines and verify expression patterns according to the reported promoter activity in multiple independent lines.

NOTE: It is important to select lines showing a representative expression pattern with a single T-DNA insertion. This might help minimize silencing and will be advantageous for genetic crosses.

1.5. Select T3 offspring that is homozygous for the marker gene.

**2. Propagation and sterilization**

2.1. Cell type-specific TRAP isolates RNA from a limited number of target cells per root. To generate the needed starting material, propagate homozygous lines using standard growth conditions with a special focus on fungal growth control.

NOTE: If single insertion lines cannot be obtained, grow batches in large populations over a few generations to avoid T-DNA-induced transgenerational silencing.

2.2. Perform sterilization of large quantities of *Arabidopsis* seeds by one round with chlorine gas and one round with 70% ethanol.

2.2.1. Spread seeds evenly on 12 x 12 cm square Petri dishes (less than 0.3 mL of seeds/plate) and stack them into a desiccator or other suitable container. Avoid clump or heap formation as the seeds need to be accessible to the gas. In a 60 L desiccator, perform gas sterilization overnight with 100 mL of bleach (13%) with 6 mL of concentrated HCl, as previously reported[52]. Defumigate for at least 1 h before collecting the seeds in a sterile container.

CAUTION: 37% HCl is highly corrosive and requires careful handling. Chlorine gas is toxic; use a fume hood.

2.2.2. Take 0.1 mL of dry, gas-sterilized seeds per plate and mix them with sterilization solution (70% ethanol, 0.01% Tween) at room temperature. Incubate for 20 min, decant ethanol, and

wash the seeds 3−4x with sterile water.

2.2.3. Transfer the soaked seeds into 50 mL tubes and dilute with sterile 0.1% agar to obtain 1 mL of imbibed seed slurry per plate (0.1 mL of seed/1 mL of slurry).

NOTE: Due to transgene integration events, plant lines can be susceptible to different sterilization techniques. ethanol incubation time was found to be especially critical. In authors' hands, The dual sterilization steps were necessary to avoid fungal contamination during the experiments. This is especially important when performing time series, because contamination of a single time point invalidates the whole experiment. Depending on local growing conditions, dual sterilization may not always be needed.

**3. Plating**

3.1. Prepare these steps in advance: Pour ½ MS plates (pH = 5.8) with 1% agar in the quantities needed for the experiment (20−30 per sample/time point). Cut 1 mL pipette tips to enlarge the tip diameter to ~3−4 mm with a razor blade and autoclave the tips. Create a template holder for plating three rows of seeds per plate with square Petri dish lids). Prepare a laminar flow hood to provide a sterile work environment and label the plates to be processed.

NOTE: If many plates are processed at the same time, colored labels can speed up the labeling.

3.2. Place the empty agar plates into the template holder and distribute 1 mL of imbibed seeds evenly onto three rows. Place the processed plates in stacks into the laminar flow until the seeds are dry (i.e., stick to the agar surface). Do not leave the plates longer, because the agar will dry out as well.

3.3. Once the seeds are sufficiently dry, close the lids and seal each plate with micropore tape. Stratify the seeds for two days at 4 °C in the dark and afterwards place them into a growth chamber.

**4. Tissue treatment (optional)**

NOTE: This protocol outlines the exogenous treatment of *Arabidopsis* roots with the synthetic auxin variant NAA. Depending on the experimental question at hand, this part needs to be adjusted or can be omitted entirely.

4.1. Prepare strips of tissue paper of 1.5−2 cm in width and 10 cm in length.

NOTE: Extended incubation times require the tissue paper to be autoclaved prior to use.

4.2. Remove the micropore tape from all plates that have to undergo the hormone treatment. Dilute 1 mL of 10 mM NAA dissolved in dimethyl sulfoxide (DMSO) in 1 L of liquid, autoclaved ½ MS solution (pH = 5.8) and soak the tissue paper in the solution (10 µM NAA).

4.3. Use tweezers to apply a strip of tissue paper onto each row of roots. Gently use the fingers to remove air bubbles. Empty the excess liquid from the plate, close the lid, and label the plate with the time. For extended incubation times, place the plates back into the growth chamber.

**5. Harvesting**

5.1. Retrieve the plates for each biological replicate, time point, or treatment. Collect liquid nitrogen in a clean Dewar vessel and label the tubes (15 or 50 mL) for the different tissue samples. Prepare an extruded polystyrene foam holder.

CAUTION: Be familiar with liquid nitrogen handling procedures and the danger it poses (e.g., aeration, frostbites, potentially exploding tubes).

5.2. Open the plate and carefully remove the tissue paper with forceps. Be careful to not detach the roots from the agar surface. With a surgical blade cut once per row along the shoot-root-junction in a single, determined stroke. Clean the blades between samples and exchange frequently to guarantee sharpness.

5.3. With tweezers swipe along the roots of each row to collect them in three bundles. Grab the roots and empty them into a 50 mL tube filled with liquid nitrogen to snap freeze.

NOTE: Do not try to assemble roots into dense structures like balls, as they are difficult to grind.

5.4. Proceed with all the plates that constitute one sample (in the order of incubation times) and pour out the excess liquid nitrogen. Use the tube lid to prevent the roots from spilling. Then close the lid and collect all the contents in the Dewar vessel. Store the root tissue at -80 °C.

**6. Immunopurification**

NOTE: This step aims to obtain high-quality TRAP/polysome RNA. Therefore, follow clean practice techniques for RNA handling. Perform all the steps in this section on a sterile bench and clean all equipment and labware with an RNase removing solution (**Table of Materials**). Wear gloves and change them immediately if they come in contact with anything that has not been cleaned (e.g., the sample, ice), to avoid contamination. Because this is crucial, a section is included on equipment reuse together with waste disposal advice.

6.1. Buffer preparation

6.1.1. Prepare stock solutions according to **Table 1** and autoclave (A) or filter sterilize (~~A~~).

NOTE: Unless otherwise specified, the solvent is RNase free water.

6.1.2. Dissolve and aliquot dithiothreitol (DTT), phenylmethylsulfonyl fluoride (PMSF),

cycloheximide (CHX), and chloramphenicol (CAM) in their respective solvents as indicated in **Table 1** and store them at -20 °C. Keep all other stocks at room temperature.

NOTE: It is possible to premix the stocks. To avoid time-consuming buffer mixing prior to every extraction, make the stocks for the wash buffer (WB) with ingredients 1−4, and for the polysome extraction buffer (PEB) with ingredients 1−6. Thus, only water and the frozen ingredients (7−10) have to be added on the day of the extraction. The premixed stocks and RNase-free water are kept at 4 °C. The DTT concentration is ⅕ of the reported concentration from Zanetti et al.[16], as the nanobody interaction with the GFP is sensitive to high DTT concentrations.

## 6.2. Tissue homogenization and grinding

6.2.1. Cool down the centrifuge and place the homogenizers and centrifuge tubes on ice. Thaw aliquots of DTT, PMSF, CHX, and CAM. Mix PEB and WB from the stock solutions in 50 mL tubes according to the number of samples and cool on ice. Add PMSF shortly before use, because the half-life of PMSF in water is only 30 min.

6.2.2. Prepare plenty of liquid nitrogen in a Dewar vessel and retrieve tissue samples from -80 °C storage. Wear cotton gloves underneath the standard lab gloves to prevent burns from cold mortars. Pour liquid nitrogen into the mortars and cool the pestles until they are cold enough to allow grinding.

NOTE: It is recommended to devise a system to distinguish the mortars (i.e., label or keep them in a certain order).

6.2.3. Empty the tissue sample into a mortar and grind carefully until all material is a white powder. If needed, add liquid nitrogen to keep the tissue frozen or to facilitate better grinding.

6.2.4. Add 5 mL of PEB to the sample and quickly mix with the powder before the buffer freezes. While this sample thaws process another sample. Mix processed samples from time to time.

6.2.5. As soon as the mixture can be transferred, empty the slurry into a glass homogenizer and keep on ice. With an additional 2 mL of PEB, rinse the mortar and pestle and add the liquid to the sample in the homogenizer.

NOTE: Avoid a completely liquid sample, because this allows RNA degradation.

6.2.6. Grind the slurry manually a minimum of 4–5x until the extract is homogenous.

NOTE: It may require some additional waiting time to allow the slurry to thaw further. Handling homogenizers is difficult. Do not apply brute force and beware of suction forces. If it is not handled carefully, misuse will lead to spillage or contamination of the sample, or destruction of the homogenizer.

6.2.7. Pour the crude root extract into a 50 mL centrifuge tube on ice.

NOTE: Usually several samples can be ground before transfer. Parallel handling of grinding, transferring, and homogenizing is required. Try to work quickly but do not rush. Always keep homogenized samples on ice.

6.3. Total RNA sample collection

6.3.1. Transfer 200 µL aliquots of each crude sample to a clean, precooled, labeled microcentrifuge tube. Proceed with the RNA extraction as detailed for TRAP samples in sections 7.1 and 7.2. Perform these steps while the samples are clearing in the centrifuge.

6.3.2. Perform a DNase treatment with the resuspended total RNA to eliminate DNA contamination and clean up the reaction using the RNase-free DNase set in conjunction with a cleanup kit (**Table of Materials**).

NOTE: Total RNA extractions usually yield high concentrations and samples need to be diluted considerably. Measuring the concentration after dilution by a sensitive fluorometer protocol is recommended.

6.4. Clearing the crude extract

6.4.1. Centrifuge the samples for 15 min, 16,000 x *g*, 4 °C.

NOTE: To balance out the centrifuge, pair samples accordingly. If this is not possible, adjust one sample by adding PEB.

6.4.2. Pour the supernatant to a fresh, precooled centrifuge tube and repeat the centrifugation (15 min, 16,000 x *g*, 4 °C).

NOTE: This transfer can be quickly performed next to the centrifuge.

6.4.3. While the crude extract is clearing, start washing the GFP beads for section 6.5.

NOTE: Keep the ice bucket rocking on the shaker but do not put it back into the sterile bench because it might be contaminated.

6.5. Bead wash

6.5.1. Aliquot magnetic GFP beads (60 µL/sample, **Table of Materials**) into a 1.5 mL tube. Place on the magnetic stand. Once the beads have collected, remove the supernatant.

6.5.2. Add 1 mL of cold WB, resuspend the beads, and collect them again. Discard the wash buffer and repeat once more with 1 mL of WB. Finally, resuspend the beads in WB to the initial volume used in step 6.5.1.

6.6. Immunopurification (IP)

6.6.1. Immediately after centrifugation, pour the cleared supernatant into labeled 15 mL tubes and add 60 µL of washed beads per sample.

6.6.2. Place all samples horizontally into the ice bucket and put it on a shaker. Let the mixture incubate for 2 h in order to bind the GFP-labeled polysomes to the beads.

6.6.3. Collect the beads on the magnetic stand for 15 mL tubes on ice and add PMSF to the remaining PEB. Discard the supernatant. Pour approximately 5 mL of PEB to the beads and resuspend them by tilting. Shake the samples for 15 min as in step 6.6.2.

6.6.4. Repeat the washes with WB for a total of three washes (1x with PEB, 2x with WB). Before each buffer exchange, add PMSF.

6.6.5. Collect the beads in 1 mL of WB and transfer them to a 1.5 mL tube. Finally, collect the beads one more time on the magnetic stand and remove all liquid. Close the tube and keep on ice until all samples are processed.

6.6.6. Transport the samples to a fume hood for RNA extraction.

6.7. Waste disposal and reconditioning of lab supplies

NOTE: If performed according to good lab practice, the sterilization procedure (step 2.2.1) yields an aqueous NaCl solution. Chlorine gas, as well as residual HCl and bleach, should be left in the fume hood.

6.7.1. For PEB and WB disposal, collect all liquids and bring to pH > 9 because CHX decomposes at high pH. Dispose of liquid waste in the halogenated chemical waste. Dispose of all solids (e.g., tissues, serological pipettes, gloves) as chemical waste.

6.7.2. Collect phenol-containing liquids, as well as phenol-contaminated material (e.g., tips, tubes, gloves) separately.

6.7.3. Hand wash mortars, pestles, and homogenizers with soap and rinse thoroughly. Then, wrap in tin foil or place into a heat-proof, covered container, and bake at >220 °C overnight.

6.7.4. Brush centrifuge tubes clean with detergent and then treat with diethylpyrocarbonate (DEPC) in the fume hood. To do so, add 1 mL of DEPC to 1 L of deionized water and mix via

shaking. Place the centrifuge tubes onto an autoclavable tray that catches spilled liquid. Pour the suspension into the tubes and leave for 3 h or overnight. DEPC decomposes in the autoclave.

CAUTION: DEPC is highly toxic.

## 7. RNA extraction and quality control

### 7.1. RNA extraction

7.1.1. Cool down the tabletop centrifuge to 4 °C.

7.1.2. Add 1 mL of acid-guanidinium-phenol-based reagent (**Table of Materials**) to each sample, invert to resuspend the beads or total RNA slurry, and incubate for 5 min on ice. Do not vortex.

7.1.3. Add 200 µL of chloroform and incubate for 3 min on ice. Then, thoroughly vortex the samples. To aid phase separation, centrifuge at maximum speed (~13,000 x *g*) for 10–15 min, 4 °C.

7.1.4. Label 1.5 mL low-retention tubes (**Table of Materials**) and aliquot 650 µL of isopropanol into each.

7.1.5. Carefully take the upper aqueous phase (~650 µL) and transfer to the prepared tubes with isopropanol. Avoid touching the pink organic phase.

7.1.6. Precipitate the RNA overnight at -20 °C.

NOTE: It is recommended to store the samples in isopropanol at -20 °C or -80 °C and only solubilize in water when needed. Aqueous RNA degrades even at -80 °C when stored for weeks or months.

### 7.2. RNA precipitation

7.2.1. Cool down the tabletop centrifuge to 4 °C.

7.2.2. Prepare fresh 80% ethanol with RNase-free water and cool down to -20 °C (keeping at -80 °C for 5 min helps to speed up the process).

7.2.3. Centrifuge the samples at maximum speed (~13,000 x *g*) for 30 min and discard the supernatant. The pellet will not be visible, so carefully pipette as if it was there. Add 1 mL of cold 80% ethanol and invert the tube 1–2x.

7.2.4. Centrifuge again for 30 min at maximum speed (~13,000 x *g*) and repeat for a total of two washes.

7.2.5. Spin down for 2 min and remove all residual ethanol with a 10 µL tip. Leave the pellet to dry for only 3–5 min at room temperature and resuspend in 20 µL of RNase-free water.

7.2.6. Keep the samples on ice and perform quality control as soon as possible. Proceed to store the samples at -80 °C. Avoid freeze-thaw cycles.

7.3. Perform quality control using dedicated equipment (e.g., Bioanalyzer, Qubit, Tapestation) according to the manufacturer's recommendations.

**8. Library preparation**

8.1. cDNA synthesis and amplification with the ultra low input RNA kit (**Table of Materials**)

8.1.1. Calculate the dilution of each sample according to the fluorometer quantification to have 1.5 ng of TRAP-RNA or total RNA in a volume of 4.75 µL. Perform all reactions in polymerase chain reaction (PCR) tubes and dilute samples with fresh aliquots of RNase-free water.

8.1.2. Perform all the steps according to the manufacturer's recommendations with ½ the reaction volumes. Amplify the cDNA with 12–13 PCR cycles.

8.1.3. Clean up the PCR by adding 0.5 µL of 10x lysis buffer and 25 µL of solid phase reversible immobilization (SPRI) beads (**Table of Materials**).

NOTE: If many samples are processed, the lysis buffer and beads can be premixed. Make sure that the beads are evenly dispersed before pipetting.

8.1.4. Proceed with the RNA kit protocol in full reaction volumes (i.e., 17 µL of elution buffer). Do not let the beads dry for more than 3 min.

NOTE: Overdried samples can potentially be rescued by prolonged incubation times.

8.1.5. Measure the sample concentrations with the Qubit HS DNA kit.

NOTE: The SMARTer v4 kit can allow for a minimum of 200 pg input. Libraries were not obtained in cases where the fluorometer values could not be determined (<250 pg, detection limit) with a 16 cycle PCR. However, the limited input material might also yield less complex libraries.

8.2. Fragmentation and adapter ligation PCR with the DNA library preparation kit (**Table of Materials**)

8.2.1. Dilute the cDNA with RNase-free water to obtain a concentration of 200 pg/µL and pipette 1.25 µL in a PCR tube.

8.2.2. Perform all steps according to the manufacturer with ¼ the reaction volumes. Amplify the

cDNA with 12 PCR cycles and compatible adapters for the samples that belong to one sequencing pool.

NOTE: With index kits A and D, up to 384 samples can be multiplexed.

8.2.3. For the PCR cleanup, add 12.5 µL of resuspension buffer and 22.5 µL of SPRI beads (0.9x ratio). Elute the sample with 22 µL of elution buffer.

NOTE: Quality control and pooling were performed by the sequencing company (**Table of Materials**) and thus no bead-based normalization was needed. The enzymatic fragmentation reaction (tagmentation) is very sensitive to material input, because every enzyme only cuts once. Therefore, do not exceed the recommended concentration.

**REPRESENTATIVE RESULTS:**

For quality assessment, the above-mentioned procedure should be probed at several intermediate steps: expression pattern validation in planta, quality control of the isolated polysomal RNA, and quality control of the final libraries. In addition, quantitative reverse transcription PCR (qRT-PCR) using known marker genes can be performed to confirm the response to the treatment condition or to fine-tune the experimental conditions.

**Confocal analysis of GFP signal distribution**

To check for both endodermal and XPP expression patterns, we analyzed homozygous lines of *pELTP::GFP-RPL18* and *pXPP::GFP-RPL18* by confocal microscopy. **Figures 2A**,**B** show representative plants with GFP signals (green) counterstained with propidium iodide (magenta) to outline the CWs. The cross section in **Figure 2B1** shows a concentric ring in the third cell layer from the outside that corresponds to the endodermis. The endodermal GFP signal begins shortly above the meristematic zone (**Figure 2B2**) and appears both in the cytosol and around the nuclei, which corresponds to ribosomes. In contrast, the XPP line exhibits two distinct poles that correspond to the XPP (**Figure 2C1**). Approximately three cells at each pole above the meristematic zone start to exhibit a GFP signal. Thus, both lines correspond to the localization pattern of the endodermis and XPP, respectively (**Figure 2A**)[53].

**Polysome RNA validation**

To determine the quality of the obtained polysome RNA we performed quality control measurements using two automated electrophoresis systems (**Table of Materials**) that work with microliter input amounts and also calculate an RNA integrity number (RIN)[54]. The proprietary algorithm assigns a RIN value between 1 and 10 to each electropherogram and is a robust and reproducible measure for RNA quality (i.e., degradation). A lower value corresponods to a more degraded sample. **Figure 3** shows examples of the measurements we obtained from polysome RNA. Most samples barely showed any degradation, with RIN values ranging from 9–10, which is in accordance with previous reports[55,56]. Any improper handling, especially periods of prolonged elevated temperatures (e.g., room temperature) or RNase contamination would be evident at this stage. Both instruments also calculate sample concentrations from their electropherograms (**Figure 3A**). These can vary substantially and are mostly at the lower detection limit. Therefore,

we advise using fluorometric measurements to accurately quantify concentrations.

**Library quality control**

Because most labs do not perform RNA sequencing in house, quality controls are often run at specialized facilities with high throughput devices (**Table of Materials**). They routinely assess the quality of the samples and quantify their concentrations by qPCR and fluorometric assays (**Table of Materials**), because accurate measurements are a prerequisite for library pooling. Nevertheless, if the library preparation is not outsourced, one can sample the outcome with specialized equipment (**Table of Materials**). **Figure 4** shows traces of successfully prepared libraries with our recommended protocol (**Figure 4A**) and highlights the robustness of the procedure despite scaled-down reaction volumes (**Figure 4B**). **Figure 4C** illustrates substandard samples that can result from over- or underfragmentation, material loss during clean up, or unsuccessful adapter removal. In the latter case, another clean up with a more stringent sample-bead-ratio could help eliminate the contamination. Completely failed samples were extremely rare in our hands and could originate at multiple points due to reasons such as too high input for the stoichiometric tagmentation reaction.

The performance of sequenced libraries is shown in **Figure 4D** for samples from the endodermis in the mutant background chosen. Libraries from wild type and/or pericycle perform similarly or even better. Ribosomal reads were on average ~2% with only a few samples >3%. Reads with an average quality score >30 were consistently >90% before the filtering. The mapping ability of the sequenced reads was equally high, on average 85%. To determine the correlation between biological replicates pairwise, Spearman coefficients were calculated for each time point. All tests resulted in high coefficient values.

**Treatment response and enrichment analysis**

Before a genome-spanning dataset is produced, TRAP RNA from a pilot experiment can be probed by qRT-PCR to validate treatment success and/or experimental conditions. We performed this type of analysis to assess auxin responses after 2 h of treatment in the XPP samples (**Figure 5A**). Three different auxin-responsive genes (GH3.3, LBD29, and GATA23) were tested via the ΔΔCt method[57]. Very strong induction was observed in all three cases after the incubation period, which suggests that the exogenous NAA application was successful.

If newly developed promoters are utilized one should also perform enrichment analysis with qRT-PCR. To this end, a known marker gene (i.e., the gene driven by the promoter used) is amplified in the TRAP and the total RNA sample and expression levels normalized to the total RNA level. If the isolation of TRAP RNA from the specific tissue was successful, a significant fold change increase should be obtained. Alternatively, equivalent information can be retrieved from the sequencing data (see **Figure 5B**). Expression of two suberin-related genes, GPAT5 and HORST, was present in all endodermis samples and notably absent from the XPP tissues. In contrast, pericycle-expressed genes PHO1 and SKOR were very poorly expressed in the endodermis and enriched in the XPP probes with an auxin-induced downregulation over the examined time frame.

**FIGURE AND TABLE LEGENDS:**

**Figure 1: Translating ribosome affinity purification (TRAP) complements the "omics" analysis portfolio.** (**A**) Increasing levels of analytical precision, down to single-cell or even subcellular resolution, can be achieved by a plethora of methods or combinations thereof. This image gives an overview of currently available tools in the plant and animal field. Tissue collection at a cellular resolution can be achieved by protocols like LCM or FACS, which are then coupled to standard transcriptome or polysome profiling/translatome analysis. TRAP and INTACT integrate both tissue capture and RNA isolation because they are based on epitope-tagging. However, INTACT samples only cell nuclei and therefore constitutes a special case of transcriptome analysis. A small rabbit icon marks newly developed methods in the animal field: While SLAM-ITseq and Flura-seq rely on metabolic targetting of nascent RNAs with modified uracil bases in cells expressing the permissive enzyme, Slide-seq makes use of a coated glass slide with DNA barcodes that provide positional information in the cellular range. A proximity-labeling approach is followed in APEX-seq to sample RNAs in specific subcellular compartments. Notably, increased resolution often requires the generation of transgenic material (asterisks) and these methods are thus predominantly used for model species. TRAP is especially suited for plant science studies involving CW or mechanic signaling as well as cell species that are difficult to release from their CW matrix. (**B**) Detailed wet lab steps of the TRAP procedure: Seedlings expressing GFP-tagged ribosomal protein in distinct cell types (e.g., root endodermis) were grown on Petri dishes for 7 days and root material was harvested by snap freezing. A total RNA control sample was collected from the homogenized crude extract before pelleting the debris via centrifugation. Magnetic anti-GFP beads were added to the cleared extract to perform immunoprecipitation. After incubation and three wash steps, the polysome-associated RNA (TRAP/polysome RNA) was directly obtained via phenol-chloroform extraction. LCM = laser capture microdissection; FACS/FANS = fluorescence-activated cell/nuclear sorting; APEX-seq = method based on engineered ascorbate peroxidase; INTACT = isolation of nuclei tagged in specific cell types; SLAM-ITseq = thiol(SH)-linked alkylation for the metabolic sequencing of RNA in tissue; Flura-seq = fluorouracil-labeled RNA sequencing (Created with Biorender.com).

**Figure 2: Cell type-specific expression of GFP-RPL18 in the *Arabidopsis* root.** (**A,B**) Confocal microscopy images of p*ELTP*::GFP-RPL18 (**A**) and p*XPP*::GFP-RPL18 (**B**) expressing roots at 6 days postgermination. CW outlines were obtained through staining with propidium iodide (magenta). Cross sections A1 and B1 are from the positions denoted with dashed lines in A2 and B2, respectively. The latter images show maximum projections (MAX) of the recorded Z-stacks. (**C**) Schematic representation of the tissue types composing the *Arabidopsis* root in longitudinal (**C1**) and cross section (**C3**) as well as in a lateral root primordium (**C2**). The image was modified with permission from F. Bouché[58]. Scale bar = 100 µm.

**Figure 3: TRAP/polysome RNA quality assessment.** (**A**) Tapestation. Representative results from 14 measured samples in gel picture representation with their respective $RIN^e$ values (top left). Electropherogram representation is shown for sample A1 (highlighted in blue). The table on the right shows the sample concentrations. (**B**) Similar traces as in panel A were obtained with the Bioanalyzer. The panels on the right show samples with increasing levels of degradation, which are reflected in their decreasing RIN values.

**Figure 4: Library profiles from TRAP/polysome samples.** (**A**) Two representative TRAP samples (left) corresponded very well with the traces for successful libraries recommended by the manufactuer's user guide. (**B**) Differing reaction volumes yielded robust library preparation outcomes. (**C**) Libraries with suboptimal outcomes: very short fragments (top left), extremely long fragments (bottom left), low concentration (top right), or complete failure (bottom right). Note the residual short fragments (blue ellipse), that had to be removed before sequencing. Bioanalyzer = red traces; LabChip = blue traces. (**D**) Selected quality measures for sequenced TRAP samples (i.e., endodermis of our lateral root-free mutant) at different time points and distribution of the Spearman correlation coefficients calculated between pairwise comparisons of all samples within a time point (n = 65).

**Figure 5: qRT-PCR and RNA-seq showed auxin-responsiveness and tissue type enrichment, respectively.** (**A**) Expression levels of three known auxin-responsive genes were assessed via qRT-PCR after 2 h of auxin treatment. Strong induction was observed in all samples. RT-PCR was performed on three independent biological replicates and normalized to the untreated samples with *UBC21* as the internal reference gene. Error bars represent the SEM. *GH3.3* = *Gretchen Hagen 3.3*; *LDB29* = *LATERAL BOUNDARY DOMAIN 29*; *GATA23* = *GATA-motif binding transcription factor 23*; *UBC21* = *UBIQUITIN-CONJUGATING ENZYME 21.* (**B**) Expression levels of four marker genes from the TRAP-seq dataset. Samples on the left were endodermis-derived (green shades), while samples on the right were XPP-derived (blue shades). Numbers represent the auxin incubation intervals in hours. Negative Z-scores reflect low expression levels and vice versa. Endodermal marker genes (*GPAT5*, *HORST*) were differentially expressed, with high levels of expression in endodermis samples. On the contrary, pericycle markers (*PHO1*, *SKOR*) were highly expressed in XPP cells and were downregulated upon auxin treatment. *GPAT5* = *glycerol-3-phosphate 2-O-acyltransferas* (suberin biosynthsis); *HORST* = *hydroxylase of root suberized tissue*; *PHO1* = *phosphate 1*; *SKOR* = *stelar $K^+$ outward rectifier*.

**Figure 6: Non-constitutive *pUBQ10::GFP-RPL18* localization patterns.** Confocal microscopy of 6 day-old seedlings. Cell wall outlines were obtained through staining with propidium iodide (magenta). Cross sections **A2** and **C1** are from the positions denoted with dashed lines in **A3** and **C2**, respectively. Images marked MAX show maximum projections of the recorded Z-stacks. **A1–A3**. Uniform localization patterns of the *UBQ10*-driven construct. **B1–C2**. Notable decrease in the signal strength in the outer tissue layers. **A**, **B**, and **C** were recorded in three different plants. Scale bars = 100 µm.

**Table 1: Buffer composition and mixing advice.** Ingredients with the given stock concentrations mixed in the given amounts yield 50 mL of WB or PEB. Tris = tris‑(hydroxymethyl)-aminomethane; EGTA = ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetra-acetic acid; PTE = polyoxyethylene-(10)-tridecyl ether; A = autoclave; ~~A~~ = filter-sterilize; *fill up to 50 mL with RNase-free water.

**DISCUSSION:**

**Verification of RPL18 localization pattern**

To avoid misinterpretation of data from any TRAP experiment the proper expression pattern of the tagged ribosomal subunit must be known. Therefore, the incorporation of GFP as an epitope tag to RPL18 allowed verification of the desired expression pattern and consecutively, pulldown of the polysome fraction from the same tissue. More invasive approaches to assure proper promoter patterns were used by Jiao and Mayerowitz requiring GUS-staining, and Tian et al. requiring immunostaining with anti-FLAG antibodies[59,60].

We strongly advise to check the localization pattern in each generation, because T-DNA transgenic lines can be prone to silencing. Thus, signal strength can deteriorate or the proportion of expressing seeds can decrease. Because the GFP-tag is incorporated into the construct, these controls are easily performed via microscopy.

However, even thorough confocal analysis can lead to false conclusions in some instances. We would like to highlight this with a description of our failed attempt to produce a control TRAP line. So far, the plant science community has not been able to create a plant line with uniform RPL18 distribution throughout all cell layers. Even the initially employed 35S promoter only showed "near constitutive" distribution with a nonuniform localization pattern[10]. Our approach was to use the promoter of *UBIQUITIN10* (*UBQ10*) to drive the *GFP-RPL18* construct. Screening in the T1 generation offered very promising localization and thus was chosen to be propagated (**Figure 6A**). However, the data of a test sequencing run did not show enrichment in comparison with *ELTP*- and *UBQ10*-driven lines for known endodermis-specific genes. Upon closer inspection of those plants, we found a decrease in signal in the outer tissue layers, whereas stele tissue showed strong expression (**Figure 6B**). Future studies need to search the promoter landscape for more suitable candidates to complement the TRAP method.

**Total RNA as control sample**

The establishment of a better TRAP control line is still pending and is highly anticipated. So far the only way to obtain a tissue-wide uniform distribution of mRNAs is to collect total RNA, as, in this case, a transcriptome is sampled. This needs to be accounted for in the bioinformatic analysis. Notably, both total RNA and polysome RNA fractions are now correlated, because they originate from the same tissue samples.

**In search for a TRAP library preparation method**

As mentioned previously, a major drawback of the TRAP approach is the variable, low RNA yields that can be obtained. With samples ranging from a few nanograms to sometimes up to 100 nanograms, a standard approach with the Illumina TruSeq kit (100 ng input requirement) was judged as too insensitive for construction of libraries of sufficient quality. A market search revealed several commercially available library preparation kits that were specified to work with as low as 5−10 ng of starting material. We did not test the protocol used by Reynoso et al.[36], which obtained TRAP samples from tomato, rice, and *Medicago* and uses the BrAD-seq approach[61].

All of our trials with subsequent test sequencing yielded unsatisfactory results. Despite the use of polyA enrichment steps, the TRAP samples suffered from high ribosomal contamination (up to

30% of reads). Furthermore, the success rate for the library preparation was variable and especially low for samples with critically low concentrations or relatively lower RIN values. Our extensive testing lead us to the conclusion that the specific RNA composition of a TRAP sample, with very high rRNA content and presumably minute mRNA concentrations, requires a more sensitive approach to obtain reliable libraries. Thus, we turned to state-of-the-art solutions for ultra-low input amounts: SMARTer v4 (TaKaRa) and Nextera XT (Illumina). Reassuringly, Song et al.[61] also found this library preparation approach to outperform competitors when they tested several methods on their sequencing output on TRAPed liver tissue[62]. The quality metrics we presented in **Figure 4D** exhibit high quality reads (Q > 30) at low rRNA mapping rates (<3%) concurrent with high gene mapping rates. Additionally, consistently high Spearman correlation coefficients show that replicates have very similar expression profiles.The use of both kits was straightforward, with modest cycle numbers, and yielded robust and reliable results. Sequencing data were of high quality with 1.5 ng of starting material. With the SMARTer kit tolerating as low as 200 pg input, the amount of plant starting material can be optimized. The applicability for rarer cell types will ultimately be determined by the feasibility of accruing enough RNA.

**TRAP complements the plant science toolkit**

The TRAP method has become increasingly popular with plant scientists[34] and we are confident that it will acquire the status of a standard technique due to several reasons.

None of the steps in the TRAP protocol need specialized equipment, like a cell sorting machine or a dedicated laser-capture microscope, which makes it possible for many labs to perform the experiments. To date, the most costly factors are the library preparation and downstream sequencing. Nevertheless, with the dynamic advancement of next-generation sequencing techniques and increasing demand for single-cell sequencing, we anticipate that costs will decrease significantly.

Furthermore, the isolation of polysome-associated RNAs means that information is gathered on the active translation status of those RNAs (translatome). Therefore, TRAP captures the output of all regulatory steps that are upstream of translation and represents a more direct proxy for the cellular protein composition. Of course, stalled ribosomes and posttranslational modifications still remain elusive and need to be addressed by other approaches (e.g., proteomics).

As stated previously, a clear advantage that TRAP has in a plant context is the preservation of CW structures and mechanical properties of the cells. As we only begin to understand the intricate connections and regulatory functions that arise through CW and mechanical signaling[31,63], approaches that preserve these structures will become more important in many different developmental contexts.

TRAP can profit from a wealth of different promoters that have already been characterized, especially in well-established model species. In *Arabidopsis*, it was possible to map the entire root in a cell type-specific manner by using 19 different marker gene promoters[30,64]. With each RNA-seq experiment, these selections will be improved and new, more specific promoters will arise

and refine the cellular resolution.

Additionally, TRAP can be combined with many promoters for cell populations where no markers are yet known. This is the case for specialized cells in the root endodermis. The so-called passage cells are characterized by the absense of the suberin layer that coats mature endodermis cells[53]. Combining suitable promoters and subsequent in silico subtraction of the distinct expression profiles will enrich for regions that harbor passage cells. Transcriptional reporter analysis will then help identify passage cell marker genes. However, whether TRAP can be used to then analyze the rare cell population on this basis remains to be determined.

In this article, we have provided a detailed description of the translating-ribosome affinity purification method, its advantages and limitations, and highlighted potential applications. In the portfolio of "omics" studies, it occupies an important niche and will help to answer many biological questions.

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The authors have nothing to disclose.

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Figure

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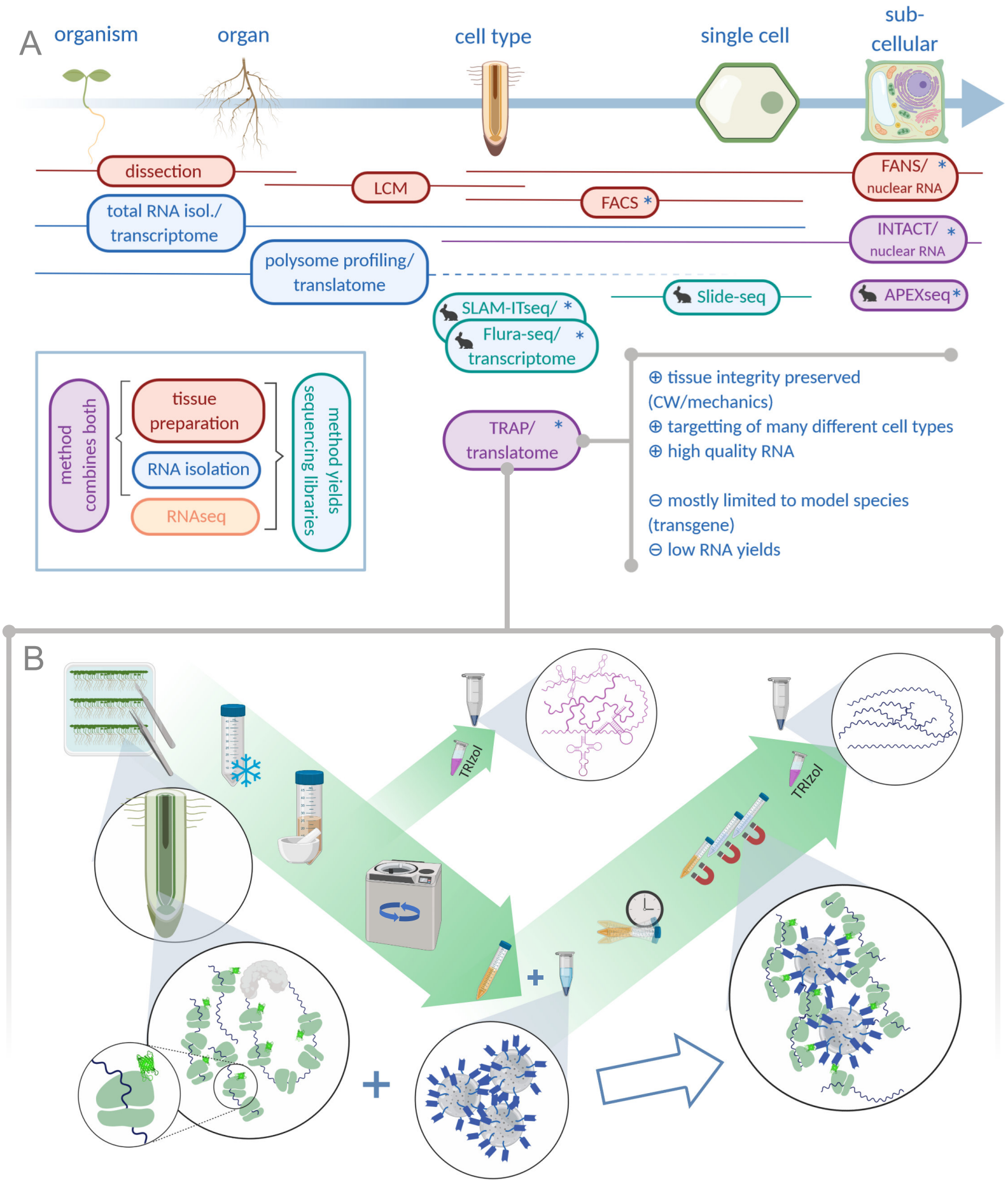

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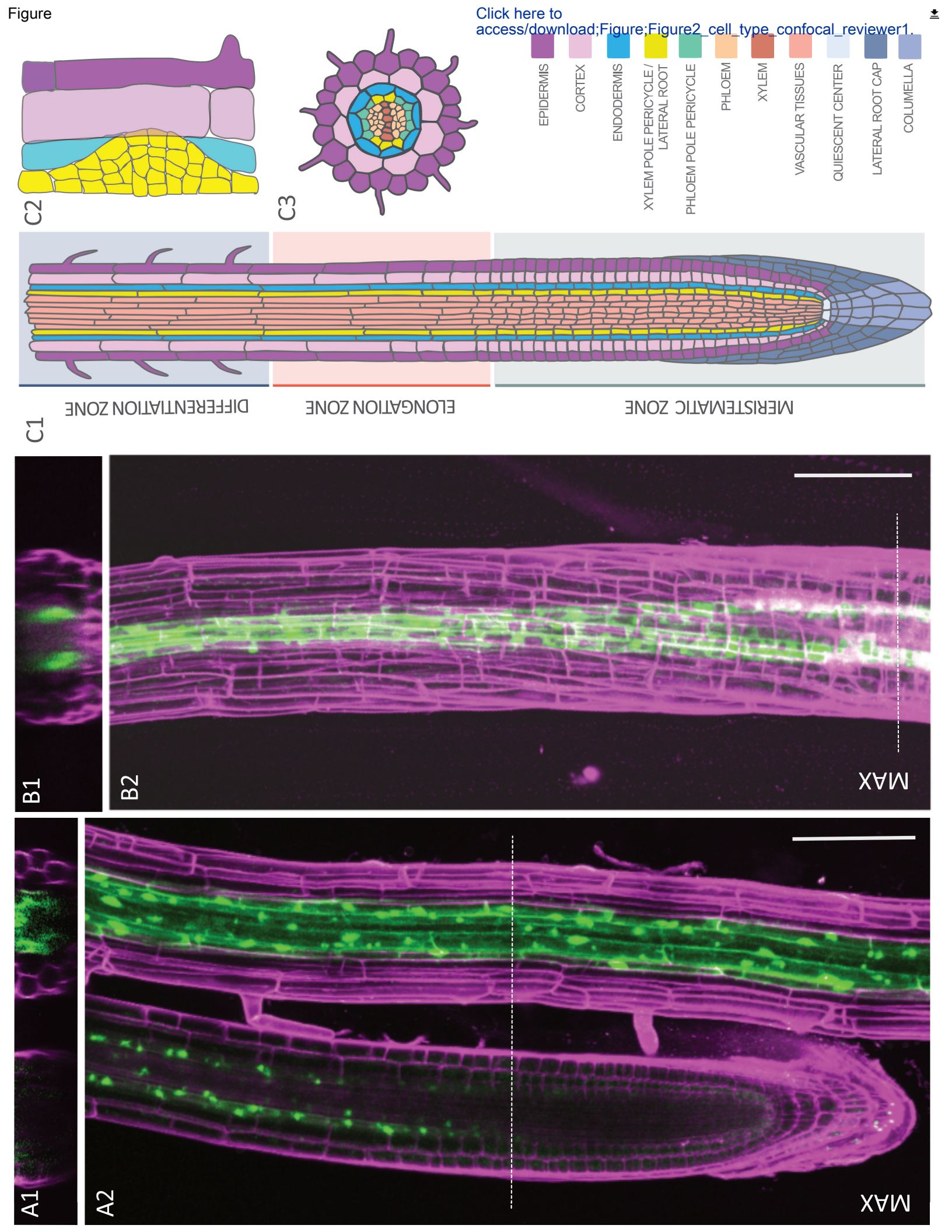

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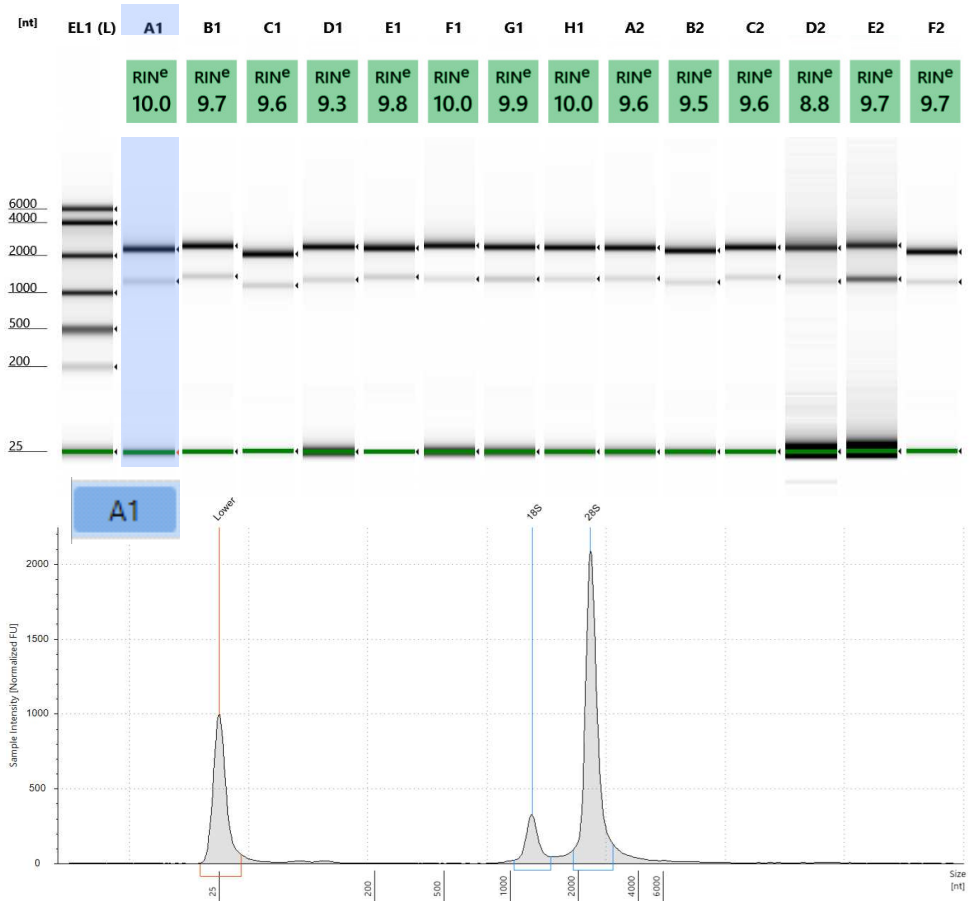
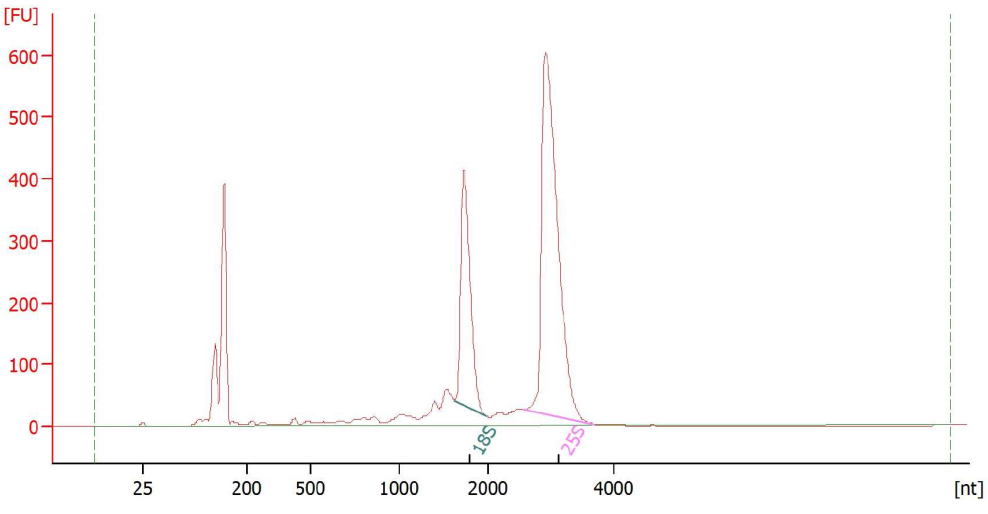
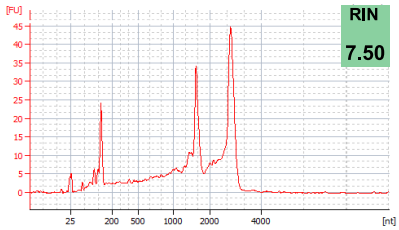
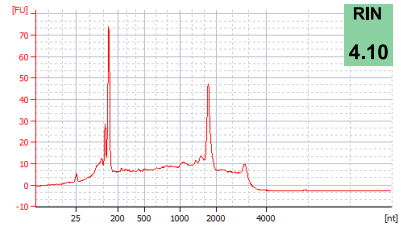


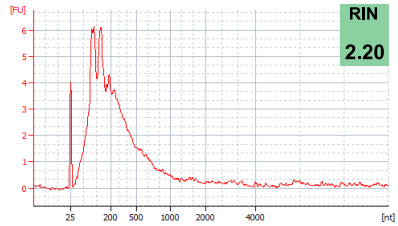

| Well | Conc. [pg/µl] |
|---|---|
| EL1 | 3750 |
| A1 | 814 |
| B1 | 2010 |
| C1 | 2860 |
| D1 | 358 |
| E1 | 4800 |
| F1 | 407 |
| G1 | 460 |
| H1 | 700 |
| A2 | 656 |
| B2 | 764 |
| C2 | 1380 |
| D2 | 136 |
| E2 | 146 |
| F2 | 1850 |

B



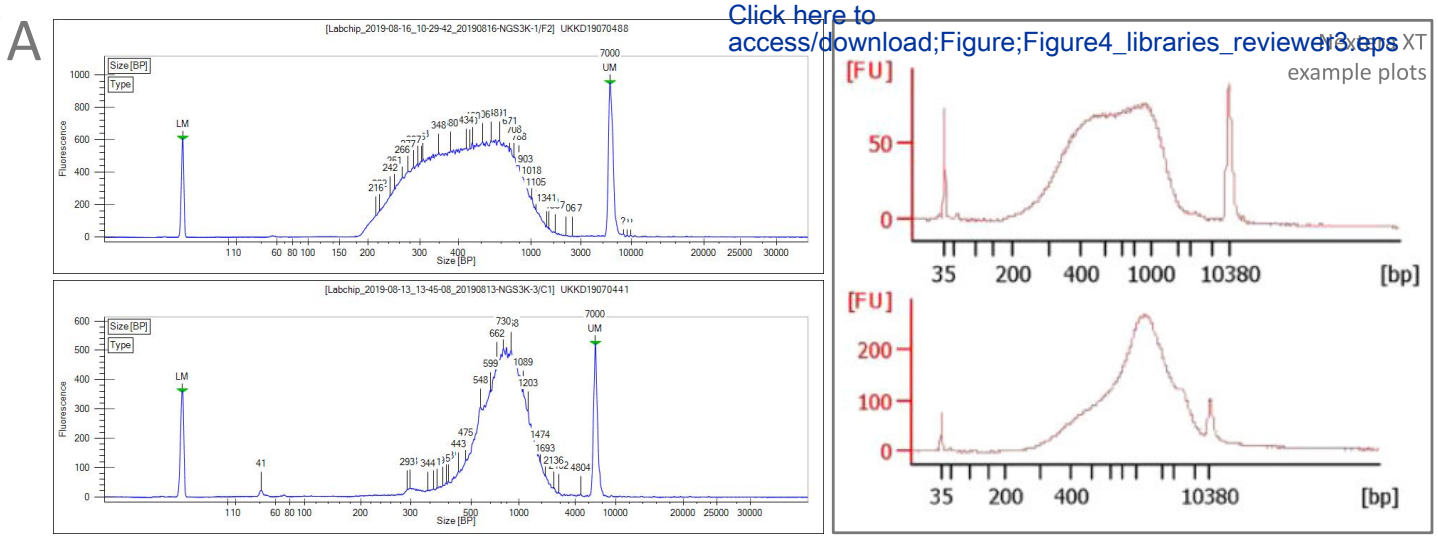



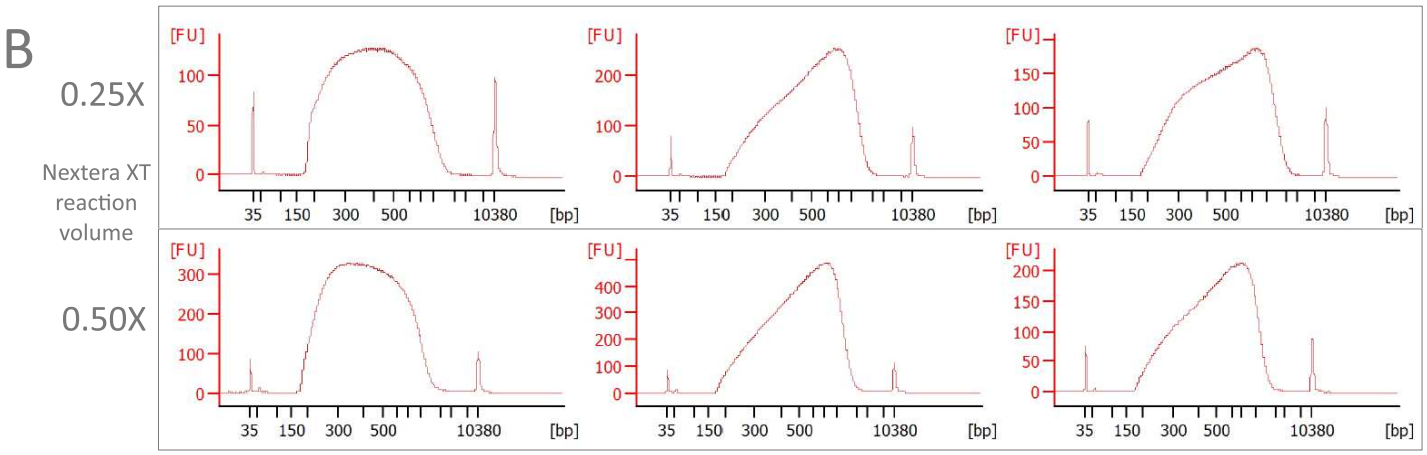

**Overall Results for sample 1 :**

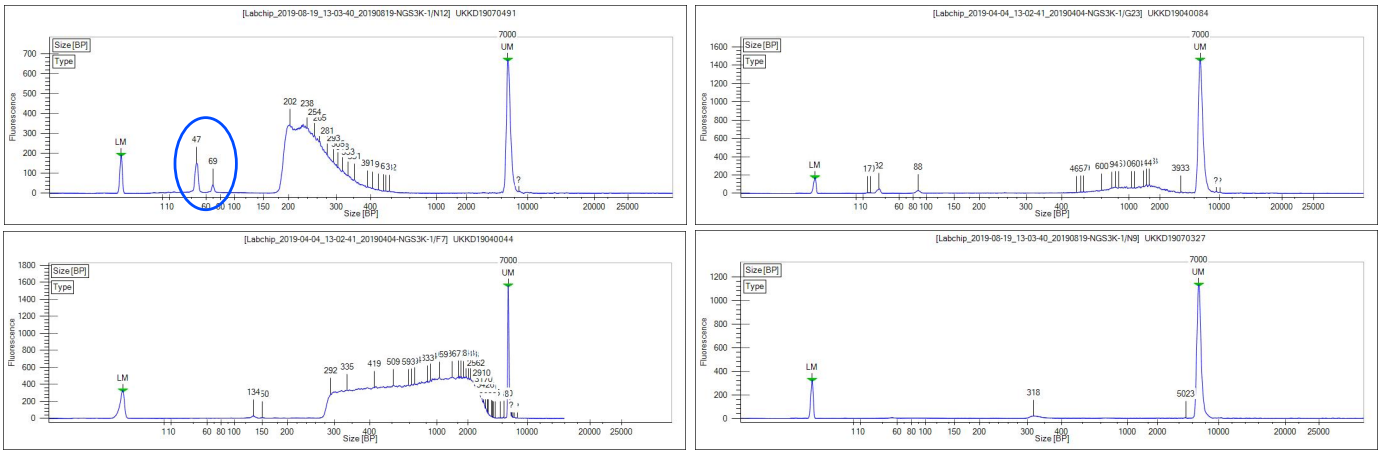
| | | | |
|---|---|---|---|
| RNA Area: | 3'404.6 | RNA Integrity Number (RIN): | 9.50 (B.02.08) |
| RNA Concentration: | 21'675 pg/µl | Result Flagging Color: | |
| rRNA Ratio [25s / 18s]: | 2.3 | Result Flagging Label: | RIN: 9.50 |

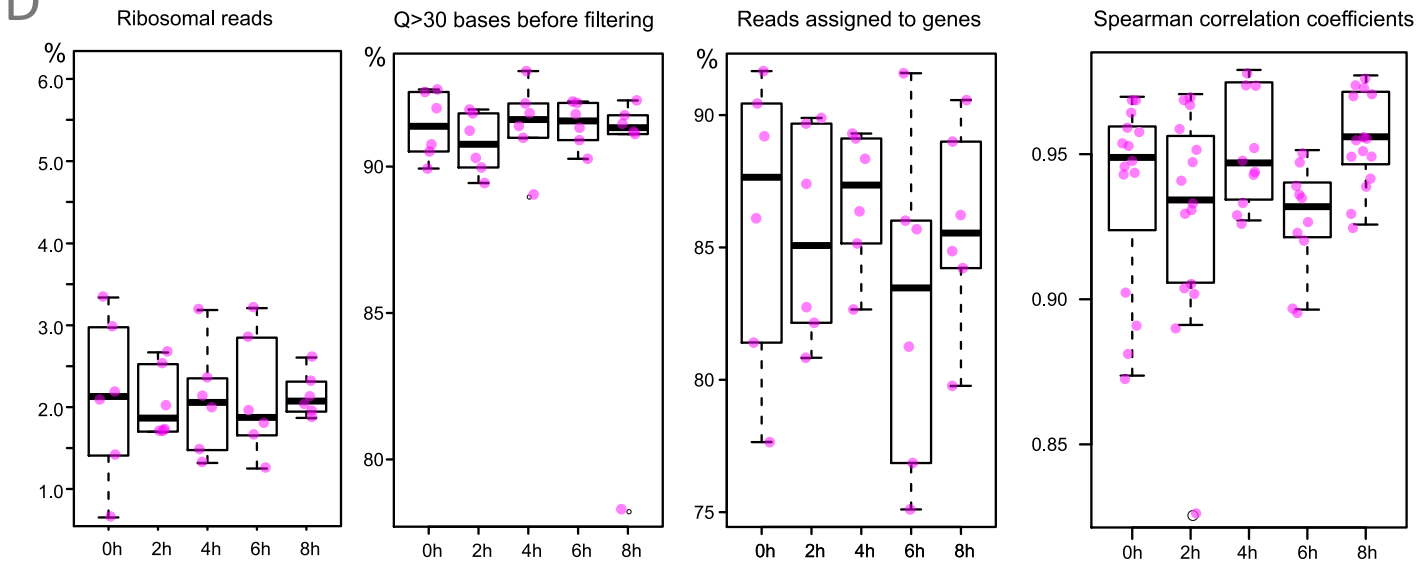
Figure

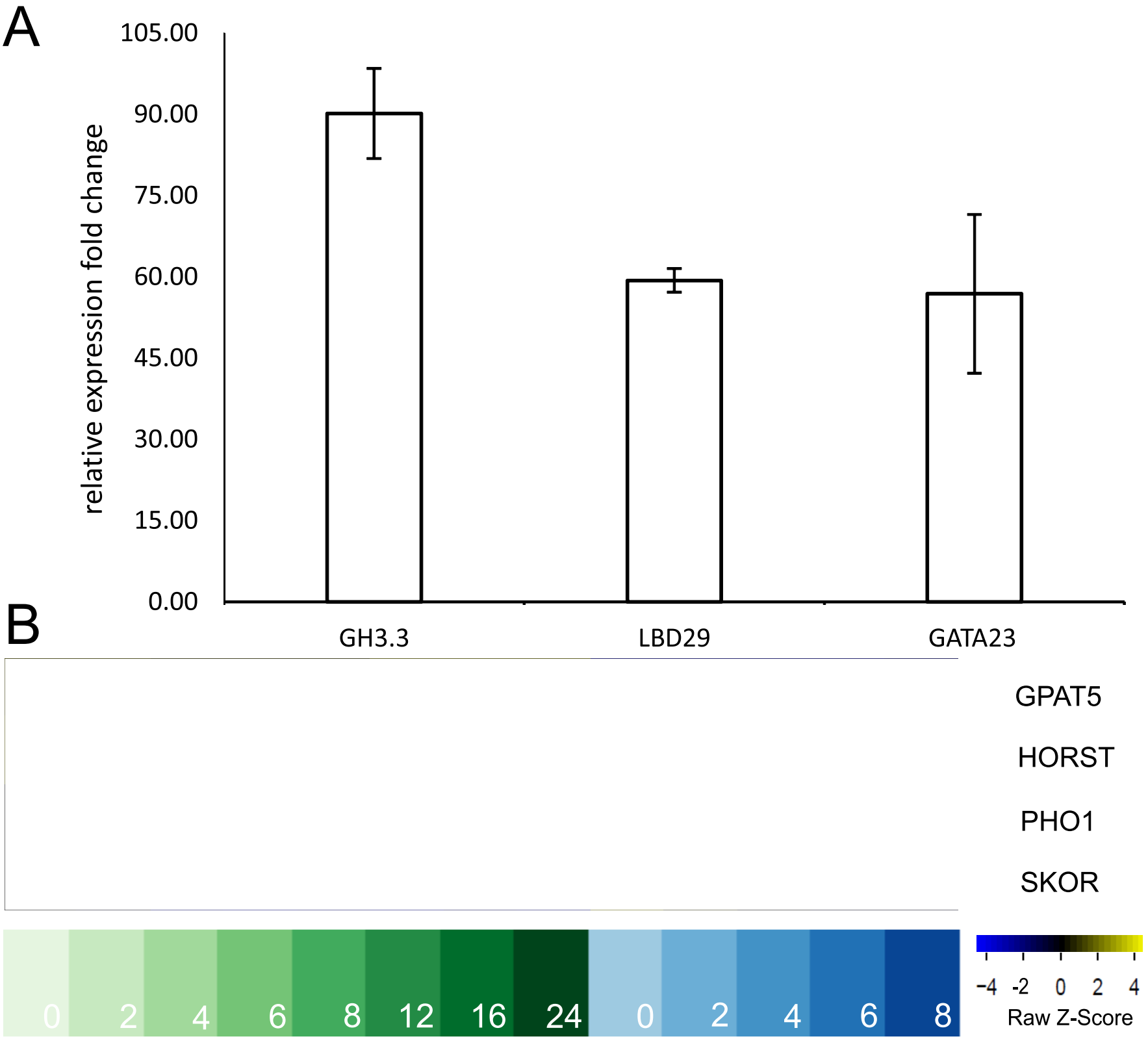
Click here to access/download;Figure;Figure4_libraries_reviewer3.eps





Figure

Click here to access/download;Figure;Figure6_UBQ.eps

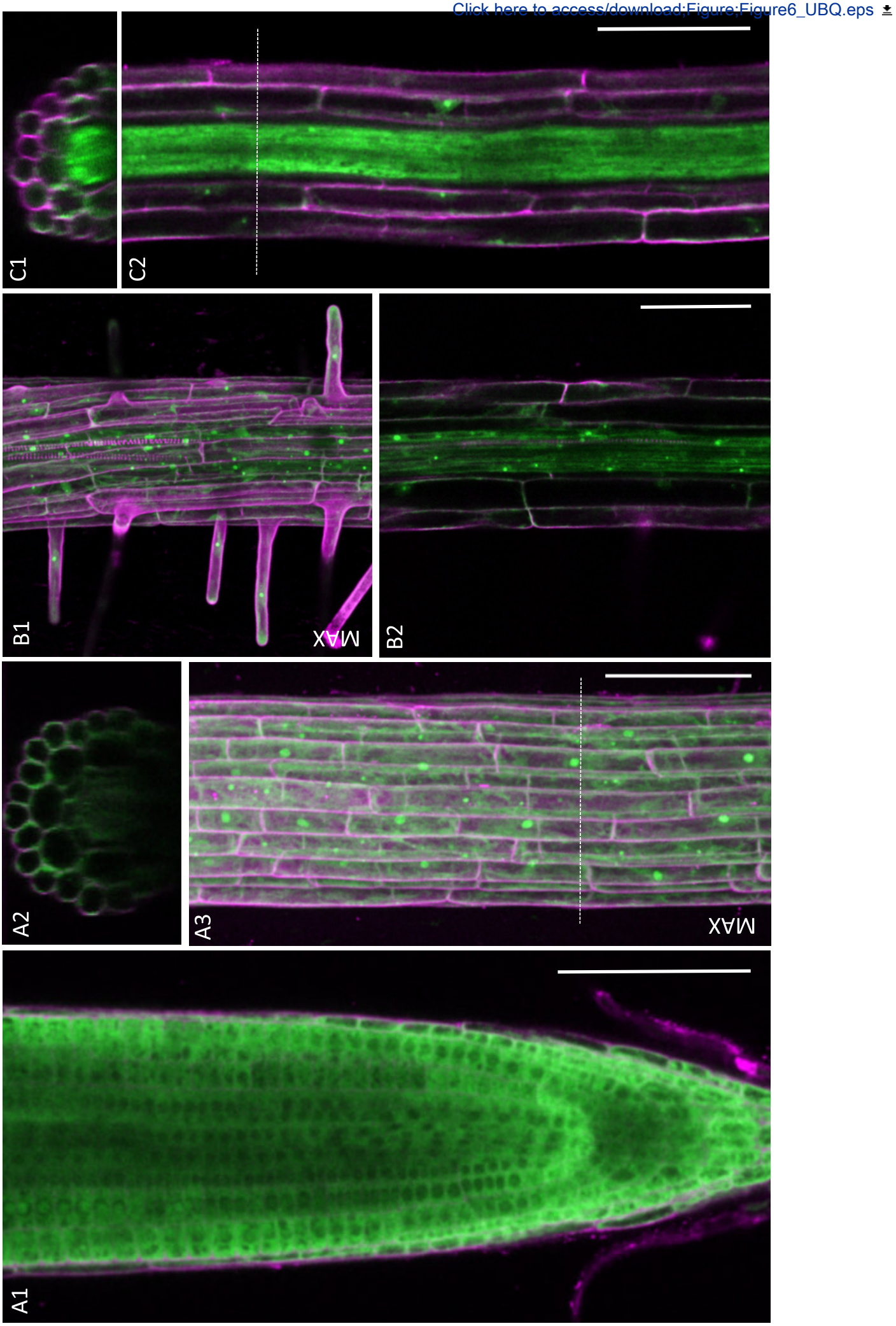


Table1 Click here to access/download;Table;60919_Table1.xlsx

| | Ingredients | | Stock concentration | Add volume in mL for 50 mL of WB* | Add volume in mL for 50 mL of PEB* |
|---|---|---|---|---|---|
| 1 | Tris, pH 9 | A | 2 M | 5 | 5 |
| 2 | KCl | A | 2 M | 5 | 5 |
| 3 | EGTA | A | 0.5 M | 2.5 | 2.5 |
| 4 | $MgCl_2$ | A | 1 M | 1.75 | 1.75 |
| 5 | PTE | A | 20% (v/v) | 0 | 2.5 |
| 6 | detergent mix | A | | 0 | 2.5 |
| | Tween 20 | | 20% (v/v) | | |
| | Triton-X 100 | | 20% (v/v) | | |
| | Brij-35 | | 20% (w/v) | | |
| | Igepal | | 20% (v/v) | | |
| 7 | DTT | ~~A~~ | 0.5 M | 0.1 | 0.1 |
| 8 | PMSF | ~~A~~ | 0.1 M (isopropanol) | 0.5 | 0.5 |
| 9 | Cycloheximide | ~~A~~ | 25 mg/mL (EtOH) | 0.1 | 0.1 |
| 10 | Chloramphenicol | ~~A~~ | 50 mg/mL (EtOH) | 0.05 | 0.05 |

Table of Materials



| Name of Material/Equipment | Company | Catalog Number | Comments/Description |
|---|---|---|---|
| | | | |
| **Sterilization** | | | |
| bleach, 13% | Sigma | 71696 | |
| beaker | VWR | 214-1172/74/75 | |
| desiccator with porcelaine plate (DURAN) | Sigma/Merck | Z317454-1EA/Z317594-1EA | |
| EtOH, p.a. | Honeywell | 02860-1L | |
| HCl, 37% | Roth | 4625.1 | |
| Tween 20 | Sigma | P9416 | |
| | | | |
| **Plate growth + harvesting** | | | |
| MS salts, basal salt mixture, incl. MES buffer | Duchefa | M0254 | |
| agar plant for cell culture | Applichem/Panreac | A2111.1000 | |
| DMSO | Sigma | D4540 | |
| forcepts | Rubis Switzerland | 5-SA model | |
| KOH | Fluka | 60370 | |
| micropore/surgical tape | 3M | 1530-0 | |
| NAA | Duchefa | N0903 | |
| petri dishes 120x120 mm | Greiner bio-one | 688102 | |
| scalpel | VWR/Swann-Morton | 233-5454 | |
| tissues, neutral, two-layered | | | any supplier of your choice |
| | | | |
| **Immunoprecipitation** | | | |
| GFP-beads: gtma-100 GFP-Trap_MA | Chromotek | e.g. gtma-100 | |
| Brij-35 | Sigma | P1254-500G | |
| centrifuge tubes (in accordance with centrifuge) | Beckman Coulter | 357001 | |
| Chloramphenicol | Applichem | C0378-25G | |
| cotton gloves | VWR | 113-7355 | |
| Cycloheximide, HPLC grade | Sigma | 01810-1G | |
| DEPC | VWR | E174 | might have long delivery times |
| DTT | Fluka | 43815 | |
| EGTA | Sigma | 3054.3 | |
| homogenizers DUALL 23 | KONTES GLASS CO (via VWR) | SCERSP885450-0023 (set) | SCERSP885451-0023 pestle only - SCERSP885452-0023 cylinder only; long delivery times |
| Igepal CA-360 | Sigma | I3021-100ml | |
| KCl | Sigma | 60130 | |
| $MgCl_2$ hexahydrat | Roth | 2189.2 | |
| mortar and pestle | VWR | 470148-960 & 470019-978 | |
| PMSF | Roche | 10 837 091 001 | |
| Polyoxyethylene-(10)-tridecylether/PTE | Sigma | P2393-500G | |
| RNase-free water | Roth | T143.3 | |
| RNAZap | Thermo Fisher | AM9780/AM9782 | for cleaning surfaces |
| Tris, >99.3% | Roth | AE15.3 | |
| Triton X-100 | Fluka | T8787-250ml | |
| Tween 20 | Sigma | P9416-100ml | |
| | | | |
| **RNA extraction** | | | |
| 2-Propanol, p.a. | Sigma | 33539-1L-GL-R | |
| Chloroform, HPLC grade | Scharlau | CL02181000 | |
| EtOH, p.a. | Honeywell | 02860-1L | |
| low-retention microcentrifuge tubes, 1.5 ml | Eppendorf/Sigma | Z666548-250EA | LoBind |
| RNase-free DNase set | Qiagen | 79254 | |
| RNeasy MiniElute Cleanup Kit | Qiagen | 74204 | |
| TRIzol reagent | ThermoFisher/Ambion | 15596018 | |
| | | | |
| **Library preparation** | | | |
| 15/50 mL Tube Magnetic Separator | Abraxis | PN 472250 | |
| AMPure beads | Beckman Coulter | A63881 | |
| Index Kit A | Illumina | FC-131-2001 | |

| | | | |
|---|---|---|---|
| Index Kit D | Illumina | FC-131-2004 | |
| neodymium magnets | Amazon/other | 6 x 1.5 mm range: N42 (NdFeB) | |
| Nextera XT kit | Illumina | FC-131-1024/1096 | https://emea.support.illumina.com/sequencing/sequencing_kits/nextera_xt_dna_kit/documentation.html |
| PCR strips | ThermoScientific | AB-0266 | |
| SMARTer v4 kit | Takara Bioscience | 634892 | https://www.takarabio.com/products/next-generation-sequencing/rna-seq/smart-seq-v4-for-mrna-seq |
| Bioanalyzer | Agilent | 2100 Bioanalyzer Instrument | specialized equipment for RNA/DNA quality control |
| Tapestation | Agilent | 4200 Tapestation Instrument | specialized equipment for RNA/DNA quality control |
| Fragment Analyzer | Agilent | 5400 Fragment Analyzer System | specialized equipment for RNA/DNA quality control (high throughput) |
| LabChip | PerkinElmer | LabChip GX Touch Nucleic Acid Analyzer | specialized equipment for RNA/DNA quality control (high throughput) |
| Qubit 4 Fluorometer | ThermoFisher | Q33239 | specialized equipment for RNA/DNA concentration determination |
| | | | |
| **qRT-PCR** | | | |
| GATA23 | Microsynth | | fwq: AGTGAGAATGAAAGAAGAGAAGGG; rev: GTGGCTGCGAATAATATGAATACC |
| GH3.3 | Microsynth | | fwd: CAAACCAATCCTCCAAATGAC; rev: ACTTATCCGCAACCCGACT |
| LBD29 | Microsynth | | fwd: TCTCCAACAACAGGTTGTGAAT; rev: AAGGAGCCTTAGTAGTGTCTCCA |
| UBC21 | Microsynth | | fwd: TGCGACTCAGGGAATCTTCT; rev: TCATCCTTTCTTAGGCATAGCG |

UNIVERSITÉ DE NEUCHÂTEL

Institut de biologie

Rue Emile-Argand 11
CH-2000 Neuchâtel

Neuchâtel, 22/11/2019

Dr. Stephanie Ray Weldon
Science editor
JoVE

**Subject: Re-submission of Thellmann *et al.,***

Dear Dr. Weldon,

Joop Vermeer
Professor
Joop.Vermeer@unine.ch
Tél : +41 32 718 2217

We want to thank you and the unanominous reviewers for constructive feedback on our JoVe manuscript: “Translating ribosome affinity purification (TRAP) to sort out *Arabidopsis thaliana* root development at a cell type-specific scale”.

Below this letter you will find our detailed responses to all the comments that were raised in the review process. We believe that this has significantly improved the manuscript and we hope you agree to consider the new version for publication.

The enclosed manuscript is not under consideration for publication in any other journal or book; its submission for publication has been approved by all authors and our institution. All persons entitled to authorship have been so named. We also assert that all authors have seen and agreed to the submitted version of the manuscript.
We thank you for your consideration of the manuscript and look forward to hearing from you.

On behalf of all authors,
Sincerely,

**Joop Vermeer**

FACULTÉ DES SCIENCES

**Institut de biologie**

Secrétariat
Rue Emile-Argand 11
CH-2000Neuchâtel
Tél : +41 (0)32 718 30 00
Fax : +41 (0)32 718 30 01
secretariat.biologie@unine.ch

www.unine.ch/biol