Journal of Visualized Experiments

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--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE61517R2
Full Title:	Laser-capture microdissection RNA-sequencing for spatial and temporal tissue-specific gene expression analysis in plants
Section/Category:	JoVE Biology
Keywords:	Laser-capture microdissection (LCM); transcriptome; tissue-specific; RNA-Seq; gene expression; spatial
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Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Bundoora, Victoria, and Australia

TITLE:

Laser-Capture Microdissection RNA-Sequencing for Spatial and Temporal Tissue-Specific Gene Expression Analysis in Plants

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KEYWORDS:

laser-capture microdissection, LCM, transcriptome, tissue-specific, RNA-seq, gene expression, spatial

SUMMARY:

Presented here is a protocol for laser-capture microdissection (LCM) of plant tissues. LCM is a microscopic technique for isolating areas of tissue in a contamination-free manner. The procedure includes tissue fixation, paraffin embedding, sectioning, LCM and RNA extraction. RNA is used in the downstream tissue-specific, temporally resolved analysis of transcriptomes.

ABSTRACT:

The development of a complex multicellular organism is governed by distinct cell types that have different transcriptional profiles. To identify transcriptional regulatory networks that govern developmental processes it is necessary to measure the spatial and temporal gene expression profiles of these individual cell types. Therefore, insight into the spatio-temporal control of gene expression is essential to gain understanding of how biological and developmental processes are regulated. Here, we describe an LCM method to isolate small number of cells from three barley embryo organs over a time-course during germination followed by transcript profiling. The method consists of tissue fixation, tissue processing, paraffin embedding, sectioning, LCM and

RNA extraction followed by real-time PCR or RNA-seq. This method has enabled us to obtain spatial and temporal profiles of seed organ transcriptomes from varying numbers of cells (tens to hundreds), providing much greater tissue-specificity than typical bulk-tissue analyses. From these data we were able to define and compare transcriptional regulatory networks as well as predict candidate regulatory transcription factors for individual tissues. The method should be applicable to other plant tissues with minimal optimization.

INTRODUCTION:

Plant development and growth involve a coordinated action of transcriptional regulatory networks within different cells that exist in a complex cellular environment. To understand the activity of these regulatory networks, we require the knowledge of spatial and temporal gene expression within different cell types across developmental stages. However, analyses of gene expression are more commonly conducted in whole organs or bulk tissue samples due to the technical challenge of isolating and analyzing small numbers of cells. The method we describe here has allowed obtaining spatial and temporal tissue-specific transcriptome analysis by coupling LCM with RNA-seq.

LCM was developed two decades ago by Emmert-Buck and colleagues[1]. The technique enabled researchers to precisely isolate single-cells or clusters of cells from their environment using direct microscopic visualization and manipulation with a narrow beam laser[1]. Since then the method has been widely used in cancer biology and pathology[2,3]. Many plant research groups have also adapted LCM for the use with different plant species and different tissue types[4-11]. Recently, several papers have also used LCM on eudicot and monocot seeds to study embryo, endosperms and other seed structures during seed development and germination[10,12,13]. Most of the other commonly used single-cell isolation methods such as micro-pipetting, cell sorting, magnetic separation and microfluidic platforms depend on the enzymatic digestion or mechanical homogenization to dissociate cells. This may perturb gene expression, introducing technical artefacts that confound data interpretation[14,15]. These methods also require previous knowledge of marker genes for each cell type to relate the dissociated cells to their spatial location and true cell-type. A further group of techniques depends on affinity-based isolation of subcellular structures instead of whole cells, for example INTACT (Isolation of Nuclei Tagged in Cell Types) and TRAP (Translating Ribosome Affinity Purification)[16,17]. However, affinity labeling and purification of nuclei or ribosomes are technically challenging in plant species that do not have well-established transformation protocols. LCM takes advantage of quick tissue fixation to preserve transcript levels and conventional histological identification by direct visualization of cells within their normal tissue/organ context, which allows discrete cells to be isolated in a short period of time[18,19].

The protocol presented here is an optimized method for the isolation of specific cells or cell types from the tissue sections of cereal seeds, which can be applicable to most of the cells that can be histologically identified. LCM provides a contact-free method of cell isolation, greatly reducing contamination and increasing integrity of recovered RNA. Furthermore, the method illustrates the power of LCM on large-scale genome wide studies starting with small quantities of biological materials. We also describe linear amplification of RNA for generating sufficient input material

for downstream transcript/transcriptome analyses.

There are ten main steps in this LCM RNA-seq protocol for spatial and temporal tissue-specific transcriptomes, including fixation of tissue samples, dehydration, paraffin infiltration, embedding, sectioning, LCM, RNA extraction, RNA amplification, RNA quantification and qRT-PCR and/or RNA-seq (**Figure 1**).

[place **Figure 1** here].

PROTOCOL:

As the final product is RNA, take care to avoid contaminating the work with RNases. Wearing gloves is a must. Use diethyl pyrocarbonate (DEPC) -treated water, buffers, etc. Autoclave buffers and bake glassware before use.

1. Tissue fixation

1.1 Prepare fixative of choice depending on the species and tissue types; for barley seed, use Farmer's fixative (75% ethanol, 25% glacial acetic acid (v/v)).

1.2 Chill the fixative on ice prior to harvesting tissues.

1.3 Collect the plant material of interest and if necessary, dissect it into pieces of appropriate size to fit into the selected embedding mold. For barley seed, cut the seed into half longitudinally to help penetration of the fixative solution and to fit into the embedding mold.

1.4 Submerge the tissue into at least 10x volume of ice-cold fixative. For barley seed, submerge the seed cut into half into the fixative.

1.5 Use vacuum infiltration to accelerate the penetration of fixative. The tissues should sink after the vacuum infiltration of the fixative. For barley seed, use 30 min of vacuum infiltration.

1.6 Replace the fixative and incubate at 4 °C to allow the fixative to fully penetrate the tissue. For barley seed, incubate the samples overnight (~12-16 h).

NOTE: Thinner or small tissues will require shorter fixation time due to the higher diffusion rate of fixative into the tissue.

1.7 Remove the tissue from fixative and transfer the tissue into cassettes and then commence tissue processing.

NOTE: Small or fragile tissue, such as leaf tissue, can be placed into cassettes for fixing to ensure it is not damaged during fixation. Biopsy bags, pads or wraps could be used to hold the tissue securely inside the cassettes during tissue fixation and tissue processing steps.

2. Tissue processing

2.1 Use an automated tissue processor in step 2 with a minimum of 10 solution chambers and 2 heated paraffin chambers (see **Table of Materials**).

2.2 Check that there are adequate amounts of solution in each chamber; replace solutions after every few uses of the tissue processor.

2.3 Place cassettes with tissue into the metal basket. Attach the metal basket to its holder above chamber 1. The holder will rotate and "dunk and dip" the cassettes into the chambers, following the designated program.

2.4 Set the program by performing button clicks on the control panels of the tissue processor which involve setting length of time for each chamber.

2.5 Press the "**Start**" button to start the processing program. The following program is designed for barley seeds and runs overnight (~18 h)

2.5.1 Perform dehydration by dipping the cassette for 1 h 30 min each in the gradient series of ethanol (75%, 85%, 100%, 100%, and 100% (v/v) ethanol).

2.5.2 Perform clearing using ethanol: xylene gradient for 1 h 30 min each at 75:25, 50:50, 25:75 (ethanol: xylene %, v/v). Then dip the cassette for 1 h 30 min each of 100% xylene then 100% xylene.

2.5.3 Perform paraffin infiltration at 55-60 °C for 1 h 30 min twice in molten paraffin.

NOTE: Temperature of paraffin heater chambers can be set at the back of the tissue processor.

2.6 The next morning, remove the cassettes from the tissue processor and proceed to paraffin embedding.

NOTE: Program time may vary between different tissue type. Vacuum and/or agitation can be used during tissue processing to accelerate the infiltration of selected solutions by pressing "**V**" and/or "**agitation**" buttons on the control panel of the tissue processor.

3. Paraffin embedding

3.1 Use an embedding machine in this step (see **Table of Materials**).

3.2 Preset the embedding machine to turn on at least a few hours prior to embedding to allow time for the paraffin in the reservoirs to melt completely.

3.3 Turn on the cold plate prior to starting.

3.4 Embed samples in molds by holding the samples in position using fine forceps and dispensing molten paraffin into the mold. Ensure proper orientation of samples for each experimental purpose. For barley seed, orientate the seed longitudinal to the cutting direction to obtain longitudinal sections.

NOTE: Embedding molds come in different sizes. Select an appropriate size to allow the sample to be positioned and embedded properly. Orientation of the samples should be considered depending on experimental needs. If longitudinal sections are required, the sample should be orientated longitudinal to the cutting direction whereas for transverse sections the sample should be orientated parallel to the cutting direction.

3.5 Place a clean cassette onto the mold and ensure sufficient paraffin fully cover the whole cassette to hold the sample onto the cassette.

3.6 Place the mold onto the cold plate and allow the paraffin to set fully (10-20 min) before releasing the block from mold.

3.7 Proceed to sectioning or transfer the blocks to 4 °C for storage.

NOTE: The protocol can be paused here. The embedded blocks can be stored at 4 °C for up to three months.

4. Preparation of polyethylene naphthalate (PEN) membrane slides

4.1 Submerge PEN membrane slides in RNase deactivating solution for 3 s followed by two brief washes in DEPC-treated water to remove RNases on the slides. Dry the slides in a 37 °C incubator to remove left over solution.

4.2 UV-treat the slides using a UV lamp in a laminar flow cabinet for 30 min to enhance hydrophilic properties for improved paraffin adhesion.

5. Sectioning

5.1 Use a microtome in the sectioning step (see **Table of Materials**).

5.2 Place a new blade into the knife holder, and always keep knife guard up when not actively sectioning

CAUTION: Microtome blades are extremely sharp and can cause significant harm when handled inappropriately.

NOTE: There are two locking mechanisms on the microtome, one is at the side of the machine and the other is on the handle of the wheel. Both are to be engaged when not actively sectioning.

5.3 Adjust the knife block to be as close to the sample as possible without touching. Ensure that the microtome arm never comes into full contact with the knife block as this will cause catastrophic structural damage to the microtome.

5.4 Turn on the cold plate prior to starting. Keep paraffin blocks on the cold plate prior to sectioning and re-cool blocks when necessary during sectioning to prevent the blocks from softening.

5.5 Fill the water bath with DEPC-treated water and heat to 42 °C prior to starting.

5.6 Trim blocks to the desired depth (where the section you are interested in) and section paraffin blocks at desired thickness (6-10 µm) using the microtome; a well-sectioned block will form a 'ribbon' at the edge of the blade. For barley seed, section with 8 µm thickness.

5.7 Gently transfer ribbons from the microtome to the water bath using fine paint brush or fine forceps, ensuring that the ribbon is flat on the surface of the water.

5.8 Hold a slide at a 45° angle, using an upward motion, lift a ribbon out of the water onto the slide and carefully remove excess water with a lint-free tissue.

5.9 Dry slides for 30 min at 37 °C to eliminate any remaining water under the paraffin.

5.10 Proceed to paraffin removal or store at 4 °C in a closed box under dehydrating conditions (to be used within several days).

5.11 Remove paraffin by washing the slides 3x for 20 s each in xylene, followed by 2x washes of 30 s in 100% (v/v) ethanol and 2x washes of 30 s in 70% (v/v) ethanol.

5.12 Proceed immediately to laser-capture microdissection after paraffin is removed.

NOTE: Cryosectioning is an alternative method that has been successfully coupled with LCM Sample preparation for cryosectioning will differ.

6. Laser-capture microdissection

6.1 Use a laser-capture microdissection microscope (see **Table of Materials**) to microdissect cells from de-paraffinized and dried tissue sections.

6.2 Load slides on the three available slots.

6.3 Use the special adhesive caps of collection tubes to collect the captured samples. Capturing without liquid ("dry" collection) minimizes RNase activity. Load the collection tubes into the available slot.

6.4 Move the stage to locate the region of the sample that needs to be cut. This can be done using mouse or joystick of the LCM machine, or the arrow keys on the keyboard.

6.5 To optimize the cutting speed, cutting energy and focus, laser pressure catapulting (LPC) energy and focus, first cut on a blank segment free of tissue on the membrane slide. For barley seed, cutting speed = 18, CutEnergy = 52 CutFocus = 63, LPCEnergy = 78, LPC focus = 61 at 10x magnification.

NOTE: Cutting focus and energy have to be adjusted for different slides, different tissues, and captured area but general rules are the catapulting power is higher than the cutting power and the laser has to be defocused for catapulting. The higher the magnification of the objective lens, the smaller the focus of the laser and the higher the energy.

6.6 Use the **Drawing tools** to select cells by outlining the area of interest.

6.7 Select **RoboLPC function** from the function toolbar to catapult cells into the adhesive caps based on the optimized parameters obtained by cutting on a black segment above.

NOTE: LCM parameters vary between tissue types as well as thickness of section, tissue hardness and objective lenses. Therefore, it is best to optimize each slide on a plain membrane area without tissue specimen before cutting the actual sample.

6.8 Use the **Flag tools** to mark regions of interest to locate them immediately by selecting that flag from the elements list.

6.9 Check by "**CapCheck**" button to inspect the adhesive cap to confirm samples were captured. Typically, LCM of 10-15 sections (~200 cells) per cap is required for RNA extraction.

6.10 Keep the captured samples on ice. Proceed immediately for RNA extraction to avoid RNA degradation.

NOTE: Some LCM microscopy are equipped with fluorescent light which allows the capture of cells labeled with fluorescent markers.

7. RNA extraction

7.1 Use a low input RNA isolation kit for RNA extraction after LCM. Such kits are designed to recover high-quality total RNA consistently from fewer than ten cells.

7.2 Isolate the total RNA from the captured cell types according to the manufacturer's instructions including the on-column DNase treatment.

NOTE: The first step of the RNA extraction where the tube is inverted and flicked is crucial to ensure the captured samples on the lid are in contact with the extraction buffer added.

8. RNA amplification

8.1 Use an antisense RNA (aRNA) amplification kit for aRNA amplification from the RNA extracted by in vitro transcription to produce sufficient aRNA for RNA-seq library synthesis.

8.2 Perform two rounds of amplification using the aRNA amplification Kit according to the manufacturer's instructions.

NOTE: It is important to preheat the thermo-cycler and lid to the temperature instructed by the kit manufacturer. An alternative approach instead of RNA amplification is to use a low input library preparation kit to synthesize the library directly from extracted RNA.

9. RNA quantification

9.1 Quantify and qualify aRNA using an automated electrophoresis system.

NOTE: An automated electrophoresis system is preferred as it requires less sample (1-2 µL) and provides a gel-like image and electropherogram for each individual sample.

10. qRT-PCR and/or RNA-seq

10.1 Synthesize cDNA from the aRNA for qRT-PCR or to make RNA-seq libraries using standard RNA-seq library kits.

REPRESENTATIVE RESULTS:

We generated spatial and temporal tissue-specific transcriptomes from barley seeds during germination using our LCM RNA-seq protocol [10]. The study was carried out by applying LCM RNA-seq to small number of cells from three embryo organs (plumule, radicle tip, scutellum) every 8 h over a 48 h time course during germination (0-48 h, 7 time points) (**Figure 2A,B**).

[place **Figure 2** here]

The fixation and embedding are critical steps as poorly fixed tissue sample can result in tissue damage and loss of RNA quantity and quality. These steps must be optimized and adjusted for different species and tissue types[19-21]. Here we used vacuum infiltration to improve the penetration of the fixatives into the seed tissues and cells. Dehydration and paraffin infiltration are conducted gradually to avoid damage to the tissue samples. We have accelerated the whole dehydration and paraffin infiltration process by using an automated tissue processor with agitation and vacuum applied at each step. This highly reduces the risk of RNA degradation occurring during the prolonged tissue processing steps conducted by a human operative. Before sectioning, it is crucial to treat the membrane slide to ensure paraffin adhesion and to make sure

it is RNase-free. If the membrane slides are not prepared properly the sample section will not adhere to the membrane, which will affect the LCM catapulting step and decrease tissue transfer to the adhesive caps because the sample will separate from the membrane (see **Figure 1**).

Another critical step in the LCM RNA-seq protocol is LCM parameter optimization. There are five main parameters to be optimized: (1) cutting speed; (2) cutting energy; (3) cutting focus; (4) LPC energy; and (5) LPC focus. It is important to adjust the cutting parameters correctly in order to cut the selected area precisely and dislodge from surrounding tissue without burning the edge of selected area (**Figure 3A,B**). The proper LPC energy and focus are essential to reliably catapult the selected area from the slide to the special adhesive caps of collection tubes, and always inspect captured samples in the caps to ensure enough material is collected (**Figure 3C**). The efficiency of LCM enables 1000 cells to be captured within one hour, which greatly reduces the likelihood of RNA degradation during sample processing. The use of collection tubes with adhesive caps allows "dry" collection without any capturing liquid, greatly reducing possible RNases activities. It is important to keep the captured samples on ice and do the RNA extraction immediately after collecting all the replicates.

RNA extraction and amplification are carried out using commercially available kits by following the manufacturers' instructions. Quantification of total RNA before RNA amplification using automated electrophoresis system will detect distinct electrophoretic bands and fluorescent peaks of 18S and 28S ribosomal subunits in good quality RNA samples (**Figure 3D**). Additional peaks corresponding to chloroplast and mitochondrial ribosomal RNA will be found before 18S and 28S peaks. Due to multiple RNA peaks in plant species, the RIN (RNA Integrity Number) values are typically lower than 9 (the expected RIN value from mammalian tissues) and do not accurately reflect the RNA integrity [22]. It is appropriate to continue to RNA amplification when clear 18S and 28S peaks are visible without sign of RNA degradation. The amount of RNA recovered in our barley seed experiment varied between three different tissue types (plumule, radicle and scutellum) and, also between early and late stages of germination, despite approximately equal numbers of cells being harvested. The amounts from barley seeds ranged from 100 pg to 20 ng from approximate 200 cells (0.5 to 100 pg per cell). The minimum amount of input RNA recommended for RNA amplification was 100 pg. The amount of RNA extracted depends on efficiency of extraction from different harvested cells and total transcript abundance in the particular cell-type at that stage of development [19,21]. It is useful to consider this when planning an LCM RNA-seq experiment, though such prior information will not be available in all cases.

Successfully synthesized aRNA will exhibit a unimodal, symmetrical size distribution from 100 to 1000 nucleotides with a peak around 300 nucleotides after two rounds of amplification (**Figure 3E**). In our barley seed experiment, we obtained 500 pg to 2 µg of aRNA after two rounds of RNA amplification[10]. Comparison between amplified and unamplified RNA has demonstrated that RNA amplification is reproducible, linear and does not introduce a systematic bias to transcript data[23-25]. The output aRNA can be used for qRT-PCR and/or RNA-seq for spatial and temporal tissue-specific gene expression analysis. Validation after RNA-seq must be conducted to corroborate results from analysis of aRNA. This can be done by examining expression of cell-type marker genes from the RNA-seq data using RNA in situ hybridization.

[place **Figure 3** here]

RNA-seq analyses were performed on all samples in biological triplicates. A multidimensional scaling (MDS) plot of genes expressed in the different tissues over 48 h of germination illustrates greater similarity between samples of a single tissue than between samples from the same time point but different tissues (**Figure 4A**). We next set out to determine how extensively gene expression was differentially regulated in individual tissues over time during germination. The number of differentially expressed genes (DEGs) increased progressively over the course of germination in each tissue, relative to that tissue's 0 h timepoint (**Figure 4B**). Twenty-five percent (910) of plumule, 34% (1876) of radicle and 41% (2562) of scutellum DEGs were found to be exclusively differentially expressed in that tissue (i.e., these genes were not differentially expressed in the other tissues, **Figure 4C**). Taken together, these results demonstrate how LCM RNA-seq can be used to understand temporal and spatial gene expression during plant development.

[place **Figure 4** here].

FIGURE AND TABLE LEGENDS:

Figure 1: Flowchart of LCM followed by RNA-seq or qRT-PCR. LCM is a spatially precise and contact-free technique to collect cells from fixed tissue sections using a laser beam under microscopic visualization. The process starts with fixation of tissue samples, followed by dehydration using a gradient series of ethanol and xylene, and finished with paraffin infiltration. The process can be fully automated by using a tissue processor. Once the tissue is infiltrated with paraffin, it is embedded in a mold with molten paraffin using an embedding station. Sectioning is carried out using microtome set to the desired thickness. Slides are prepared and LCM conducted immediately before RNA is to be extracted from captured cells. Capture is followed directly by two rounds of RNA amplification prior to qRT-PCR and/or RNA-seq.

Figure 2: Cells collected from barley seeds by LCM. (**A**) Longitudinal cross-section of a barley seed and, inset, an enlarged cartoon indicating the regions from which cells were captured. Blue = plumule, magenta = radicle, green = scutellum. (**B**) LCM of plumule, radicle, and scutellum. Top panel, tissue before laser pressure catapulting (LPC). Bottom panel, tissue after LPC. Cells were captured from 5 consecutive longitudinal sections of each sample with three biological replicates per sample. Each replicate consisted of approximately 200 cells (~0.05 - 0.15 mm^2 total area). Bar = 100 µm. E = endosperm, C = crushed cell layer, SE = scutellar epithelium, S = scutellum. Figure reproduced from previous data in The Plant Journal [10].

Figure 3. Representative result of LCM. (**A**) Tissue after cutting with proper cutting energy and focus. A clear fine line can be seen where the cut was made. The selected area will be dislodged from surrounding materials. Bar = 100 µm. (**B**) Tissue after cutting with incorrect cutting energy and focus. The selected area is still connected to surrounding materials. Bar = 100 µm. (**C**) Examining captured cells in special adhesive caps of collection tubes. Bar = 100 µm. (**D**) An example of total RNA profile before RNA amplification (left, gel-like image; right,

electropherogram) with distinct electrophoretic bands and fluorescent peaks of 18S and 28S ribosomal subunits. Unlabeled peaks correspond to additional ribosomal RNA including chloroplast and mitochondrial ribosomes. (**E**) An example of a typical aRNA profile after RNA amplification (left, gel-like image; right, electropherogram). A distribution of sizes from 100 to 1000 nucleotides, with a peak around 300 nucleotides, was detected.

Figure 4. Analysis of RNA libraries of LCM samples showing distinct separation of different tissues over 48 h of barley germination. (**A**) Multidimensional scaling (MDS) plot of genes expressed in the different tissues over 48 h of germination. Each point represents 1 sample, and the distance between 2 points reflects the leading log fold change (FC) of the corresponding RNA samples. The x, y, and z axis represent first, second, and third dimensional logFC. P = plumule, R = radicle, S = scutellum, 0-48h = h of germination, R1-3 = Replicate 1-3. (**B**) Number of significant up-regulated (red) and down-regulated (blue) differentially expressed genes (DEGs) for six time points compared to time point 0 h for three tissue types. (**C**) Venn diagram showing number of DEGs in three tissue types. Genes in overlapping sets show the differential expression in two or three tissue types.

DISCUSSION:

Many tissue-specific gene expression studies have been limited by hand dissection of samples, which is time-consuming, labor intensive, has a high risk of contamination and can only utilize samples that a human operative is sufficiently dexterous to harvest. LCM is a precise and contact-free technique to collect cells from fixed tissue sections using a mechanically operated laser beam under microscopic visualization.

Good sample preparation is critical for LCM. The process relies upon proper fixing and embedding of samples to maintain a good balance between tissue morphology and integrity of RNA. The protocol presented here provides optimized fixing and embedding steps for barley seeds which includes longer fixing time and vacuum infiltration of fixatives. Analysis of different tissues or species will require optimization of the conditions, as these typically differ between tissues. Another important aspect of this protocol is the RNA amplification step which ensures sufficient amounts of RNA are generated for RNA-seq library construction. We find that RNA amplification results in high-quality libraries, but alternatively low input library preparation methods might be employed[25]. Histological identification of target cells is a critical step in our method and may provide challenges for rare or poorly characterized cell-types. Target cell recognition may be improved by using traditional histological stains or immunofluorescence stains as part of the preprocessing step or, alternatively, by using transgenic lines that express fluorescently labeled cell-specific markers[26,27].

The LCM technique was first developed to be used on animal-derived samples for cell-type-specific transcript analysis[1]. However, a broad range of genomic, proteomic, and epigenetic analyses have subsequently been developed. Adapting these methods for application in plant biological studies is an ongoing challenge[18-20,28]. Schad et al. demonstrated that proteins and metabolites from *Arabidopsis* vascular bundles could be analyzed by using LCM with 2-D gel electrophoresis and mass spectrometry[29,30]. Recently, Latrasse et al. carried out chromatin

immunoprecipitation (ChIP)-qPCR analysis on LCM-dissected stamens and carpels using LCM, establishing that tissue homogeneity improves the resolution of cell-specific epigenetic events[31]. Turco et al. also coupled LCM with bisulfite sequencing to study DNA methylation events during vascularization in sorghum, highlighting the tissue-specific difference between vascular and nonvascular tissues[32]. Interestingly, several studies have applied LCM to explore plant-microbe and plant-pathogen interactions[33-35]. The ability of LCM to visualize and capture cells at different infection stages provided information about the spatial and temporal responses of infected cells.

Our LCM protocol coupled with RNA-seq presented here will facilitate the spatio-temporal global profiling of gene expression of distinct cell types. With improved purification methods for chromatin and proteins along with enhanced mass spectrometry instruments, LCM will be an emerging tool to facilitate cell-type-specific epigenomic and proteomic studies in plants.

ACKNOWLEDGMENTS:

This work was supported by the Australian Research Council Centre of Excellence in Plant Energy Biology (CE140100008) to JW. M.G.L was supported by a La Trobe University starting grant. We thank the La Trobe Genomics Platform for their support in high-throughput sequencing and data analysis.

DISCLOSURES:

The authors have nothing to disclose.

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590

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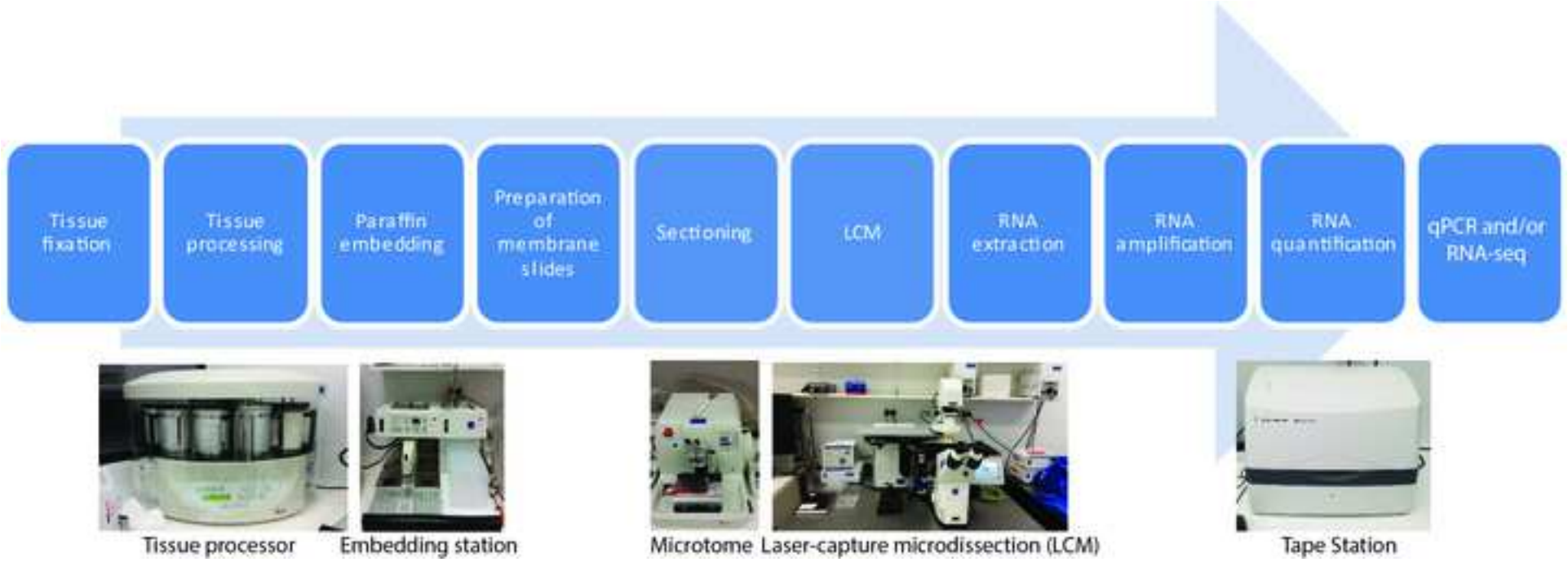

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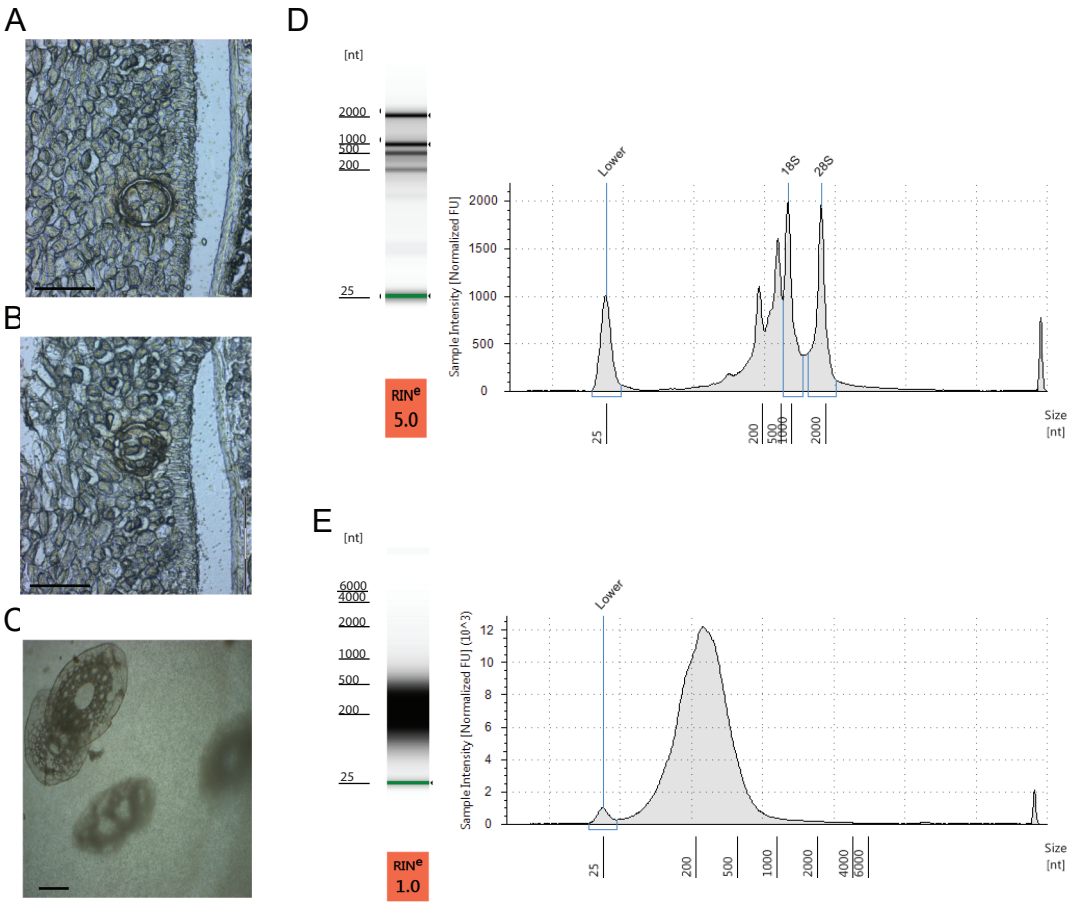

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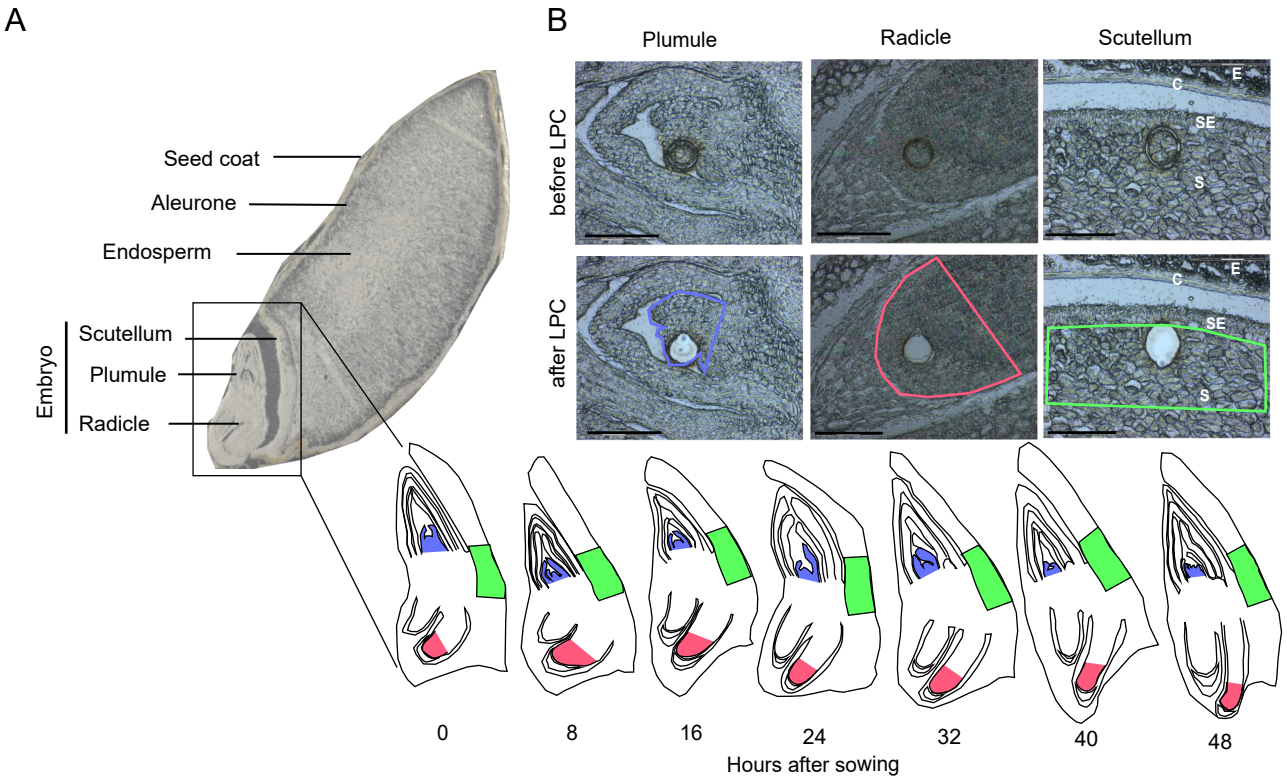

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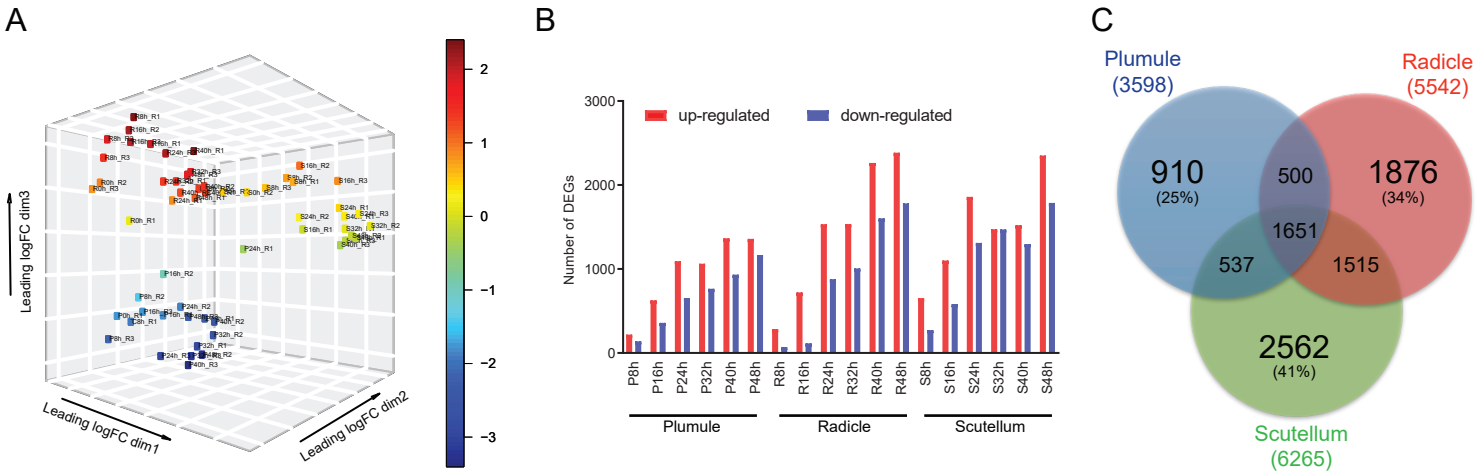

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Table of Materials

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Name	Company	Catalog Number	Comments/Description
Acetic acid 100 % ACS/R.	AnalaR NORMAPUR (BioStrategies)	VWRC20104.323	
AdhesiveCap 200 opaque	Zeiss	415190-9181-000	
Clear base moulds 8 X 10	Leica	3803015	
Diethyl pyrocarbonate	Sigma-Aldrich	40718-25ML	
High Sensitivity RNA ScreenTape	Agilent	5067-5579	
Low-profile disp.blades DB80LS	Leica	14035843489	
MembraneSlide 1.0 PEN	Zeiss	415190-9041-000	
MessageAmp II aRNA Amplification Kit	Ambion (ThermoFisher)	AMB17515	
On-Column DNase I Digestion Set	Sigma-Aldrich	DNASE70	
Ovation RNA-Seq System V2	NuGen (Integrated Science)	7102-08	
Paraffin (Surgipath Paraplast)	Leica	39601006	
PicoPure RNA Isolation Kit	ABI (ThermoFisher)	KIT0214	
RNaseZap RNase Decontamination Solution	Ambion (ThermoFisher)	AM9780	
Xylene	AnalaR NORMAPUR (BioStrategies)	VWRC28975.360	
Leica Benchtop Tissue Processor	Leica Biosystems	TP1020	
Leica Heated Paraffin Embedding Module	Leica Biosystems	EG1150H	
Leica Cold Plate	Leica Biosystems	EG1150C	
Safemate Class 2 Biological Safety Cabinets	LAF Technologies Pty Ltd	Safemate 1.5	
Leica Fully Automated Rotary Microtome	Leica Biosystems	RM2265	with PALMRobo v 4.6 software
Zeiss PALM MicroBeam LCM system	Zeiss miscroscopy		
TapeStation	Agilent	TapeStation 2200	

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