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Introduction

Arabidopsis thaliana is an important plant model that has been used to study the molecular mechanisms that occur during various stages of a plant's life. Laser microdissection is commonly performed to isolate pure cell populations from *Arabidopsis* seedlings, allowing for cell-specific molecular profiling. When the specificity of a pure cell population is not required, a crude microdissection can be performed on the live plant tissue. This technique eliminates the need for fixation and sectioning of the plant tissue, reducing time-consuming sample preparation.

The Veritas™ Microdissection System employs a state of the art, solid state UV laser, which has the power and precision necessary for the microdissection and capture of large regions of plant tissue, with a thickness of up to 400µm, in a single pass. This system enables the analysis of live plant tissue, which cannot be performed using standard laser microdissection methods. This protocol describes the microdissection of live plant specimens using the Veritas™ Microdissection System.

Tissue Preparation

Arabidopsis seeds were grown over wet Whatman filter paper inside sterile petri dishes and allowed to grow at 4°C. The seedlings were harvested when the first leaves (cotyledons) appeared.

Note: This protocol describes the microdissection of plant material ≤400µm thick. If thicker sections are desired, manual dissection with a scalpel is recommended. To prepare fixed plant tissue sections, please see Arcturus Protocol #7 at <http://www.arctur.com>, or visit <http://research.yale.edu/tnelsonLCM>.

Sample Preparation

Step	Procedure
1.	Snip leaf/root from plant.
2.	Clean off soil and debris with water or PBS.
3.	Dab the sample dry with Kimwipe or similar lint-free cloth. Note: Excess water will inhibit laser cutting.

Slide Preparation

Step	Procedure
1.	Place the plant tissue on the “flat” side of the framed membrane slide (see Figure 1).
2.	Make frame slide “sandwich” using a regular glass slide. Note: The tissue must stick tighter to the framed membrane slide than to the glass support slide. Pretreatment of the membrane slide with Poly-L-Lysine may increase adherence properties of the biological tissue to the membrane and aid in this manner.

Figure 1. Frame Slide “Sandwich”

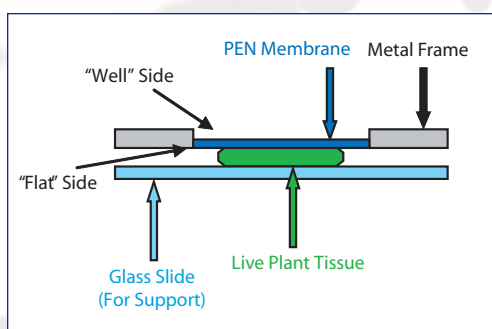
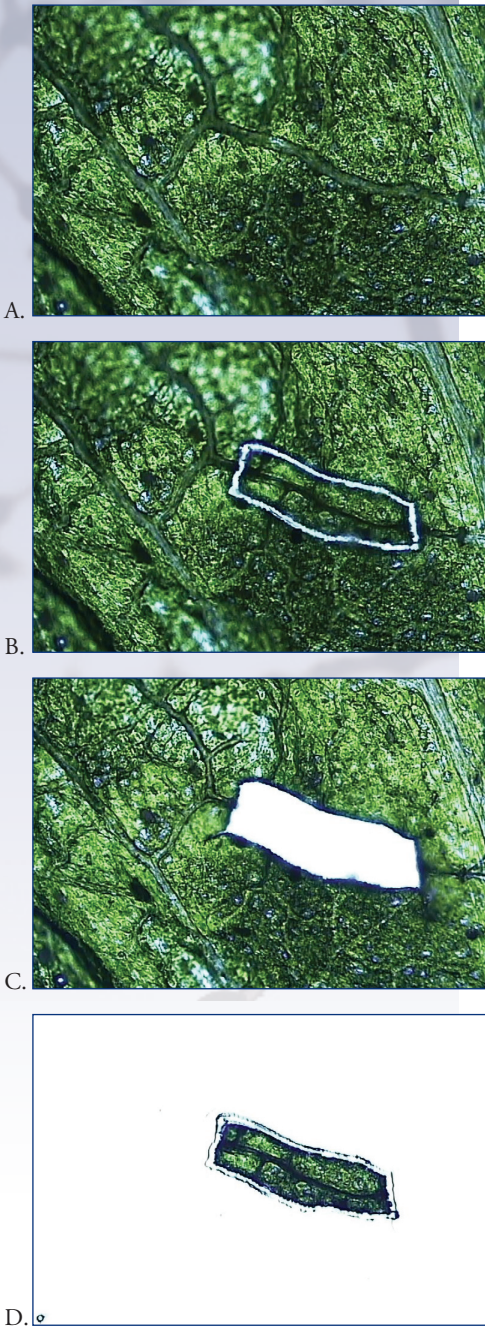


Figure 2. Microdissection of live Arabidopsis leaf using the Veritas™ Microdissection System: A. Before microdissection. B-C. After microdissection. D. Captured leaf tissue on CapSure cap.



Microdissection Procedure

Note: Perform entire microdissection procedure at 10X objective for optimum visualization.

Step	Procedure
1.	Set laser cutting power to lowest possible setting that will still allow full cutting through the plant tissue. Test the UV laser settings by applying a few test pulses. The laser cutting power will be dependent on thickness, and as a guide, will generally fall between the high 20s to low 30s.
2.	Within the desired capture group, set laser cutting properties to the following parameters: Spacing = 700, Number of Tabs = 2, Size = 2.
3.	Use default laser capture properties. Note: You may require additional LCM spots to achieve enough adhesion to pick up the thick section.
4.	Scan slide for desired cells. While scanning, store the location(s) of interest to be retrieved later for collection by using “Stored Position” feature in the “Microscope” window.
5.	Place a CapSure® Macro LCM cap onto field containing desired area(s) for collection.
6.	Draw around the cells that will be collected.
7.	Activate the laser cutting process with LCM cap on top of sample.
8.	Manually place LCM spot(s) to attach the plant cells to LCM cap.
9.	Lift the cap off the PEN membrane to process microdissected cells for downstream analysis.

RNA Extraction

RNA is extracted from the microdissected live plant tissue on the CapSure® Macro LCM cap using the PicoPure® RNA Isolation Kit. Isolated RNA can now be used for downstream analysis.

Acknowledgements:

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Please visit www.arctur.com to view and obtain the latest Arcturus application notes and protocols.



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