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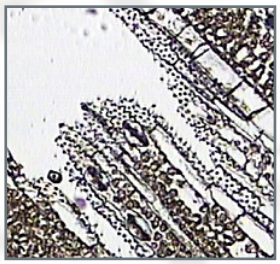
Laser Microdissection of Stomatal Cells from Rice Leaf Sections for Molecular Analysis

PROTOCOL #7

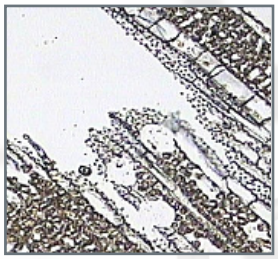
Introduction

Rice is one of the world's major cereal crops and an important plant model system representing the Poaceae (grass) family. Study of pure cell populations from rice can aid tremendously in our understanding of the molecular mechanisms that take place at the cellular level during various stages of plant life. Pure cell populations from a variety of paraffin-embedded plant tissues have been isolated successfully using laser capture microdissection (LCM). In this application note, the rice plant is used as a model to demonstrate the ability of the UV cutting laser, available on the Veritas™ Microdissection System, to isolate single cells from a mixture of cells in a tissue section.

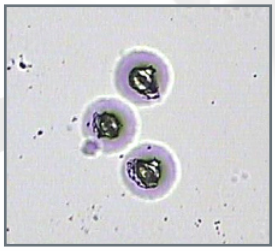
A: Before microdissection.



B: After microdissection.



C: Captured stomatal cells on CapSure LCM cap.



Tissue Preparation

Seedlings from *Oryza sativa japonica* were grown using standard techniques. The seedlings were harvested at approximately 5 days old, when 5 to 7 cm, then fixed in 3:1 Ethanol/ Acetic Acid (Farmer's Fixation) and processed into paraffin blocks. Using a standard rotary microtome, sections were then cut from the paraffin blocks and mounted onto polyethylene naphthalate (PEN) membrane slides. Sections were deparaffinized just prior to laser microdissection. Further information on the general processing of plant tissues can be found at the following web site: http://research.yale.edu/tnelsonLCM/

Deparaffinization

Step	Process	Time
1.	Dip slide in Xylene 2 times	2-5 minutes per dip
2.	Air dry slide in fume hood for a minimum of 5 minutes	

Note: If microdissection is not performed immediately following deparaffinization, place slides in a room temperature dessicator for storage. It is recommended to use stored slides within one week of deparaffinization.

Laser Microdissection

Area(s) of interest was collected by laser cutting following the procedure detailed below, with no automated LCM "tack points" placed post-cutting.

Microdissection Procedure

Step	Procedure
1.	Set Laser Cutting power to lowest possible setting that will still allow full cutting through the PEN membrane.
2.	Within the appropriate capture group, set laser cutting properties such that no tabs will be generated during the cutting process.
3.	Set laser capture properties such that no automatic LCM spots are generated after the cutting process.
4.	Scan slide for desired cells. While scanning, store the location(s) of interest to be retrieved later for collection by using the "**Stored Position**" feature in the "**Microscope**" window.
5.	Place a CapSure® Macro LCM cap onto field containing desired area(s) for collection.
6.	With the mouse and pointer, draw around the cells, marking them for microdissection.
7.	Activate the laser cutting process with LCM cap on top of sample.
8.	Manually place LCM spots to attach the stomatal cells to LCM cap.
9.	Lift the cap off the PEN membrane to process microdissected cells for downstream analysis.

RNA Extraction

Microdissected rice stomatal cells are now captured on the CapSure LCM cap. RNA from the ethanol/acetic acid-fixed cells is extracted and isolated using the PicoPure® RNA Isolation kit and protocol. Isolated RNA can now be used for downstream molecular analysis.

Acknowledgements

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