Application Note
iST Sample Preparation for Plant Tissues

PREOMICS

AUTHORS

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OBJECTIVE

To develop an easy-to-use, sensitive and fast sample preparation workflow compatible with shoot and root tissue from *Arabidopsis thaliana*.

KEYWORDS

Proteomics; sample preparation; mass spectrometry; plant tissue; *Arabidopsis thaliana*; sulfur; root; shoot

INTRODUCTION

Inherent biochemical complexity within plant tissue presents a significant challenge for extracting proteins and generating clean peptides suitable for mass spectrometry-based proteomics. The presence of cell walls and fibers requires harsh mechanical shearing forces. Additionally, plant tissue contains high amounts of interfering secondary compounds such as phenolics, pigments and polysaccharides, which detrimentally affect sample quality. To overcome these challenges, sample preparation of plant material typically employs multiple sample processing and transferring steps, making these workflows prone to sample loss and compromise their reproducibility (1).

The PreOmics' iST technology has become a standard solution when addressing proteomic sample preparation challenges for mammalian cell lines, tissues, liquid biopsies and many model organisms such as bacteria, yeast or flies. In this application note, we now demonstrate that the PreOmics iST Sample Preparation Kit enables a streamlined preparation of two different plant tissues for LC-MS/MS analysis (**Figure 1**). The whole workflow takes less than 5 hours from cryogenic grinding of plant material to ready-to-measure peptides, providing significant time savings compared to traditional sample preparation protocols. The optimized and patented peptide washing procedures eliminate both hydrophobic and hydrophilic contaminants, including secondary metabolites. Thus, the resulting clean samples help to decrease MS downtime and enable more reproducible and reliable results.

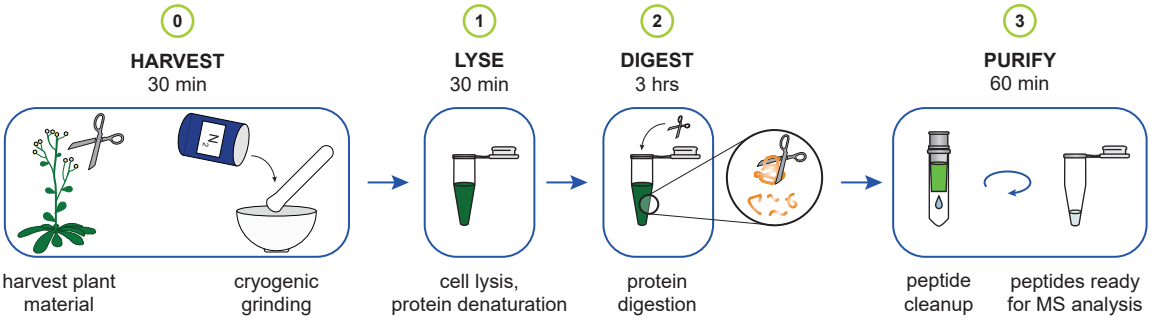

Figure 1 - iST workflow for plant tissues

MATERIAL AND METHODS

Arabidopsis thaliana plants were in the Columbia (Col-0) ecotype. Plants were grown under short-day conditions in hydroponic ½ Hoagland solution (pH 5.8) with full sulfate supply (500 µM $MgSO_4$) for two weeks. Subsequently, seedlings were transferred into larger pots containing 6 L ½ Hoagland solution (+S: 500 µM $MgSO_4$ / -S: 1 µM $MgSO_4$) for another five weeks and then transferred to fresh media 24 hours before being harvested. 50 mg of shoot and 30 mg of root tissue (wet weight) were harvested and ground in liquid nitrogen using an electronic mortar (Geneo, Heidolph RZR 2041). The resulting powder was frozen at -80°C until further use. For the following steps, all buffers described were produced by PreOmics GmbH. Each sample was resuspended in 100 µl LYSE buffer, boiled at 95°C for 10 min and then sonicated using the Diagenode Bioruptor® Pico (10 cycles, 30 sec ON/OFF). After the heat and sonication treatment, 50 µL of DIGEST was added and incubated at 37°C for three hours. Next, 100 µL STOP buffer was added and the samples were then centrifuged for one minute at 13,000 rpm to clear tissue debris. Next, the supernatant was transferred to CARTRIDGES, before continuing with the peptide cleanup according to iST Sample Preparation Kit`s instructions. In addition, 200 µL plant-specific WASH0 buffer was used per sample to remove secondary metabolites. MS analysis was performed on a LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific) with standard settings. Data analysis was performed using MaxQuant (3), statistical analysis was done using Perseus (4). Biological quadruplicates were used throughout the experiment.

RESULTS

As a proof-of-principle, we used both root and shoot tissue from *A. thaliana* plants grown under normal (+S) or sulfur-deprived (-S) growth conditions. Our LC-MS/MS analysis resulted in the identification of about 24,000 peptides and 4,000 proteins in total (**Figure 2A+B**). While the differences for the two distinct tissue types were minimal at the protein level (**Figure 2A**), peptide identifications showed more variation and were decreased for plants grown under sulfur-deprived conditions (**Figure 2B**). We assessed the reproducibility of our experiments across the different biological replicates and found overall very good correlation for both root (R^2: 0.93) and shoot (R^2: 0.89) tissues (**Figure 3A+B**). Next, we performed Gene Ontology analysis of our proteomic dataset compared to the total *A. thaliana* proteome to identify potential bias in functional protein class enrichment. Several protein classes were found enriched, including cytoplasmic, organellar and membrane-associated terms, indicating adequate extraction of proteins across distinct subcellular localizations (**Figure 4**). Finally, we asked whether our analysis could identify proteins with significantly altered expression upon sulfur deprivation. As an example, we found two proteins (SULTR1.1 and SULTR1.2) specifically upregulated in the S- condition in root but not shoot tissue (**Figure 5**). The high-affinity H^+/sulfate co-transporters SULTR1.1 and SULTR1.2 mediate the major uptake of elemental sulfate in root tissue and play a central role in the regulation of sulfate assimilation for *A. thaliana* (5). Thus, the iST workflow is able to identify the specific upregulation of both sulfate transporters in root tissue upon environmental stress conditions.

CONCLUSION

We have demonstrated that the iST Sample Preparation Kit enables robust and reproducible sample preparation of root and shoot tissue from the plant *A. thaliana*. In comparison to multi-step processing and transferring workflows, the iST Kit is is significantly faster and easy-to-use even for non-proteomics experts. While it permits unbiased proteome identification of plant material, the iST technology also allows the sensitive detection of specific protein regulation upon abiotic stress.

REFERENCES

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PRODUCTS

Product	Quantity	Manufacturer	Product Code
iST Kit 8x*	8 reactions	PreOmics GmbH	P.O.00001
iST Kit 96x*	96 reactions	PreOmics GmbH	P.O.00027

*plant-specific buffers upon request

CONTACT INFORMATION

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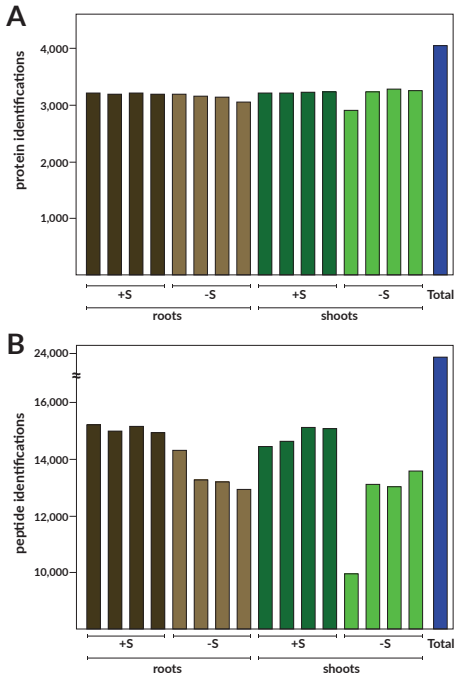

Figure 2
Comparison of protein (A) and peptide (B) identifications from root and shoot tissue in presence or absence of sulfur during plant cultivation.

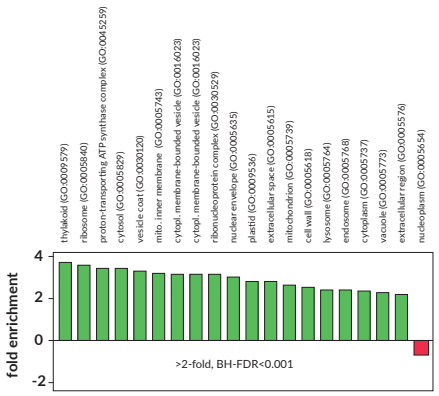

Figure 4
Distribution of functional protein classes compared to total *A. thaliana* proteome (GO_CC, >2-fold enrichment and BH-FDR <0.001).

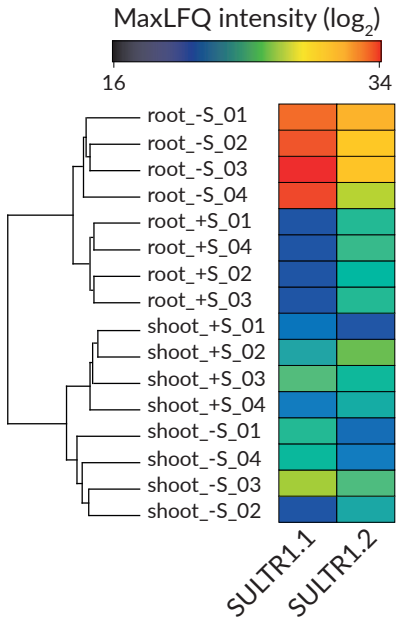

Figure 5
Upregulation of the two sulfate transporters SULTR1.1 and SULTR1.2 in root tissue upon sulfur deprivation.

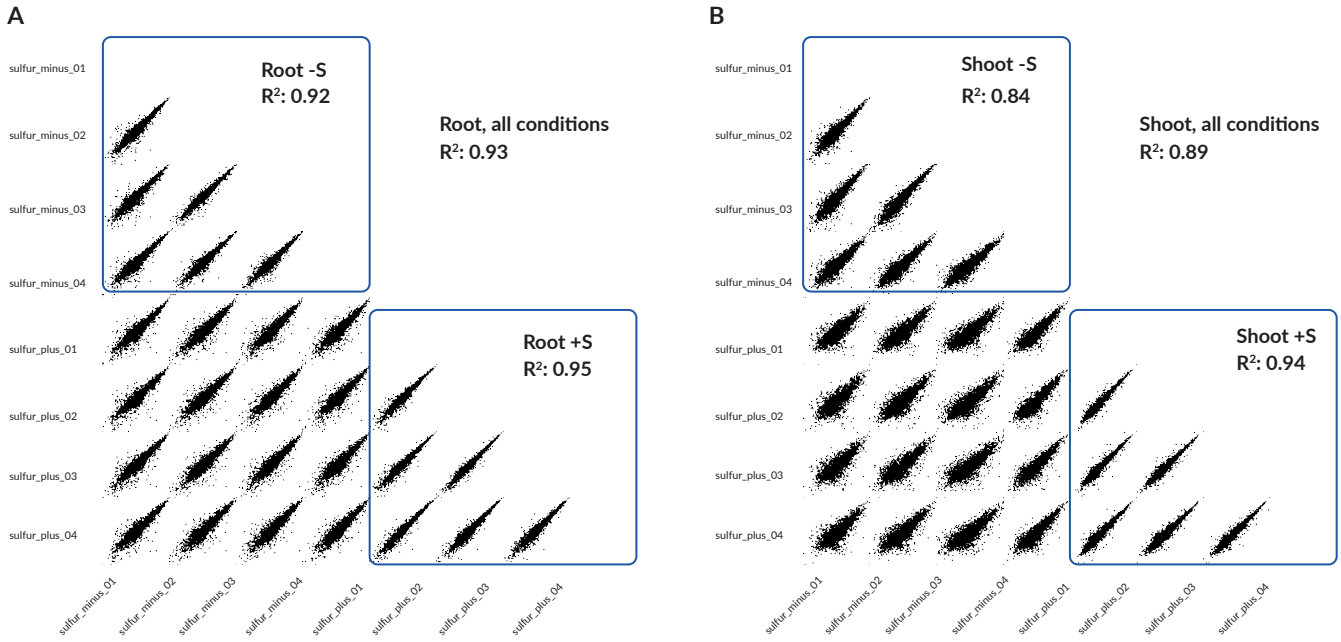

Figure 3
Reproducibility of protein intensities across different replicates for both (A) root and (B) shoot tissue.