Improved iST workflows for high-throughput preparation of chemical labeling samples or plant tissues

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1 Introduction

The recently described in-StageTip (iST) method enables proteomic sample processing in a single, enclosed volume (Kulak et al., 2014). Here, we present further developments of the iST technology that facilitate fast & reproducible 96-well preparation of chemical labeling samples. In addition, we have developed a novel and streamlined iST application for different plant tissues providing significant time savings, as well as both high protein extraction and peptide recovery rates.

2 Workflow & comparison to other sample preparation methods

Sample collection

iST sample preparation

UPLC-MS/MS

identified proteins (+fractionation)

more IDs & no functional bias



3 Compatibility with chemical labeling (iTRAQ, TMT)



<3.5 hrs total workflow time including labeling reaction

labeling efficiency of > 98%, high reproducibility of R² > 0.94



- fast & reproducible workflow, 5 hrs from harvest to ready-to-measure peptides
- >4,000 proteins from A. *thaliana* roots and shoots identified (A+B)

• environmental stress-mediated protein upregulation in specific tissues detected (C)



5 Conclusions

more protein & peptide identifications

compared to alternative workflows

 dual clean-up to remove contaminants (hydrophilic & hydrophobic)

works with standard lab equipment

- intuitive for every non-expert
- ultrapure MS-grade reagents
- validated in multiple publications



6 References & Contact

Doll et al. (2018), Mol Oncol Geyer et al. (2017), Mol Syst Biol Kulak et al. (2014), Nat Methods

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