

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

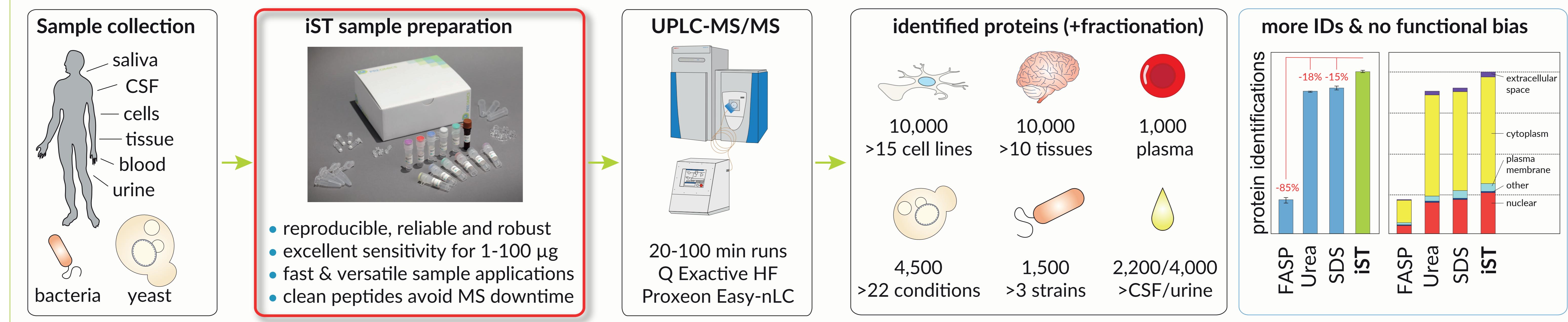
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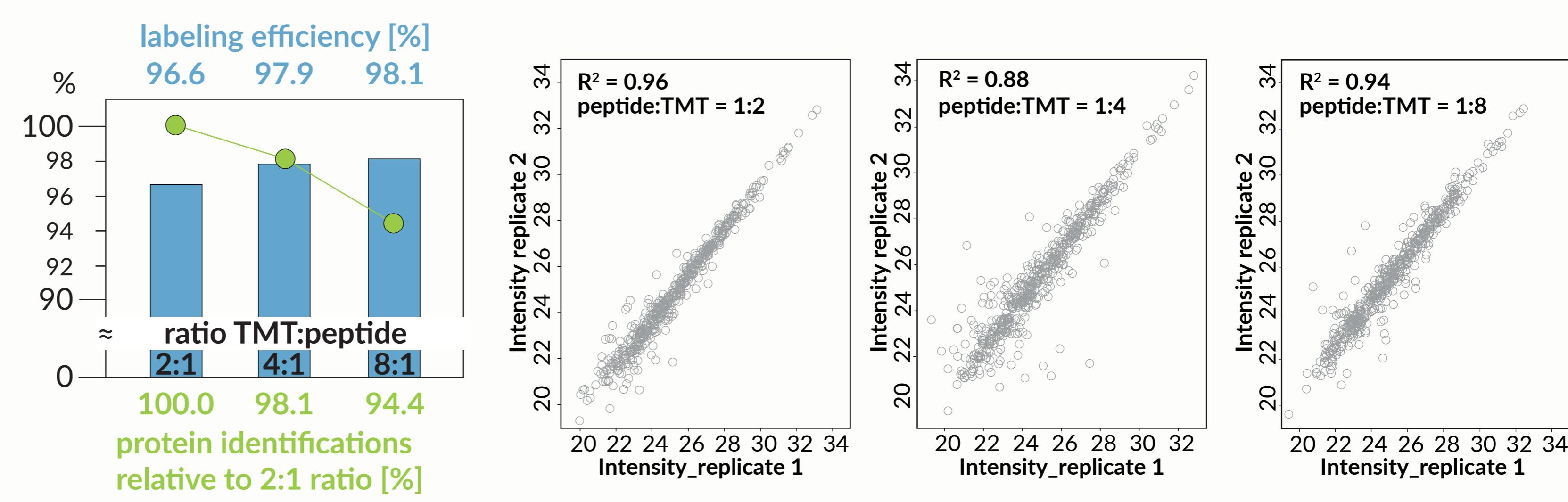
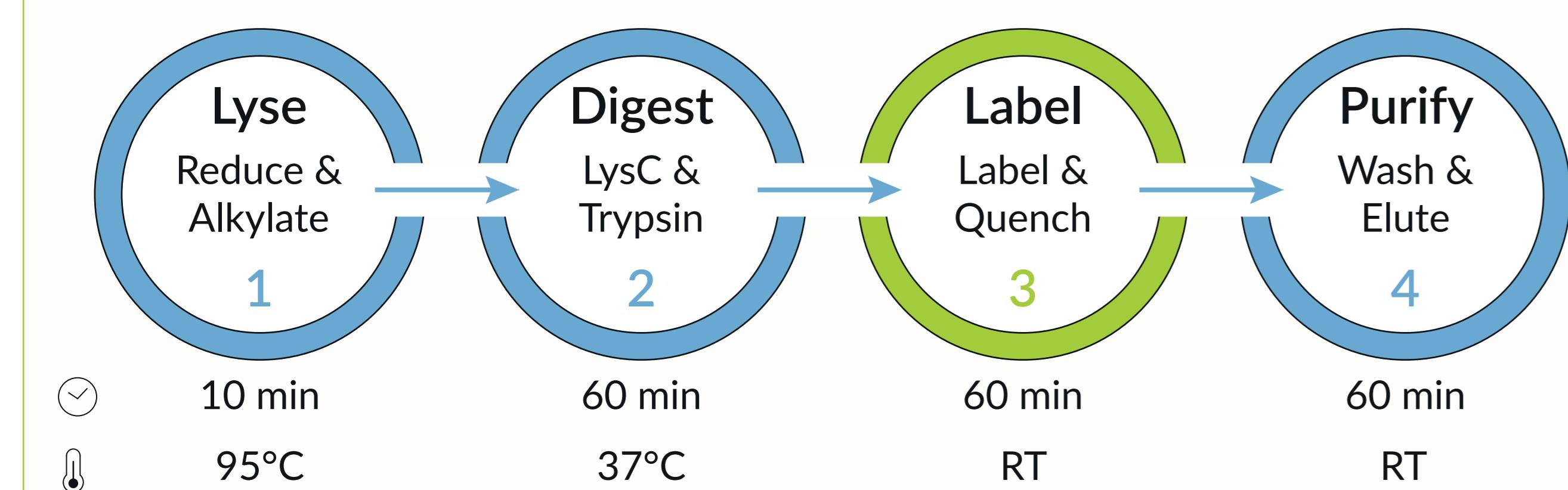
## 1 Introduction

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

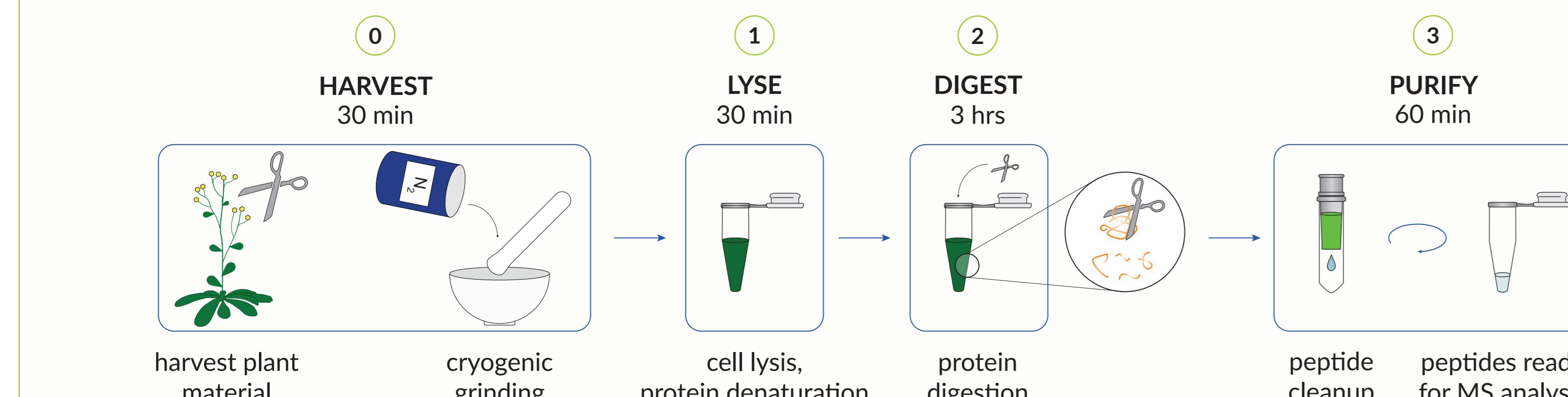


## 3 Compatibility with chemical labeling (iTRAQ, TMT)

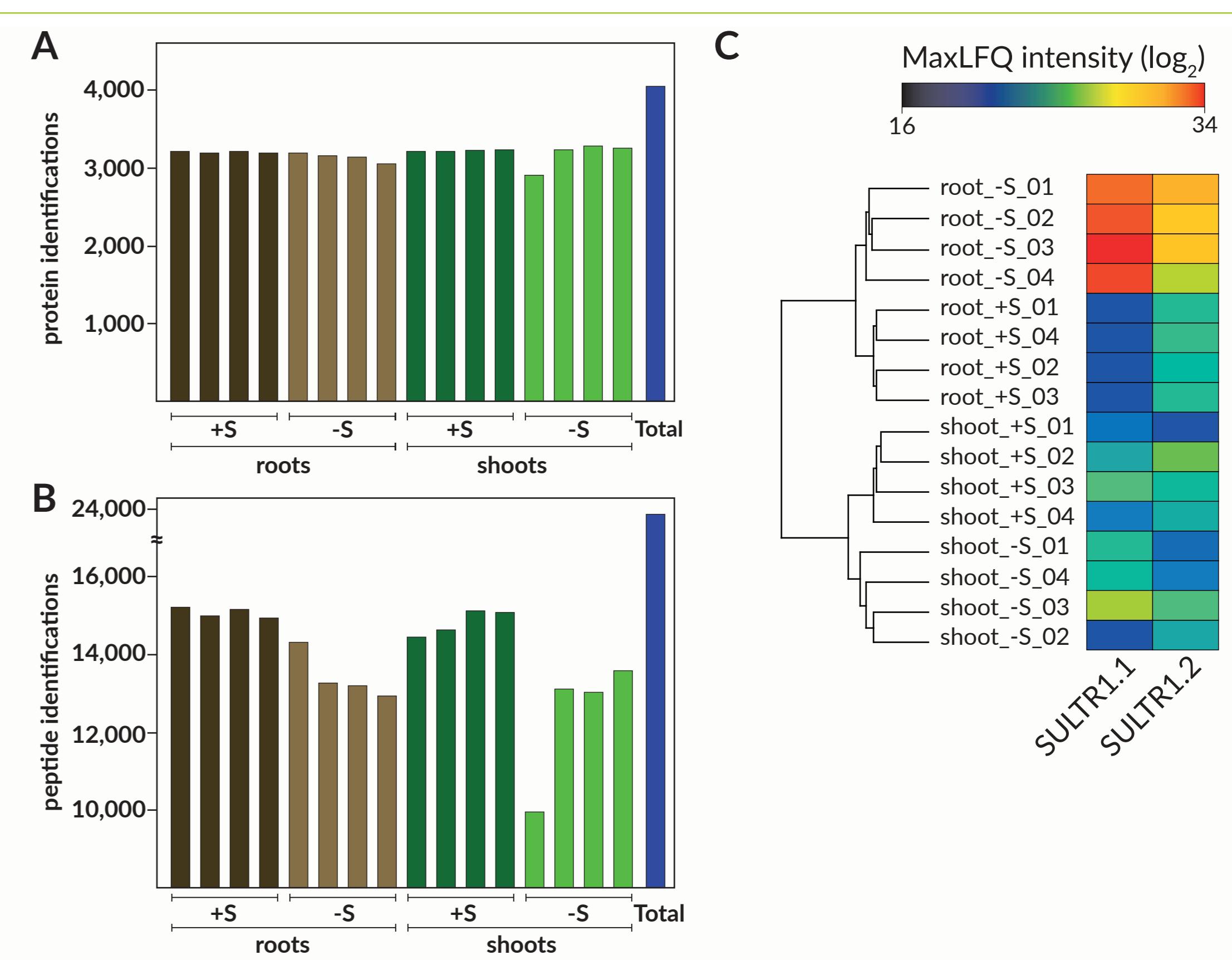


- <3.5 hrs total workflow time including labeling reaction
- labeling efficiency of > 98%, high reproducibility of R<sup>2</sup> > 0.94

## 4 Compatibility with different plant tissues

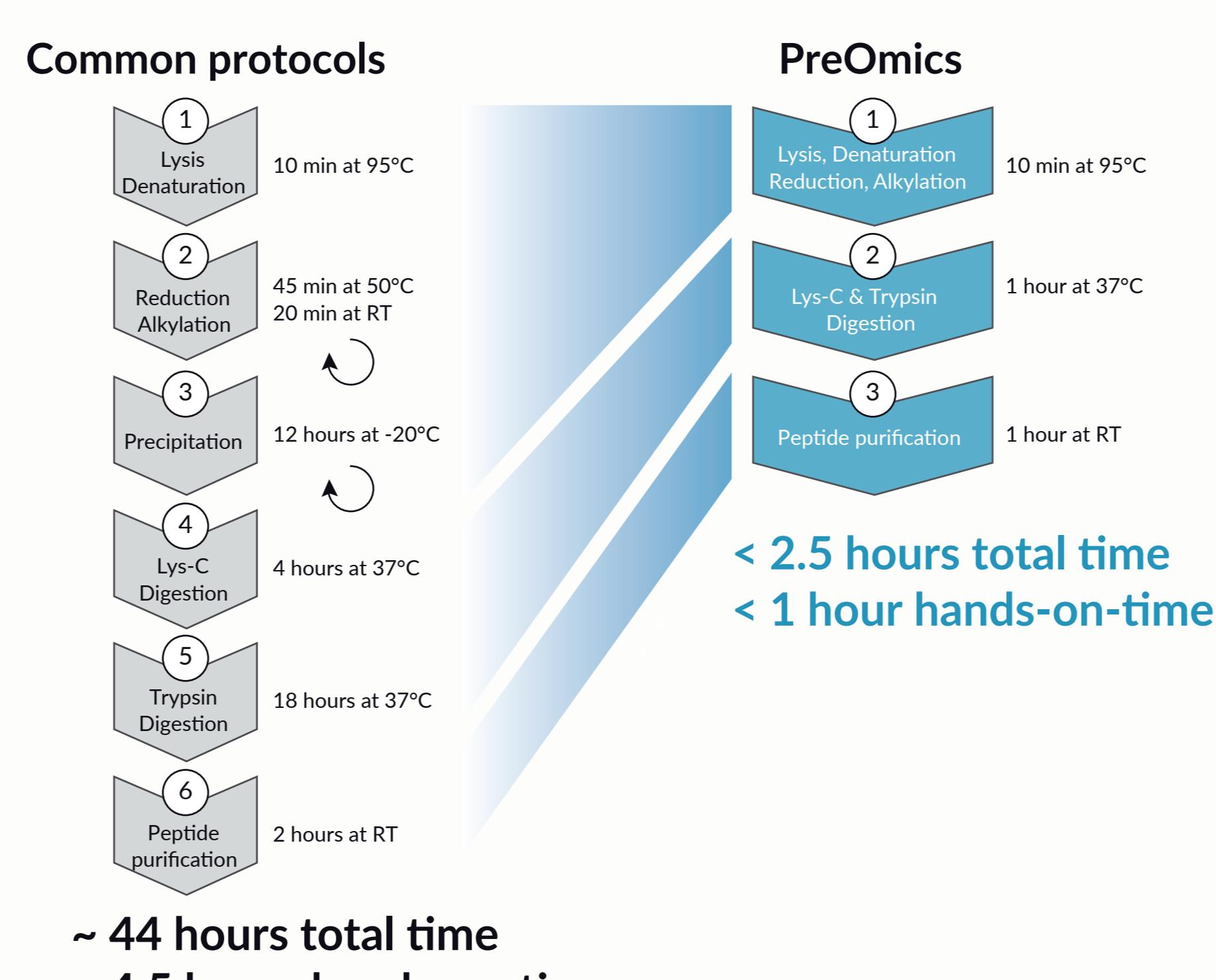


- fast & reproducible workflow, 5 hrs from harvest to ready-to-measure peptides
- >4,000 proteins from *A. thaliana* roots and shoots identified (A+B)
- environmental stress-mediated protein upregulation in specific tissues detected (C)



## 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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