Hands-off: automated & TMT-compatible sample preparation on the PreON platform employing the iST-NHS technology

PREOMICS

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1 Introduction

Sample preparation is an important component of the overall MS-based proteomics workflow and remains to be a limiting factor for high-throughput analyses. Here, we present an automated end-to-end solution for standardized sample processing, including cell lysis, digestion, TMT labeling & peptide cleanup by combining the NHS adaption of the iST technology (Kulak et al., 2014) with a newly developed automation platform called PreON.

2 Material & Methods

Sample preparation was done employing iST or iST-NHS kits, either by a manual operator or on the PreON platform. The instrument includes a built-in centrifuge and can process up to 12 samples per run in a fully automated fashion from lysis to cleaned up peptides. MS runs were performed on a QExplus instrument, data analysis by PD and TPP.

3 Performance

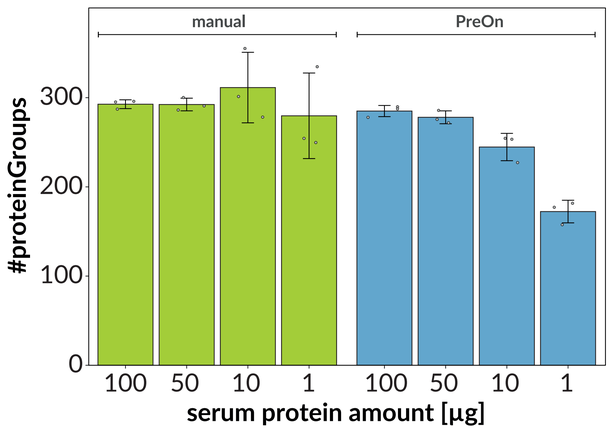

Fig. 1 | Dilution series of human serum. Manual iST sample preparation vs. operation on PreON.

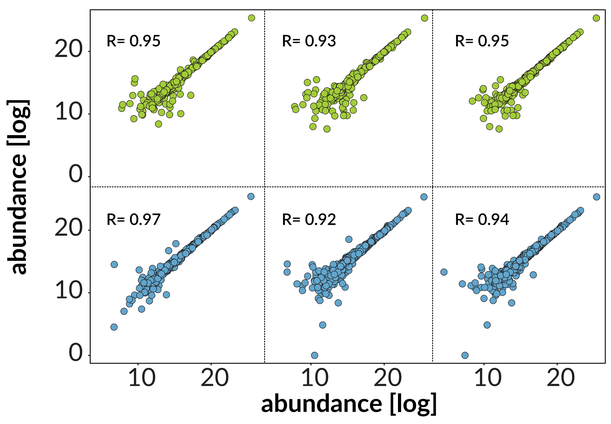

Fig. 2 | Quantitative reproducibility of triplicates from Fig. 1 for 100 µg serum input.

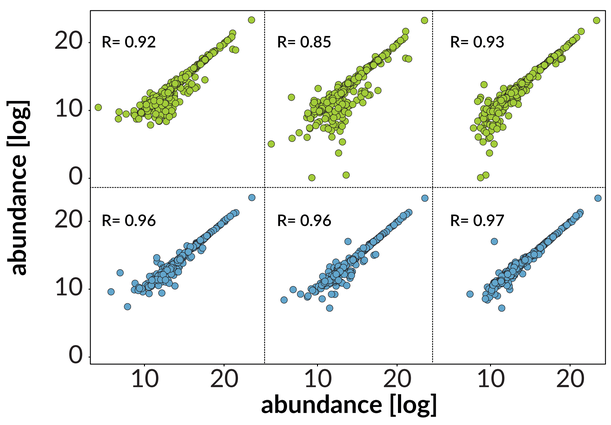

Fig. 3 | Quantitative reproducibility of triplicates from Fig. 1 for 10 µg serum input.

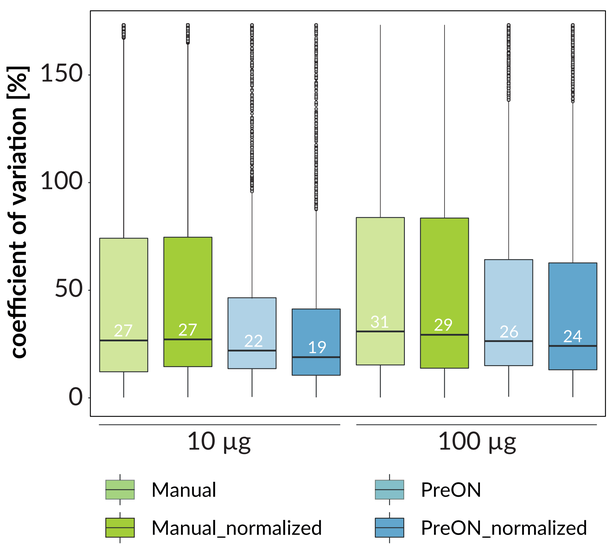

Fig. 4 | Technical variability of triplicates from Fig. 1 for 10 & 100 µg serum input, with or without normalization at peptide level.

4 Sensitivity & Sample Types

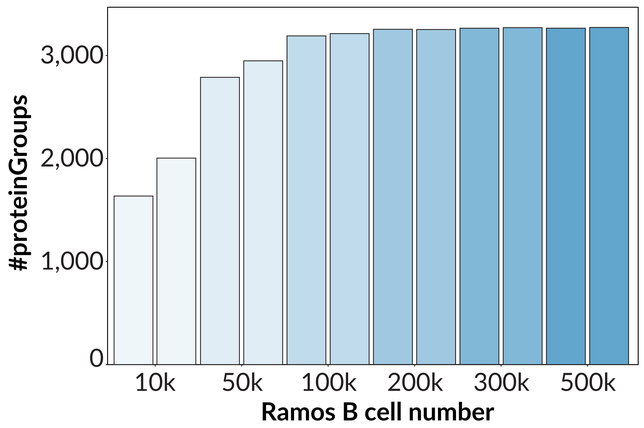

Fig. 5 | Dilution series for 10k-500k human Ramos B cells; operation on PreON. 500,000 Ramos B cells equal ~100 µg protein input.

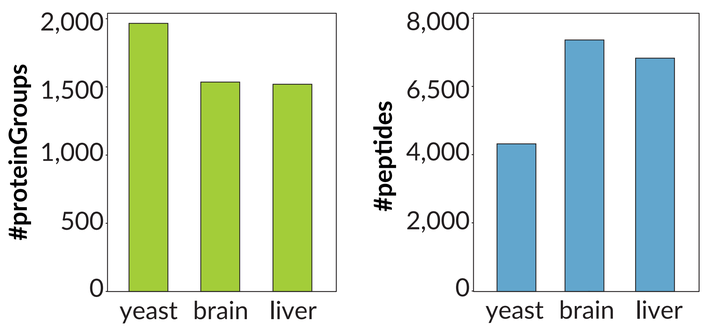

Fig. 6 | Sample prearation of yeast (*S.cerevisiae*), mouse brain and liver tissue, 100 µg input each; all samples processed on PreON. For tissue samples, lysis was done off-board with additional ultrasonication for enhanced tissue disruption (10 cycles 30 sec on/off; Diagenode Bioruptor® Pico). Single-shot MS analysis on LTQ-Orbitrap.

5 TMT Compatibility

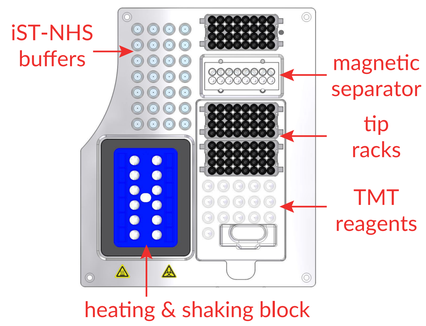

Fig. 7 | Workdeck operation when employing iST-NHS technology on the PreON. All TMT reagents, acetonitrile for label hydrolyzation and hydroxylamine for quenching are placed on the bottom right deck. TMT labels are covered with punchable membranes to prevent hydrolyzation on board during lysis and digestion time. All iST-NHS buffers are placed on the upper left deck.

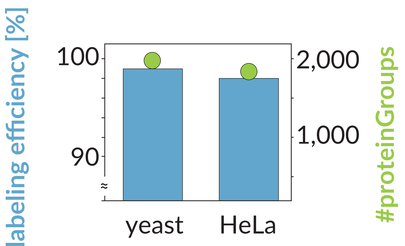

Fig. 8 | Number of proteinGroups identified & TMT labeling efficiency for yeast and HeLa samples, 100 µg protein input. Lysis, digestion, TMT-zero labeling and peptide cleanup all performed on PreON instrument without manual interference.
TMT 11-plex experiments on the PreON are currently in progress.

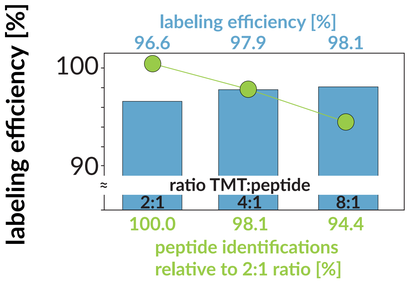

Fig. 9 | Inverse correlation between labeling efficiency and peptide identifications. The recommended TMT:peptide ratio of 8:1 results in slightly higher labeling efficiency at the cost of reduced peptide identifications. Use of a 4:1 TMT:peptide ratio results in a more effective balance between labeling efficiency and peptide identifications.

6 Conclusions

- low technical variability
- high reproducibility R > 0.9
- automation for label-free & TMT on one instrument
- flexibility to operate 4-12 samples
- ease-of-use, plug & play methods
- optical & ultrasonic sensors control workflow quality
- fully complementary with ready-to-go iST and iST-NHS kits

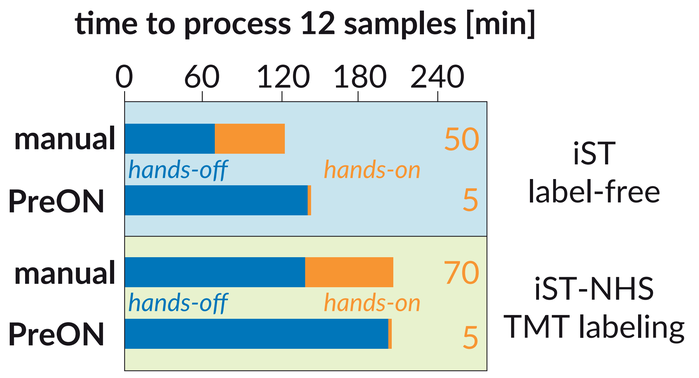

Fig. 10 | Comparison of hands-on and hands-off times for a manual operator vs. operation on the PreON platform. Top chart for label-free applications, bottom chart for TMT labeling.

7 Outlook

We foresee the PreON evolving as the flexible platform for reproducible and standardized sample processing. Label-free and chemical labeling workflows are the first in a pipeline of applications that allow walk-away automation for mass spectrometry-based protein analysis.

8 Contact

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