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NEXT GENERATION PROTEOMICS

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A Universal Workflow for Fully Automated Sample Preparation in Large-scale Proteomics

Abstract ID number: 313085

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Introduction

Sample preparation is a crucial step in bottom-up proteomics and comprises many critical steps, from the quantitative extraction of proteins to generation of a clean peptide sample, ready to be measured in a performance LC-MS/MS. As any multi-step process, the several liquid transfers and timed incubations are crucial in ensuring the quality and reproducibility of the results. Hence, large-scale proteomics studies are often impeded by the introduction and propagation of technical variability over time and the extensive hands-on time required. Automation of sample preparation addresses and improves these drawbacks. Here we present a completely autonomous platform capable of processing liquid as well as solid tissues at high throughput and outstanding quality.

Methods

We developed a sample preparation system that is equipped with an 8-channel liquid handler which is robotically linked to different devices performing ultrasonication, absorbance measurement, sealing, and peeling. The platform allows automated sample preparations from biological specimen input to MS-ready peptide output that can be directly loaded on a LC-MS/MS. The method includes sample denaturation, protein concentration determination, reduction, alkylation, proteolytic digestion, peptide clean-up, concentration determination, and normalization. The system is designed for parallelized processing of up to two 96-well plates in a single run and is flexible to work with a variety of different sample types and can modularly be coupled to other processing steps such as sample depletion of highly abundant proteins in biofluids.

Preliminary Data

The automated system was stress-tested with a sample matrix of 192 samples consisting of HeLa lysates and plasma with highly varying protein loads and extensive replicates. Overall, the system provided samples of similar or better quality to our manual processing workflow. For example, processing of 50 µg protein input and subsequent single-shot injection using Biognosys' TrueDiscovery Ultra Deep solution led to the identification of 8300 protein groups (n = 6) with a median coefficient of variation (CV) of 4.1% and processing of 100 ng of proteins led to the identification of 5515 protein groups (n = 6, CV = 5.3%), showcasing the methods sensitivity. The reproducibility within and between the two prepared 96-well plates was

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assessed with technical replicates (n = 24), which showed stable identifications and high quantitative precision (r = 0.998, n = 24). Furthermore, we processed the same sample on five different days and could assess overall low-degree of technical variability, lower than in a comparable experiment with our manual workflow.

In addition, we modularly coupled the above described workflow to an automated, parallel plasma depletion solution. Together, this allowed to run a series of complex processing steps all within the same platform while increasing identifications and quantitative precision. For instance, from a cohort of healthy (n = 10) and pancreatic cancer (n = 10) plasma samples, we identified 3'618 protein groups which is equivalent to an increase of 20% to our currently best performing workflow (column-based serial LC depletion), while reducing hands-on and total processing time by over 90%.Overall, the flexible end-to-end automated system allows us to implement most of our in-house workflow with a high-degree of standardization and significantly improves laboratory efficiency , while ensuring sample quality and enhancing reproducibility of preparations over time.

Novel aspect

Development of a novel workflow for fully-automated sample preparation in bottom-up proteomics with excellent sensitivity ,reproducibility, flexibility and processing efficiency.