Automated sample preparation workflows for quantitative proteomics applications

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Overview

Three reasons for automating workflows in a proteomic laboratory:

- Reduce time spent on repetitive work
- Increase consistency
- Increase reproducibility

WORKFLOW in a PROTEOMICS lab

<table>
<thead>
<tr>
<th>Reduce time investment</th>
<th>More time for important steps in the analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Automation</td>
<td>Automation</td>
</tr>
<tr>
<td>In-solution digest</td>
<td>In-solution digest</td>
</tr>
<tr>
<td>Isothiocyanate dimethyl labelling</td>
<td>Isothiocyanate dimethyl labelling</td>
</tr>
<tr>
<td>Phospho-peptide enrichment</td>
<td>Phospho-peptide enrichment</td>
</tr>
</tbody>
</table>

Recently, we presented the successful automation of in-solution and in-gel digestion on a PAL setup (CTC; Axel Semrau; presented on the ASMS 2014). We achieved medium-high throughput with high reproducibility and very good quality data. Here, we extended our automated sample preparation repertoire by the digestion on a PAL setup (CTC, Axel Semrau; presented on the ASMS 2014). Recently, we presented the successful automation of in-solution and in-gel digestion on a PAL setup (CTC, Axel Semrau; presented on the ASMS 2014). We achieved medium-high throughput with high reproducibility and very good quality data.

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Isotopic dimethyl labelling

Similar to a SILAC approach, dimethyl labelling (DNL) is used for quantitative shotgun proteomics where SILAC is not applicable (e.g. tissue samples are difficult to be metabolically labelled). DNL is a post-lysis labelling where peptides are modified with light (L) or heavy (H) isotopic variants of formaldehyde. This introduces a mass shift of 28 Da (L, 32 Da (H) or 36 Da (M)), which can be analysed similarly to a SILAC sample. Here, the change in protein abundance of mass isotopemers was determined upon treatment of the cells with particles from ship engine exhaust fumes.

Automation: Schematics of the RTO-PAL setup for the dimethyl labeling.

Application of the automated dimethyl labeling

Impact of heavy and light dimethyl labelling on macroparticles

MDC

Quantification

Reproducibility of mass spectrometer calibration and statistical analysis. Outliers represent potentially regulatory changed proteins.

Gene ontology analysis

Phospho-peptide enrichment

Protein function can be then be regulated by cofacial modification of amino acids. These post-translational modifications (PTMs) act as switches for the activity of cellular proteins like phosphorylation, methylation, or acetylation. They are responsible for a variety of cellular functions (e.g., cell cycle control, cell growth, cell division, and cell death) and can be the targets of various diseases (e.g., cancer, diabetes, and neurodegenerative disorders).

Phosphorylation occurs on serine, threonine and tyrosine residues and is involved in all cellular processes. The phosphorylation status of proteins strongly regulates their function (especially in intracellular signaling) and is therefore studied thoroughly especially to understand signaling-dependent diseases. Recent developments in the field of proteomics allow the large scale identification of phospho-peptides leading to an understanding of regulatory networks within the cell (Olsen et al. 2006).

Manual

Automated

We compared a manual, stage 2-based procedure with the automated procedure on the PAL. A 28-run format was compared with a 28-run format. While in the manual procedure, peptide isolation is used to enrich selected peptides, in the automated version on the PAL, a vacuum chamber is used.

We observed comparable numbers of phospho-peptides compared to the manual version.

The PAL system provides an affordable and reliable platform optimised for medium-throughput peptide preparation for shotgun-proteomics based mass spectrometry.