Automated protein digestion workflows for MS-based proteomics applications

Gunnar Dittmar¹, Oliver Popp¹, Guenter Boehm², Andreas Bruchmann³

¹Max Debrück Center for Molecular Medicine, MDC, Berlin, Germany; ²CTC Analytics, Zwingen, Switzerland; ³Axel Semrau GmbH, Sprockhövel, Germany

Overview
- Three reasons for automating workflows in a proteomic laboratory:
  - Reduce hands-on time on repetitive work
  - Increase consistency
  - Increase reproducibility

WORKFLOW
- Reduce time investment: More time for important steps in the analysis
- In-solution digest
- In-gel digest

Mass spectrometry (MS) based bottom-up proteomics is built upon large scale identification of peptides, and depends on proteins being efficiently converted to peptides by a protease of known specificity. The most common preparation methods are digestion in solution (ISD) or digestion of proteins separated on an SDS-PAGE gel, in-gel digestion (IGD). Both methods consist of a lengthy sequence of washing and chemical modification steps. To increase throughput and reproducibility, automation of these processes is highly desired. Contrary to other “omic” applications, proteomics analysis by MS remains time-intensive, making the measurement the rate-limiting step in the pipeline. Thus the preparation of samples does not highly desired. Contrary to other “omic” applications, proteomics analysis by MS remains time-intensive, making the measurement the rate-limiting step in the pipeline. Thus the preparation of samples does not

In-solution digest
- Mass spectrometry (MS) based bottom-up proteomics is built upon large scale identification of peptides, and depends on proteins being efficiently converted to peptides by a protease of known specificity. The most common preparation methods are digestion in solution (ISD) or digestion of proteins separated on an SDS-PAGE gel, in-gel digestion (IGD). Both methods consist of a lengthy sequence of washing and chemical modification steps. To increase throughput and reproducibility, automation of these processes is highly desired. Contrary to other “omic” applications, proteomics analysis by MS remains time-intensive, making the measurement the rate-limiting step in the pipeline. Thus the preparation of samples does not highly desired. Contrary to other “omic” applications, proteomics analysis by MS remains time-intensive, making the measurement the rate-limiting step in the pipeline. Thus the preparation of samples does not high throughput and reproducibility, automation of these processes is highly desired. Contrary to other “omic” applications, proteomics analysis by MS remains time-intensive, making the measurement the rate-limiting step in the pipeline. Thus the preparation of samples does not

In-gel digest
- Peptides are generated in a multi-step digestion procedure. The first steps occur at highly denaturing conditions (8 M urea). Proteins are reduced (TCEP) and blocked by alkylation (chloroacetamide). These conditions ensure complete unfolding of the protein as all parts of polypeptide are accessible for the protease. Since the protease has only limited activity in 8 M urea, the solution is diluted and a second protease, trypsin, is added. An additional incubation at 37°C finishes the digest and allows reproducible production of small peptides. The PAL robot performs all liquid solvent transfer steps on a 96-well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the

Sample preparation
- The PAL robot performs all liquid solvent transfer steps on a 96-well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the well plate equipped with a PTFE membrane that keeps the gel in the

Peptide sequences identified after digestion with the PAL are slightly decreased. However, the overall reproducibility, as reflected by the standard deviations (SD), is increased.

Peptide sequences identified after digestion with the PAL are slightly decreased. However, the overall reproducibility, as reflected by the standard deviations (SD), is increased.

The setup allows extensive washing of the gel bands by transfer of organic and inorganic solvent in alternating steps for up to 20 times, each followed by 15 min of incubation.

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

Density plot
- Density plot of the log10 intensity of identified peptides. The density plot shows the distribution of the MS data of peptides for the contamination test and the control experiment. Missed cleavages are slightly elevated in the automatic procedure. This can be explained by a better mixing of the samples in the manual procedure compared to the automated procedure where reactions take place in a liquid plate.

Cross-contaminations
- Cross-contaminations were slightly elevated in the automatic procedure. This can be explained by a better mixing of the samples in the manual procedure compared to the automated procedure where reactions take place in a liquid plate.

Accuracy
- A representation of an ITO-PAL setup. Each module is variable and thus the robotic setup can be extended by introducing new tools. Transfer of liquids is achieved by a syringe pump. Syringes of different volumes can be automatically exchanged during the process and are washed in a washing station with organic solvent and water.

Quality control
- Heat maps were digerised both manually and using the PAL. In manual digestion, an overall wash is increased. Missed cleavages are slightly elevated in the automatic procedure. This can be explained by a better mixing of the samples in the manual procedure compared to the automated procedure where reactions take place in a liquid plate.

Peptide sequences identified after digestion with the PAL are slightly decreased. However, the overall reproducibility, as reflected by the standard deviations (SD), is increased.

Peptide sequences identified after digestion with the PAL are slightly decreased. However, the overall reproducibility, as reflected by the standard deviations (SD), is increased.