# Automated in-gel digestion on a commercial autosampler directly coupled to nanoLC-MS/MS

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## Overview

Automated in-gel digestion of proteins and extraction of peptides on a commercially available autosampler coupled to nano LC-MS/MS for high-throughput proteomics enables continous data production with high reproducibility.

## Introduction

SDS-PAGE separates protein samples from LC-MS incompatible contaminations, and due to its separation efficiency, ease of use and low cost, it is frequently used to fractionate proteins of entire proteomes for bottom-up proteomics. One disadvantage is that each gel lane, containing a proteome, has to be cut into many slices, followed by in-gel digestion of proteins and extraction of peptides. The number of gel slices of a comparative proteome study goes into the hundreds, rendering this process very repetitive and prone to mistakes and errors during sample handling. It is therefore beneficial to automate the in-gel digestion process, in order to reduce such risks and improve reproducibility.

## Methods

We have adapted a manual in-gel digestion protocol, including reduction, alkylation and acid-labile detergent assisted trypsin digestion of proteins, to be performed on a PAL RTC autosampler system (CTC Analytics AG, Switzerland). Peptide extracts in HPLC vials were transferred to a nanoLC-orbitrap XL mass spectrometer (Thermo Scientific, Germany). Direct injection by the PAL system is planned. Proteins were identified and quantified with MaxQuant software.

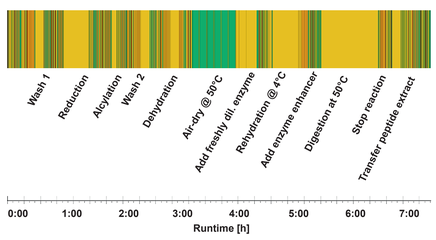



**Figure 1**

Run Schedule of the automated in-gel protein digestion. The current PAL RTC setup treats batches of 6 gel samples within 7 hours.

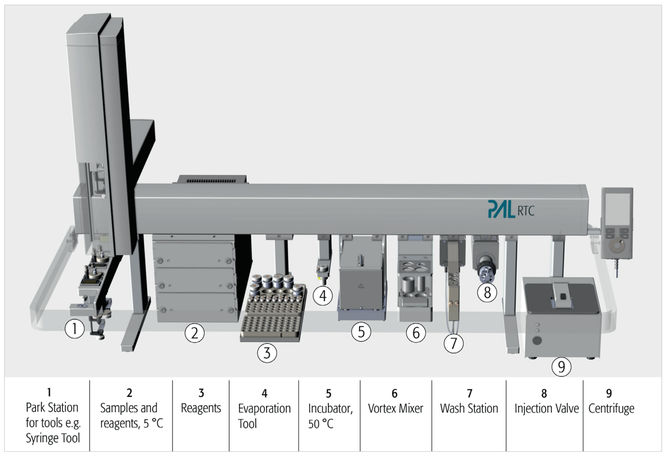



**Figure 2**

The system is equipped with a park station for syringe tools, a cooled tray stack and a tray holder for samples/reagents, an evaporation tool, a heatable incubator with orbital shaking capability, a vortex mixer, a syringe wash station, a centrifuge, and a LC injection valve.

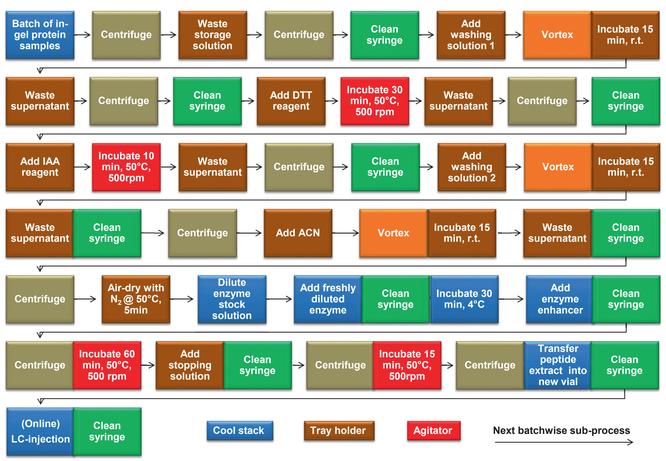



**Figure 3**

Schematic Overview of the automated batchwise in-gel digestion method.

The caps of the vials used were made of steel and the syringe tools were fitted with four magnets. This enabled a reliable transport of the vials between the various modules.

Potential blocking of syringes with gel pieces during the aspiration of supernatants was avoided by sweeping away the gel pieces from the syringe with aspiration ejection cycles with a few microliters.

Gel pieces potentially adhering to the outer surface of the syringe were stripped off on the cap, when the syringe was pulled out of the vial. In order to save the gel piece, vials had to be centrifuged after each aspiration task.

## Results

A 12-protein molecular weight standard (Bio-Rad) was separated over the entire width of a 12.5% SDS-PAGE at two concentrations corresponding to 1ng or 5ng of each protein standard present in vertical slices of 1.5mm width cut from the gel. Four batches, including three slices of each protein concentration, were processed by an experienced human operator or on the PAL RTC system during four consecutive days. Protein yields were determined by identification and quantification with MaxQuant software.

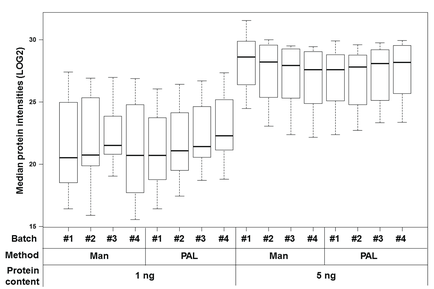
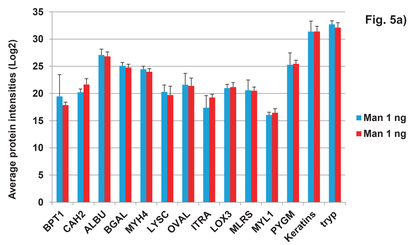



**Figure 4**

Boxplot of the non-normalized median protein intensities of batches, methods and processed protein contents.

One-way Anova tests were used to compare MaxQuant derived non-normalized protein intensities of batches and procedures with Tukey's honestly significant difference criterion to evaluate statistical significance (alpha = 0.05). No statistically significant differences in protein yield between batches and between procedures were found. The average coefficients of variation of the 12 standard proteins were 19.4% and 11.8% at 1ng protein, and 5.8% and 6.5% at 5ng for the manual and the automated procedure, respectively.



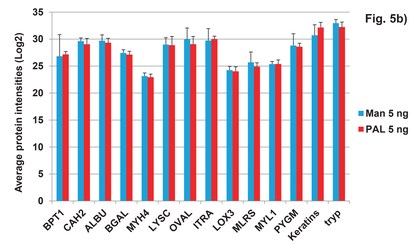




**Figure 5**

Barplots of the non-normalized median protein intensities of individual molecular weight standard proteins and the contaminating keratin and trypsin proteins, methods and processed protein contents (5a = 1ng, 5b = 5ng).

The low molecular weight proteins (Mr <20 kDa) were not consistently identified from the 1ng samples with both procedures. This is a known problem with the gel-LC-MS/MS approach.

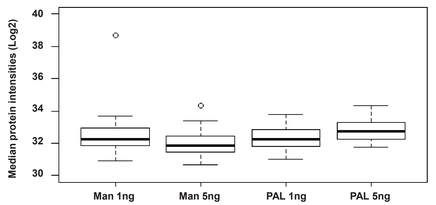



**Figure 6**

Boxplot of the non-normalized median keratin protein intensities of batches, methods and processed protein contents.

Keratin contaminations were very similar with both procedures and protein concentrations, indicating that keratin proteins were introduced into the samples before the in-gel digestion process.

## Conclusions

These results indicate that the PAL RTC automated in-gel digestion protocol performs as well as an experienced human operator, with potentially a better reproducibility achieved on the PAL system when dealing with low protein concentrations. The next step is the direct coupling of the PAL system to the nanoLC-MS/MS system, which will enable to operating on a 24/24 hours, 7/7 days schedule.