

Authors:

Jürgen Schäfer, Christian Baumann, Marco Schärfke, Karsten Kuhn, Thorsten Prinz,
Christian Hamon, Josef Schwarz, Sandra Steiner
Proteome Sciences R&D GmbH&Co KG, Frankfurt, Germany

Titel:

Differential proteomics using qPST with a defined biological system –a validation study.

Introduction:

Stable isotope coding is an emerging and promising MS based quantification method [1] for differential protein profiling. Our Quantitative Protein Sequence Tag (qPST) approach is a further development of our previously published PST work [2] and contains a powerful quantitation feature [3]. Here we describe the validation of the qPST technology for protein profiling. Two yeast cultures grown under different conditions (Galactose vs Ethanol) were chosen as a well described biological system.

Methods:

The qPST procedure uses two different isotopomeric dimethylglycine tags. Compared to the light the heavy PST tag contains four ¹³C and one ¹⁵N atom, resulting in a mass difference of 5 amu. The quantification is performed in MS mode done on a Q-ToF 2 instrument equipped with a Cap LC HPLC system. The MS data were analyzed by our proprietary software package. The selection module generates include lists containing regulated and exclusive signal peaks for MS/MS experiments, which were analyzed with SEQUEST. A peak matching software module performs the assignment of the regulation to the identification data.

Preliminary data:

The principle of the qPST technology is based on isolation and identification of the N-terminal peptide from polypeptide fragments using a proprietary capping chemistry. qPST combines the features of relative protein quantification via specific isotopomeric peptide pairs in MS mode and a MS/MS based identification. A key feature of the qPST approach is that the labeling is performed at the protein or large peptide level and hence sample pairs can be combined at an early stage of the analysis process.

Data to be presented:

- Our qPST results are in good agreement with literature data.
- A List of regulated peptides and proteins will be presented.
- Reproducibility data of the MS runs from replicate qPST procedures will be shown.
- Reproducibility data of the quantitation of the qPST approach will be shown.

This validation study shows that the qPST approach is highly reproducible and a powerful tool for differential proteomics biomarker discovery.

Literature

- [1] Thompson, A.; Schäfer J.; Kuhn, K.; Kienle, S.; Schwarz, J.; Schmidt, G.; Neumann, T.; Hamon, C. Anal. Chem. 2003, 75, 1895-1904
[2] Kuhn, K.; Thompson, A.; Prinz, T.; Müller, J.; Baumann, C.; Schmidt, G.; Neumann, T.; Hamon, C. (2003) J. Proteome Res., 2003, 2, 598-609
[3] Jürgen Schäfer, Christian Baumann, Christian Hamon, Josef Schwarz, ASMS 2004, Poster TPT 389