Evaluating the reproducibility of bottom-up proteome sample preparation in the ProTrap XG through quantitative mass spectrometry

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Summary

With advances in LC-MS instrumentation, the precision of quantitative proteomics is increasing. However, sample preparation strategies remain a significant source of variance particularly due to biases in recovery and variable digestion completion.

The ProTrap XG is a semi-automated filtration cartridge that facilitates a rapid precipitation-based sample preparation, enabling the inclusion of SDS for complete proteome solubilization. The present study evaluated the repeatability of bottom-up proteome sample preparation in the ProTrap XG using a rapid precipitation strategy developed by the Doucette lab.

The repeatability of bottom-up peptide and protein identifications and quantitation were benchmarked against the inherent variance of replicate LC-MS detection and compared to tradition solution and in-gel digestion approaches. Protein quantitation in the ProTrap XG was 25% more precise than the in-gel approach and 50% more precise than in-solution digestion, which was attributed to variable recovery and digestion completion.

Methods

Proteome solubilization

S. cerevisiae was cultured, harvested and lysed under liquid nitrogen. The lysate was aliquoted for one part to be extracted in 100 mM Tris-HCl (pH 8) and the other extracted in 5% SDS.

Bottom-up sample preparation

Solution Digests: The aqueous extract was reduced and alkylated with DTT and IAA. Four aliquots were combined with trypsin at a 50:1 substrate-to-enzyme ratio and digested overnight at 37 °C followed by quenching with 0.1% TFA.

In-Gel Digests: Four 50 µg aliquots of the SDS extract were combined with 5x Laemmli buffer and electrophoresed across ~1 cm of a 12% T SDS PAGE gel. The lanes were excised and digested with trypsin at a 50:1 substrate-to-enzyme ratio overnight at 37 °C. Peptides were subsequently extracted from the gel and dried by SpeedVac.

ProTrap XG Digests: The SDS extract was reduced and alkylated with DTT and IAA. Eight 100 µg aliquots were combined with 4 volumes acetone and 100 mM NaCl and precipitated for 2-5 min at room temperature. Pellets were re-solubilized in 8 M urea for 1 h with periodic vortex mixing.

Re-solubilized pellets were diluted to 1.5 M urea and combined with 50 mM Tris-HCl (pH 8), 50:1 trypsin, and digested overnight at 37 °C, and quenched with 0.1% TFA. Four digests were subject to additional de-salting in the associated SPE cartridge.

LC-MS/MS Replicates: A single aliquot of the aqueous extract was prepared in the ProTrap XG and five replicate LC-MS/MS injections were done to benchmark the instrumental error.

LC-MS/MS

LC-MS/MS analysis of proteome digests was conducted by Allumiqs Inc. (Sherbrooke, CA) according to their standard protocols. Online reversed phase separation was done on a Kinetex XB C18 column at 60 °C across a 60-min ethanol gradient. MS acquisition was performed on an ABSciex TripleTOF 6600 via electrospray. Information Dependant Acquisition (IDA) mode was used to establish the ion library. The samples were analyzed in SWATH acquisition mode.

Data analysis

Bottom-up peptide and protein identifications were compared across replicate preparations to determine qualitative repeatability. Peptides were also sorted based on miscleavage frequency to estimate relative digestion completion.

Precursor intensities from MS1 were normalized across all data. Quantitative precision across preparative and instrumental replicates were compared at the peptide and protein level using peptides in common between all samples. Bottom-up protein precision was estimated based on the most precise associated peptide.

Results

Repeatability of bottom-up identifications is optimal in the ProTrap XG

Bottom-up protein identifications

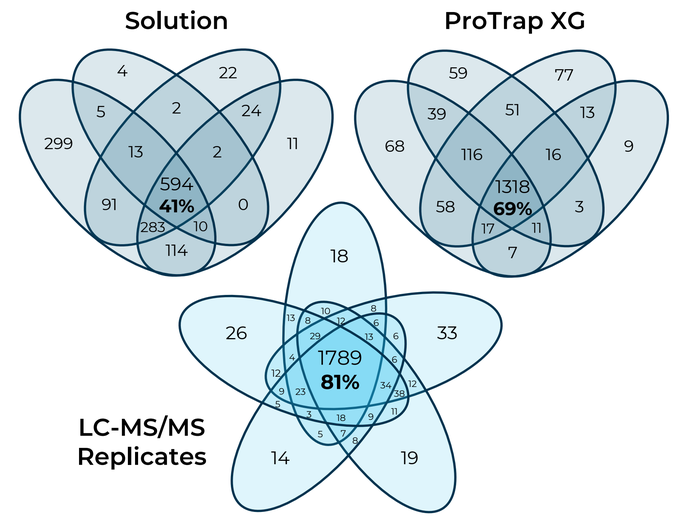

Figure 1. Venn diagrams of bottom-up protein identifications following replicate sample preparation of an *S. cerevisiae* lysate by in-solution digest, precipitation in the ProTrap XG and replicate LC-MS/MS injections.

Summary of bottom-up IDs

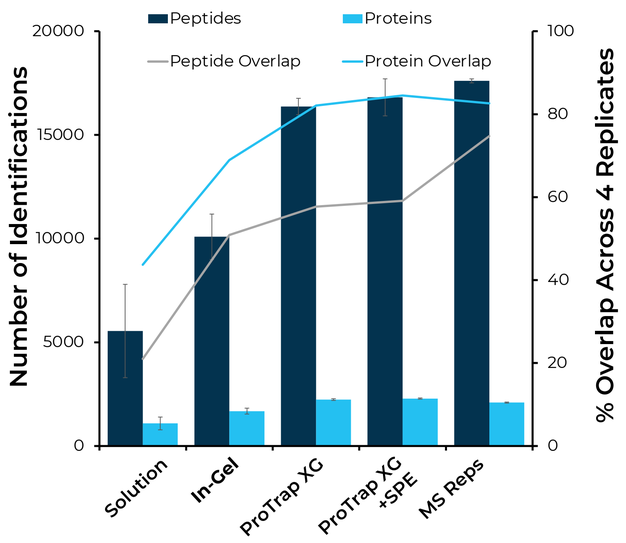

Figure 2. Summary of bottom-up peptide and protein identifications following sample preparation of an *S. cerevisiae* lysate by in-solution digestion, in-gel digestion, precipitation in the ProTrap XG (± SPE).

Miscleavage analysis

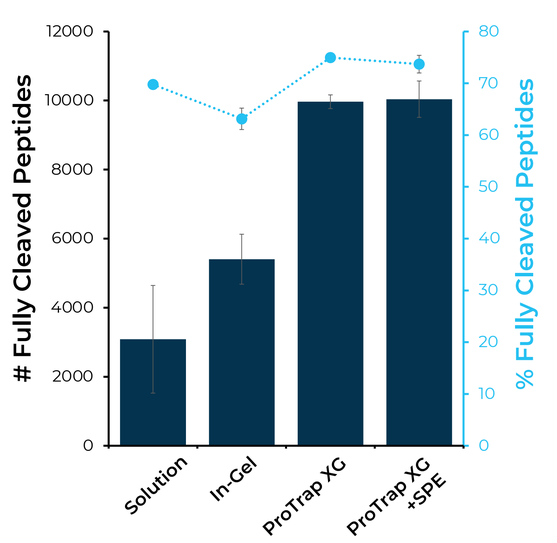

Figure 3. Miscleavage analysis following digestion of *S. cerevisiae* lysate in-solution, in-gel, and in the ProTrap XG.

Bottom-up quantitative precision is optimized in the ProTrap XG

LOD, LOQ

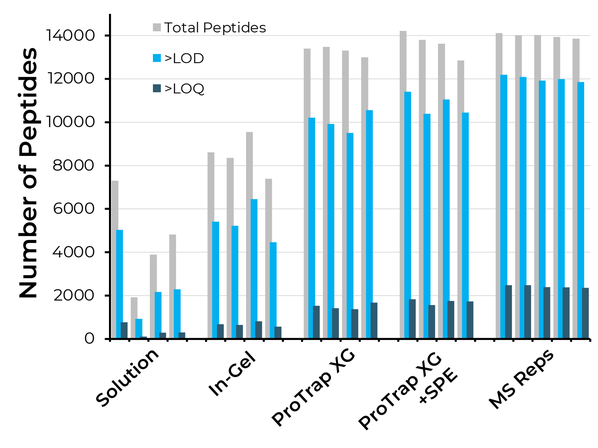

Figure 4. Characterization of identified peptides based on the LOD and LOQ determined from modelling measurement error. High recovery and digestion efficiency in the ProTrap XG enables more peptides to be quantified.

Quantitative precision: Peptides

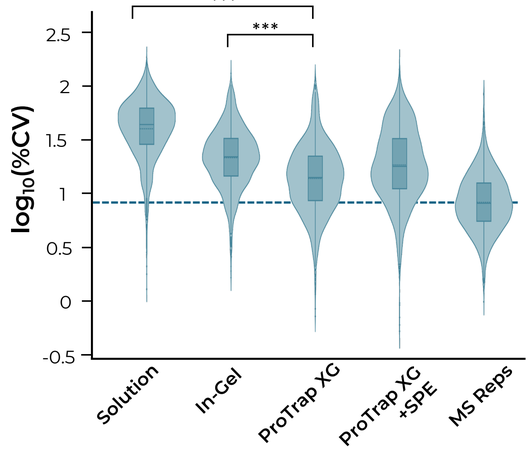

Figure 5. Violin plot comparing the distribution of coefficients of variation following bottom-up peptide quantitation across four replicate preparations.
*** p <0.0001

Quantitative precision: Proteins

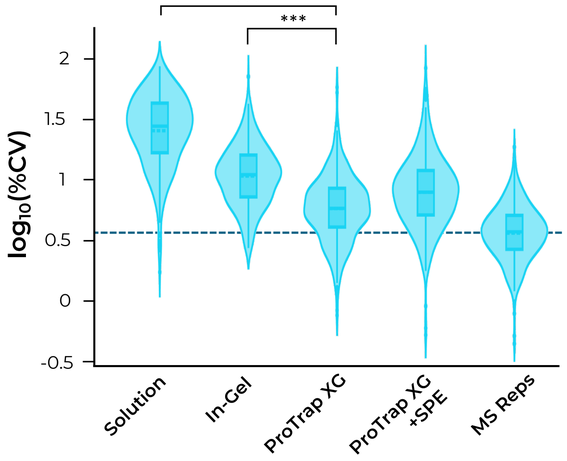

Figure 6. Violin plot comparing the distribution of coefficients of variation following bottom-up protein quantitation across four replicate preparations.

PCA analysis of peptide intensities

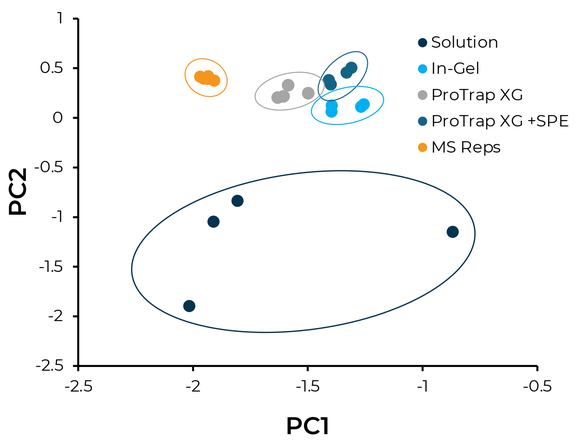

Figure 7. Principal components analysis of normalized peptide intensities, showing high repeatability for in-gel and ProTrap XG-based sample preparation strategies.

Conclusions

- Bottom-up sample preparation strategies all introduce variance at the qualitative and quantitative level.
- Unbiased recovery and digestion completion are critical factors in maximizing quantitative precision at the peptide and protein level.
- Both qualitative and quantitative repeatability are optimized by conducting a precipitation-based sample preparation in the ProTrap XG cartridge compared to traditional solution and in-gel approaches.

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