# In-depth analysis and optimization of the ProTrap XG sample preparation device using quantitative DIA mass spectrometry.

Jean-Philippe Couture[1], Jean-François Noël[1], Victoria Miller[2], Sara Lahsaee Little[2], and Hugo Gagnon[1].

1- PhenoSwitch Bioscience, Sherbrooke, QC, Canada. 2- Proteoform Scientific, Halifax, NS, Canada.

## OVERVIEW

The ProTrap XG is an all-in-one, fast, and robust proteomics sample preparation device that performs close to our optimized and fine-tuned protocol.

## MATERIALS AND METHODS

**Side-by-side variations in the protocols**

| PhenoSwitch No Device | Proteoform ProTrap XG | Modified ProTrap XG |
|---|---|---|
| No salt during precipitation | 50 mM NaCl during precipitation | 50 mM NaCl during precipitation |
| Protein pellet wash with methanol | Protein pellet wash with acetone | Protein pellet wash with methanol |
| Reduction with 10 mM DTT | Reduction with 20 mM DTT | Reduction with 10 mM DTT |
| Alkylation with 15 mM IAA | Alkylation with 100 mM IAA | Alkylation with 15 mM IAA |
| Quench with 10 mM DTT | No quenching | Quench with 10 mM DTT |
| Digestion in 0.75 M urea + 50 mM Tris pH 8.0 | Digestion in 2 mM CaCl2 + 50 mM Tris pH 8.0 | Digestion in 0.8 M urea + 50 mM Tris pH 8.0 |
| 30:1 protein:enzyme ratio | 100:1 protein:enzyme ratio | 30:1 protein:enzyme ratio |
| Stop digestion with 2% FA | Stop digestion with 0.1% TFA | Stop digestion with 2% FA |

**Side-by-side variations in the SPE**

| Steps | ProTrap XG SPE | Phenomenex SPE |
|---|---|---|
| Organic priming 1 | 100% MeOH | 100% ACN |
| Organic priming 2 | 50% ACN + 0.1% TFA | N/A |
| Aqueous wash | 5% ACN + 0.1% TFA | 2% FA |
| Sample loading | Sample in 5% ACN + 0.1% TFA | Sample in 2% FA |
| First Wash | 5% ACN + 0.1% TFA | 0.1% TFA |
| Second Wash | N/A | 2% FA |
| Elution | 50% ACN + 0.1% TFA | 40% ACN + 2% FA |

**Mass Spectrometry Method**

Sample acquisition was performed on a TripleTOF 6600 (Sciex) coupled to an Eksigent MicroLC200. Peptides were separated on a Kinetex XB column (150 x 0.3 mm, 2.6 µm particle) over a 60 minute LC gradient (A: 3% DMSO + 0.2% FA in water, B: 3% DMSO + 0.2% FA in ethanol). Acquisitions were performed in data independent acquisition, with 10 m/z windows ranging from 350 to 1250 m/z. Peptides were quantified using either the SWATH 2.0 micro app (Peakview, Sciex) and DIA-NN (Demichev et. al. Nature methods, 2020).

## RESULTS

Fig.1: Normalized peptide signals over a 60 minutes gradient, for the different sample preparation protocols. Each protocol was repeated 4 times.

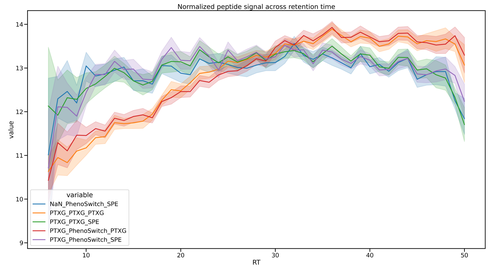


Fig.2: Global comparison of the different sample preparation protocols.

TEST A: No device, Protocol Standard PhenoSwitch, SPE Phenomenex
TEST B: PTXG Device , PTXG Protocol , PTXG SPE
TEST C: PTXG Device , PTXG Protocol , Phenomenex SPE
TEST D: PTXG Device, PTXG Modified Protocol, PTXG SPE
TEST E: PTXG Device, PTXG Modified Protocol, Phenomenex SPE

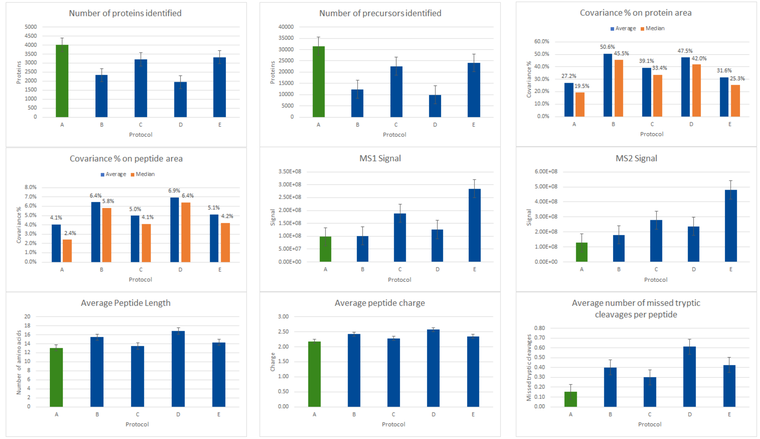


Fig.3: Heatmap clustering analysis of peptides with different PTMs or miscleavages/missed cleavages, for all different sample preparation protocols.

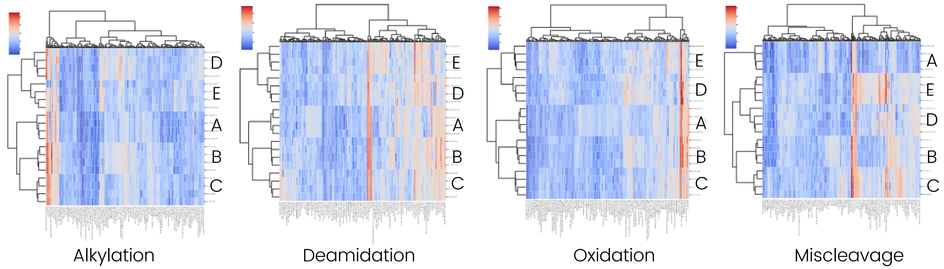


Fig.4 : Head-to-head comparison of the ProTrap XG and PhenoSwitch Bioscience's current optimized protocol.

| Comparisons | ProTrap XG | PhenoSwitch |
|---|---|---|
| Complete workflow time | 2 days | 2 days |
| Hands-on time | 2.5 hours | 5 hours |
| Pricing | $$ | $$$ |
| Results | Comparable | Optimized |
| Convenience | Everything in one device | Precipitation, digestion, and SPE on 3 different devices |

## CONCLUSIONS

- The ProTrap XG device performs similarly to PhenoSwitch Bioscience's current optimized protocol.
- The biggest differences are attributable to the SPE step (more miscleavages/missed cleavages, longer peptides).
- Given the savings in preparation time and resources, we conclude that the ProTrap XG device is a viable sample preparation tool for whole cell proteomics.

PhenoSwitch BIOSCIENCE