Towards a comprehensive targeted proteogenomic assay repository for the liquid fraction of sheep blood

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25th Australian and New Zealand Society for Mass Spectrometry
6th Asia Oceania Mass Spectrometry
CONFERENCE 19-22 July 2015

INTRODUCTION

Proteogenomics tools to interrogate the circulating acellular proteome (CAP) in veterinary species are currently scarce. This emerging area of research at the interface of proteomics and genomics is vital in identifying and quantifying proteins with the aid of advances in tandem mass spectrometry (MS/MS), making it an attractive alternative to antibody ELISA technology. Alternatives to expensive and low throughput ELISA are sorely lacking for diagnosis and monitoring of animal diseases. Proteogenomics technologies provide a promising option, but they remain underutilised and challenging in veterinary science largely due to lack of complete genome sequencing data and relevant expertise. We are developing a proteogenomic assay that is capable of simultaneously measuring several hundred CAP proteins, using sheep as a model. Our methods employ shotgun proteomics approach MS/MS^{ALL} with SWATH™ Acquisition. We have constructed a comprehensive peptide spectral library to be used with SWATH™ that could be implemented to understand the body's response to illness or stress, for example. Access to such tools is important for researchers, who often wish to measure circulating levels of many different proteins. This work is of practical significance to the veterinary industry and also to scientists who seek to use sheep or other ruminants as a model for studying human disease.
The additional prevailing constraints of this proof-of-concept work in veterinary science applications include the wide dynamic range of CAP protein concentrations and lack of species-specific abundant protein depletion kits.

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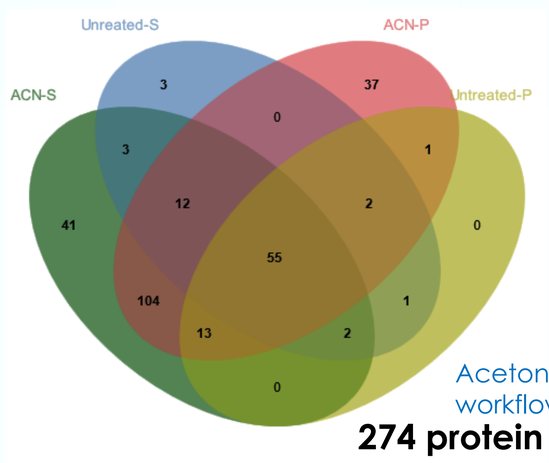
METHODS

Sheep plasma and serum samples were subjected to 4 pre-fractionation techniques prior to protein reduction, alkylation and in-solution trypsin digestion as follows:

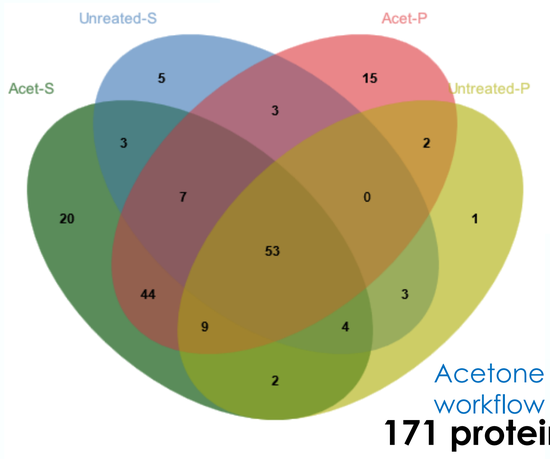
- Acetonitrile treated serum (ACN-S) and plasma (ACN-P) vs untreated-S&-P.
- Acetone treated serum (Acet-S) and plasma (Acet-P) vs Untreated-S&-P.
- ProteoMiner™ treated serum (ProtM-S) and plasma (ProtM-P) vs Untreated-S&-P.
- ProteoMiner™ treated serum (ProtM-SG) and plasma (ProtM-PG) vs untreated-serum (S) and plasma (P), respectively for in-gel workflow for the peptide library.

The digests were then analysed by LCMS/MS on a TripleTOF 5600+ instrument platform using a 90 min method and 15 cm analytical column at 40°C. Peptide MS/MS spectra were searched against a custom composite database of ox, sheep, goat and common contaminants using ProteinPilot ™5.0, Mascot 2.5.1 and X!Tandem Pipeline 3.3.4.

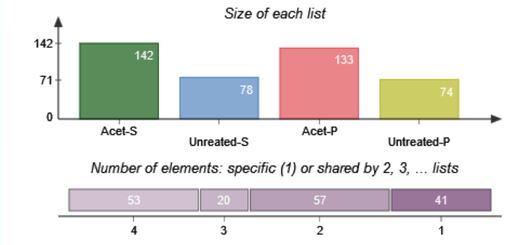
Preliminary Results*



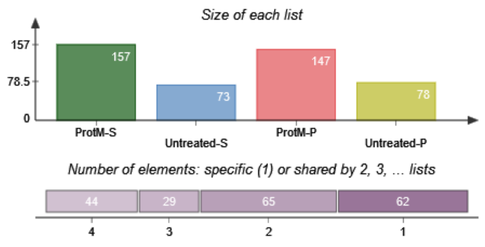
Acetonitrile in-solution workflow
274 protein groups

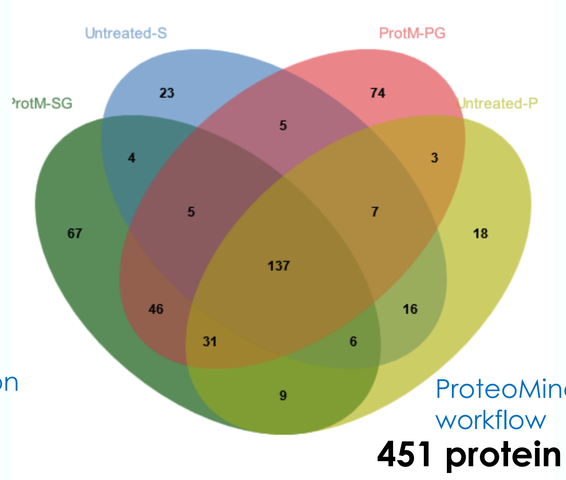


Acetone in-solution workflow
171 protein groups

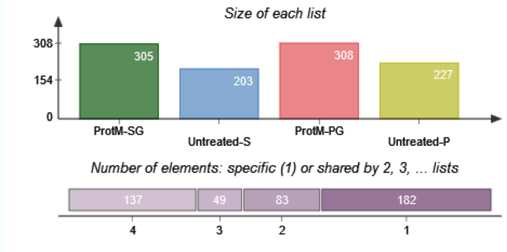


ProteoMiner™ in-solution workflow
200 protein groups





ProteoMiner™ in-gel workflow
451 protein groups



*Results Quality: Detected Protein Threshold [Unused ProtScore (Conf)] >: 0.05 (1% FDR filter).

FURTHER WORK

We are presently applying MS/MS^{ALL} with SWATH™ Acquisition to analyse digests of plasma and serum from sheep exposed to endotoxin to identify a larger number of sheep proteins across a broad dynamic range.

Conclusions

Performance ranking based on in-solution protein identifications (1=best):
1-Acetonitrile treatment - best candidate for SWATH™ workflow.
2-ProteoMiner™. In-gel workflow good for protein library coverage.
3-Acetone treatment by analysing precipitate and supernatant.
4- Untreated samples.
This is the first study to generate the most extensive dataset that has identified at least 450 protein groups of the circulating acellular proteome in sheep.

Acknowledgement of funding sources: APA Scholarship; The University of Queensland; Proteomics and Small Molecule Mass Spectrometry, CARF, Queensland University of Technology.