Sheep proteogenomics

Advancing veterinary clinical pathology & diagnostics

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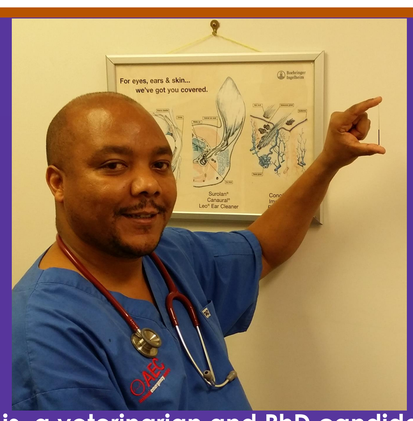

Saul is a veterinarian and PhD candidate at The University of Queensland. His interests include large animals used as models of intensive care. Collaborative research with QUT is driven by the state-of-the-art instrumentation and expertise in the field of proteomics at CARF.

INTRODUCTION

Proteogenomics tools available for veterinary applications are comparatively lagging behind those for humans. Alternatives to expensive and low throughput ELISA assays are urgently needed for diagnosis and monitoring of animal diseases. Proteogenomics technologies provide a promising option but they remain underutilised in veterinary science due to a lack of complete genome sequencing data and relevant expertise. Here, we present a proof of concept work on the development of a mass spectrometry-based assay aiming to explain a differential response to a simulated disease challenge in sheep. Our work could be beneficial to the veterinary industry and in the long term, it might benefit animal welfare and production.

STRATEGY

Our earlier work has shown that despite having similar breed characteristics, sheep that we were taking care of at QUT Medical Engineering Research Facility (QUT-MERF) responded differently to *Escherichia coli* endotoxin challenge. We hypothesised that serum proteogenomic biomarker change patterns contribute in defining and predicting sheep response to endotoxin. We are developing a stable isotope dilution(SID) multiple reaction monitoring (MRM)-based assay to monitor the levels of 5 cytokines in serum collected from healthy and endotoxin-treated animals on two instrument platforms available at CARF (AB Sciex 5600+ and Shimadzu LCMS-8050). In this initial stage, we have tested the recently implemented compatibility of Skyline software platform with Shimadzu instrument.

PRELIMINARY RESULTS

Using Skyline, we predicted 2 best peptides to be monitored for 5 cytokines (putative inflammation biomarkers) using SID-MRM-MS assay. We then synthesized 10 selected peptides in their unlabeled and stable isotope-labeled version. Finally, for each peptide we selected 3 MRM transitions (60 transitions in total) for which we optimized liquid chromatography and mass spectrometry conditions with the support from Skyline.

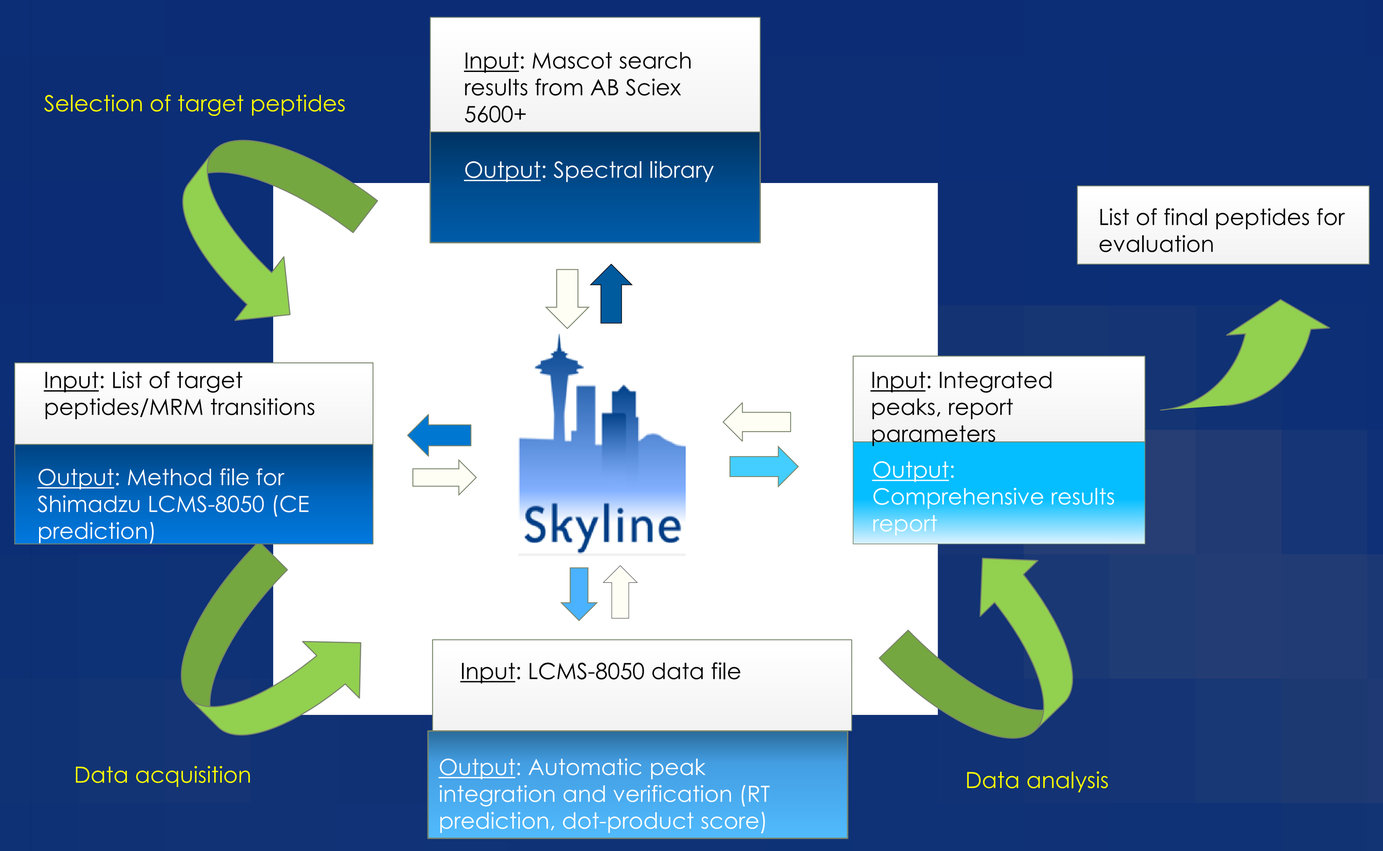

Figure 1. Skyline software (MacCoss Lab) is the heart of our peptide SID-MRM-MS workflow. The workflow starts with collecting peptide ion fragmentation spectra on AB Sciex 5600+ instrument. Spectra are then annotated with amino acid sequences using Mascot search engine (Matrix Science) and imported into Skyline as spectral libraries. Based on these libraries, Skyline helps us to choose the optimal combination of peptide ions and fragment ions (transitions) used for quantification of proteins of interest. Skyline then exports a method file for Shimadzu LCMS-8050 instrument, predicting optimal fragmentation energies (CEs). After initial data has been acquired using LCMS-8050, Skyline can read it and perform automatic peak integration and identity verification. Finally, a list of peptides is exported for evaluation.

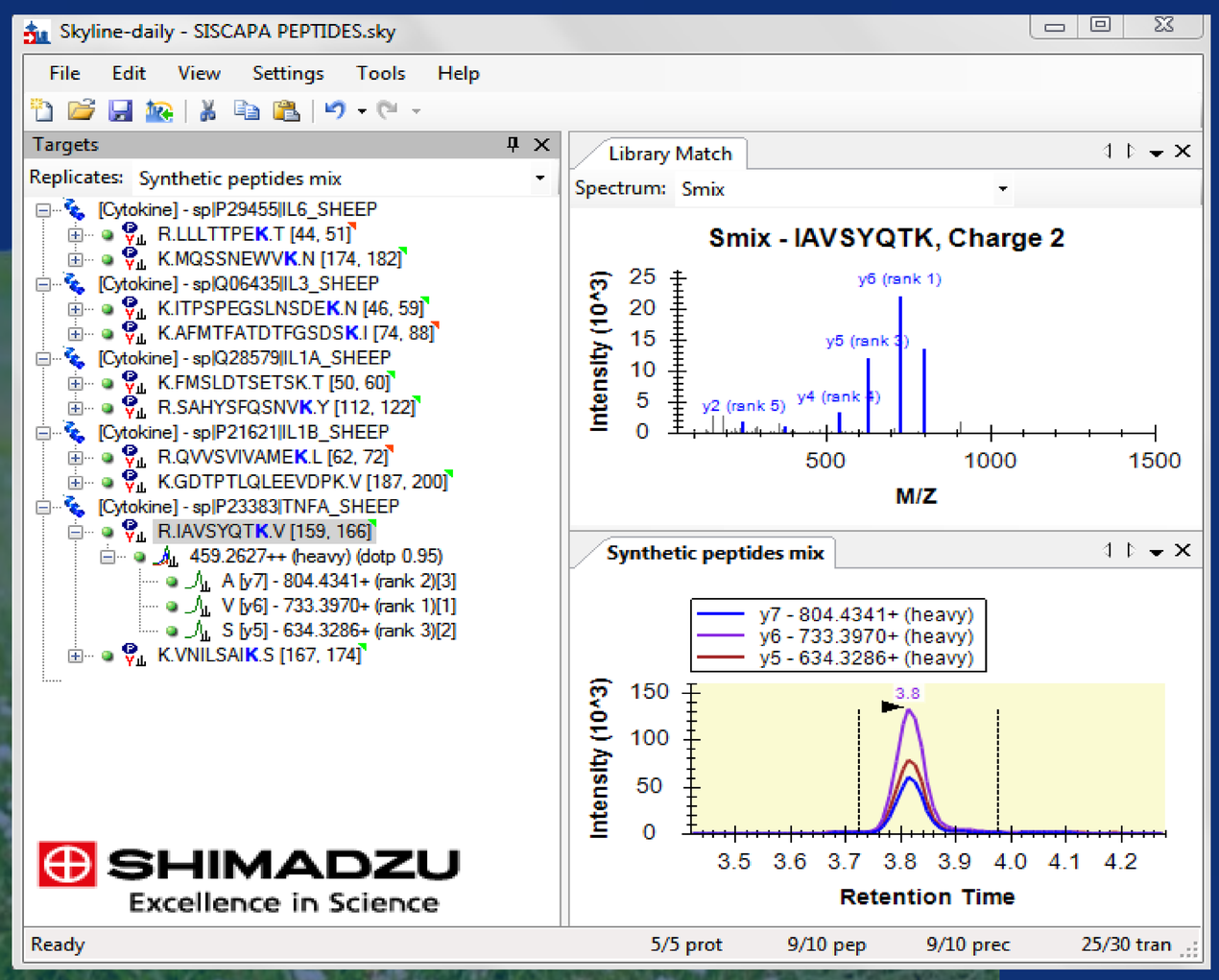

Figure 2. Skyline software considerably simplifies the development of peptide SID-MRM-MS assays using Shimadzu instrument available at QUT-CARF. The left panel shows a list of target peptides in a Skyline window. The upper right panel shows library spectrum generated using the 5600+ instrument for the highlighted heavy-labeled synthetic peptide. The lower right panel shows an MRM chromatogram for 3 transitions monitored using LCMS-8050 instrument for the same peptide.

FURTHER WORK

We are currently generating calibration curve data with and without sheep serum background to verify that the selected transitions can be used for quantitation of selected putative biomarkers in the biological samples.

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