Evaluation of cross-instrument data for SWATH peptide library construction and quantification

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Introduction

Data independent mass spectrometry such as SWATH requires a spectral library with matching retention times to extract peak areas to identify and quantify peptides. Because the library needs to be comprehensive for discovery purposes and extensive data acquisition using the same mass spectrometer is not always possible, it is desirable and sometimes necessary to use LC-MS/MS data obtained from different mass spectrometers. We have previously described the SwathXtend bioinformatics tool to accommodate this application [1, 2]. In this presentation, we demonstrate the use of SwathXtend to process SWATH data using libraries built from different MS instrument architectures.

Testing Samples

- *Staphylococcus aureus* cultured in RPMI or in Serum;
- Samples are tryptic digested and desalted

SWATH experiment aim

Identify proteome profile differences due to cell culture buffer differences

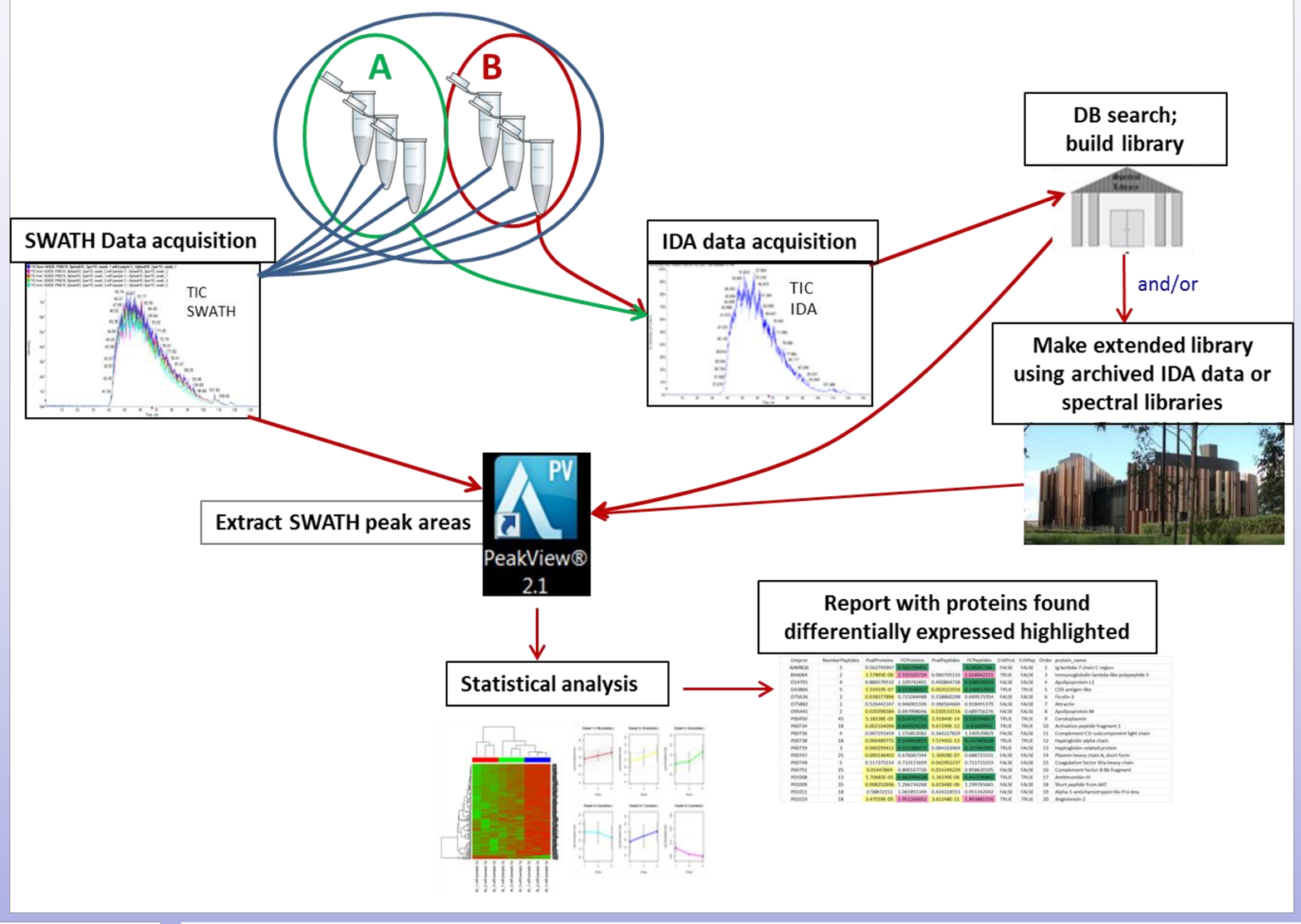
SWATH and 1D LC seed library data (APAF)

- Acquired using a 6600 TripleTOF (SCIEX) coupled with Eksigent nanoLC;
- Processed using ProteinPilot and PeakView

2D LC external library data (MBPF)

- Acquired using QExactive Plus coupled with Dionex Ultimate 3000 RSLCnano;
- Processed using MaxQuant and Spectronaut

SWATH Workflow for Discovery



Correlations between seed and external libraries

Seed Lib Peptides	Addon 2D Lib Peptides	Overlap Peptides	Seed Lib Proteins	Add on Proteins	Overlap Proteins	R2	RMSE	RIICor. median
2206	58285	2022	528	2365	526	0.95	2.43	0.714

Peptide relation time mapping

SWATH extraction results from 1D library and extended 2D library Compared by number of samples passing FDR<0.01

Quantification consistency measured by peak area CV using extended library

Differentially expressed proteins detected by 1D seed and extended libraries

All commonly detected up regulated and down regulated proteins are consistent for the two library processed results.

Conclusion

- MS/MS spectral libraries generated by QExactive Plus mass spectrometer (Thermo) can be added to seed library generated by 6600 TripleTOF mass spectrometer (SCIEX) to extract SWATH data. The extended library increased the depth of proteome profile analysis with consistent quantification results.
- SwathXtend was used to combine seed and external libraries.
- Differentially expressed proteins between two cell culturing conditions were successfully detected using extended library.
- This test proved that archived spectra data can be used to extend SWATH spectra library even for data acquired using different types of mass spectrometers.

References

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2. Jemma X. Wu, Dana Pascovici, Vera Ignjatovic, Xiaomin Song, Christoph Krisp and Mark P. Molloy* Improving Protein Detection Confidence Using SWATH-Mass Spectrometry with Large Peptide Reference Libraries, Proteomics 2017, 17, 1700174, DOI: 10.1002/pmic.201700174

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