Multi-Attribute Method reporting with Panorama and Skyline

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http://panoramaweb.org/

Overview

Panorama is a web-based data management system for targeted mass spectrometry data. It integrates closely with the popular Skyline desktop analysis tool. The Multi-Attribute Method (MAM) is designed for improved simultaneous detection, identification, quantitation, and quality control (monitoring) of molecular attributes. Panorama has recently added MAM-specific reporting to its existing set of analytics, offering immediate results and data sharing to any imported data. Additionally, Panorama and AutoQC's automated workflow provide longitudinal tracking of MAM-related metrics for QC purposes.

Introduction

Create Skyline document

- Skyline, a Windows application, supports all major mass spec vendors
- The document captures the peptides, transitions, and modifications to be monitored

Set up Panorama folder

- Panorama organizes data into folders, typically one per instrument being monitored
- A new MAM-oriented variant includes predefined reports that are unique to MAM analysis

Analyze and integrate results

- Web-based interface is ideal for sharing collaborators, including non-Skyline users
- Results can be easily leveraged elsewhere, such as Spotfire visualizations or tracking via Panorama's system suitability monitoring

Methods

Panorama uses a relational database to store targeted mass spectrometry data, importing data from Skyline's XML file format. Panorama has long-offered a variety of views and reports over this data, and has now added standardized versions of MAM reports such as peptide maps and the percentage of a peptide with post-translational modifications across a variety of experimental conditions. Users can now configure how peptides are grouped, allowing this type of reporting for sequence variants and other non-PTM scenarios as well.

Results

Skyline document

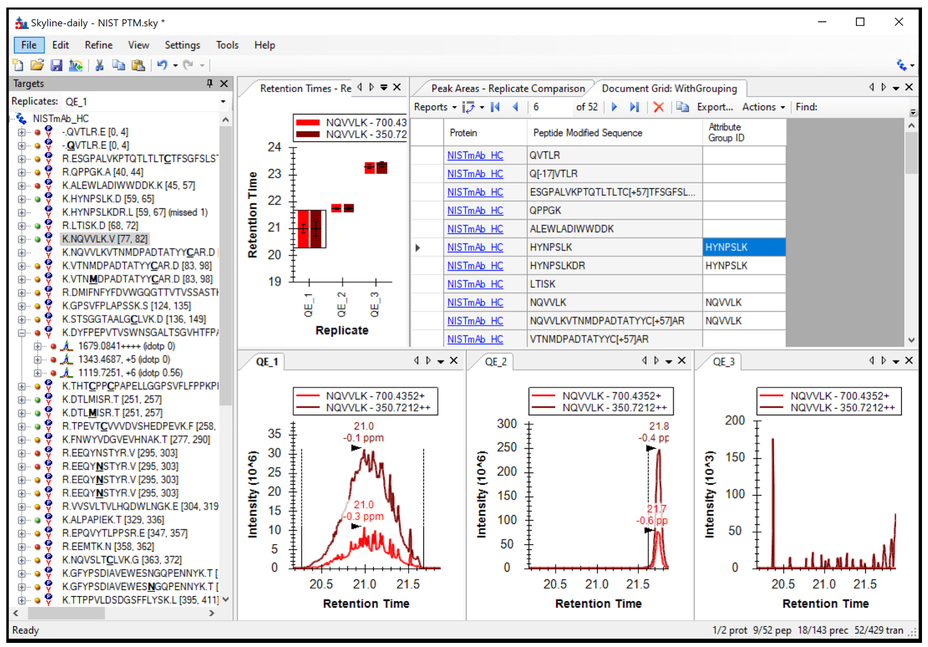

Figure 1: Skyline lets users easily set up the proteins and peptides to analyze, and pull in raw data from all of the major mass spec vendors without needing to do a separate conversion step. Here, a Skyline document shows a reference NIST monoclonal antibody (Dong et al) results as analyzed on a Orbitrap Lumos (Levy et al). The document grid in the upper right includes the Attribute Group ID column, which lets users associate peptide variants that do not share a common unmodified sequence. Here, the user has manually grouped four peptides into two different groups to capture missed cleavages as a single group.

Panorama Multi-Attribute Method folder

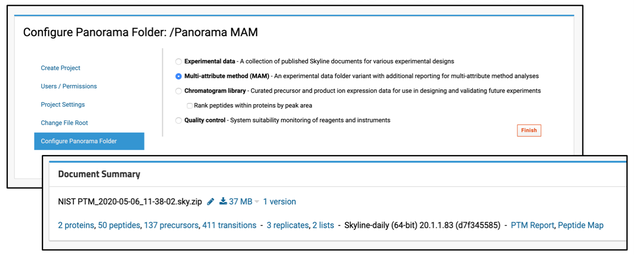

Figure 2: Panorama organizes data into folders, and offers different folder types that are optimized for different workflows and analyses. A new MAM folder type introduces predefined reports guides the user to them through links in the Document Summary view.

Post-translational modification report

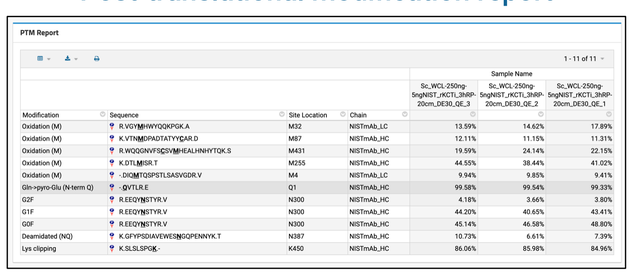

Figure 3: The post-translational modification (PTM) report shows the proportion for each peptide variant's peak area across samples. Panorama automatically groups peptides with identical unmodified sequences, but a user can configure alternative groupings within the Skyline document, to group splice variants, for example.

Peptide map report

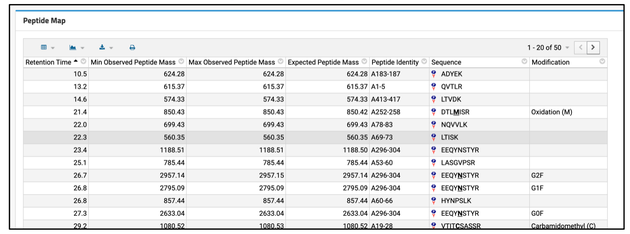

Figure 4: The peptide map report shows the observed peptides in the MAM analysis, sorted by retention time. It compares the observed and expected masses, and the modifications for each peptide variant.

Additional reporting

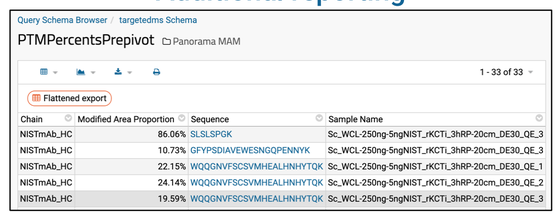

Figure 5: The Panorama team is adding more MAM-focused reporting. Additionally, because Panorama uses a relational database for its storage, the data is easily presented in alternative formats, integrated with other data sources, and made available to other tools. For example, PTM percentages can be used as a metric in a Panorama QC folder for system suitability monitoring. Data can be pulled into Spotfire, Tableau, or R for additional visualization.

Conclusions

Groups first started creating MAM-related reports in Panorama via custom SQL queries in 2015, but these were not built-in as standard reports or widely distributed. As interest in MAM has grown, the Panorama team saw the need to generalize them and make them broadly available. The initial set of reports include post-translational modification percent and peptide map reports. A collaboration between users and the Skyline and Panorama development teams resulted in new capabilities to override how peptides and their variants are grouped when calculating the percentage that each variant represents of the whole. This flexible approach means that users can either use the default, which groups all PTM variants together, or group based on sequence variants or other factors. Supporting these reports directly also means that database schema changes and precalculation can speed up query times by 20x or more. Additionally, because Panorama can track a wide variety of data for automated longitudinal tracking, these MAM-related metrics can be easily incorporated into QC folders where they can be analyzed with statistical process control techniques like Levey-Jennings, CUSUM, and moving range plots. As of January 2020, more than 400 labs are using Panorama projects free of charge to manage targeted mass spectrometry assays on http://panoramaweb.org/, a server hosted by the MacCoss lab at the University of Washington. Additionally, major pharmaceutical companies and other organizations have deployed their own in-house installations of Panorama.

References

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