Absolute Quantification using Targeted Mass Spec Assays with Panorama and Skyline

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Panorama

http://panoramaweb.org/

Overview

Panorama's new support for absolute quantification further extend its capabilities for managing targeted mass spectrometry assays, including data management and visualization, and secure sharing with internal and external collaborators. The popular Skyline targeted mass spec desktop application added support for quantification assays in 2016, and this recent work closely integrates Panorama, ensuring that the full analysis capabilities are available in both environments. Additionally, Panorama's improved plotting and analysis tools facilitate downstream analytics in interactive charts or via custom scripts in languages like R. Developed as a module in the LabKey Server web-based data management platform, Panorama is freely available, open source and may be installed and managed by any laboratory or organization. Free, hosted Panorama projects are also available on panoramaweb.org.
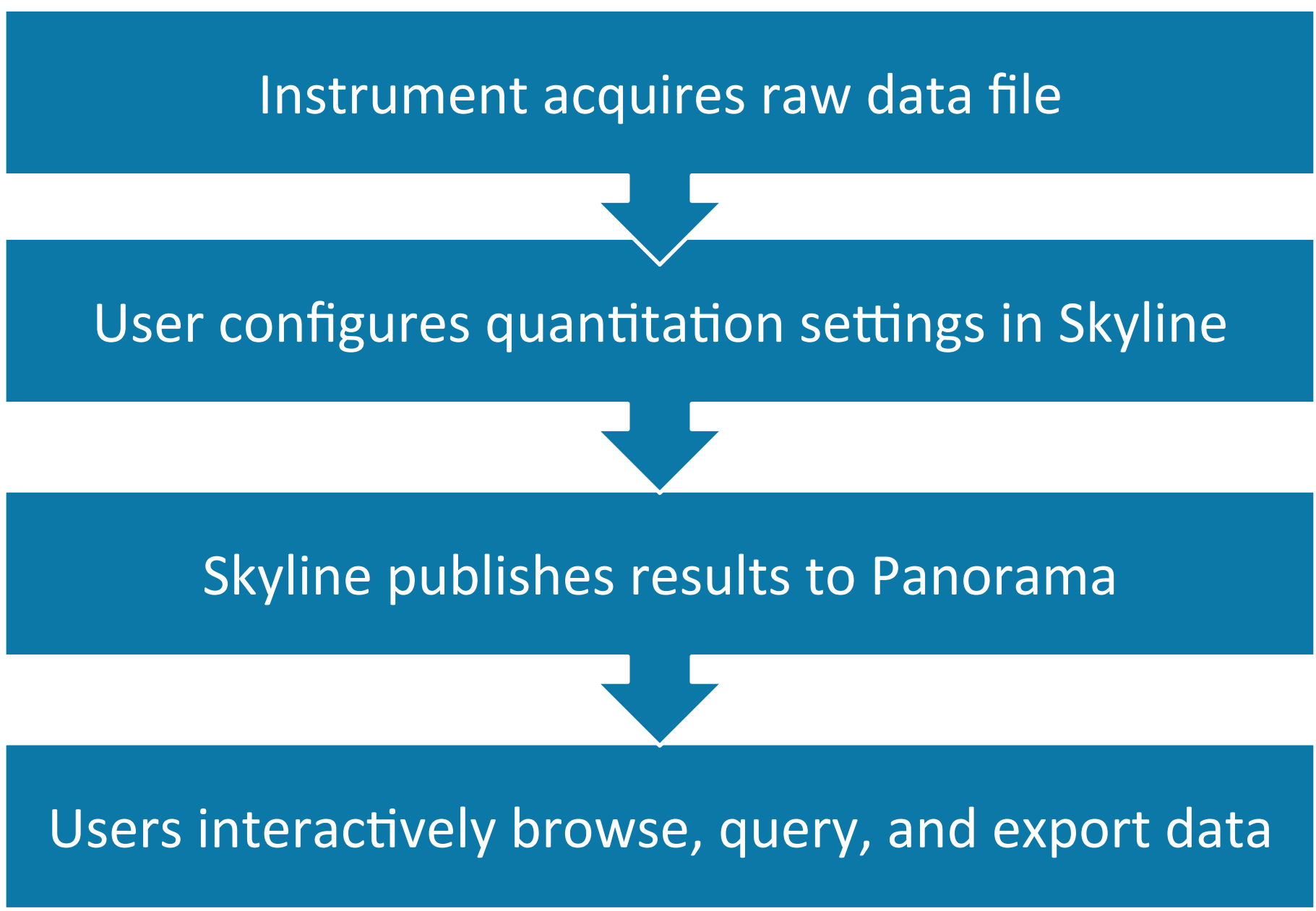
Introduction

ANALYSIS WORKFLOW

Figure 1: The workflow begins with data acquisition. Users configure Skyline with the calibration curves, QC samples, and unknowns for a given assay design, and then use Skyline to analyze the file and publish to Panorama. Panorama uses the same algorithm as Skyline to perform the regression and fit QC and unknown samples to the curve, making it available for reporting, plotting, and querying.

Methods

Skyline and Panorama combine to provide an integrated workflow for managing and analyzing LCMS-based quantitation assays experiments. Skyline captures regression fit parameters, which are then used by both Skyline and Panorama. Panorama utilizes a variety of JavaScript libraries including D3 to provide built-in visualizations, and includes wizards to configure custom reports. LabKey Server's client APIs also enable developers to create their own types of visualizations and reports, add their own analysis algorithms in languages like R, JavaScript, and Java, and enable automated or manually curated analysis.

All tools are released under the Apache 2.0 open source license.

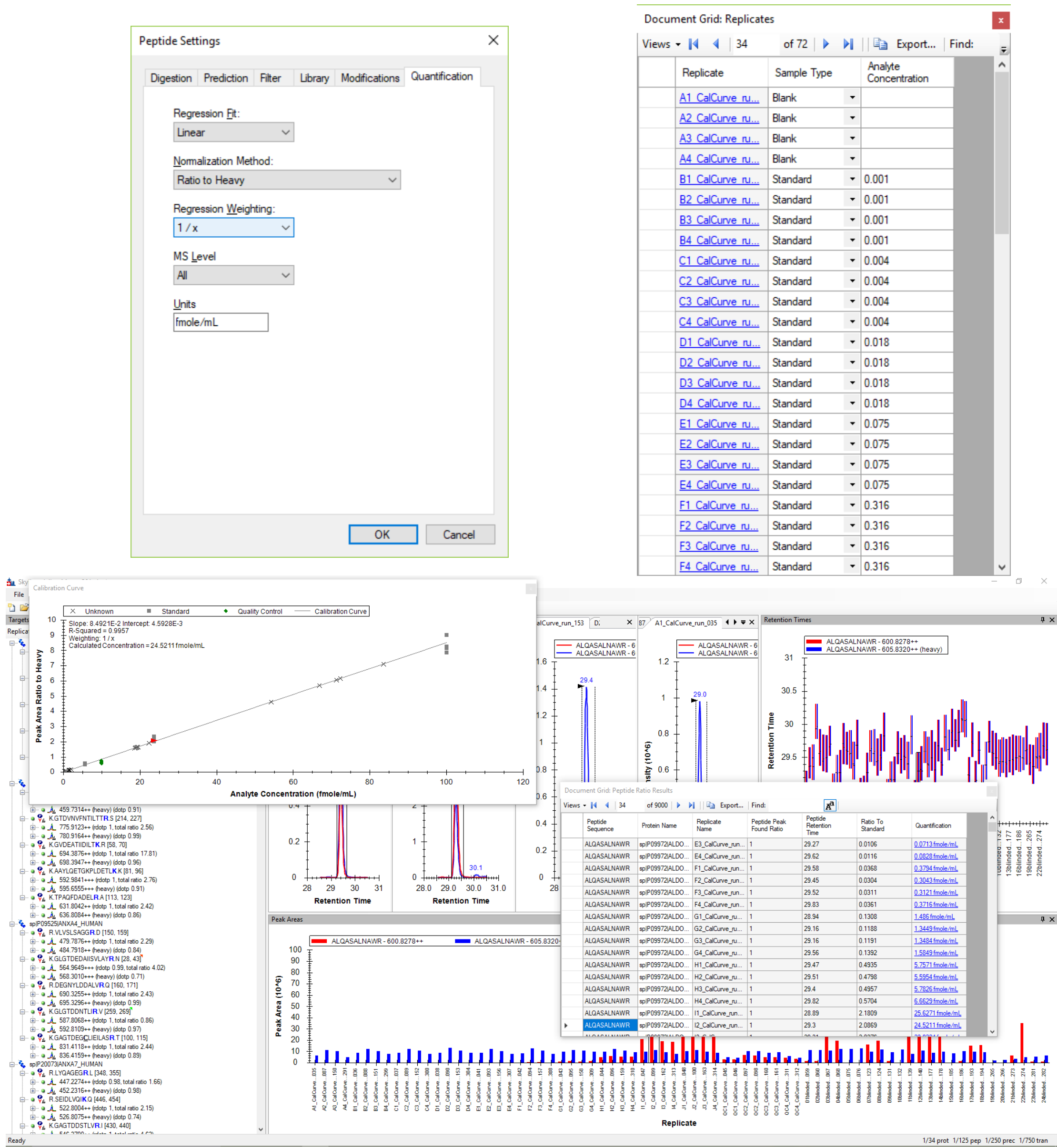## Results

CONFIGURE QUANTITATION IN SKYLINE

Figure 2: Calibration curves are defined within Skyline. Users select regression fit options, and configure which peptides or small molecules to use for fitting the curve. Data shown is from CPTAC Study 9.1, site 56A taken from PanoramaWeb.org.

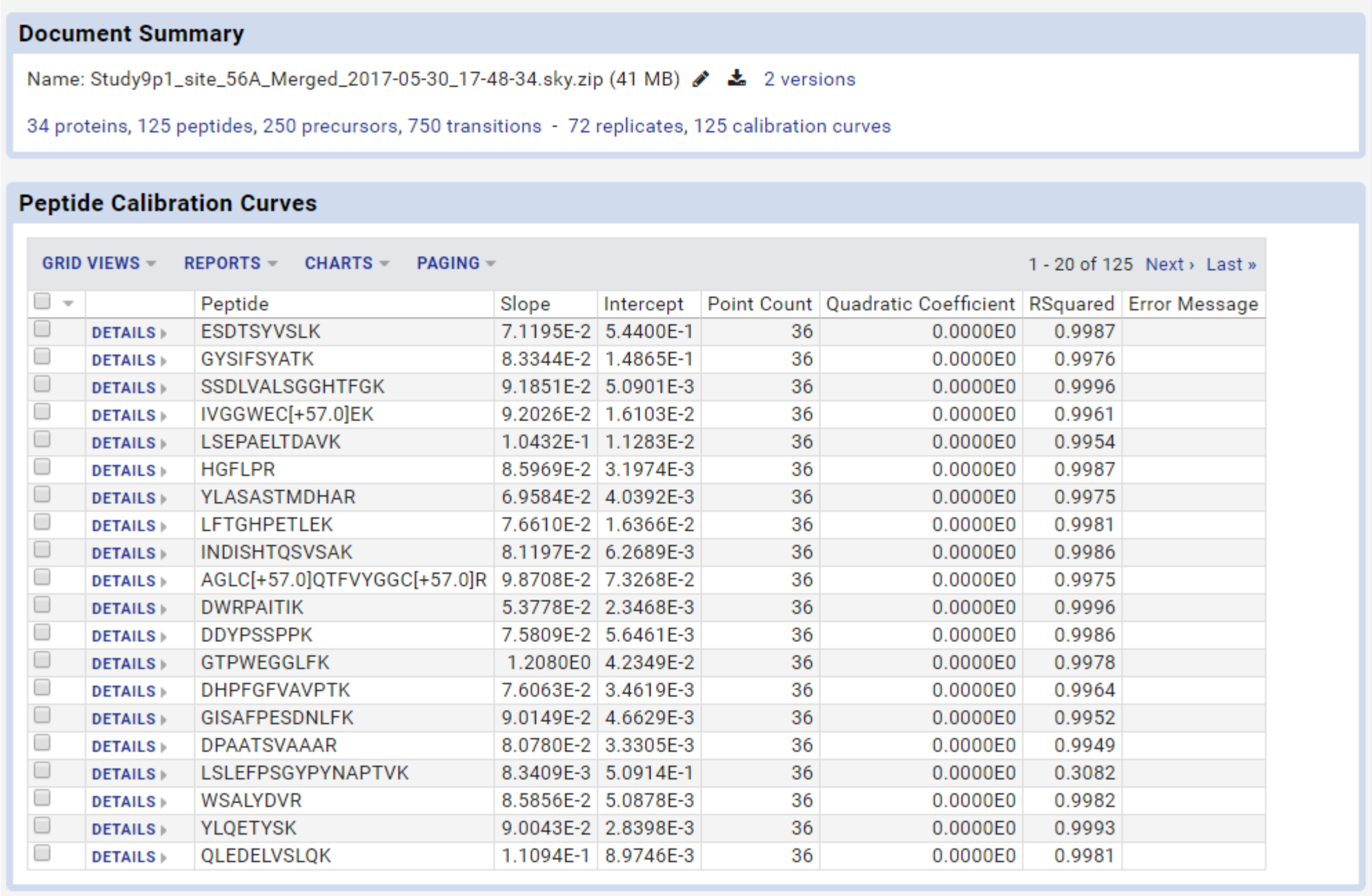### PUBLISH TO PANORAMA AND VIEW DATA

Figure 3: Panorama shows a revamped document summary, including counts of key data while using less screen space and offering simpler navigation. Calibration curves and replicates are now both included, and clicking on any of the counts shows a grid with all of the related data. Calibration curves show the calculated regression, or any problems the fit. This screenshot shows results from a linear fit, as previously configured in Skyline.

SEE ALSO

WP 320 - TargetedMSQC: an R package for peak quality assessment and interference detection in targeted mass spectrometry proteomics datasets
Shadi Toghi Eshghi; Kristin Wildsmith; Paul Auger; W. Rodney Mathews
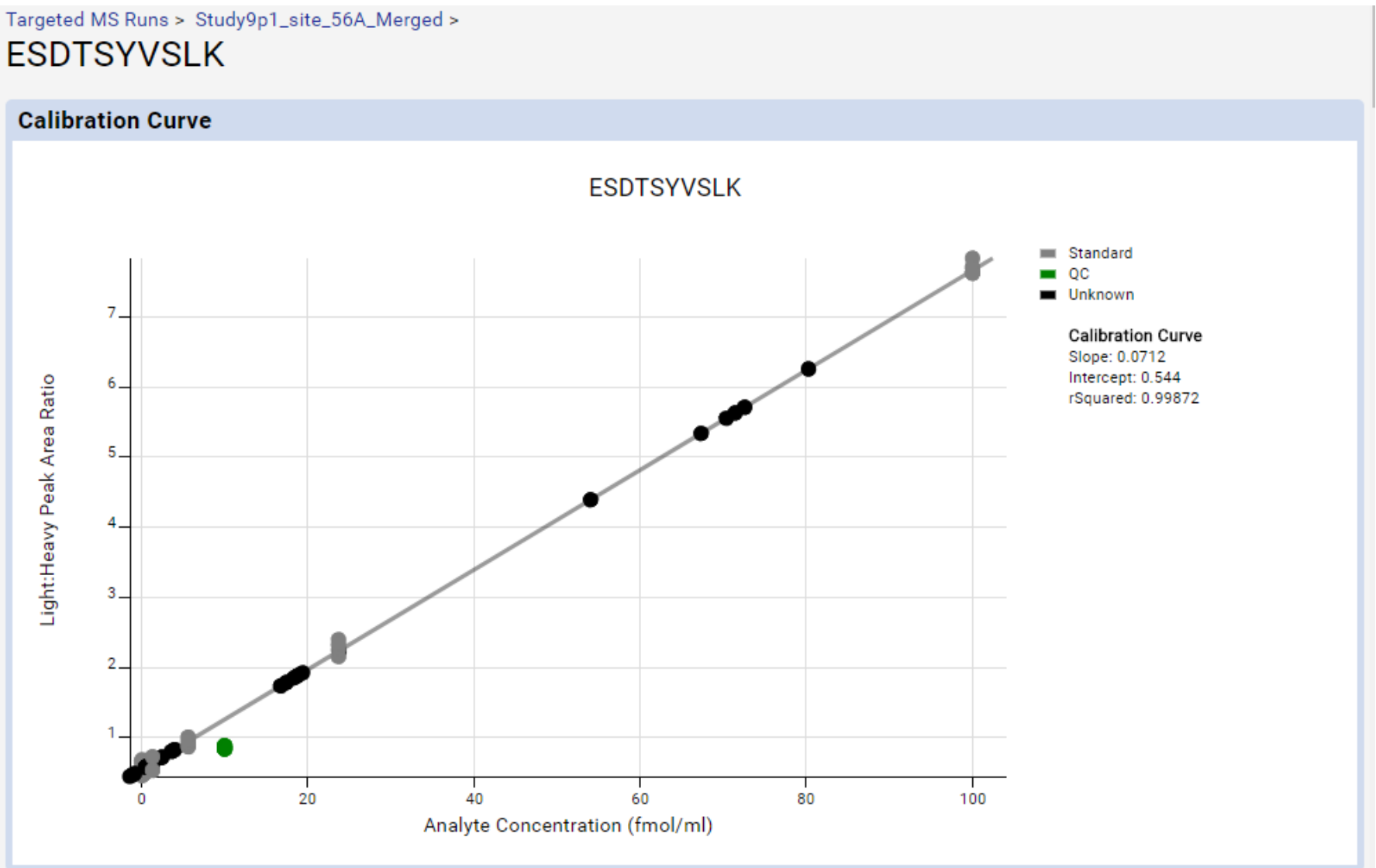
VIEW CALIBRATION CURVES

Figure 4: Panorama renders web-based plots, familiar to Skyline users. They can also be exported to PDF or PNG format for embedding in presentations or publications. This plot shows an example peptide from a document that contains only standards.

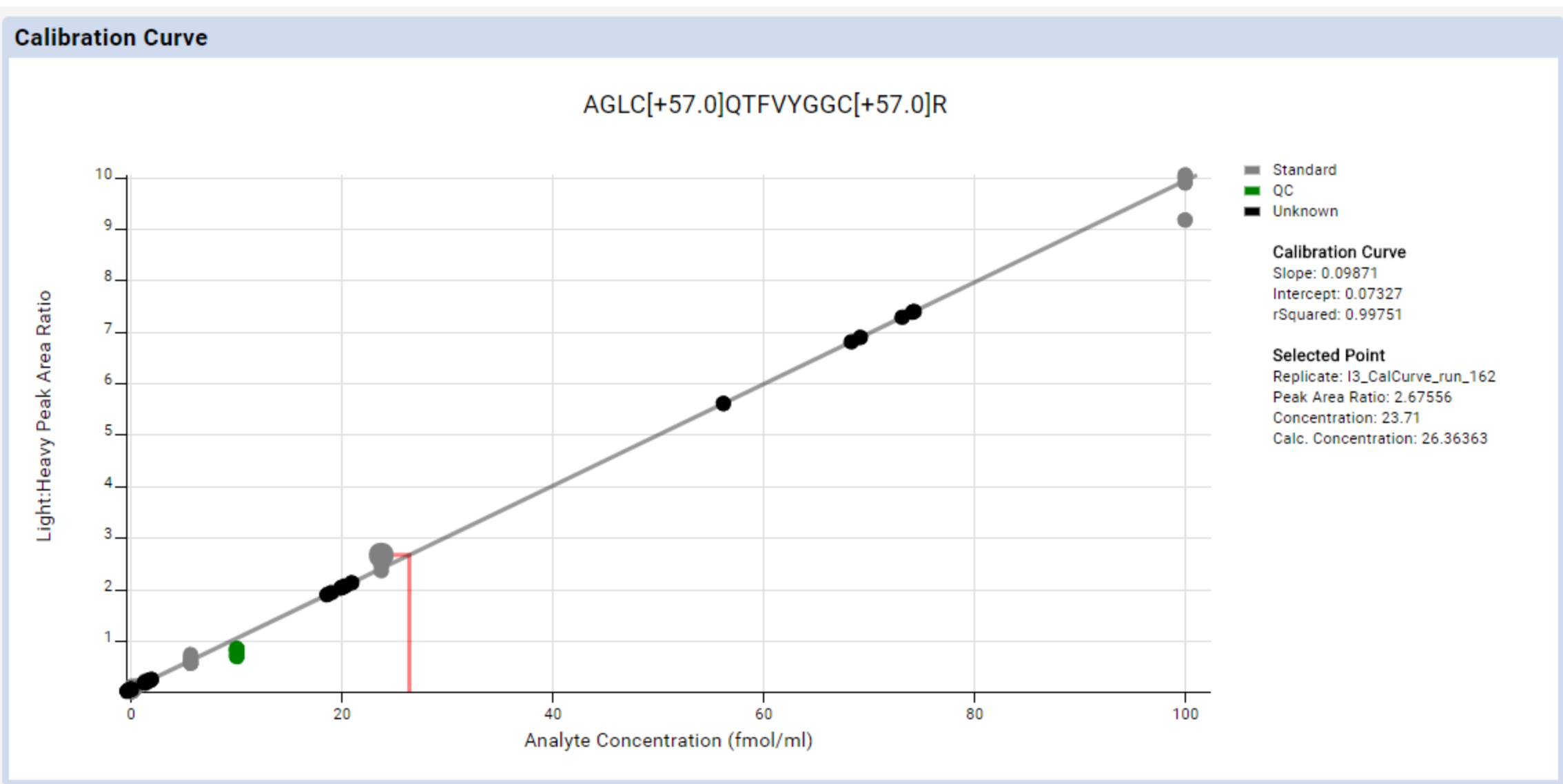### INTERACT WITH PLOTS AND DATA

Figure 5: This plot shows data for a small molecule, including standards, QC, and unknowns. Clicking on any data point shows details on how it intersects the regression fit.

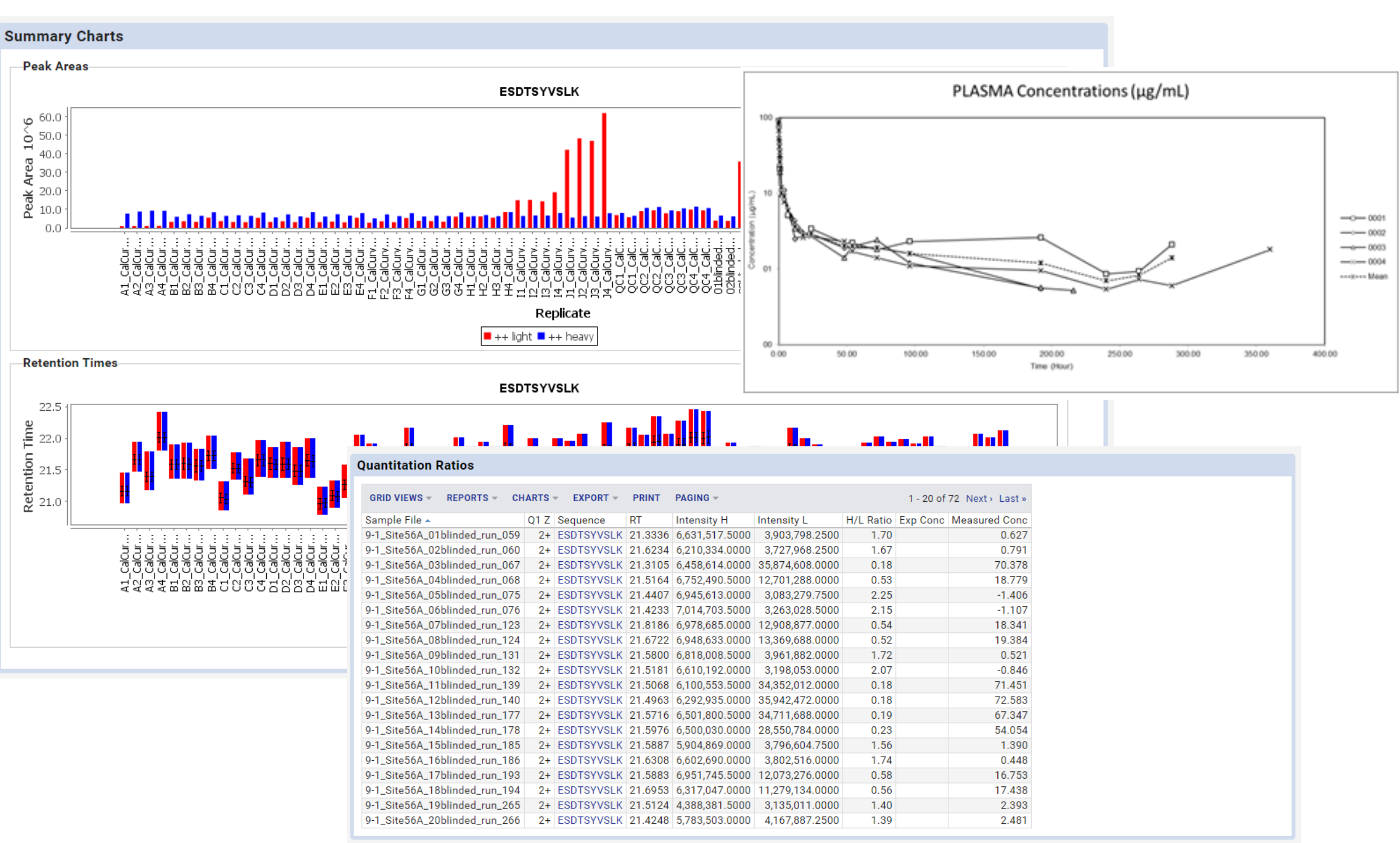### CREATE CUSTOMIZED DASHBOARDS

Figure 6: Panorama offers a wide range of reporting options. Many commonly used plots and reports are pre-built and immediately available, while assay-specific reports, leveraging customizable entries like replicate annotations, can be easily configured.
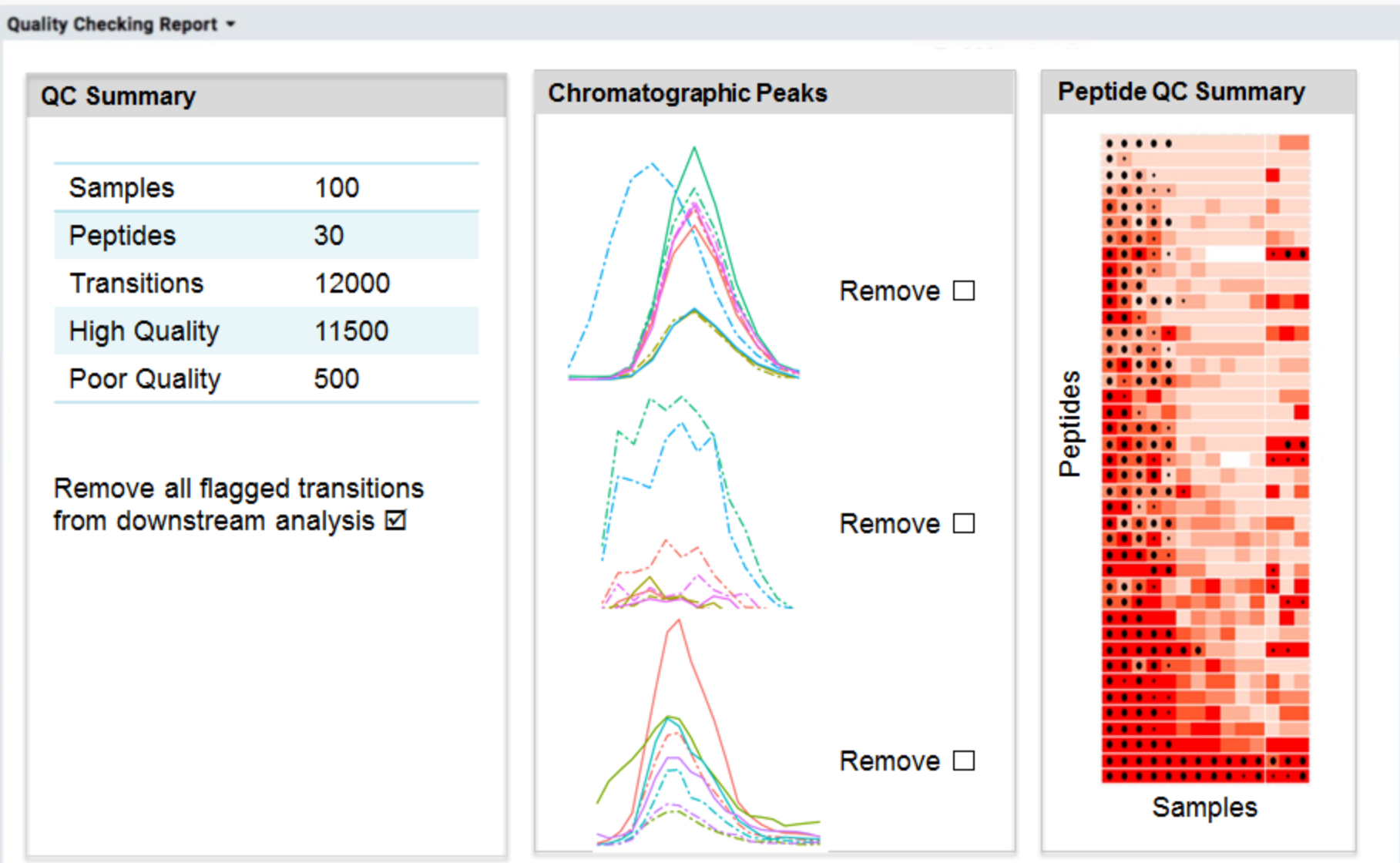
BUILD CUSTOM WORKFLOWS

Figure 7: In addition to custom reports, Panorama leverages LabKey Server's full APIs and module architecture, enabling the development of rich downstream workflows. Here, custom visualizations combine with tools to automatically and manually perform quality control on chromatographic peaks, currently in development by Shadi Toghi Eshghi at Genentech.

Other recent Panorama work includes additional built-in quality control visualizations such as moving range and CUSUM plots, performance improvements, and more.

Conclusions

Panorama now imports quantification data as configured in Skyline, loading it into its relational database. Panorama can also perform curve fit and other calculations using the same algorithms as Skyline, enabling interactivity with the data, as well as enabling custom analysis scripts in R and other programming languages to make further downstream use of the information. Additionally, new data views within Panorama generate figures of merit and assist users in performing quality control of their quantitative results. Panorama has also been recently optimized to better facilitate large and complex targeted mass spec datasets. More than 200 labs are using Panorama to manage targeted mass spectrometry assays on the panoramaweb.org server hosted by the University of Washington. Additionally, major pharmaceutical companies and other organizations have deployed their own in-house installations of Panorama.

References

Sharma V, MacLean B, et al. J. Proteome Res. 2014/08; 10.1021/pr5006636.
Abbatiello S, Carr S et al. Mol & Cell Proteomics. 2015/02; mcp.M114.047050.
Bereman MS, MacCoss MJ et al. J. Proteome Res. 2016 / 15; 10.1021/6b00744