Repository for
Targeted Proteomics Assays

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Skyline Users Group - June 9, 2013
Goals

• Organize and reuse targeted assays
  – Search and review a large body of data
  – Use previous results to inform new experiments

• Facilitate data sharing
  – Private, with access for internal/external collaborators
  – Public, for published data

• Excellent integration with Skyline
  – Import and retain the complete data model
  – Support data from various workflows
Skyline/Panorama Workflow
History

• Work started in late 2011
• Announced at ASMS 2012
• Publicly released in December 2012
  – Part of LabKey Server version 12.3
• Released to PanoramaWeb.org in February 2013
  – Hosted server managed by MacCoss Lab
  – Recorded webinar has full details
• Second public release in April 2013
  – Added search features, reporting enhancements, fixes
• Next release scheduled for July 2013
LabKey Server

- Open source (Apache 2.0 license)
- Web user interface
- Powerful security and permissions system
- Other assay data
  - MS/MS peptide search
  - Flow cytometry
  - Sequencing
  - Plate-based assays
- Specimen tracking
- Clinical study data
- Collaboration tools
- Extensive client APIs
LabKey Software

• Co-developers with MacCoss Lab on Panorama
• Consulting company focused on LabKey Server
  – 25 employees in Seattle and San Diego
  – 8 years history working with scientists
• Strength in computer science and data management
• Partner with researchers to develop software
• Related service offerings include:
  – Training
  – Technical support
  – Customization
  – Hosting
Panorama - Setup

Configure Targeted MS Folder

- **Experimental data** - a collection of published Skyline documents for various experimental designs
- **Chromatogram library** - curated precursor and product ion expression data for use in designing and validating future experiments

- Rank peptides within proteins by peak area

**Finish**
Publishing to Panorama - Setup

Publishing to Panorama
Viewing Runs - Precursors

### Document Summary
- **Name**: Stergachis - Supplementary Data 2_2013-06-02_17-14-10.zip
- **Protein Count**: 96
- **Precursor Count**: 1475
- **Peptide Count**: 1475
- **Transition Count**: 10806

### Precursor List

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Peptide Annotations</th>
<th>Missed Cleavages</th>
<th>Peptide Neutral Mass</th>
<th>Rank</th>
<th>Precursor</th>
<th>Precursor Annotations</th>
<th>Label</th>
<th>Precursor Neutral Mass</th>
<th>Q1 m/z</th>
<th>Q1 Transition Count</th>
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### Table Headers
- **Peptide**: The amino acid sequence of the precursor.
- **Peptide Annotations**: Quality Score and number of missed cleavages.
- **Peptide Neutral Mass**: The calculated mass of the peptide.
- **Rank**: The rank of the precursor in the list.
- **Precursor**: The name of the precursor.
- **Precursor Annotations**: Details about the precursor.
- **Label**: The label associated with the precursor.
- **Precursor Neutral Mass**: The calculated mass of the precursor.
- **Q1 m/z**: The mass-to-charge ratio of the first fragmentation.
- **Q1 Transition Count**: The count of transitions for the first fragmentation.
Viewing Runs - Transitions
Viewing Proteins
Viewing Peptides

Peptide Summary
- Name: Human_plasma.zip
- Group: A2MG
- Sequence: SSSNEEVMFLTVQVK
- Neutral Mass: 1696.8342
- Avg. RT: 26.6774
- Predicted RT: 26.3900
- Precursors: SSSNEEVMFLTVQVK - 853.4315++ (heavy)

Chromatograms
- A1 SSSNEEVMFLTVQVK
- A1 SSSNEEVMFLTVQVK - 849.4244++
- A1 SSSNEEVMFLTVQVK - 853.4315++ (heavy)

Peak Areas
- SSSNEEVMFLTVQVK
- Group By: Condition
- CV Values: []
- Width: 500
- Height: 300
- Annotation: ++ heavy ++ light
Protein and Peptide Search

Mass Spec Search

Protein Search Peptide Search Modification Search

Protein Name *?
Search in subfolders?

SEARCH

Mass Spec Search

Protein Search Peptide Search Modification Search

Peptide sequence *?
Exact matches only?
Search in subfolders?

SEARCH

Targeted MS Peptides

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Distinct Count: 1

Distinct Count: 2
Modification Search

Targeted MS Modification Search

- Search By:
  - Delta Mass
  - Modification Name

- Type:
  - Names used in imported experiments
  - Common Unimod modifications
  - All Unimod modifications

- Include:
  - Structural
  - Isotope Label

Custom Name:

Search in Subfolders:

- Label: 13C(6)15N(2)
- Carbamidomethyl (C)
- Heavy K
- Heavy R
- Label: 13C(6)15N(4)
- Phospho (S,T)
- Phospho (Y)

Search

Targeted MS Peptides

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Customizing Views, Sorts, Filters

Targeted MS Peptides

**Available Fields**
- Protein / Label
- Peptide
- Precursor
- File
- Predicted RT
- Decoy
- Score
- RT
- Predicted RT
- Avg. Measured RT
- Missing Cleavages
- Mass
- Neutral Mass

**Selected Fields**
- Predicted RT
- Decoy

**Targeted MS Peptides**

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<th>Protein / Label</th>
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Export and Client APIs
Permissions

Permissions for /MacCoss/brendanx

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<tr>
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<tr>
<td><strong>Author</strong></td>
<td>MACCOSS MEMBERS, <a href="mailto:ABSTERGA@UW.EDU">ABSTERGA@UW.EDU</a></td>
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<td><strong>Assay Designer</strong></td>
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</table>

* indicates permissions are inherited
Chromatogram Library Export

Library Statistics:
The library contains 270 proteins with 1,762 ranked peptides. The 1,762 ranked peptides contain 12,173 ranked transitions.

Download Library:

Revision 9
ARCHIVED REVISIONS

You have chosen to open:

- Rat_rev9.clib
  which is a .clib File
  from: https://panoramaweb.org:9443

What should Firefox do with this file?

- Open with Browse...
- Save File
- Do this automatically for files like this from now on.
Chromatogram Library Import
Chromatogram Library Usage

The image shows a software interface with chromatogram data. The interface includes a table of targets and a graph with retention time on the x-axis and intensity on the y-axis, comparing in vivo versus in vitro samples. The graph displays peak areas and intensity data for different samples, indicating the usage of the Chromatogram Library for analysis.
How to Get Panorama

• PanoramaWeb (panoramaweb.org)
  – Hosted at University of Washington
  – Request access for a lab or organization
  – Data can be private or public

• Create your own installation
  – Download and documentation at labkey.com
  – Install on Windows, Linux, OSX, etc

• Use LabKey Software’s private hosting
Future Plans

• Release chromatogram library support
  – July 2013
  – Will coincide with new Skyline release
• Store and calculate normalized retention times (iRT)
• Store and view QC runs
• Show more Skyline graphs
• Support response curves with QuaSAR
More Information

• Poster tomorrow
  – Sharing Targeted Proteomics Assays using Skyline and Panorama
  – MP 382

• panoramaweb.org
  – View recorded webinar
  – Request a project

• labkey.org/labkey.com
  – General information
  – Downloads for installers and source code
Questions

Josh Eckels
jeckels@labkey.com

Skyline Users Group - June 9, 2013