A Workflow for Quality Assessment, Quantitation and Statistical Inference of Targeted Proteomics Data using Skyline and Panorama

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Skyline User Meeting
June 4, 2017
Data analysis is a challenge for development of clinical biomarkers using targeted mass spectrometry

Drug development

<table>
<thead>
<tr>
<th>Research</th>
<th>Pre-IND</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III (Pivotal)</th>
<th>Clinical Practice</th>
</tr>
</thead>
</table>

An ideal data analysis pipeline would be:

- High throughput
- Reproducible
- Objective
- Automated

Targeted MS for development of clinical biomarkers

- 100 patients
- 2 timepoints
- 40 target analytes
- 5 transitions per analyte
- 8,000 peak groups
- 40,000 transitions

Triple Quad (High sensitivity)
Panorama and skyline simplify data management and analysis for targeted MS assays

- Centralized archiving model
- Distributed analysis model

JASMS, November 2016
Enabling targeted MS post-processing on Panorama centralizes data archiving and analysis

- Centralized archiving and analysis model
- Automated and objective QC
- Generation of results within minutes post data acquisition

JASMS, November 2016
Targeted MS data analysis pipeline

- Peak Integration
- Quality Checking
- Quantitation
- Statistical Inference
- Data Integration

Skyline

Skyline/Panorama (MP 637)

TargetedMSQC (WP 320) [Panorama compatible R package]

MSstats [Panorama compatible R package]
Samples 100
Peptides 30
Transitions 12000
High Quality 11500
Poor Quality 500

Remove all flagged transitions from downstream analysis ☑
**Profile Plots**

All proteins

<table>
<thead>
<tr>
<th>Sample</th>
<th>Condition</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0 h</td>
<td>0.13</td>
</tr>
<tr>
<td>B</td>
<td>48 h</td>
<td>0.48</td>
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</tbody>
</table>

Rv2031c_hspX

**Group Comparison**

48h-00h

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sample</th>
<th>Condition</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pep 1</td>
<td>A</td>
<td>0 h</td>
<td>0.13</td>
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<tr>
<td>Pep 2</td>
<td>A</td>
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<tr>
<td>Pep 1</td>
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<tr>
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</tbody>
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- Brendan MacLean (UW)
- Vagisha Sharma (UW)
- Nick Shulman (UW)
- Michel Petrovic (Roche)
- Daniel Spellman (Merck)

Related ASMS Posters:
- TargetedMSQC: WP 320
- Absolute Quant on Panorama: MP 637