



Skyline Tutorial Webinar #8

DDA to Targeted: Differential Statistics with Skyline

With
Brendan MacLean (Principal Developer)

Agenda

- ▶ Welcome from the Skyline team!
- ▶ **DDA to Targeted: Differential Statistics**
- ▶ Introduction with Brendan MacLean
 - ▶ Workflow and data set overview
- ▶ Tutorial with Brendan MacLean
 - ▶ DDA data processing review
 - ▶ Hypothesis generation from DDA data
 - ▶ Initial data review and Chorus Cloud Extraction
- ▶ Audience Q&A – submit questions to Google Form:
<https://skyline.gs.washington.edu/labkey/qa4skyline.url>



Chromatography-based Quantification

- ▶ Hypothesis testing (Verification)
- ▶ SRM
- ▶ **MS1 chromatogram extraction**
- ▶ Targeted MS/MS (PRM)
- ▶ Data independent acquisition (DIA/SWATH)



| Acquisition | Targeted | Survey |
|----------------|----------|------------|
| More Selective | PRM | DIA |
| Less Selective | SRM | MS1 |

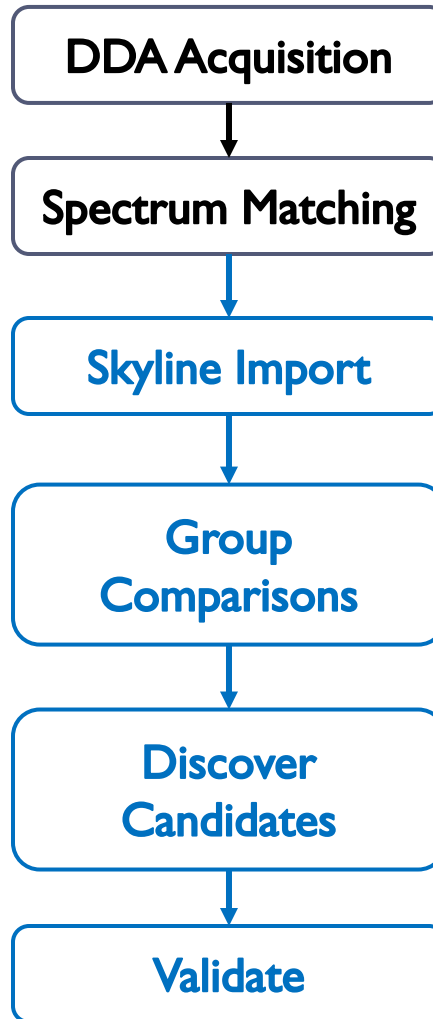
Got **HYPOTHESIS??**



Multiple Instrument Vendors



Discovery to Targeted with Skyline



Got HYPOTHESIS!!



Case Study: ABRF iPRG 2014

| | Fake Accession | Name | Origin | Molecular Weight |
|---|-----------------------|----------------------|---------------------|-------------------------|
| A | P44015 | Ovalbumin | Chicken Egg White | 45KD |
| B | P55752 | Myoglobin | Equine Heart | 17KD |
| C | P44374 | Phosphorylase b | Rabbit Muscle | 97KD |
| D | P44983 | Beta-Galactosidase | Escherichia Coli | 116KD |
| E | P44683 | Bovine Serum Albumin | Bovine Serum | 66KD |
| F | P55249 | Carbonic Anhydrase | Bovine Erythrocytes | 29KD |



Sample Preparation

| | A | B | C | D | E | F (fmol) | |
|----------|----|----|----|----|-----|----------|-----------------------|
| Sample 1 | 65 | 55 | 15 | 2 | 11 | 10 | + 200 ng yeast digest |
| Sample 2 | 55 | 15 | 2 | 65 | 0.6 | 500 | + 200 ng yeast digest |
| Sample 3 | 15 | 2 | 65 | 55 | 10 | 11 | + 200 ng yeast digest |



Group Comparisons

| | A | B | C | D | E | F | (fold change) |
|------------|------|-------|------|------|-------|-------|-----------------------|
| Sample 1-2 | 0.85 | 0.27 | 0.13 | 32.5 | 0.055 | 50 | + 200 ng yeast digest |
| Sample 1-3 | 0.23 | 0.036 | 4.33 | 27.5 | 0.91 | 1.1 | + 200 ng yeast digest |
| Sample 2-3 | 0.27 | 0.13 | 32.5 | 0.85 | 16.7 | 0.022 | + 200 ng yeast digest |



Group Comparison Maxima

| | A | B | C | D | E | F | (abs log2 fold change) |
|----------------|------------|------------|------------|------------|------------|------------|------------------------|
| Sample 1-2 | 0.2 | 1.9 | 2.9 | 5.0 | 4.2 | 5.6 | + 200 ng yeast digest |
| Sample 1-3 | 2.1 | 4.8 | 2.1 | 4.8 | 0.1 | 0.1 | + 200 ng yeast digest |
| Sample 2-3 | 1.9 | 2.9 | 5.0 | 0.2 | 4.1 | 5.5 | + 200 ng yeast digest |
| Maximum | 2.1 | 4.8 | 5.0 | 5.0 | 4.2 | 5.6 | |



DDA Runs Searched

| | Identified yeast proteins |
|------------|---------------------------|
| sample 1-a | 3016 |
| sample 1-b | 3073 |
| sample 1-c | 2905 |
| | |
| sample 2-a | 2916 |
| sample 2-b | 2984 |
| sample 2-c | 2907 |
| | |
| sample 3-a | 2883 |
| sample 3-b | 2972 |
| sample 3-c | 2913 |

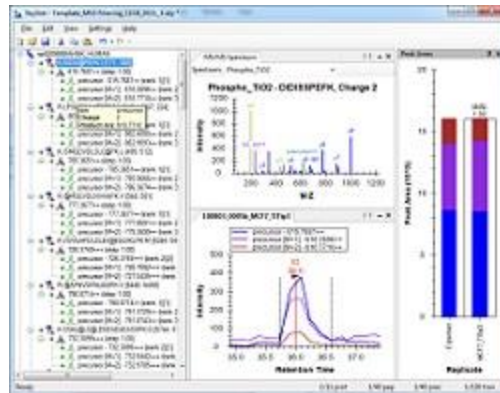
Comet, OMSA, MSGF+ - iProphet



Keys to Success with MS1 in Skyline

- ▶ Use Import DDA Peptide Search wizard
- ▶ Make sure you have ID annotations
 - ▶ Diagnose with Spectral Library Explorer
 - ▶ <http://tinyurl.com/Skyline-missing-ids>
- ▶ Review RT alignment in alignment viewer
- ▶ **Got HYPOTHESIS??**
- ▶ Review and manually adjust <5% of peaks

- ▶ Do the tutorial



Tutorial Discovery with Skyline

- ▶ Import DDA Peptide Search
- ▶ Data analysis



Four Processing Workflows Compared

- ▶ **Peptides adjusted p value 0.05 – remove single hits**
 - ▶ 6 proteins + 1 false discovery (easily discounted)
- ▶ **Peptides adjusted p value 0.01 – remove single hits**
 - ▶ 5 proteins (missing A)
- ▶ **Proteins adjusted p value 0.05**
 - ▶ 5 proteins (missing A) + 3 false discoveries (discounted)
- ▶ **Proteins adjusted p value 0.01**
 - ▶ 4 proteins (missing A & B) + 3 false discoveries (discounted)



Discovery versus Validation

- ▶ **Discovery**

- ▶ asking your data what changed

- ▶ **Validation**

- ▶ asking if there is evidence you candidates changed

Got HYPOTHESIS??



Learn More

- ▶ Webinars #1 (DDA) and #6 (Processing) - tutorials
- ▶ Webinar #9: TBD
 - ▶ Tuesday, July 14
- ▶ Workshop in Rio de Janeiro, August 31-September 2
- ▶ Workshop in Puerto Vallarta, November
- ▶ Weeklong Course at IIT-Bombay
 - ▶ December 10-14
- ▶ Weeklong Course in San Francisco
 - ▶ January?



Questions?

- ▶ Ask any questions you have on differential statistics at the following form:

<http://tinyurl.com/QA4Skyline>

- ▶ Take the post-webinar survey:

<http://tinyurl.com/Survey4Webinar>





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This ends this Skyline Tutorial Webinar.

Please give us feedback on the webinar at the following survey:

<http://tinyurl.com/Survey4Webinar>

A recording of today's meeting will be available shortly at the Skyline website.

We look forward to seeing you at a future Skyline Tutorial Webinar.

