Skyline Tutorial Webinar #6

Welcome to the sixth in a series of tutorial webinars designed to help you get the most out of Skyline proteomics software.

We'll begin shortly …

▸ **Webinar Audio:** <mark>Make sure you have audio during the webinar.</mark>

Click on the telephone icon in the JoinMe toolbar - **circled** here →
You then have the choice to either:
  ▸ Connect by phone (US and International numbers)
  ▸ Connect by internet – a short, temporary software download is required.

▸ **Submit Skyline Questions:** Use this form to submit Skyline questions to the team – we will try to answer as many as possible:

▸ The QA link will also be available in the chat window – to open your chat window click the bubble icon – **circled** here →

Skyline Tutorial Webinar #6
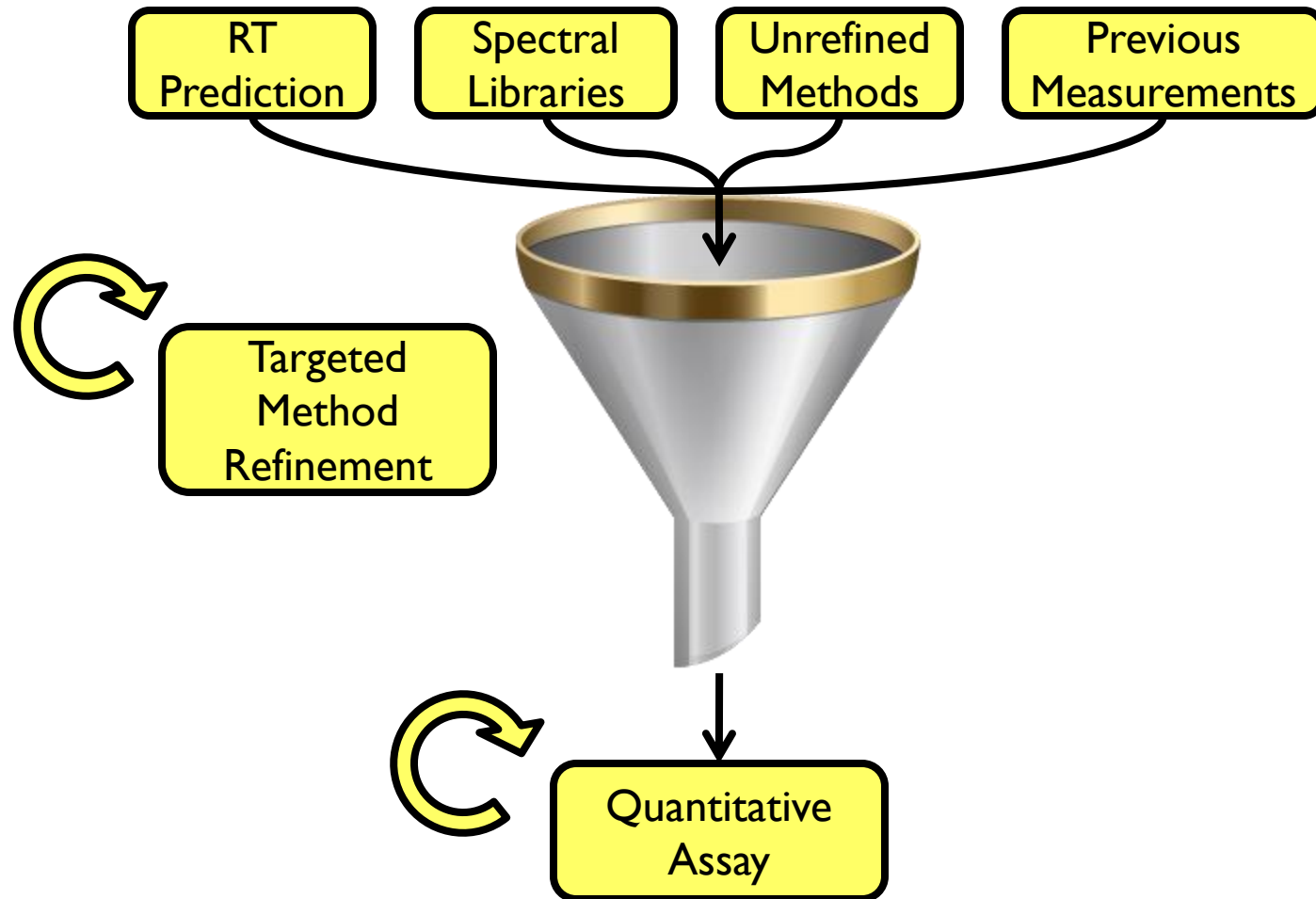
Effective Data Processing and Interrogation with Skyline
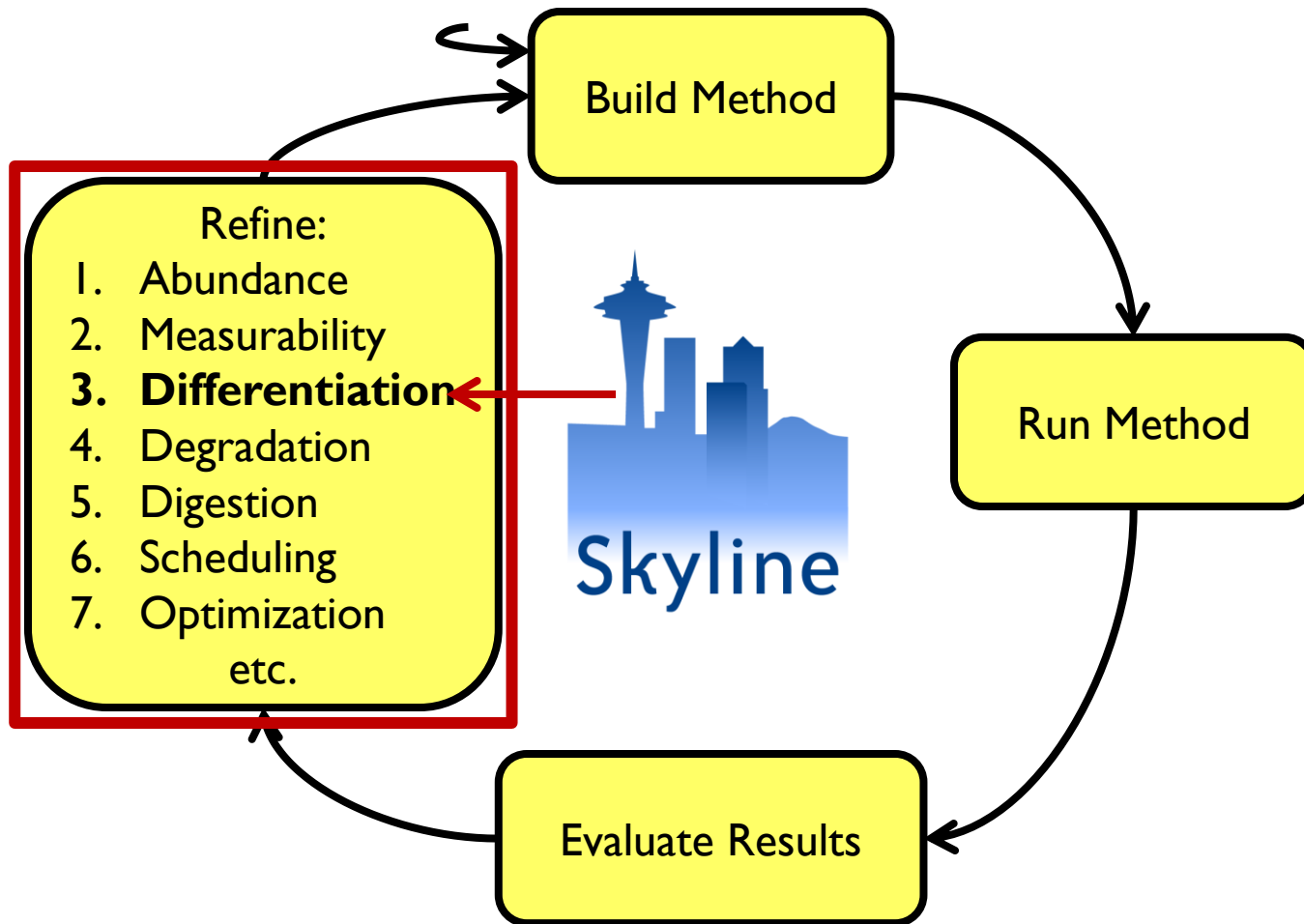
With
Brendan MacLean (Principal Developer)

Agenda

▸ Welcome from the Skyline team!

▸ **Effective Data Processing and Interrogation**

▸ Introduction with Brendan MacLean

  ▸ Brief overview of refinement by peptide/protein differentiation

▸ Tutorial with Brendan MacLean

  ▸ Effective grouped study data processing and QC

  ▸ Sneak peak: Group Comparison features in Skyline 3.1

▸ Audience Q&A – submit questions to Google Form:

http://tinyurl.com/QA4Skyline

Supporting a Broader Hypothesis

Targeted Method Refinement

Build Method

Refine:
1. Abundance
2. Measurability
3. **Differentiation**
4. Degradation
5. Digestion
6. Scheduling
7. Optimization
   etc.

Skyline

Run Method

Evaluate Results

Identifying Plasma Proteins with Altered Levels in a Dahl Salt Sensitive Rat Model

7 Rats on a Low Salt Diet

7 Rats on a High Salt Diet


109 proteins reported in literature as related to heart disease

Phases of Refinement: Detection

Verification: Skyline refinement and method development

Detection
Proteins: 109
Peptides: 2,165
Transitions: 12,194

151 unscheduled methods

Refinement

Differentiation
Proteins: 49
Peptides: 135
Transitions: 780

1 scheduled method
(x 42 injection)

Refinement

Validation

Investigation

1 scheduled method
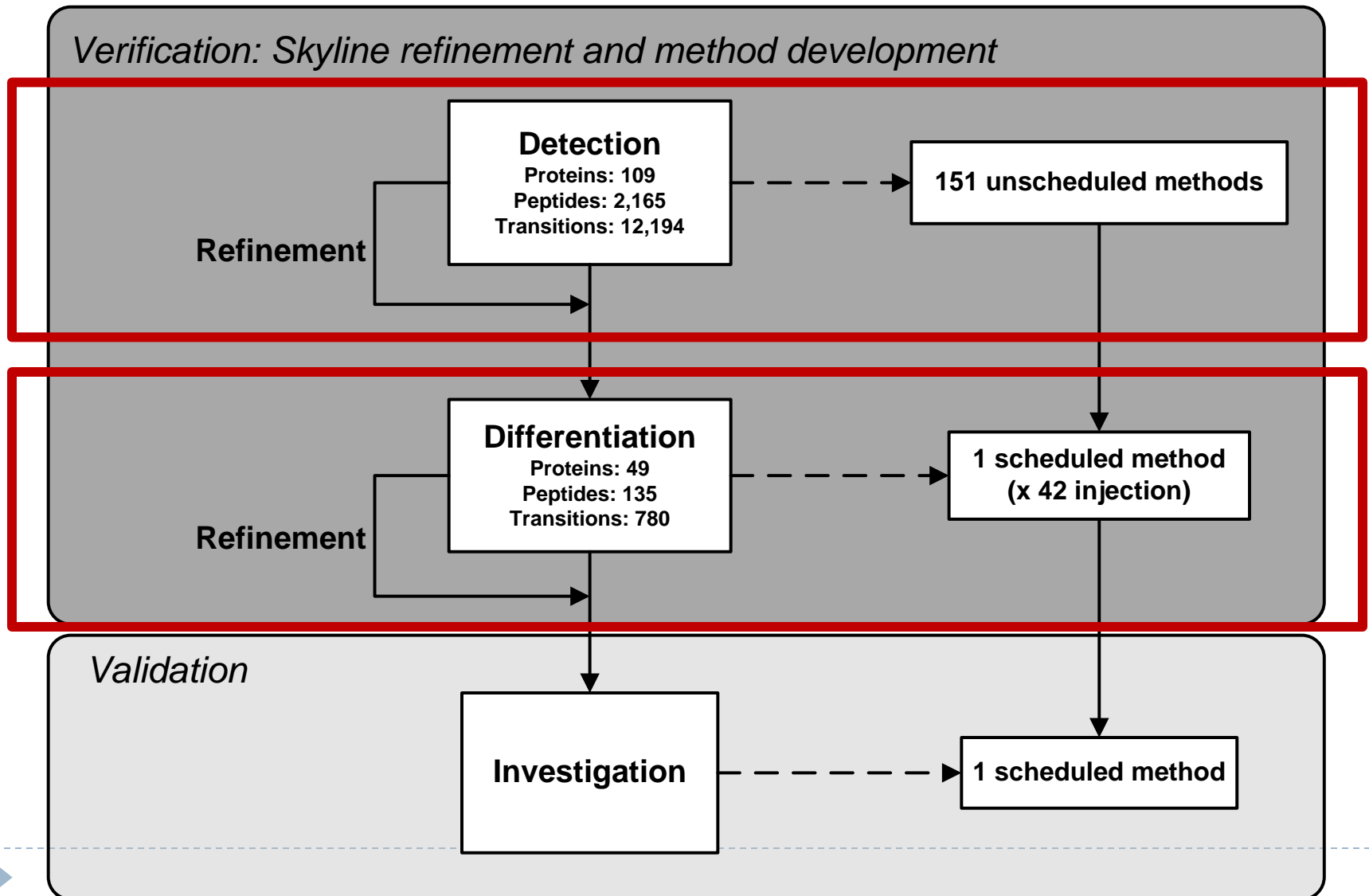
Scheduling Multi-Replicate Assays

- 49 Proteins

- 135 Peptides

- 780 Transitions

- 6 Unscheduled Injections
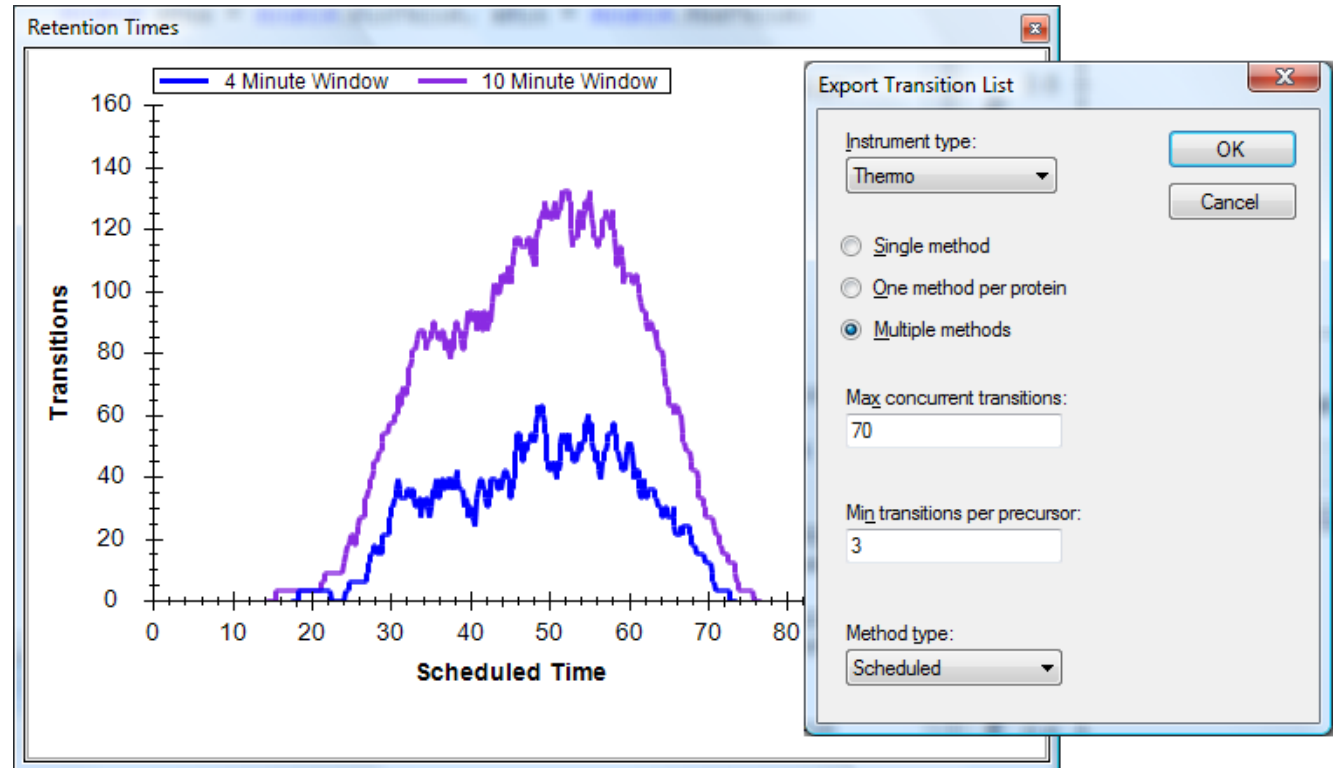
Prior Knowledge and Consistency

▸ Based on empirical measurement
▸ Powerful enough to be used cross-lab / cross experiment
▸ More powerful run-to-run

▸ Relative ion abundance
  ▸ Spectral and chromatogram libraries
▸ Retention time
  ▸ iRT
▸ Optimal collision energy
▸ Ion mobility
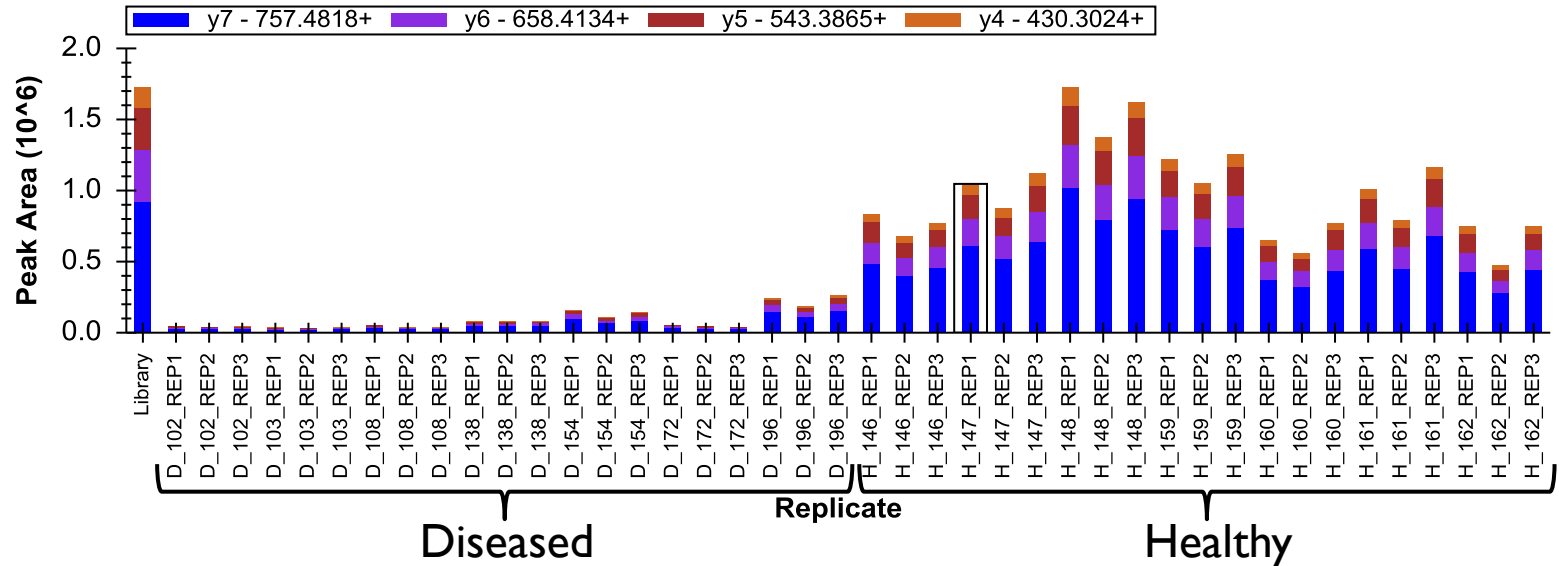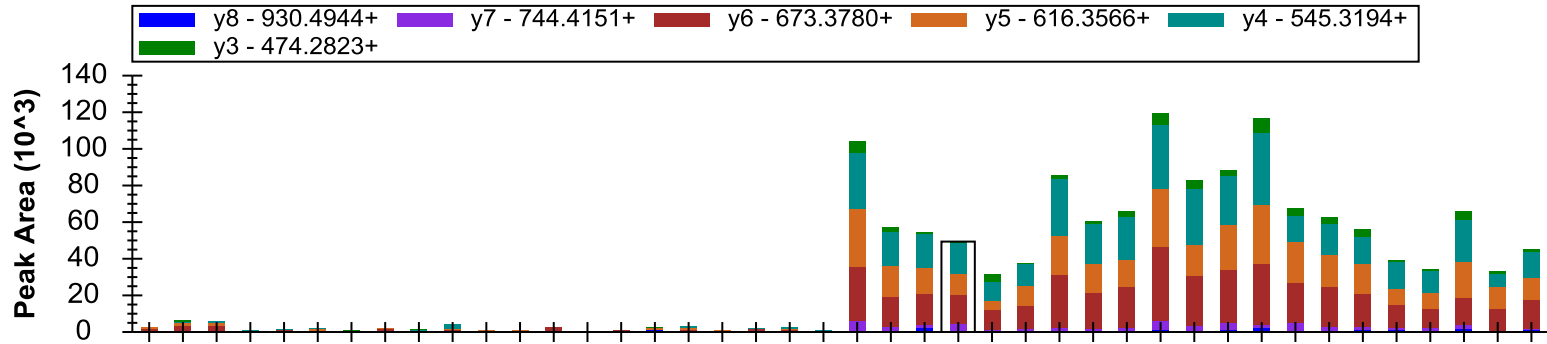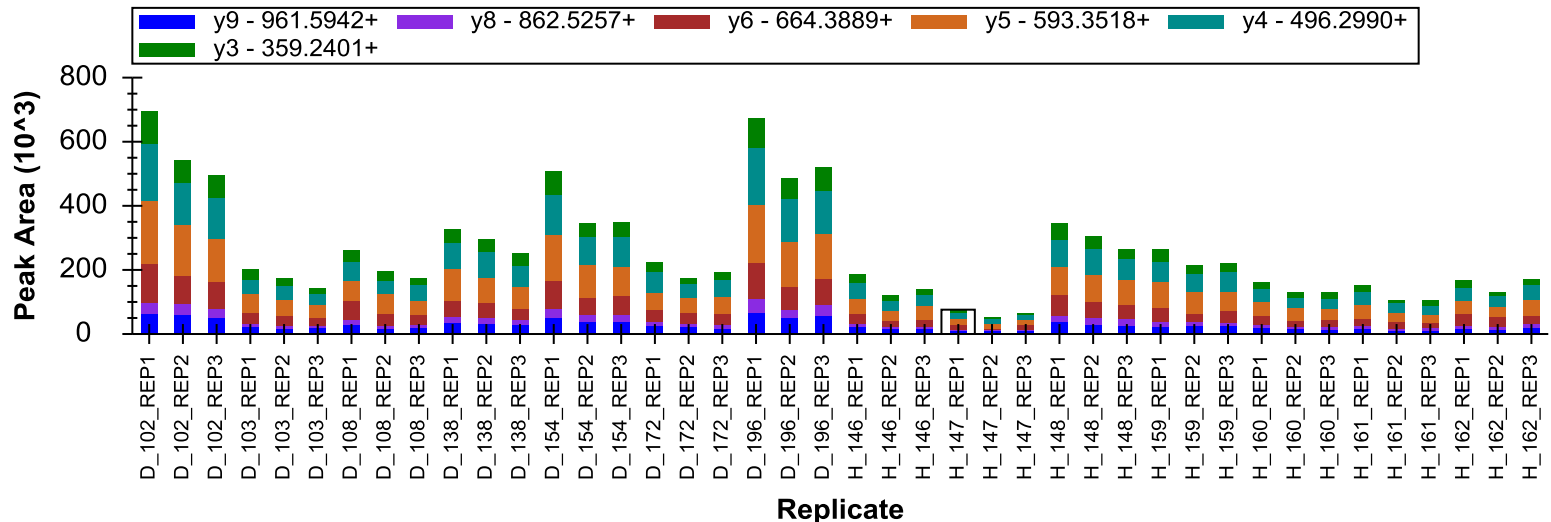 ▸ Collisional cross-section

Haptoglobin

LQTEGDGIYTLNSEK
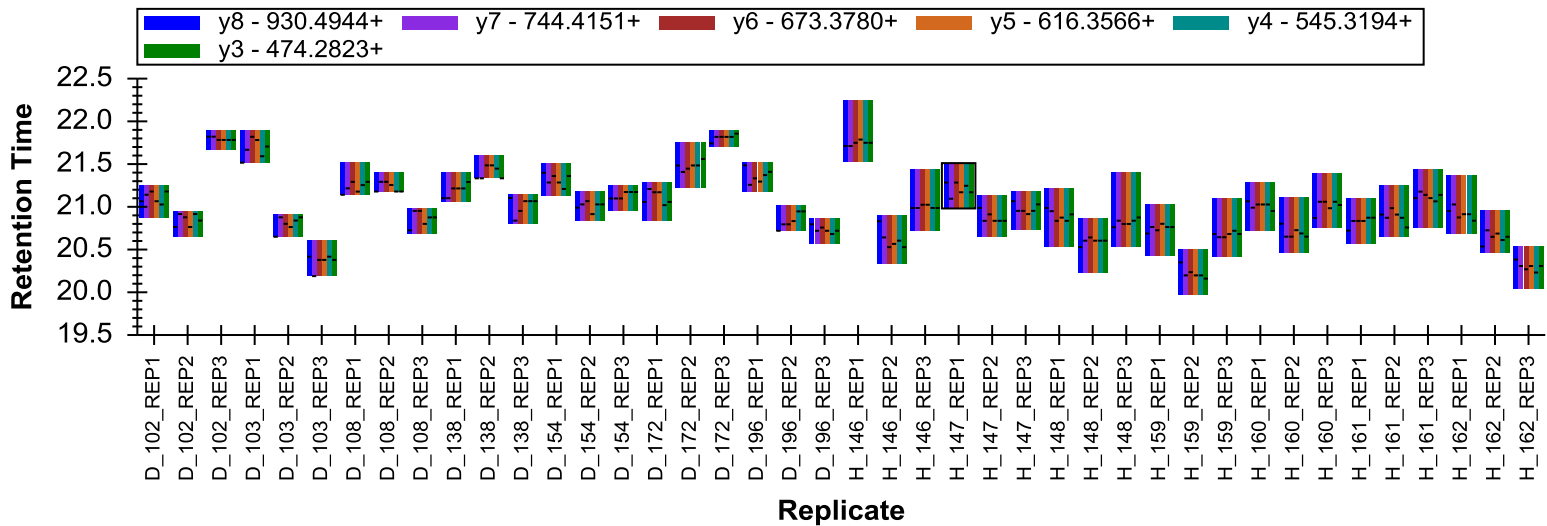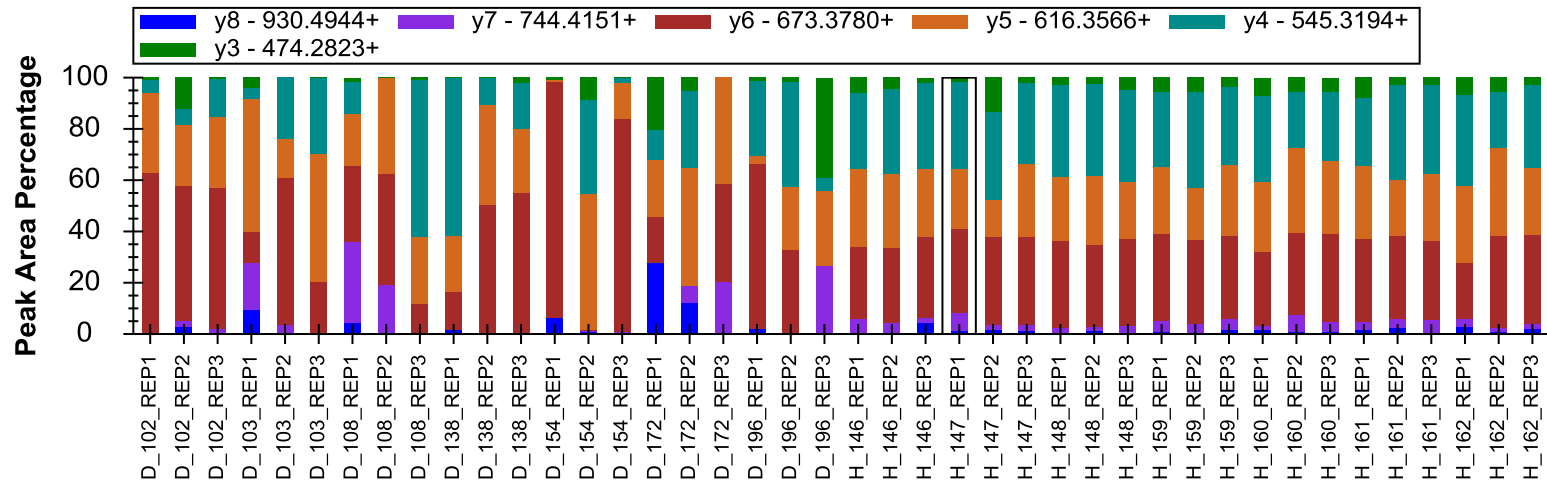
Haptoglobin

Mitochondrial 39S ribosomal protein L9

CSSLLWAGAAWLR

Mitochondrial 39S ribosomal protein L9

CSSLLWAGAAWLR
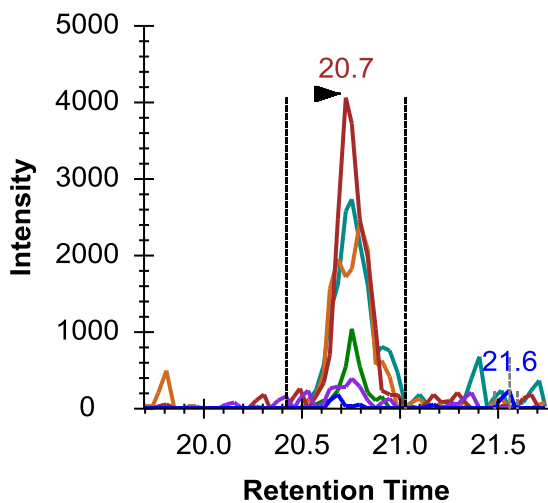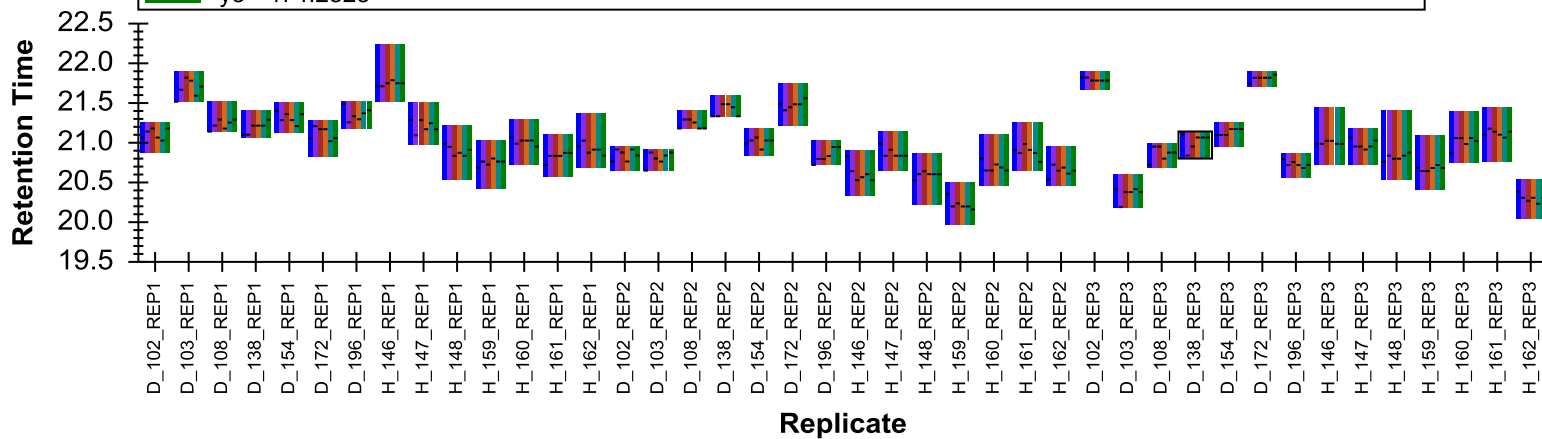
Truncated and Missing Peaks
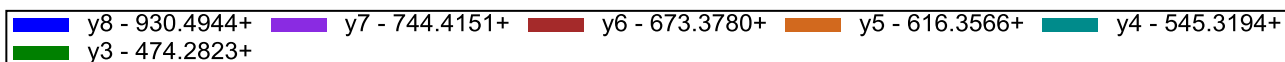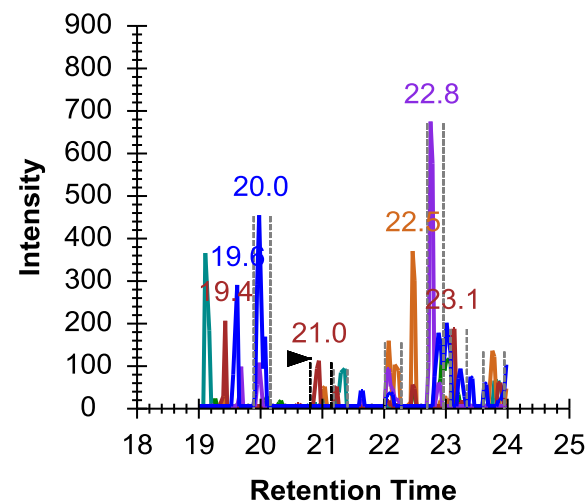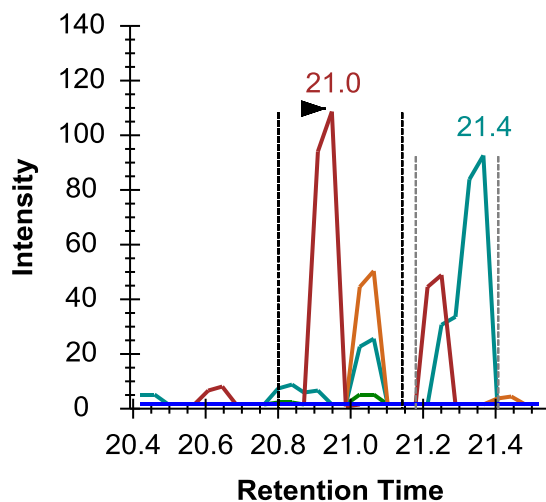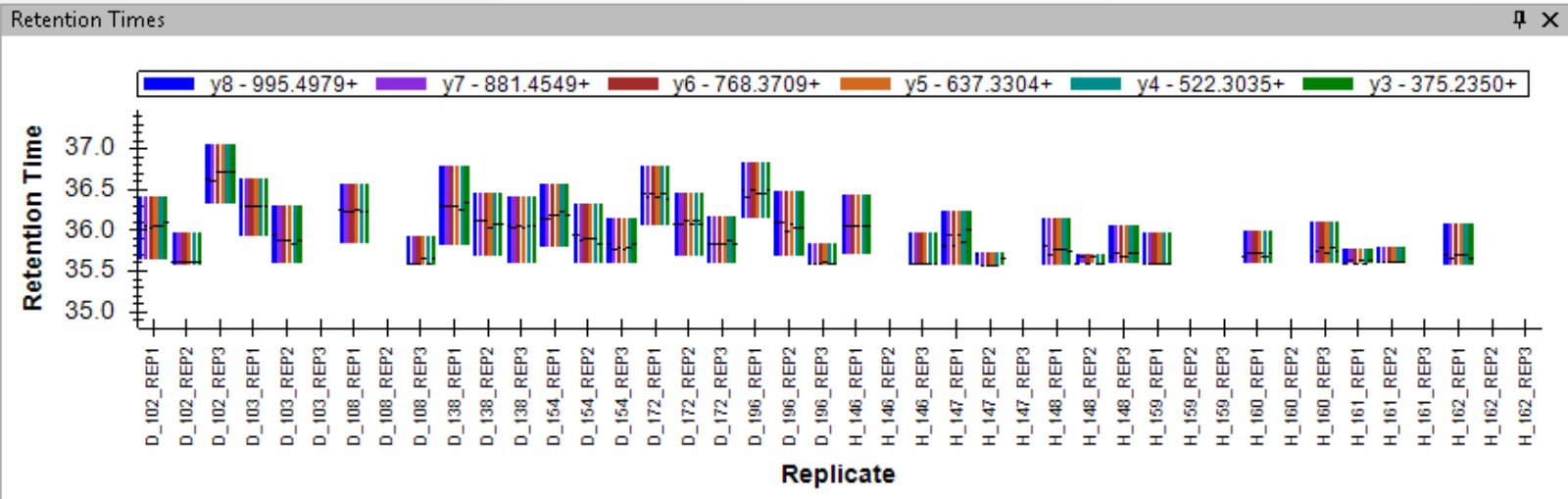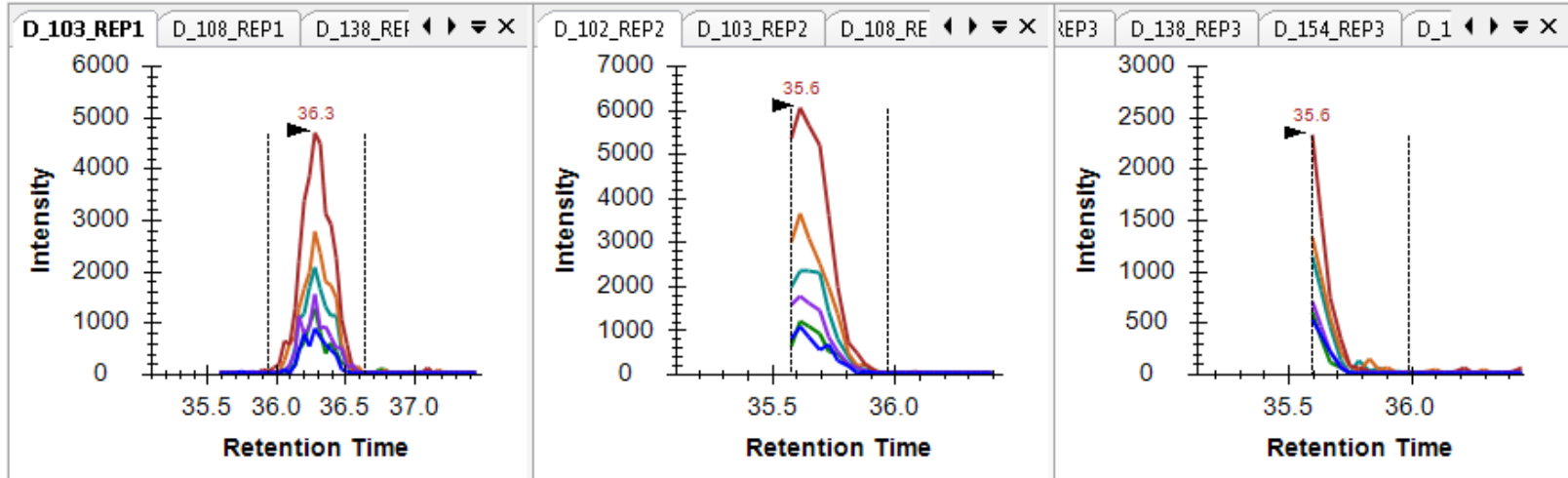
TGTNLMDFLSR

Tutorial

- Effective Data Processing and Interrogation with Skyline

Learn More

- **NEW!** Grouped Studies Tutorial

- Webinar #7: iRT or Isotope Labeled Standards
  - Tuesday, May 12

- Workshop and ASMS
- Skyline User Group Meeting at ASMS
  - May 31 at Old Post Office, St. Louis, MO
- Workshop in Rio de Janiero, August 31-September 2
- Workshop in Puerto Vallarta, November
- Weeklong Course at PRBB, Barcelona,
  - November 15-20

Questions?

▸ Ask any questions you have on this month's topic, Effective Data Processing, at the following form:

http://tinyurl.com/QA4Skyline

▸ Take the post-webinar survey

http://tinyurl.com/Survey4Webinar

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.