

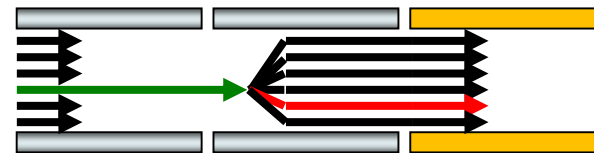
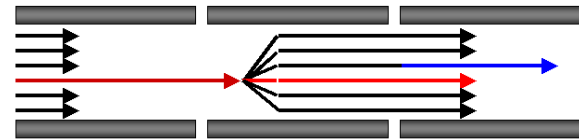


Skyline

Targeted Proteomics Environment

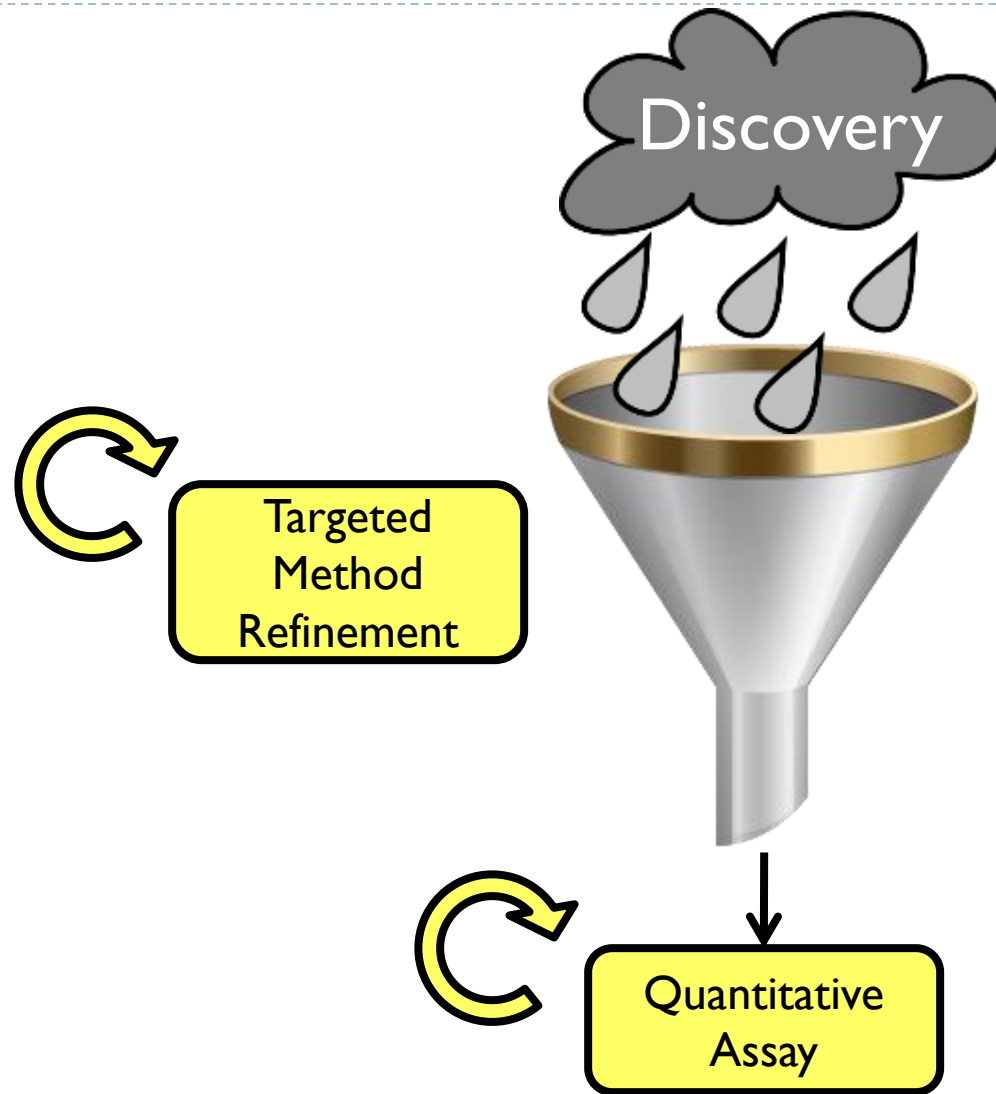
Tutorial: Grouped Study Data Processing

Brendan MacLean
MacCoss Lab

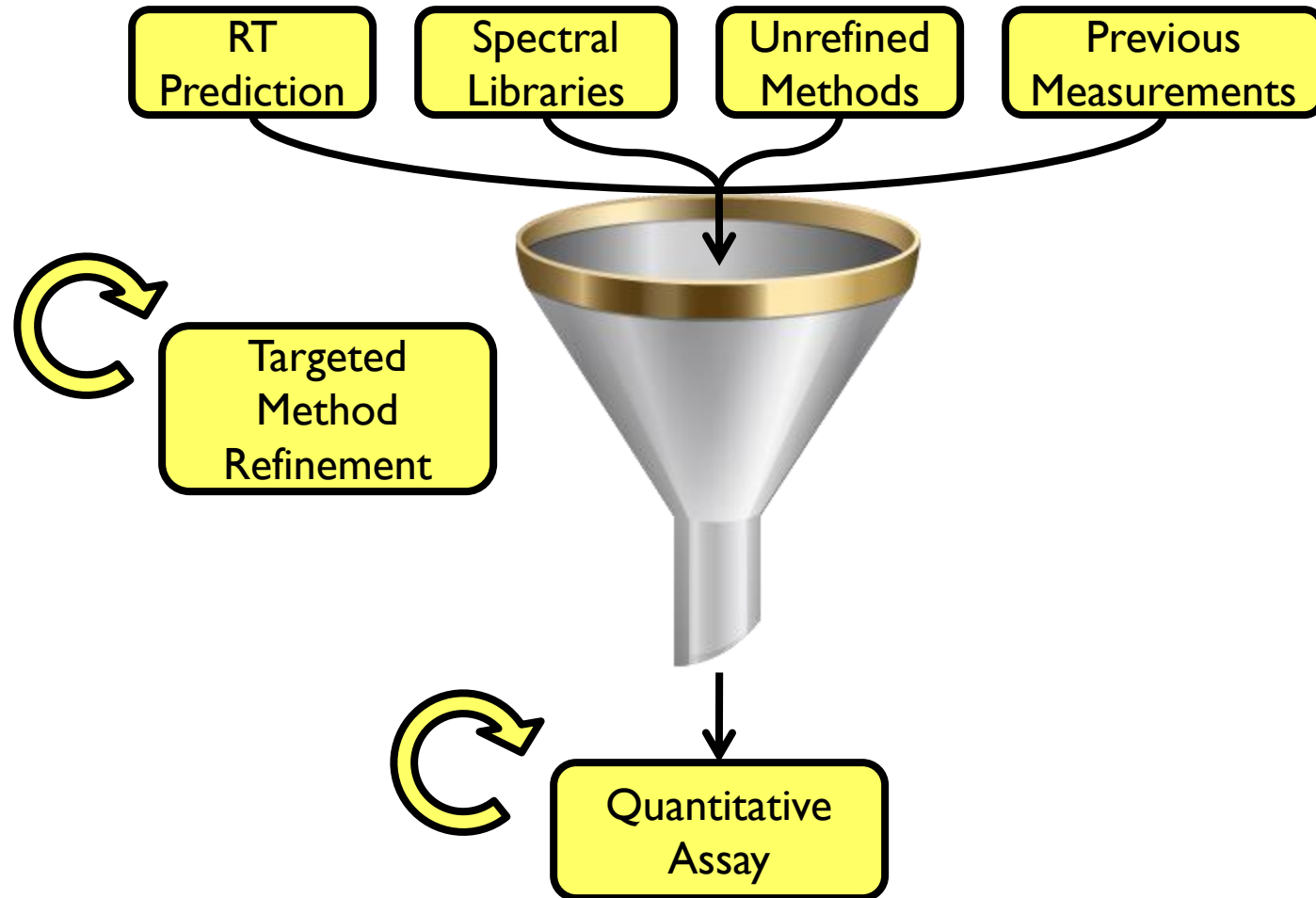


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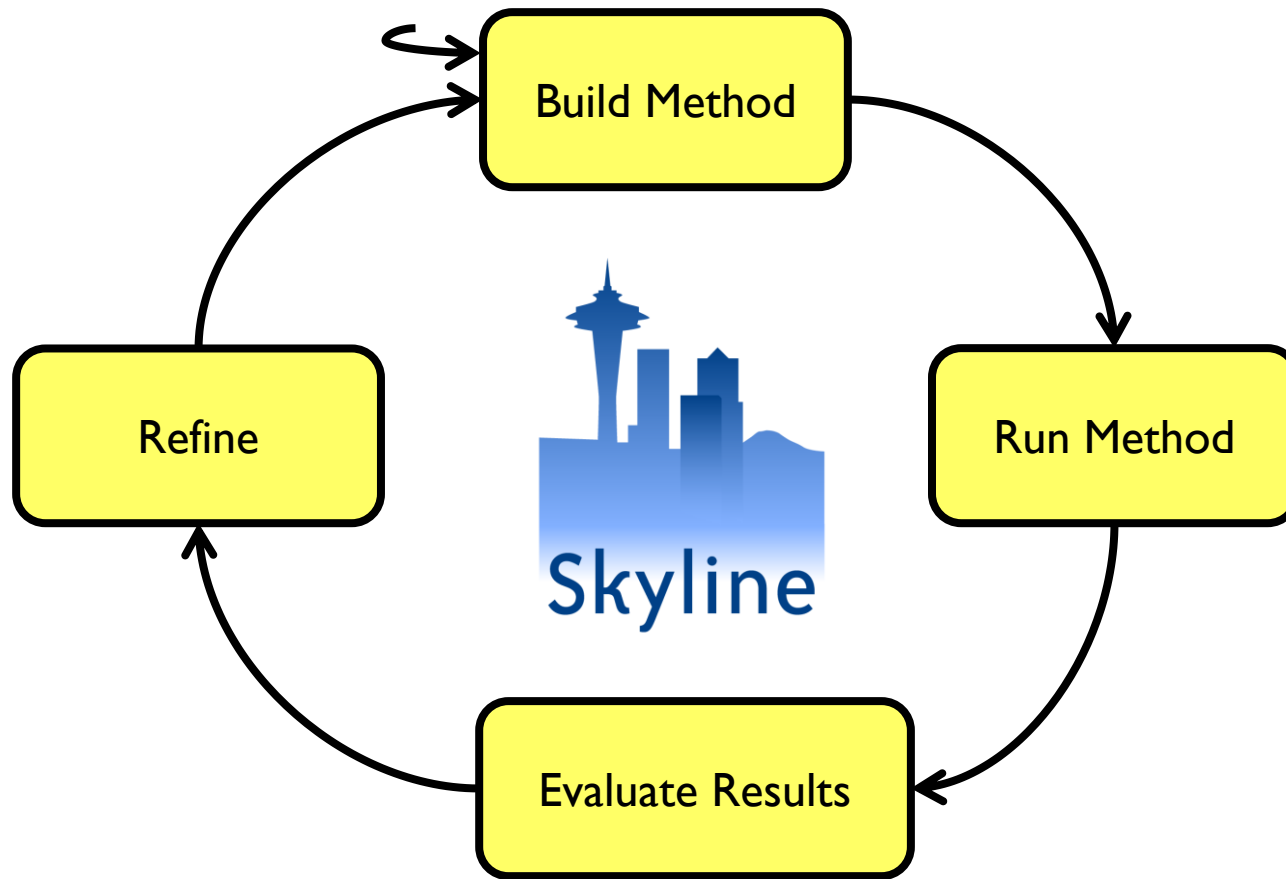
Supporting a Broader Hypothesis



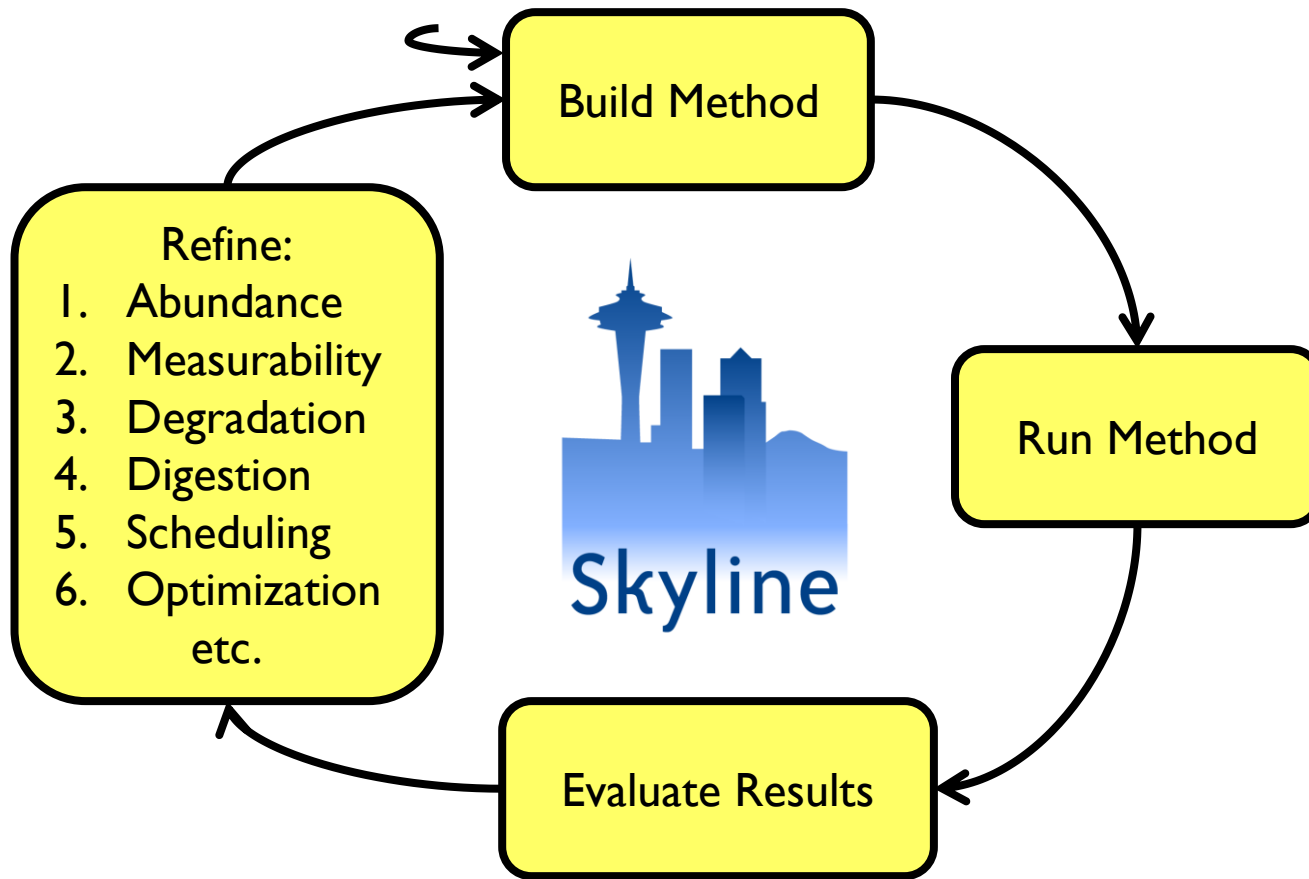
Supporting a Broader Hypothesis



Targeted Method Refinement



Targeted Method Refinement



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

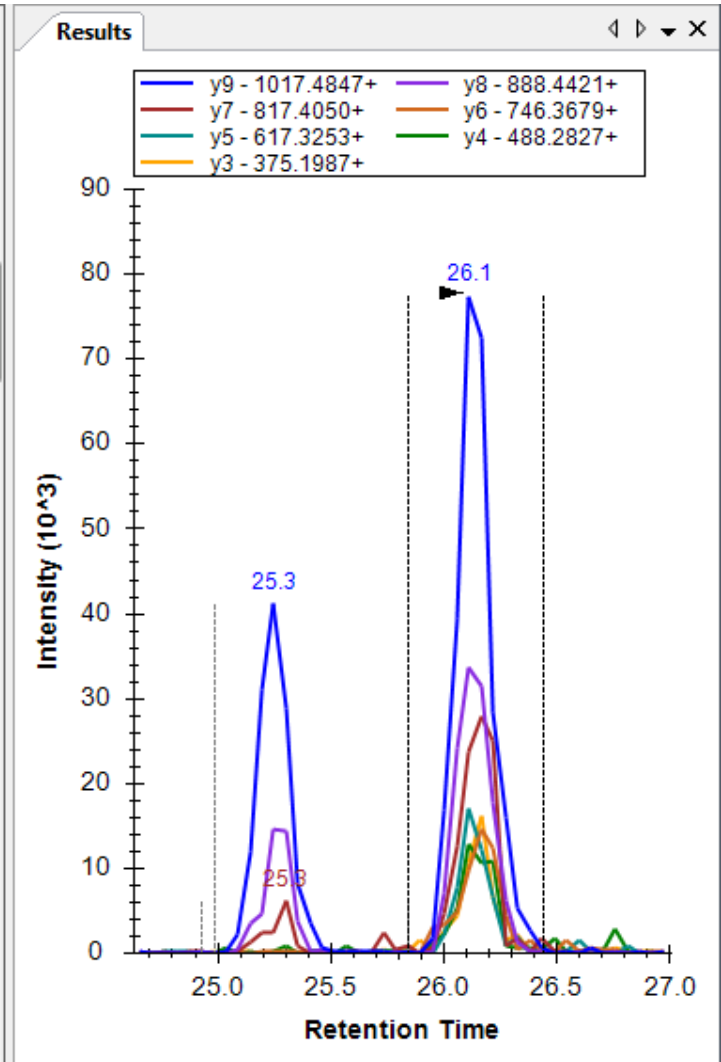
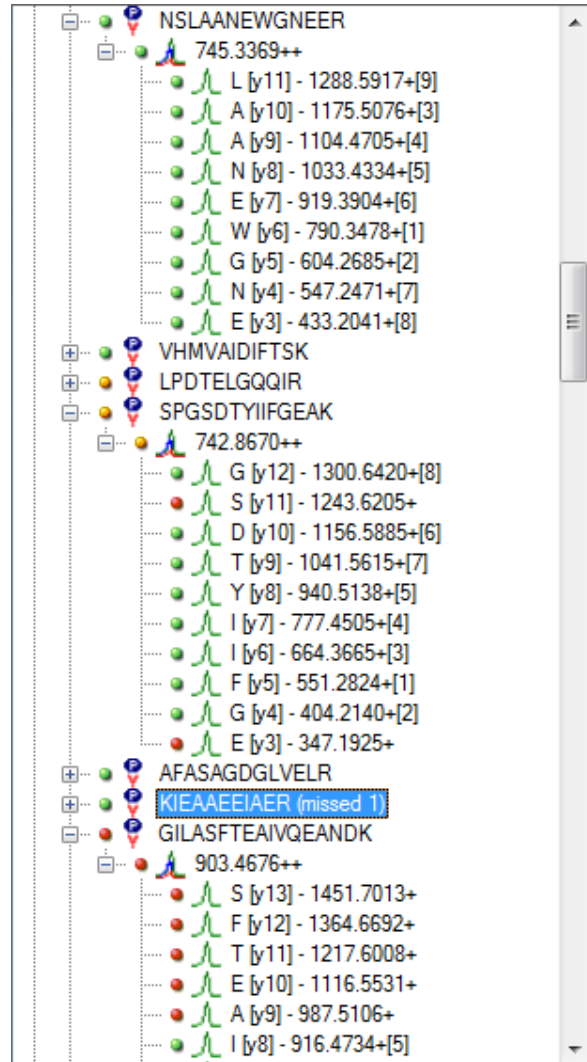


109 proteins reported in literature as related to heart disease



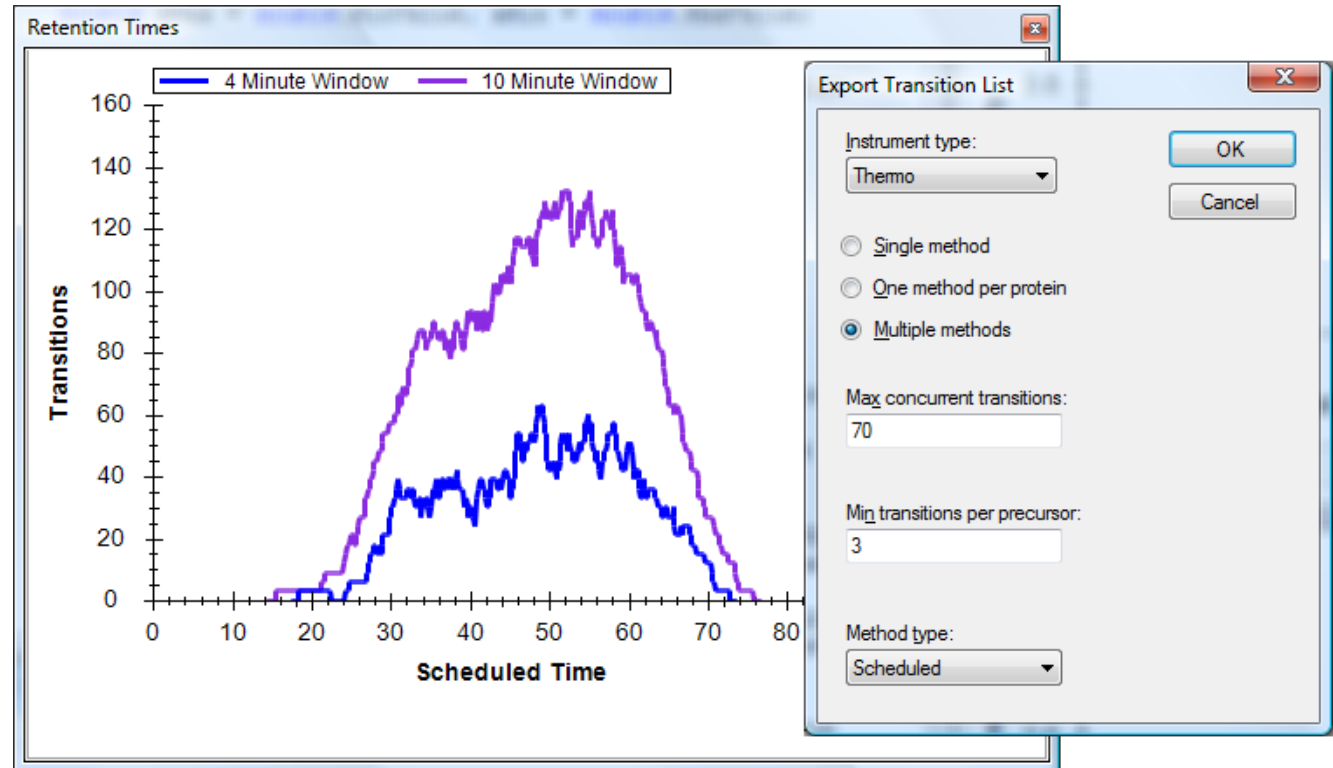
The Unrefined Method

- ▶ 109 Proteins
- ▶ 2165 Peptides
- ▶ 12,194 Transitions
- ▶ 151 Sample injections

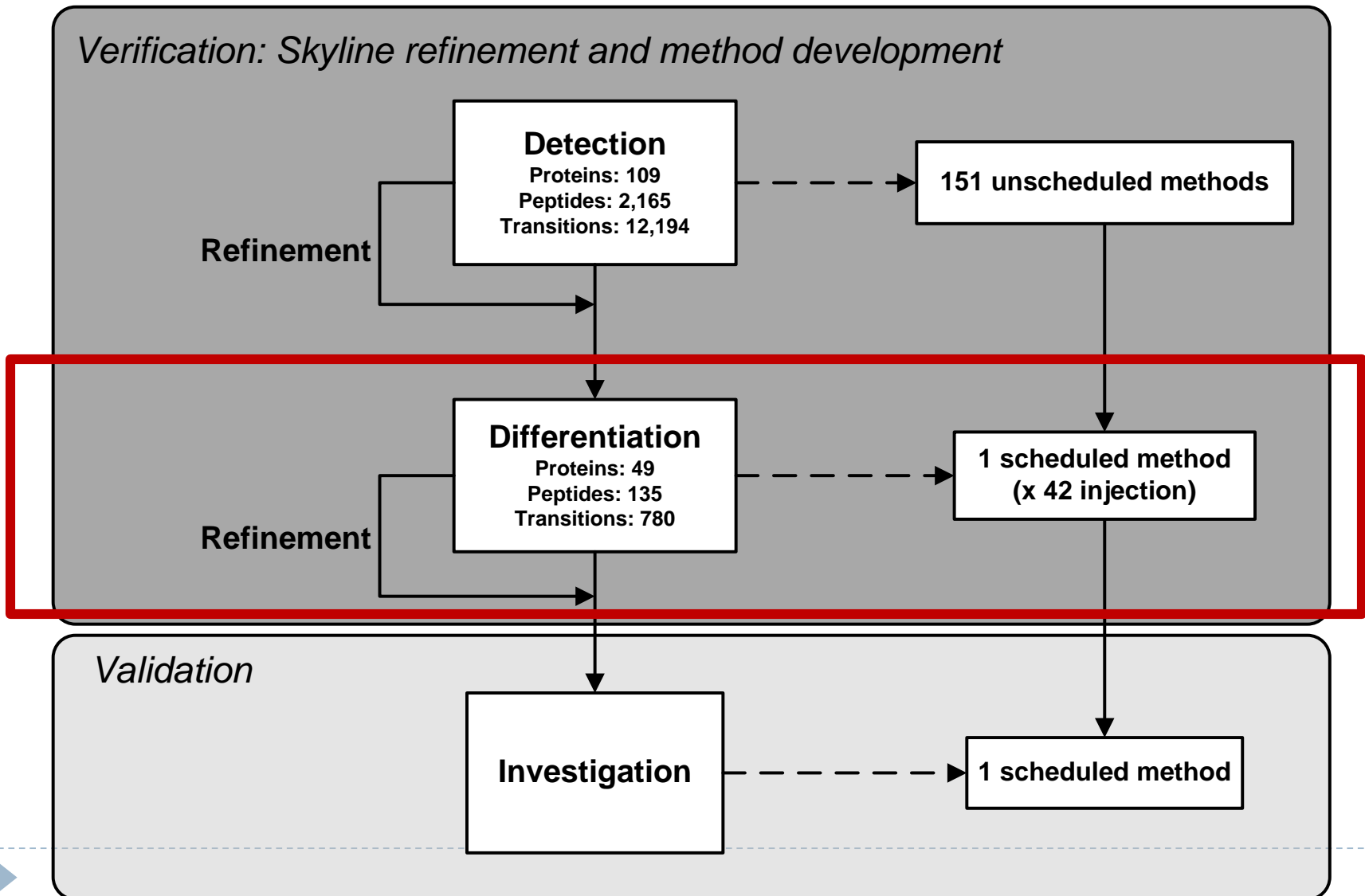


Scheduling Multi-Replicate Assays

- ▶ 49 Proteins
- ▶ 135 Peptides
- ▶ 780 Transitions
- ▶ 6 Unscheduled Injections



Phases of Refinement: Differentiation



Experimental Data

- ▶ **Rat plasma experiment**
 - ▶ Daniela Tomazela at University of Washington
- ▶ **14 subjects (7 diseased, 7 healthy)**
- ▶ **Technical triplicate**
- ▶ **Method design**
 - ▶ Proteins from literature
 - ▶ No labeled reference peptide
 - ▶ Sparse spectral library coverage
 - ▶ Exhaustive measurement of tryptic peptides
 - ▶ Exhaustive measurement of $y_3 - y_{(n-1)}$ product ions
 - ▶ Refinement



Prior Knowledge and Consistency

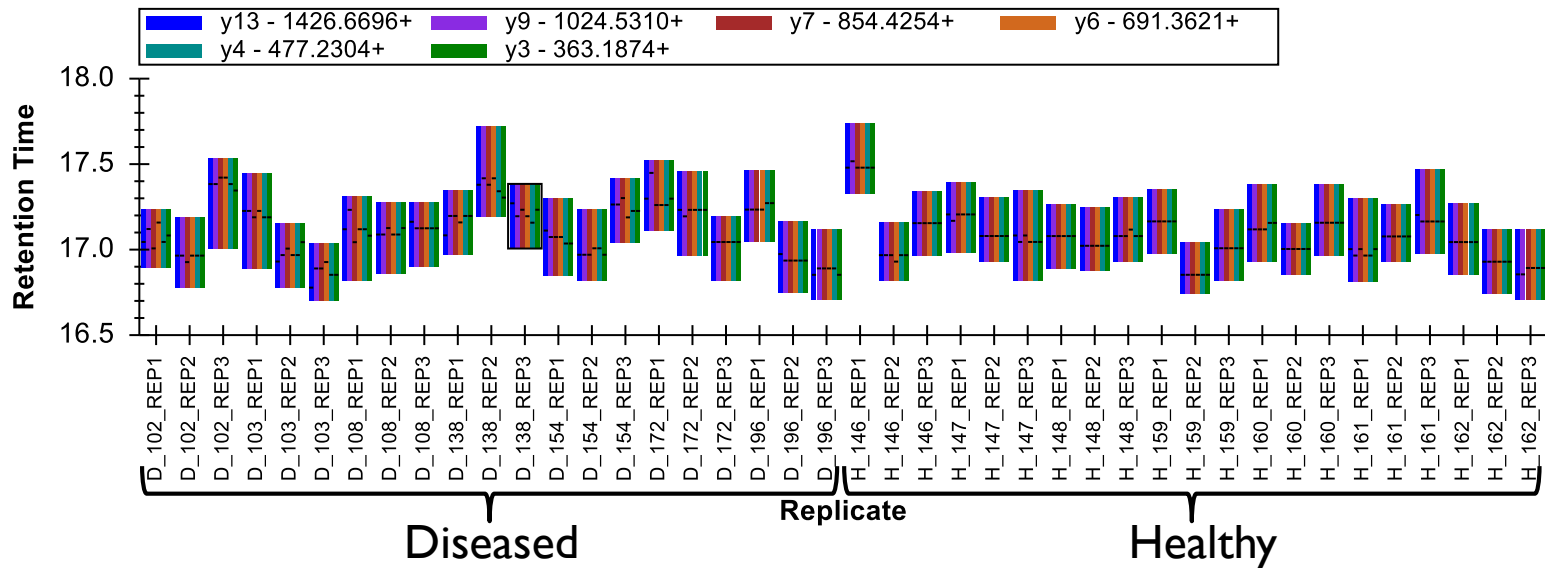
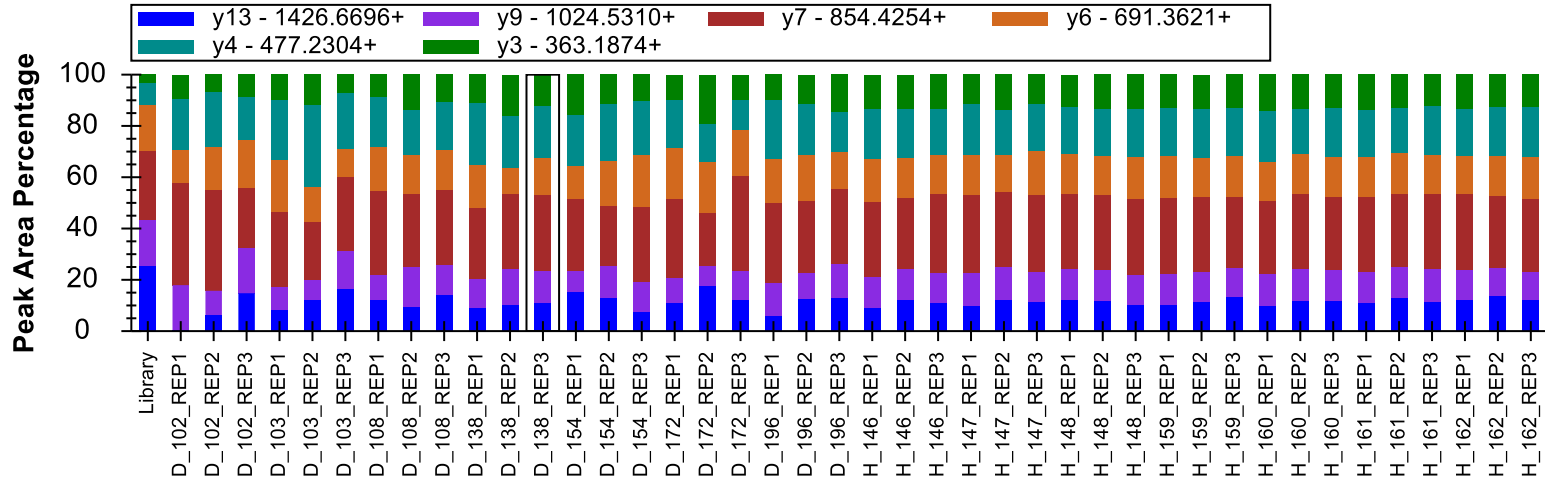
- ▶ Based on empirical measurement
- ▶ Powerful enough to be used cross-lab / cross experiment
- ▶ More powerful run-to-run

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



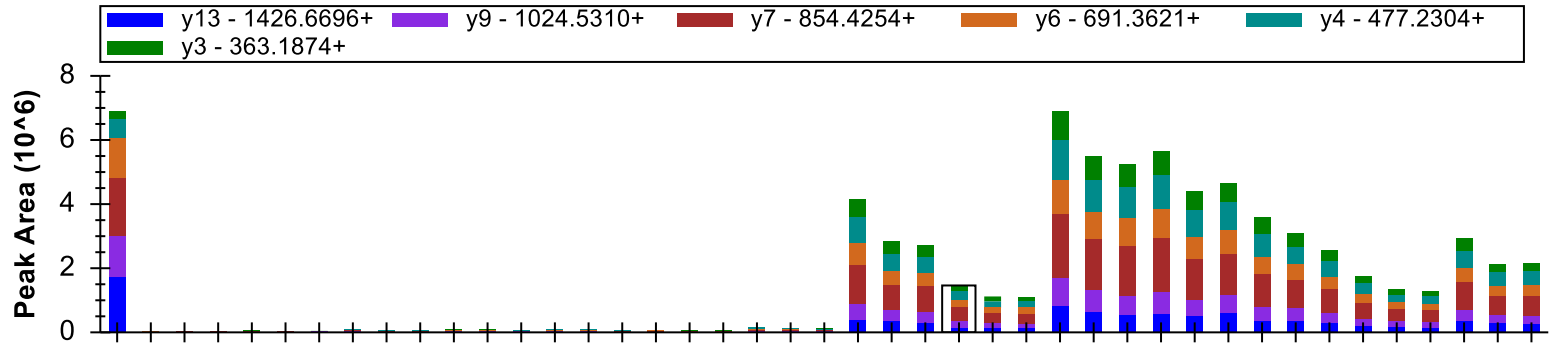
Haptoglobin

LQTEGDGIYTLNSEK

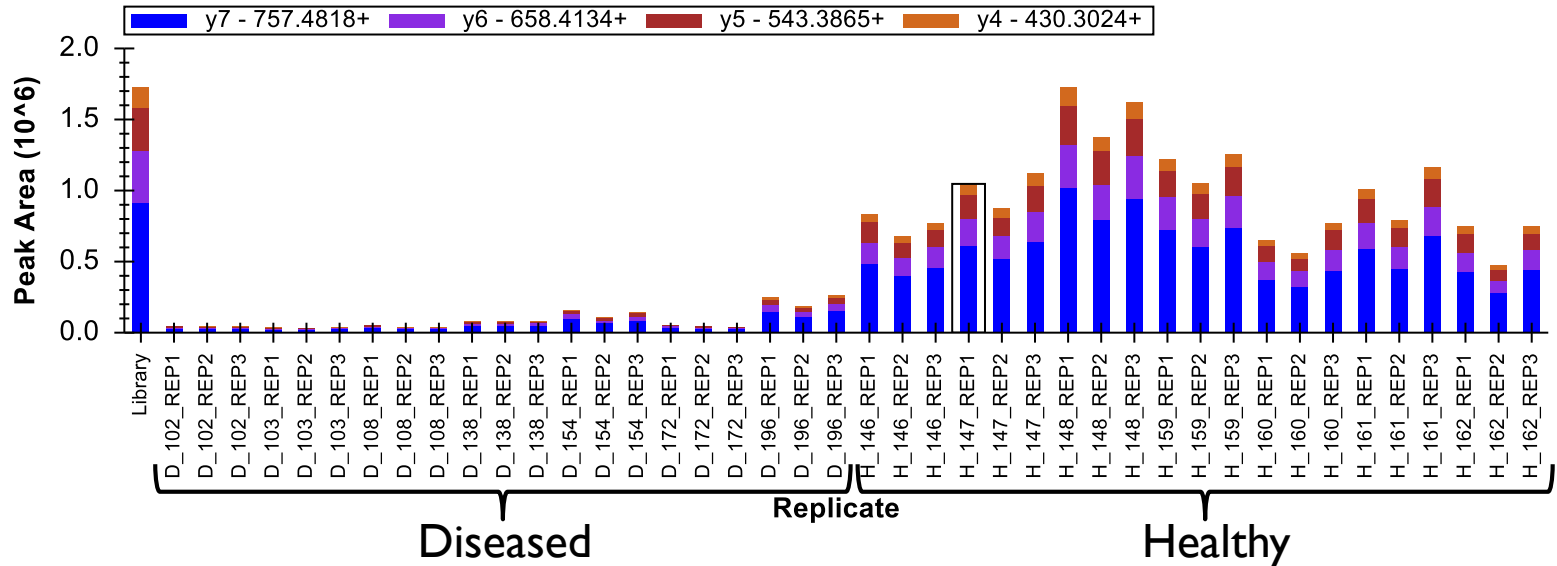


Haptoglobin

LQTEGDGIYTLNSEK

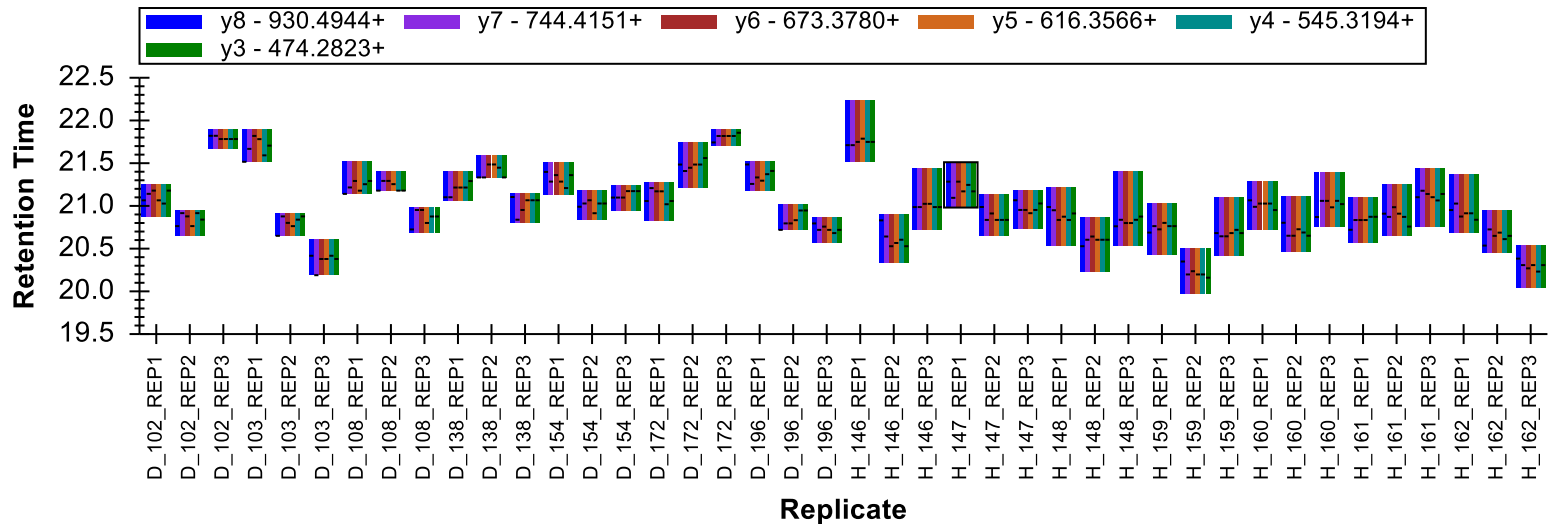
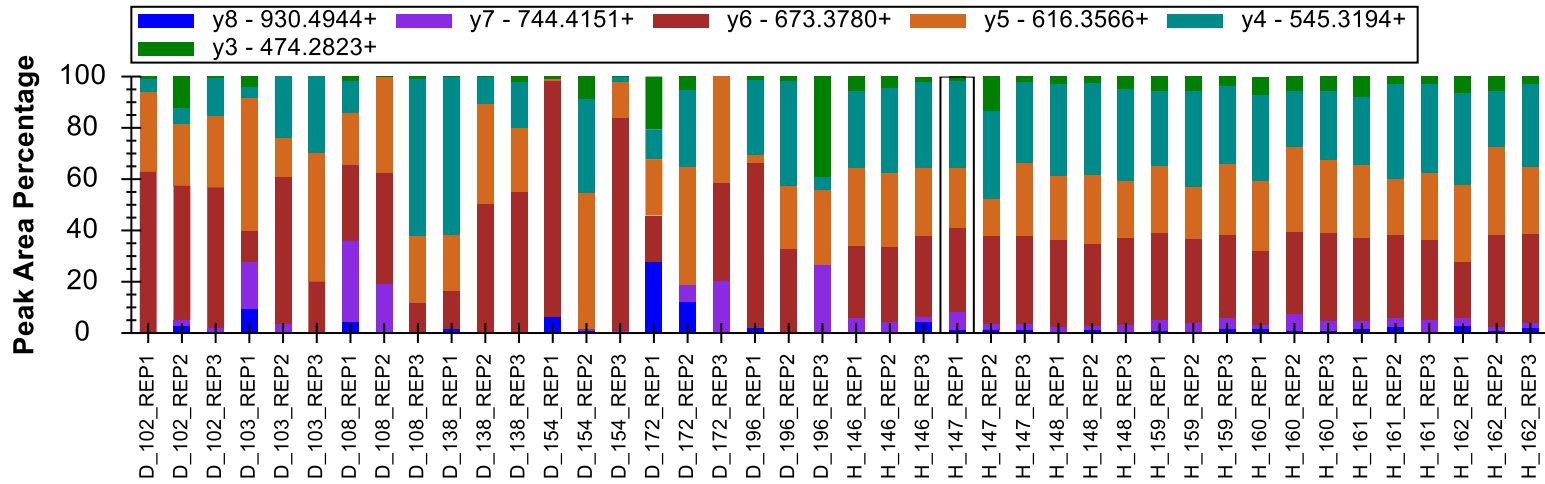


SVVDIGLIK



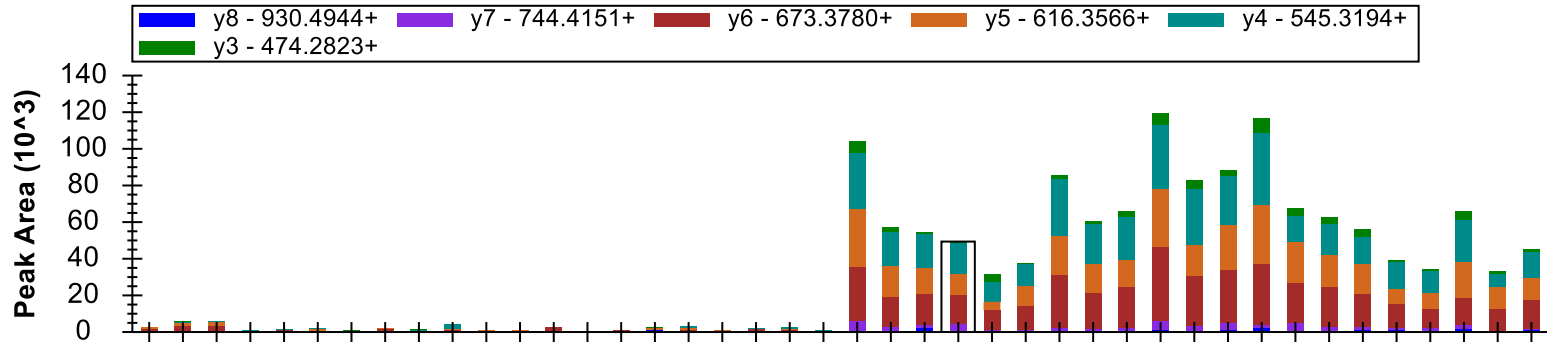
Mitochondrial 39S ribosomal protein L9

CSSLWAGAAWLR

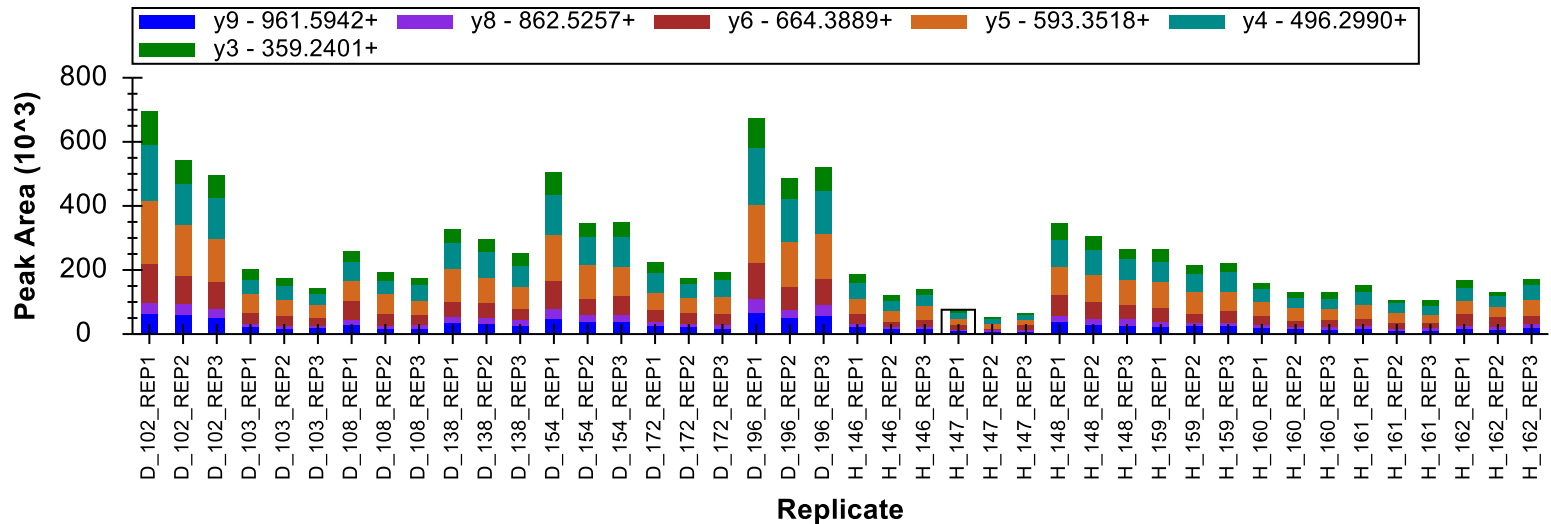


Mitochondrial 39S ribosomal protein L9

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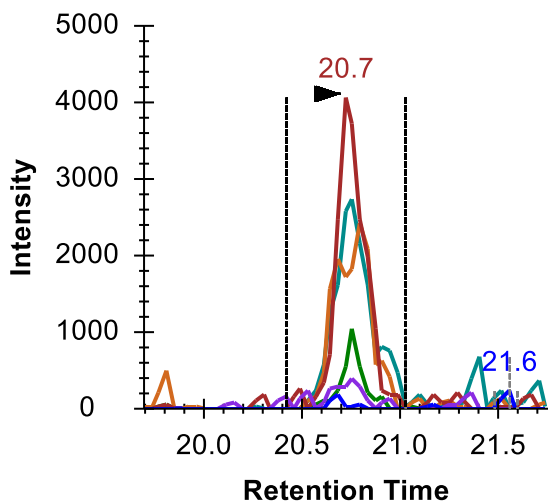


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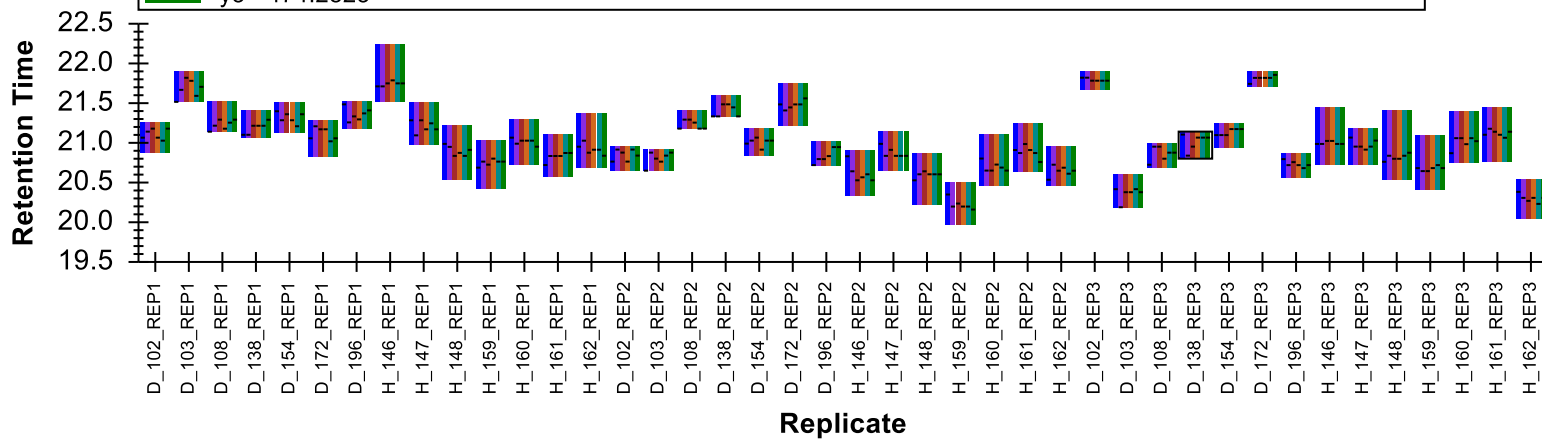
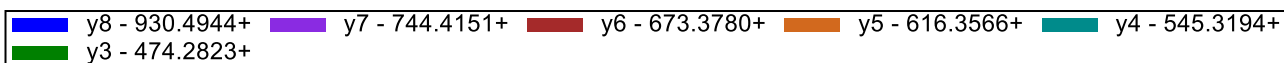
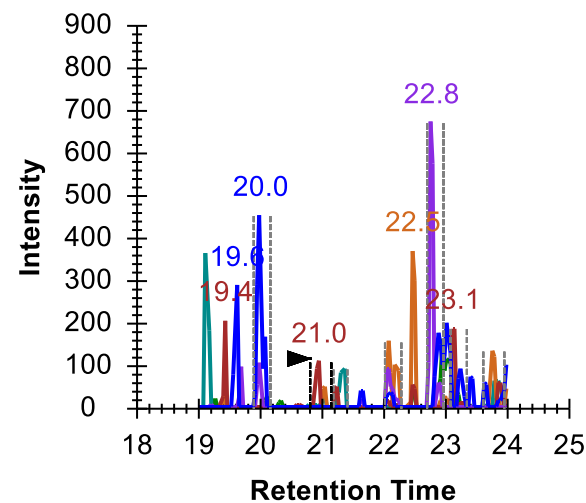
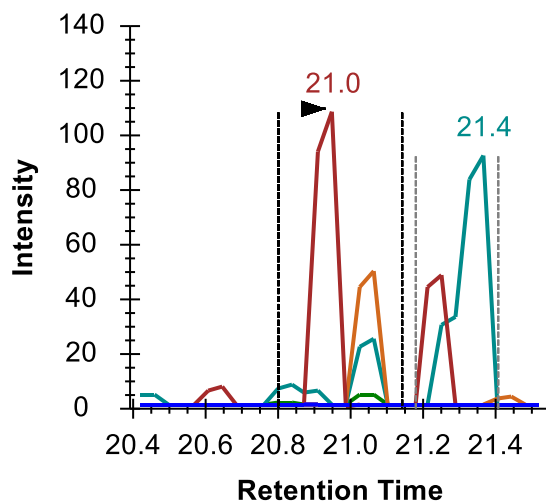


CSSLWAGAAWLR

H_159_REP1



D_138_REP3



Truncated and Missing Peaks

TGTLNLMDFLSR

