Using Skyline to Monitor Long-Term Performance Metrics of High-Resolution Mass Spectrometers

J. Will Thompson and M. Arthur Moseley

Duke Proteomics Core Facility

Skyline Users Meeting – ASMS 2012

DUKE INSTITUTE FOR GENOME SCIENCES & POLICY

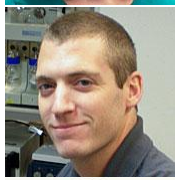
Skyline

The Duke Proteomics Core Facility

- **Arthur Moseley, PhD, DPCF Director (2007)**

- **Will Thompson, PhD, Senior Laboratory Administrator (2007)**

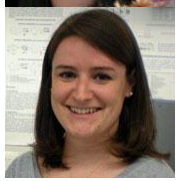
- **Laura Dubois, BS, Laboratory Analyst II (2008)**



- **Erik Soderblom, Laboratory Analyst II (2008)**

- **Matt Foster, PhD, Assistant Research Professor - Pulmonology, 20% DPCF (2009)**

- **Meredith Turner, BS, Research Technician II (2010)**



- **Brenna Richardson, PhD, Laboratory Analyst II (2011)**

From the GLP world:

Operational Qualification (OQ):

"Establishing confidence that equipment and sub-systems are capable of consistently operating with established limits and tolerances."

Performance Qualification (PQ):

"Demonstrate compliance with all requirements…including tests designed to verify the satisfactory performance of the equipment."

System Suitability:

"The process of validating whether your system is acceptable for providing useful analytical data without any bias."

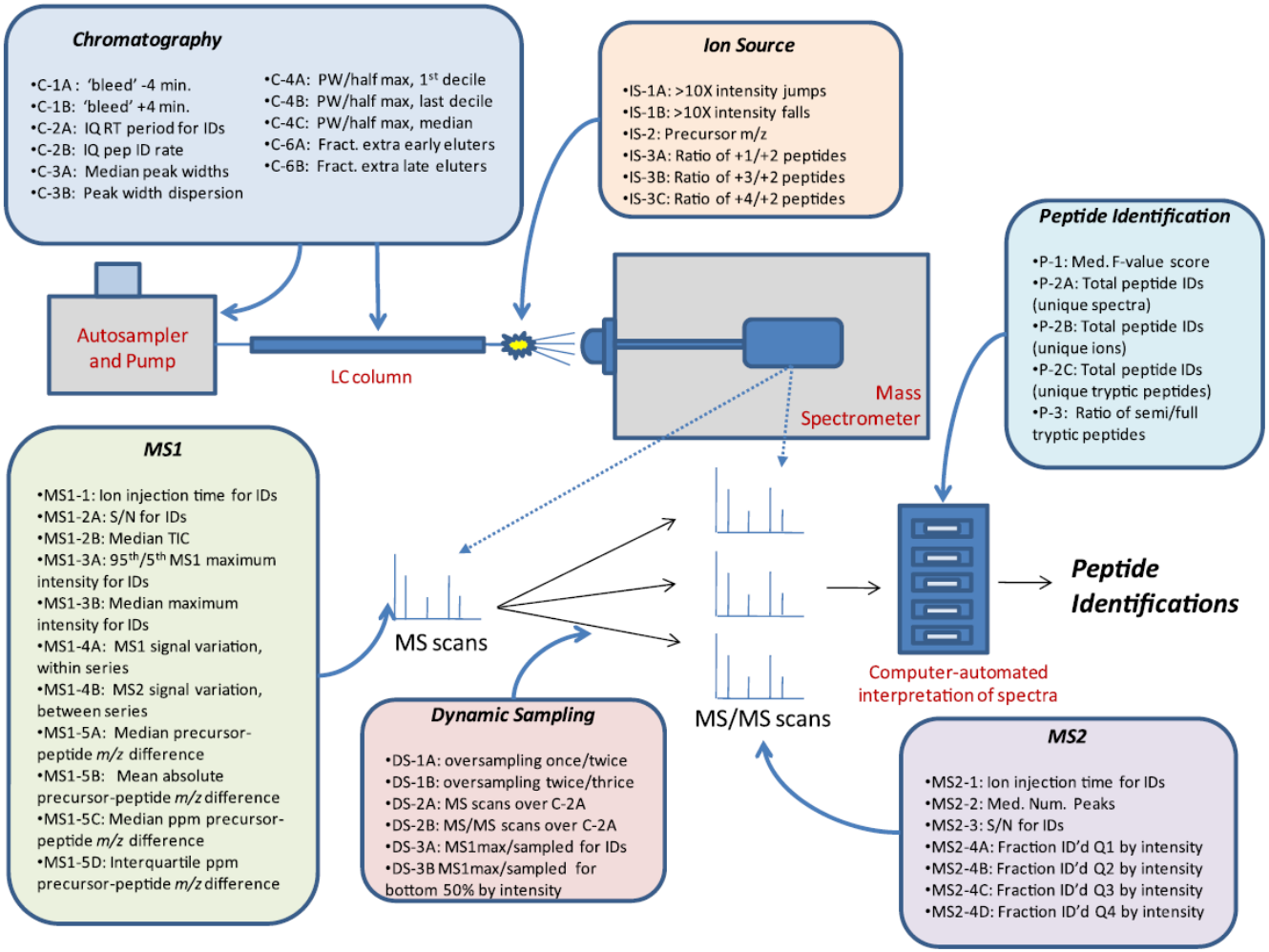
We know this is important, but how do we go about doing this for a *proteomic* analysis?

What Metrics are Important?

Performance Metrics for Liquid Chromatography-Tandem Mass Spectrometry Systems in Proteomics Analyses*

Molecular & Cellular Proteomics 9.2

Paul A. Rudnick,[a]
Pedatsur Neta,[a] N
David M. Bunk,[c] H



http://peptide.nist.gov/software/nist_msqc_pipeline/NIST_MSQC_Pipeline.html

What Type of Sample?

Single Peptide? Single Protein? Few Proteins? Lysate?

TIME

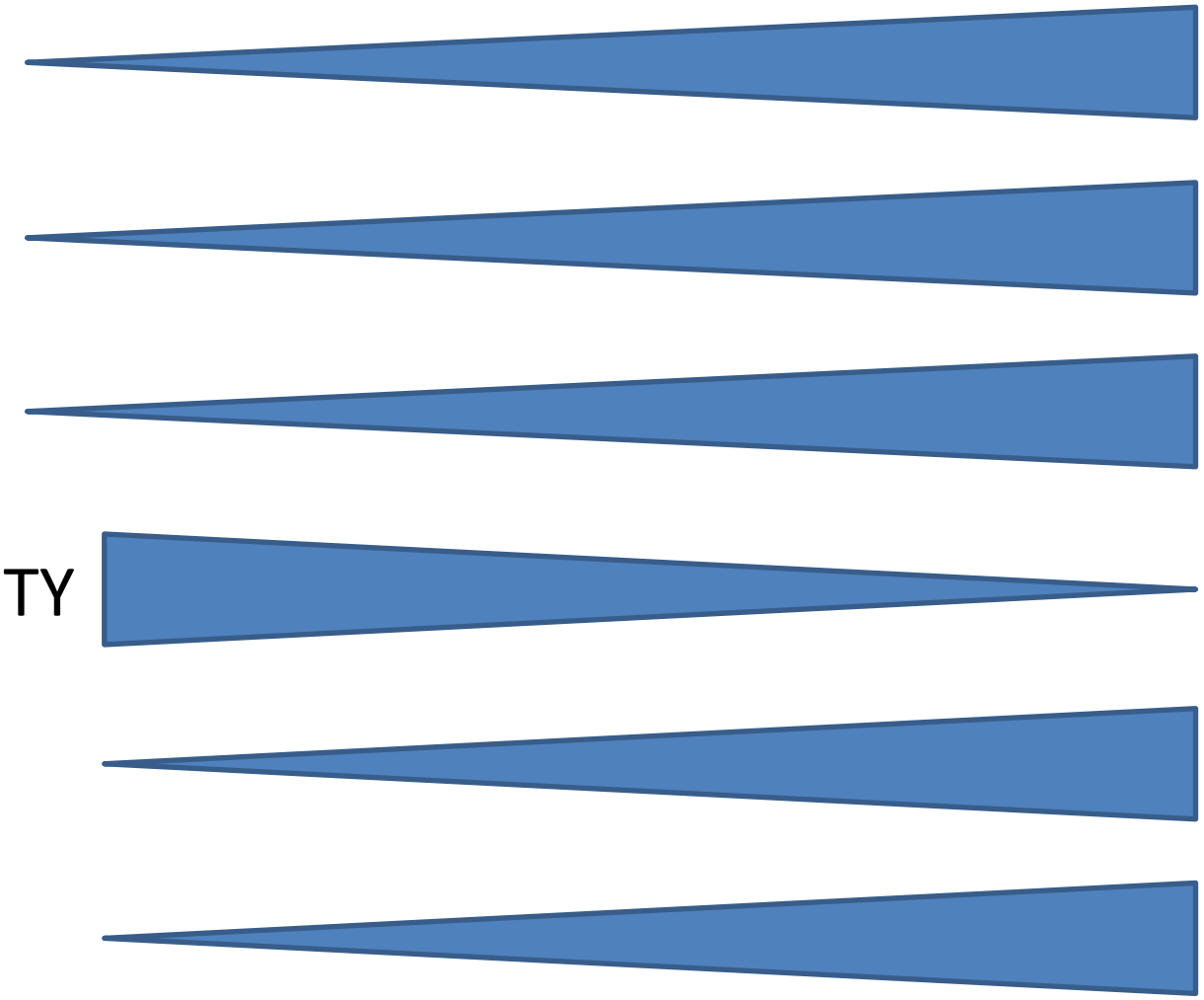
COST

CARRYOVER

REAGENT STABILITY

SENSITIVITY

RELEVANCE



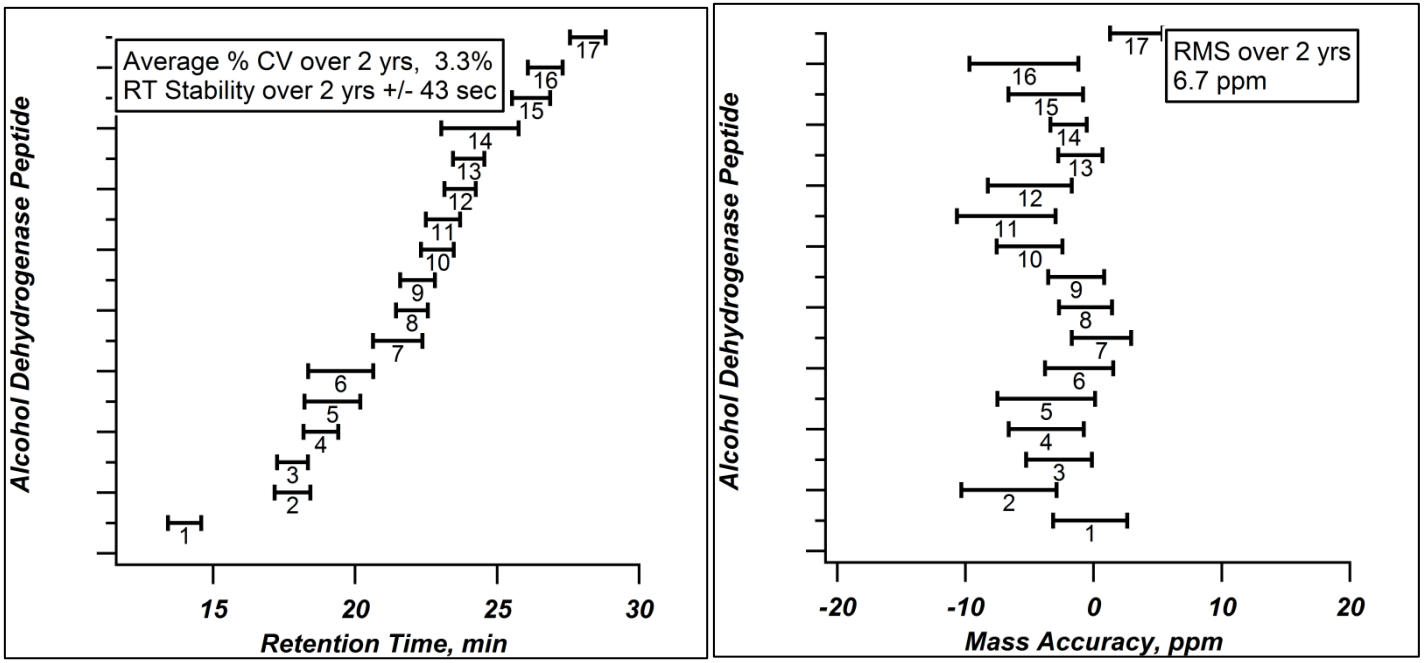
ABRF-Sponsored Longitudinal Variability Study (2012-2013)

The 2012 PRG study: Assessing longitudinal variability in routine peptide LC-MS/MS analysis

http://www.abrf.org/ResearchGroups/Proteomics/Studies/ABRF2012.pdf

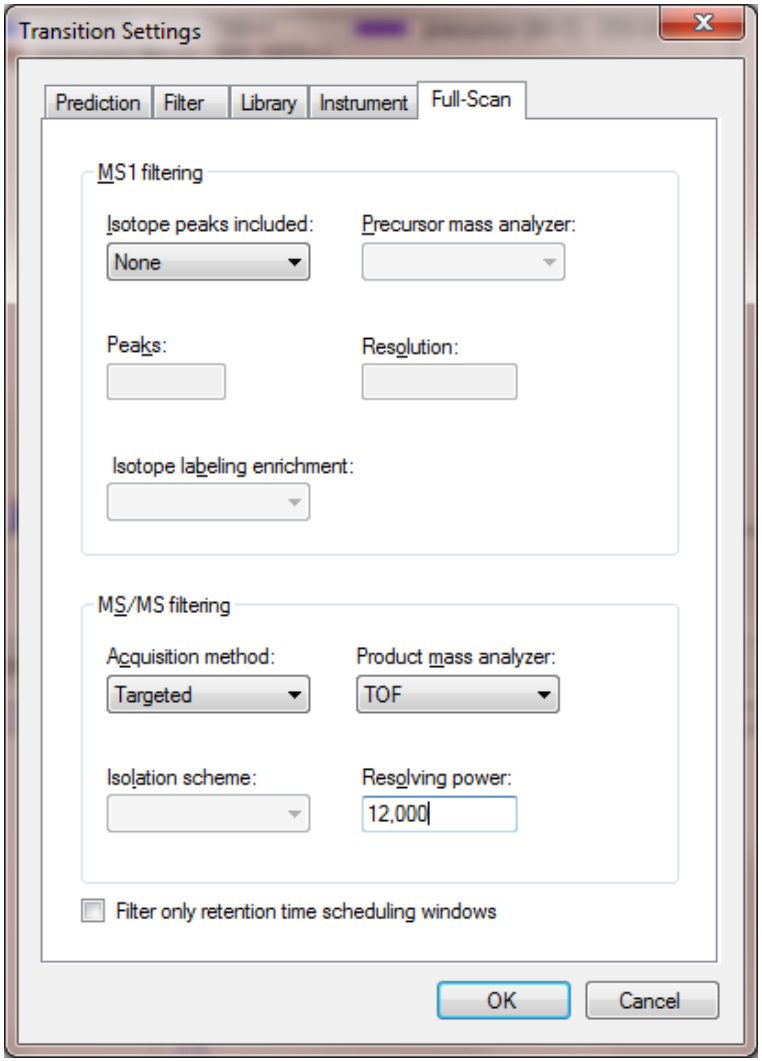
So, Just Start Somewhere…

- In 2007, the DPCF started using 50 fmol injections of yeast alcohol dehydrogenase (Waters MassPrep)
- Primary check was mass accuracy and sequence coverage, using database search results



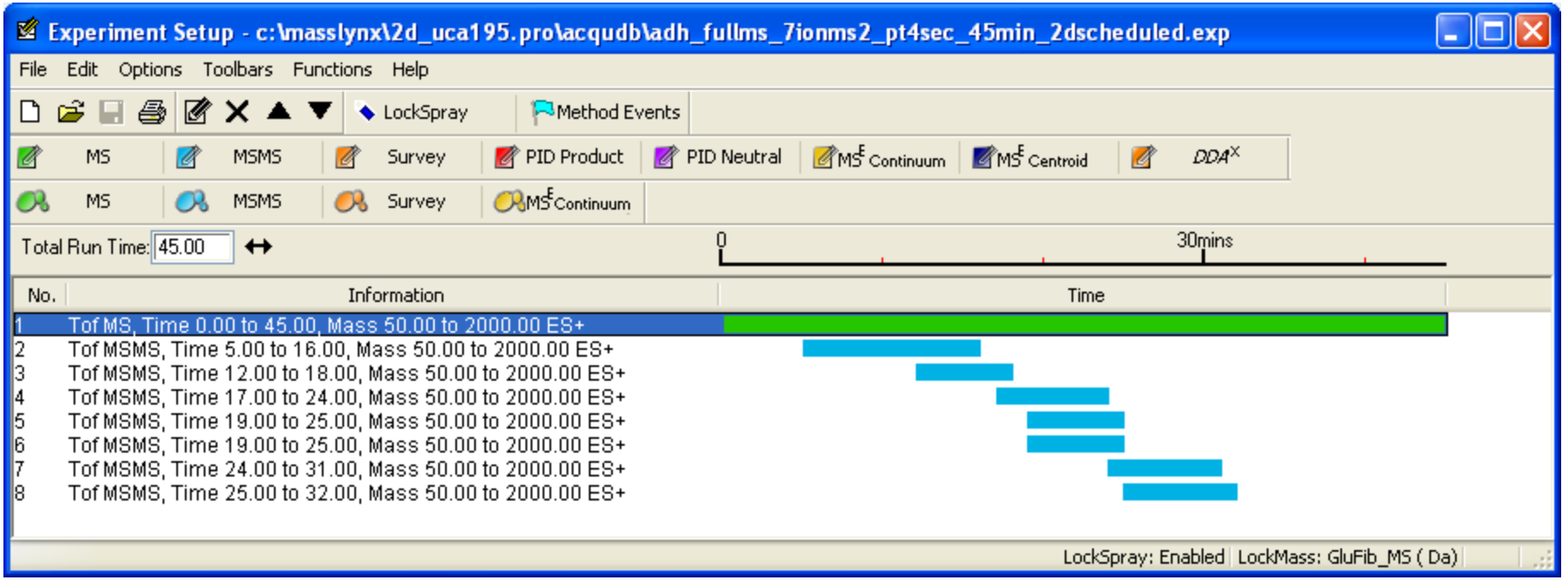
- In May 2011 the DPCF started using Skyline to analyze and track SS data from our QToF instruments using a Full Scan MS2 method targeting 7 analytes

Skyline Setup for Full Scan MS/MS Analysis

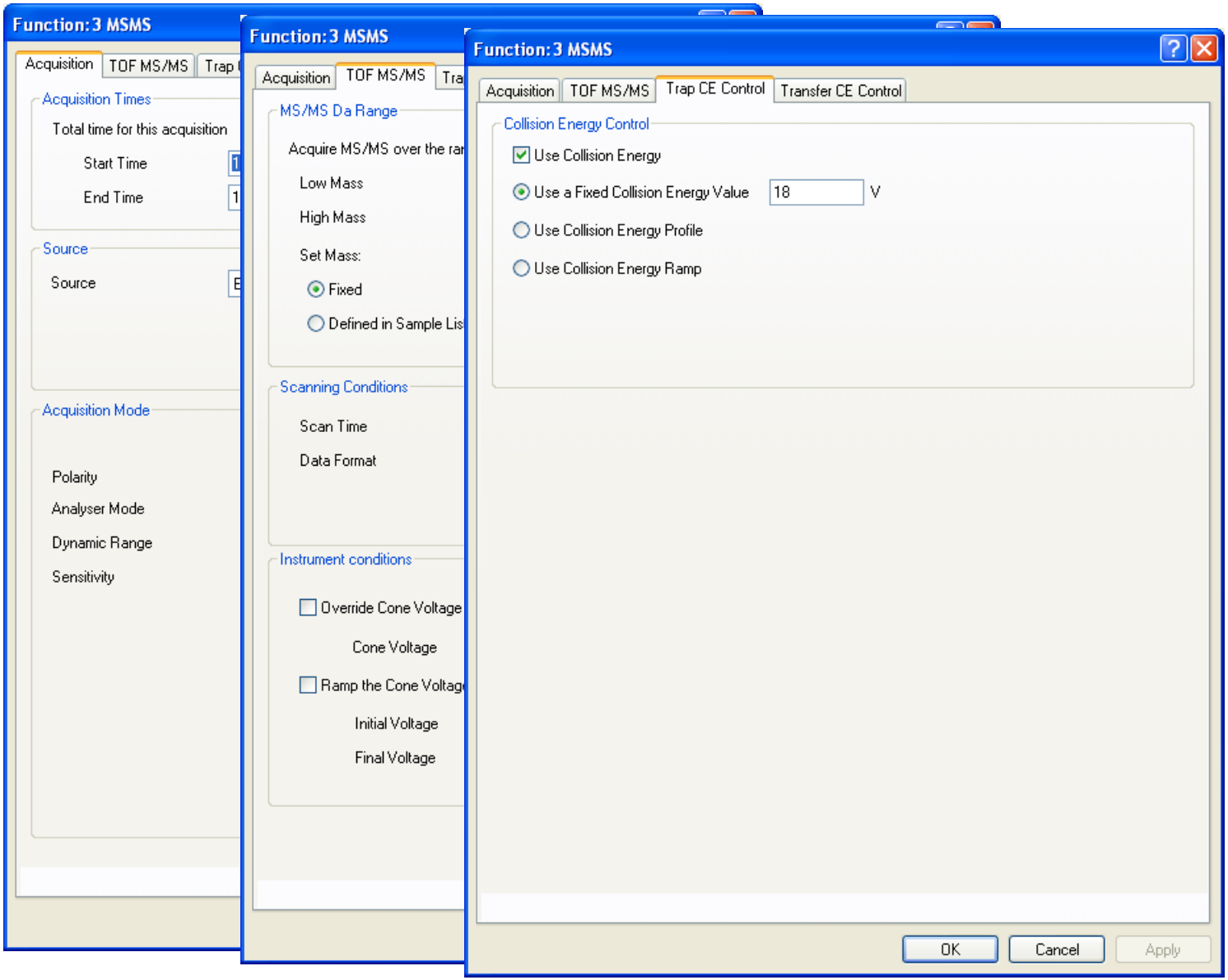


https://skyline.gs.washington.edu/labkey/wiki/home/software/Skyline/page.view?name=tutorial_targeted_msms

Targeted MS/MS (Pseudo-MRM) Method on a QToF

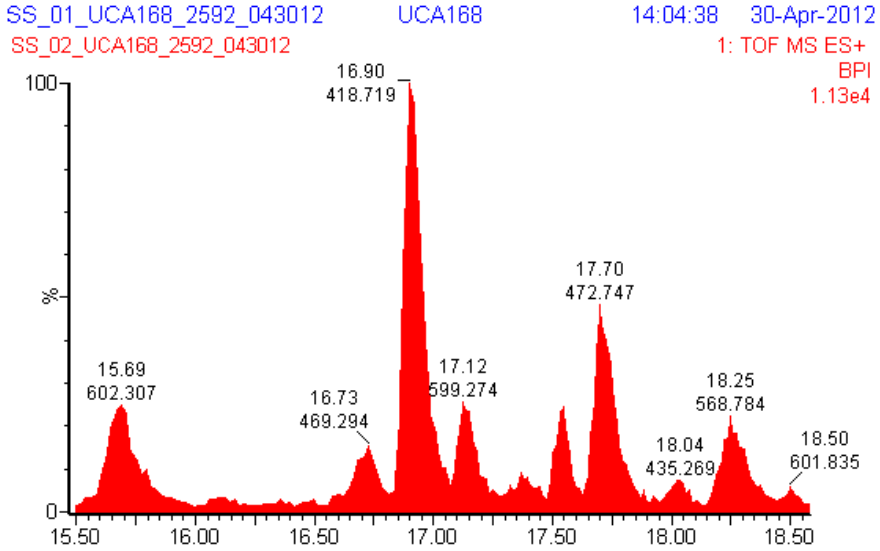


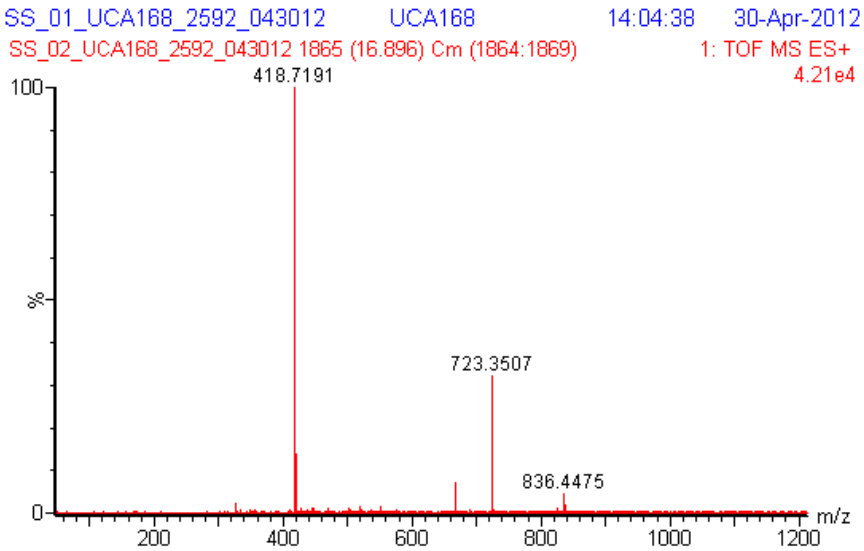
Acquisition Method Details, Continued…

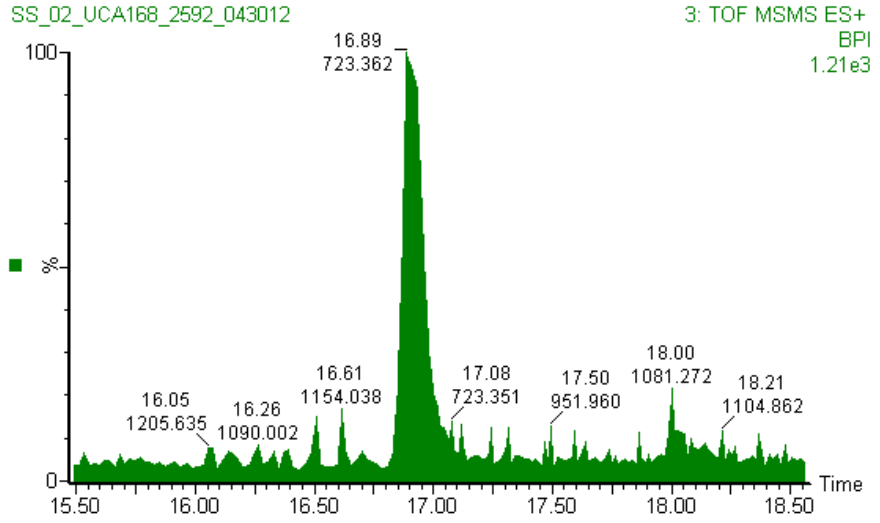


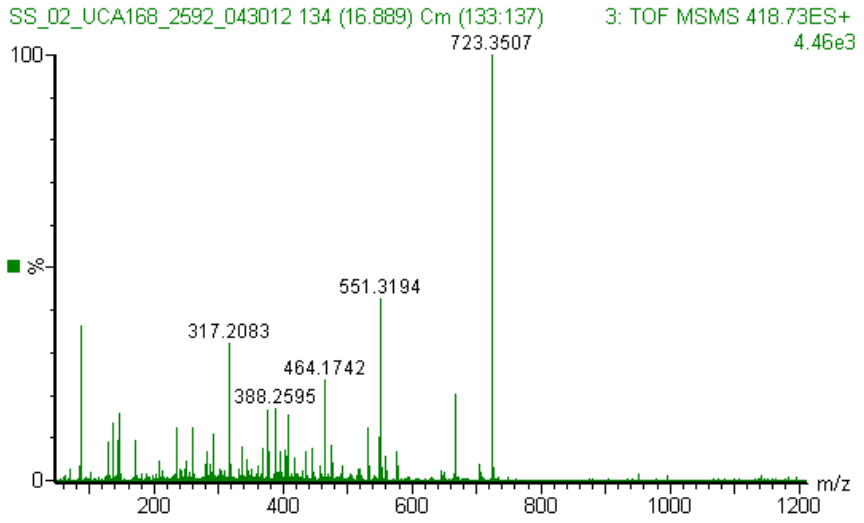
Example Raw Data

50 fmol ADH1_YEAST digest, Synapt G2 HDMS

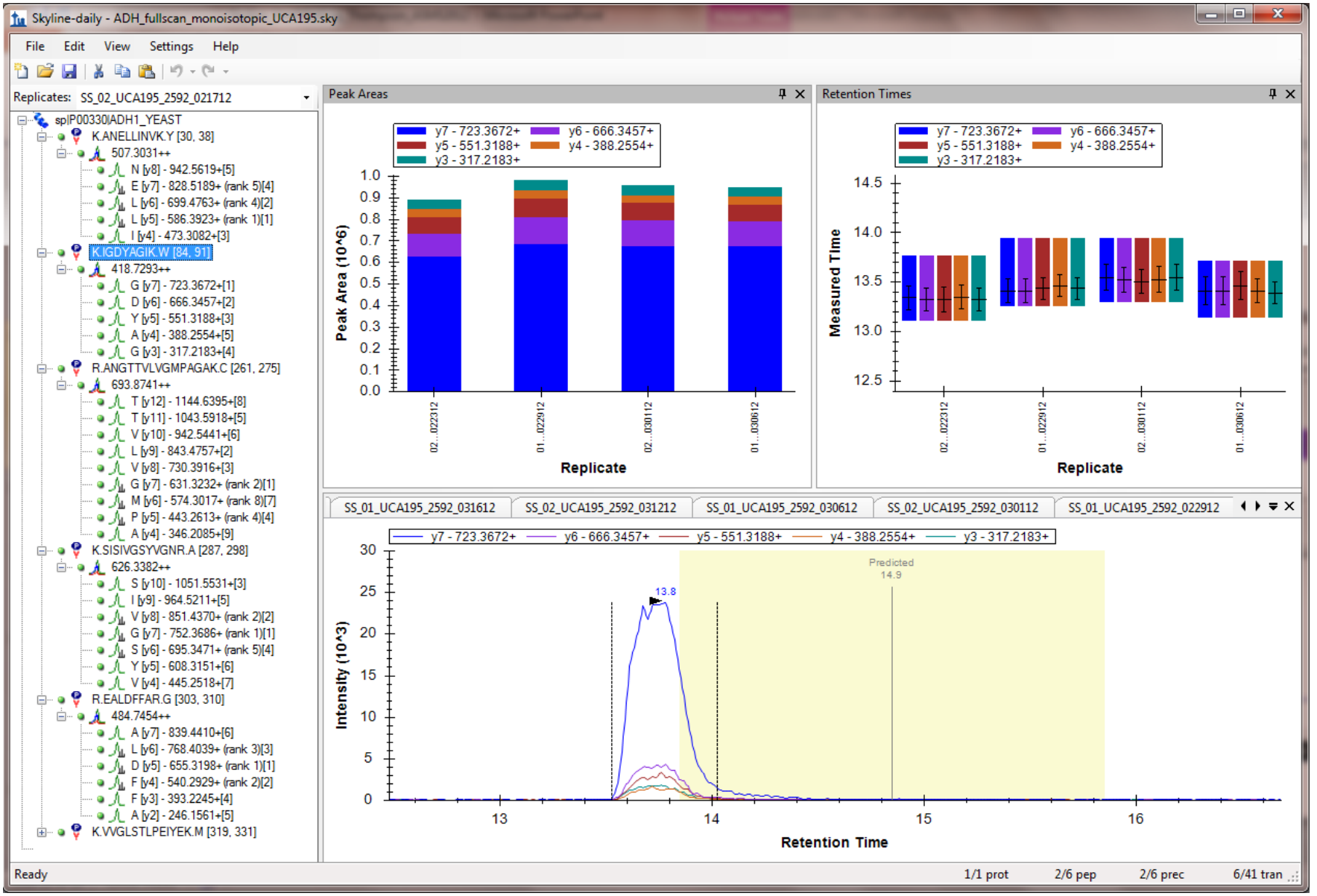




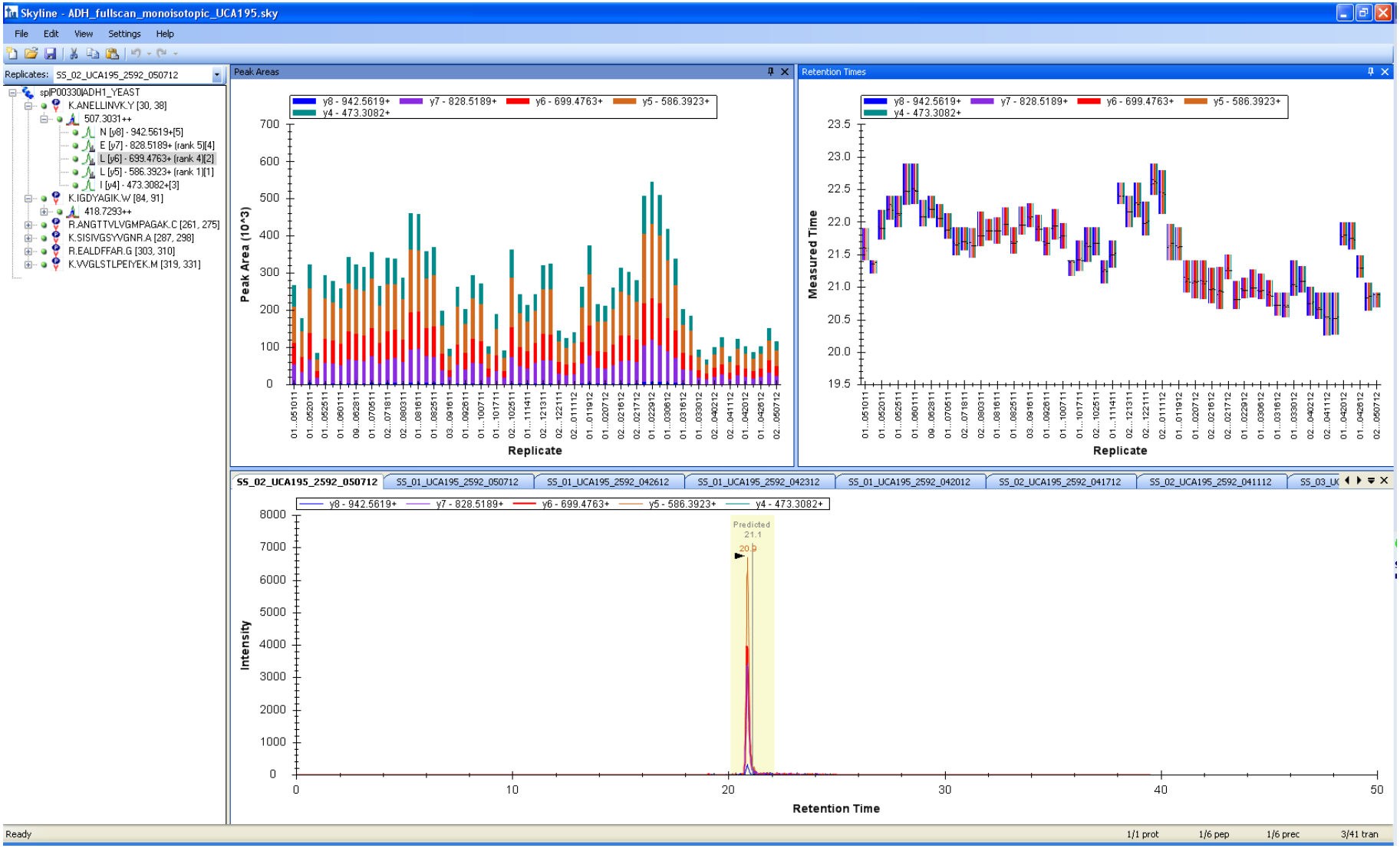




Skyline Customizable View



Longitudinal Measurements over 1 Year



Peak Intensity versus Sequence Coverage

Nominal mass (M_r): **37282**; Calculated pI value: **6.21**
NCBI BLAST search of ADH1_YEAST against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Saccharomyces cerevisiae

Fixed modifications: Carbamidomethyl (C)
Variable modifications: Deamidated (NQ),Oxidation (M)
Semi-specific cleavage, (peptide can be non-specific at one ter
Cleavage by semiTrypsin: cuts C-term side of KR unless next res
Sequence Coverage: **48%**

Matched peptides shown in **Bold Red**

```
    1 MSIPETQKGV IFYESHGKLE YKDIPVPKPK ANELLINVKY SGVCHTDLHA
   51 WHGDWPLPVK LPLVGGHEGA GVVVGMGENV KGWKIGDYAG IKWLNGSCMA
  101 CEYCELGNES NCPHADLSGY THDGSFQQYA TADAVQAAHI PQGTDLAQVA
  151 PILCAGITVY KALKSANLMA GHWVAISGAA GGLGSLAVQY AKAMGYRVLG
  201 IDGGEGKEEL FRSIGGEVFI DFTKEKDIVG AVLKATDGGA HGVINVSVSE
  251 AAIEASTRYV RANGTTVLVG MPAGAKCCSD VFNQVVKSIS IVGSYVGNRA
  301 DTREALDFFA RGLVKSPIKV VGLSTLPEIY EKMEKGQIVG RYVVDTSK
```

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Semi-specific cleavage, (peptide can be non-specific at one termi
Cleavage by semiTrypsin: cuts C-term side of KR unless next resid
Sequence Coverage: **46%**

Matched peptides shown in **Bold Red**

```
    1 MSIPETQKGV IFYESHGKLE YKDIPVPKPK ANELLINVKY SGVCHTDLHA
   51 WHGDWPLPVK LPLVGGHEGA GVVVGMGENV KGWKIGDYAG IKWLNGSCMA
  101 CEYCELGNES NCPHADLSGY THDGSFQQYA TADAVQAAHI PQGTDLAQVA
  151 PILCAGITVY KALKSANLMA GHWVAISGAA GGLGSLAVQY AKAMGYRVLG
  201 IDGGEGKEEL FRSIGGEVFI DFTKEKDIVG AVLKATDGGA HGVINVSVSE
  251 AAIEASTRYV RANGTTVLVG MPAGAKCCSD VFNQVVKSIS IVGSYVGNRA
  301 DTREALDFFA RGLVKSPIKV VGLSTLPEIY EKMEKGQIVG RYVVDTSK
```

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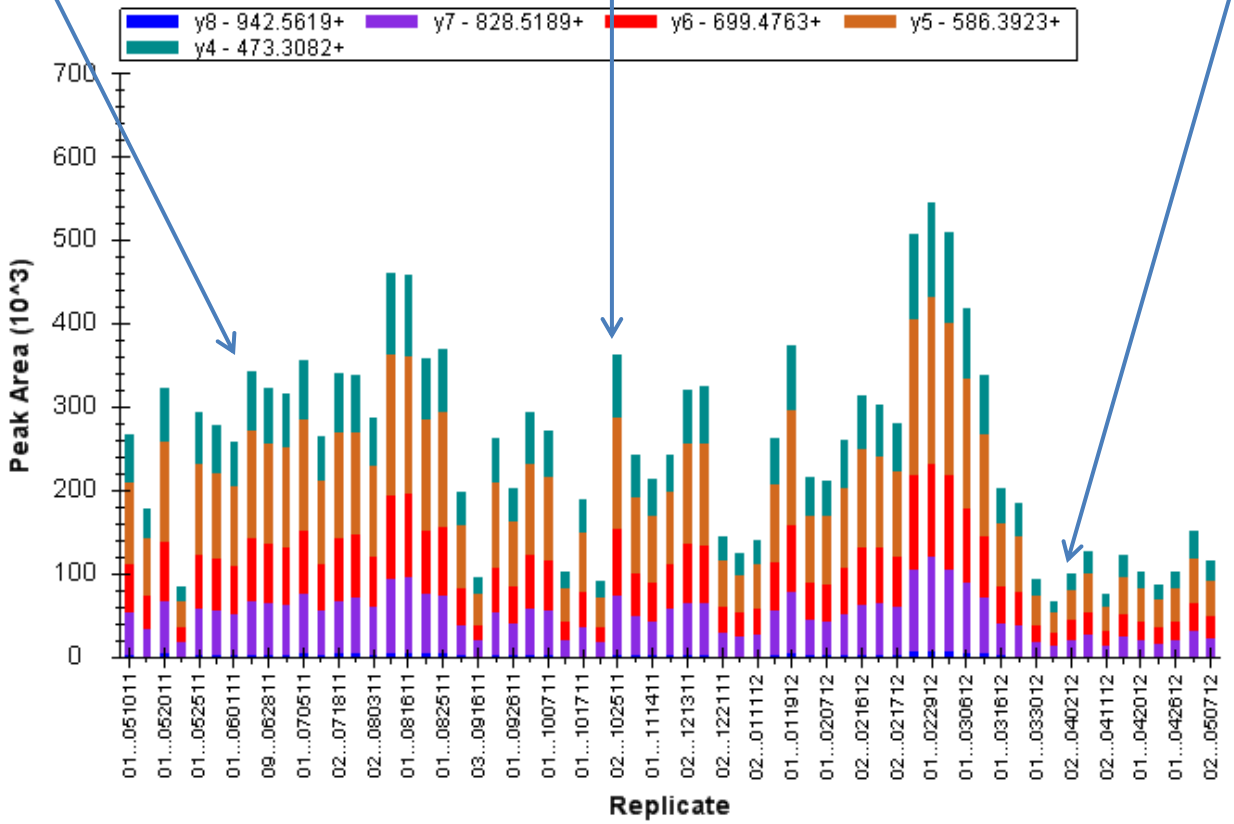
Unformatted sequence string for pasting into other applications

Taxonomy: Saccharomyces cerevisiae

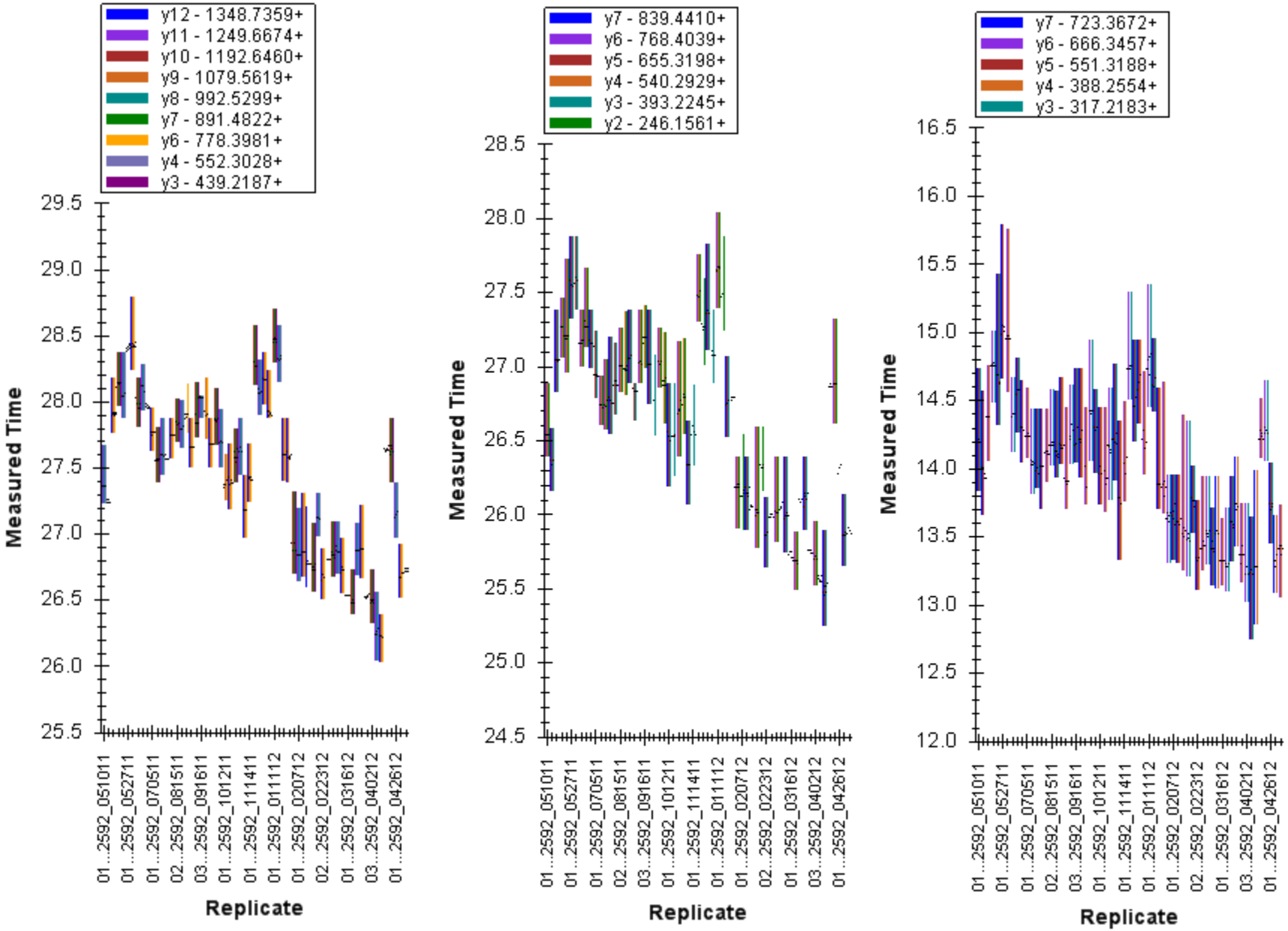
Fixed modifications: Carbamidomethyl (C)
Variable modifications: Deamidated (NQ),Oxidation (M)
Semi-specific cleavage, (peptide can be non-specific at one term
Cleavage by semiTrypsin: cuts C-term side of KR unless next resi
Sequence Coverage: **49%**

Matched peptides shown in **Bold Red**

```
    1 MSIPETQKGV IFYESHGKLE YKDIPVPKPK ANELLINVKY SGVCHTDLHA
   51 WHGDWPLPVK LPLVGGHEGA GVVVGMGENV KGWKIGDYAG IKWLNGSCMA
  101 CEYCELGNES NCPHADLSGY THDGSFQQYA TADAVQAAHI PQGTDLAQVA
  151 PILCAGITVY KALKSANLMA GHWVAISGAA GGLGSLAVQY AKAMGYRVLG
  201 IDGGEGKEEL FRSIGGEVFI DFTKEKDIVG AVLKATDGGA HGVINVSVSE
  251 AAIEASTRYV RANGTTVLVG MPAGAKCCSD VFNQVVKSIS IVGSYVGNRA
  301 DTREALDFFA RGLVKSPIKV VGLSTLPEIY EKMEKGQIVG RYVVDTSK
```

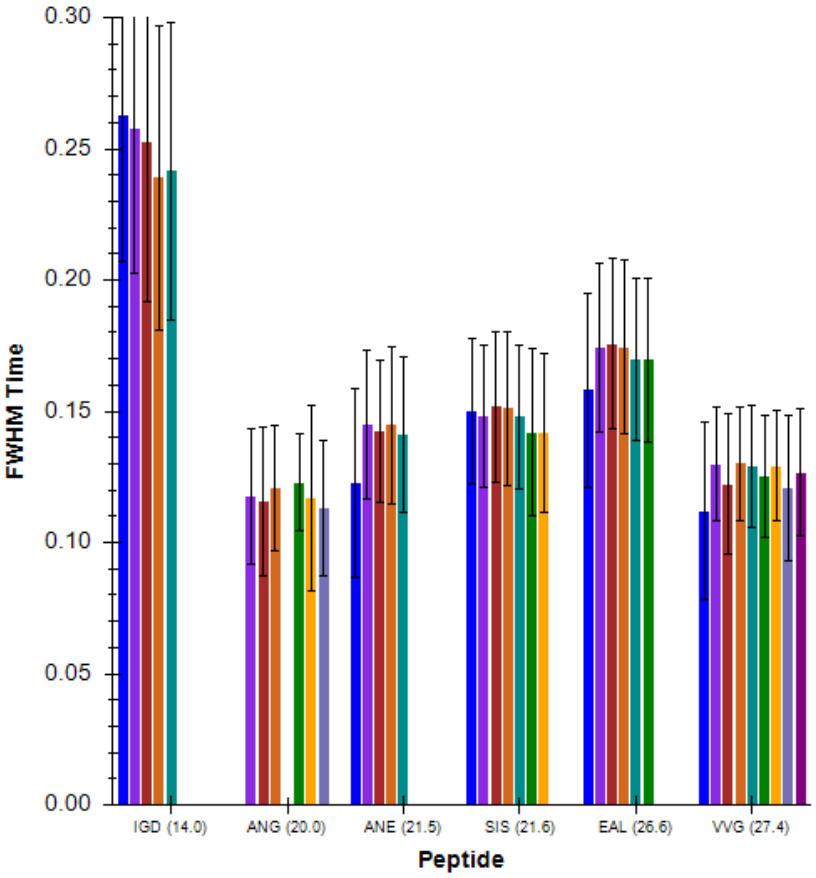


LC Retention Time Consistency

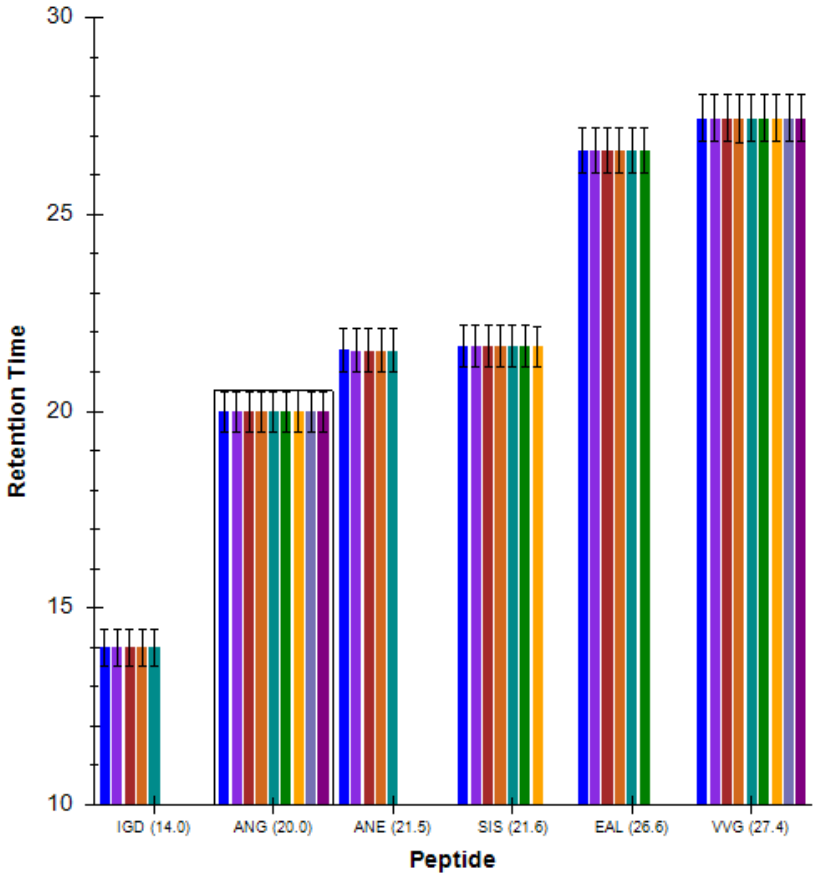


Other Chromatography Metrics

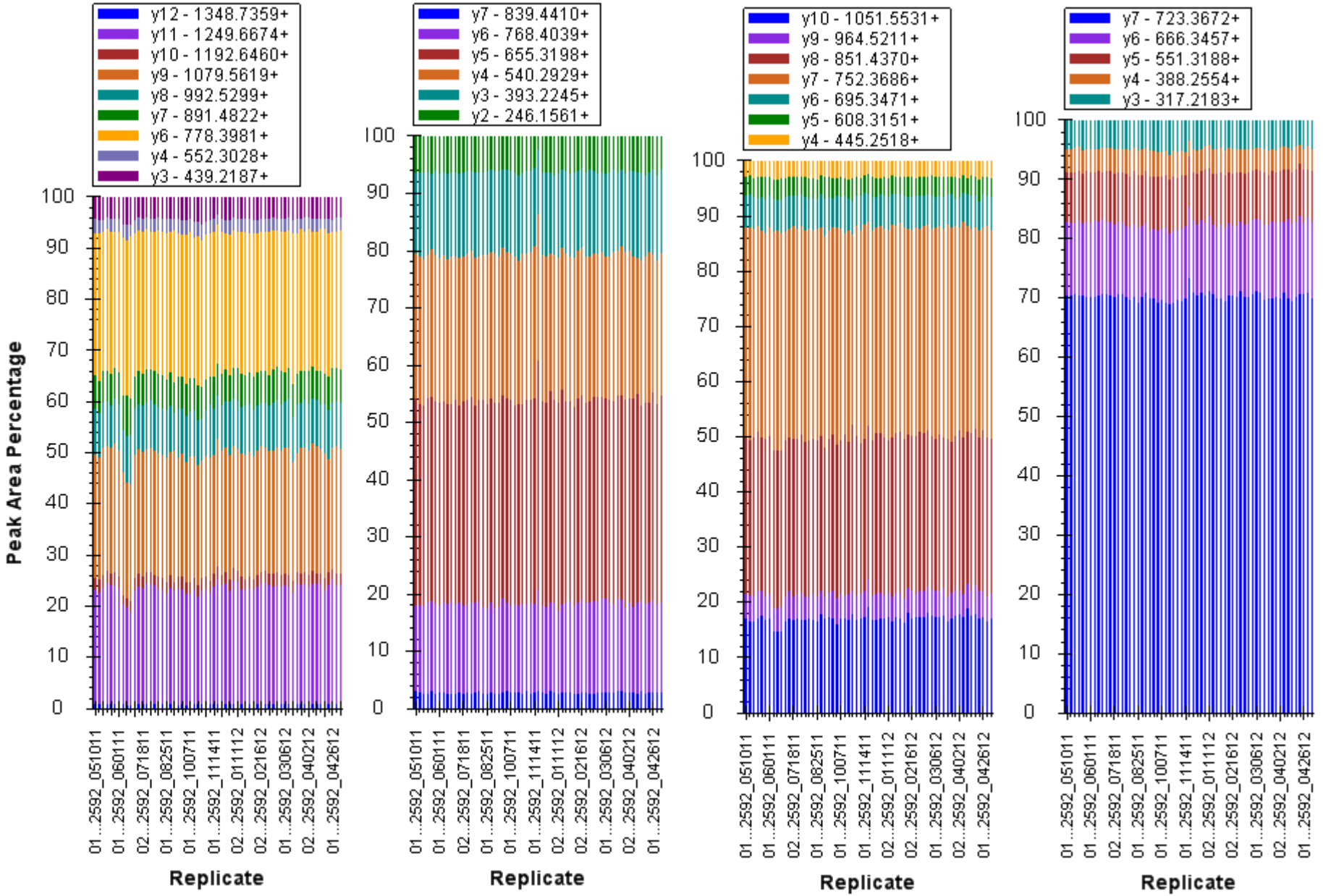
Average Peak Widths (with CV)



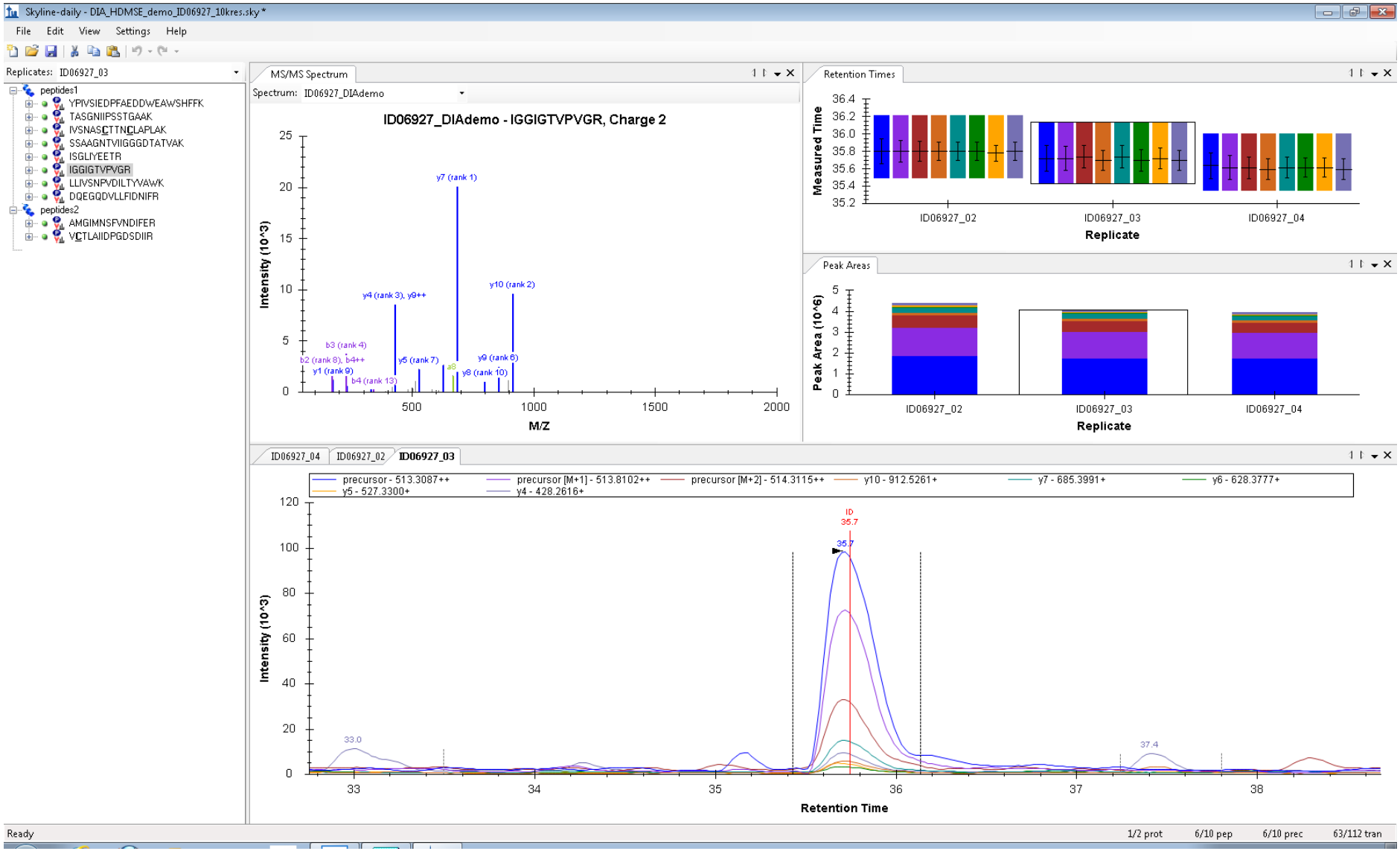
Average Retention Time (with CV)



MS/MS Fragmentation Consistency



Example of Precursor and Product Ion Extraction in Skyline, Ion Mobility-Data Independent Acquisition



Summarization

- Skyline provides a fast, efficient way to gain many system suitability metrics from most MS raw data formats
  - Thermo, Waters, AB/Sciex, and Agilent
- Easy to visualize performance over time, with data at the individual peptide or aggregate level
- Can utilize Full Scan MS1, MS/MS, MRM, or DIA approaches for system suitability
- Ability to set/visualize “thresholds” for performance metrics once they are established would be a plus