

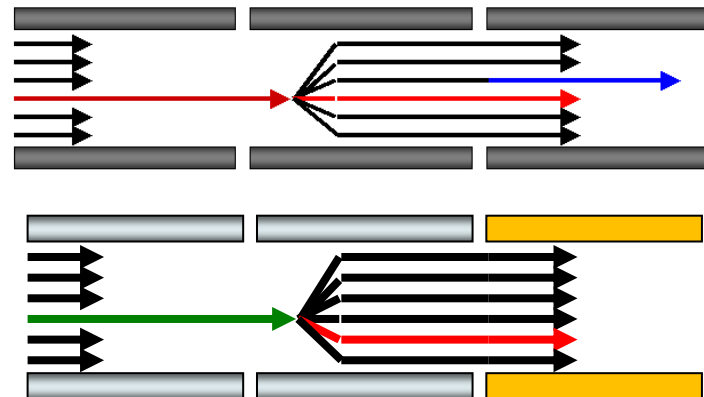


# Skyline

## Targeted Proteomics Environment

Status of the Skyline open-source software project  
five years after its inception

Brendan MacLean



# User Community After 4 Years

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- ▶ 560 registered users
- ▶ 150 registered for this meeting
- ▶ 4 new papers in May
  - ▶ Platform Independent and Label-free Quantitation of Proteomic Data using MSI Extracted Ion Chromatograms in Skyline – Mol. Cel. Prot.
  - ▶ Label-Free Quantitation of Protein Modifications by Pseudo-Selected Reaction Monitoring with Internal Reference Peptides – J. Prot. Res.
  - ▶ Using iRT, a Normalized Retention Time for More Targeted Measurement of Peptides - Proteomics
  - ▶ The Development of Selected Reaction Monitoring Methods for Targeted Proteomics via Empirical Refinement - Proteomics
- ▶ 25 abstracts at ASMS mention Skyline
- ▶ 75 citations of original paper (after 2 ½ years)
  - ▶ 30 in 2012



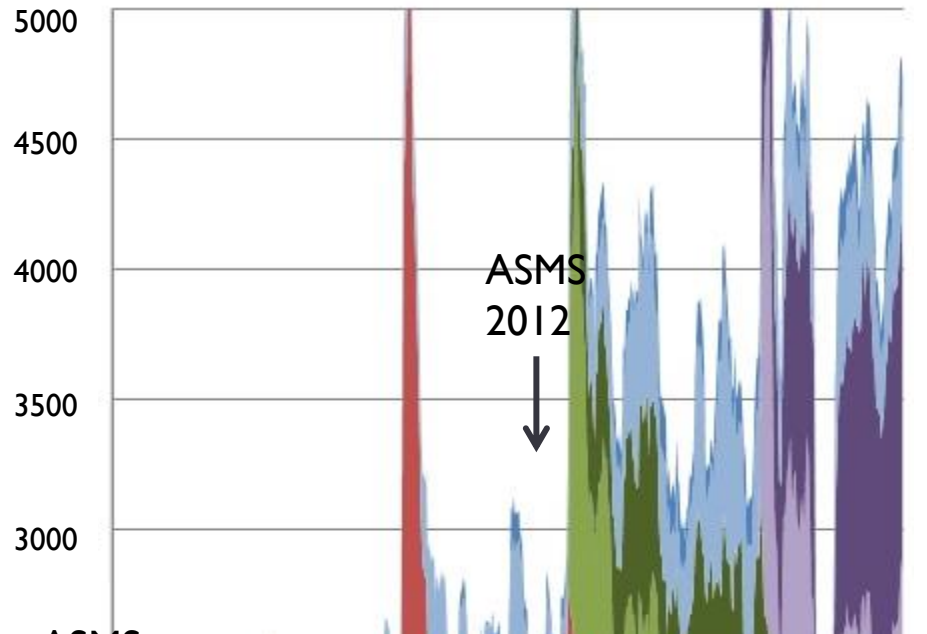
# User Community After 5 Years

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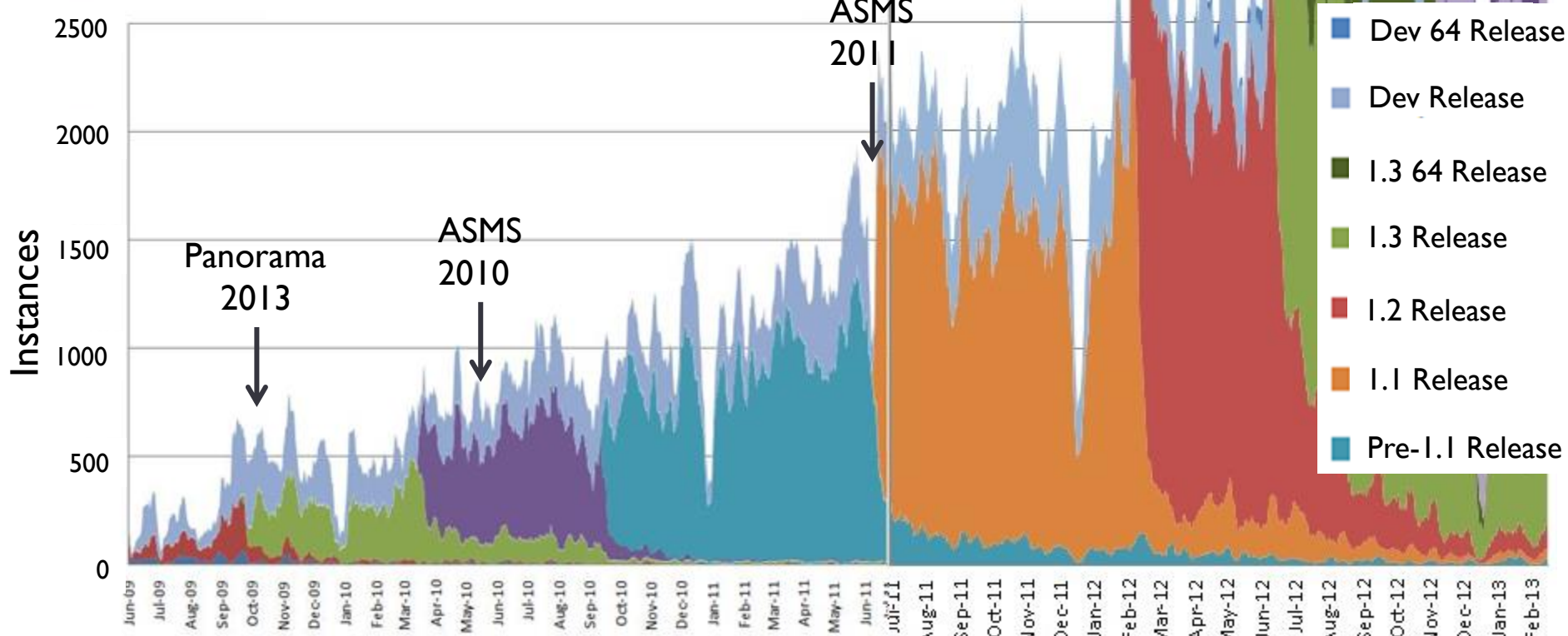
- ▶ 1000+ registered users
- ▶ 250 registered for this meeting
- ▶ More papers and press
  - ▶ Targeted proteomics Nature Methods Method of the Year in 2012
  - ▶ MSI Label-free Quantification Using Ion Intensity Chromatograms in Skyline (Research and Clinical Applications) (book chapter)
  - ▶ Design, Implementation, and Multi-Site Evaluation of a System Suitability Protocol for the Quantitative Assessment of Instrument Performance in LC-MRM-MS - MCP
  - ▶ Viewing the Targeted Proteomics Horizon with Skyline – eProtein feature
- ▶ 37 abstracts at ASMS mention Skyline
- ▶ 200+ citations of original paper



# Skyline Use

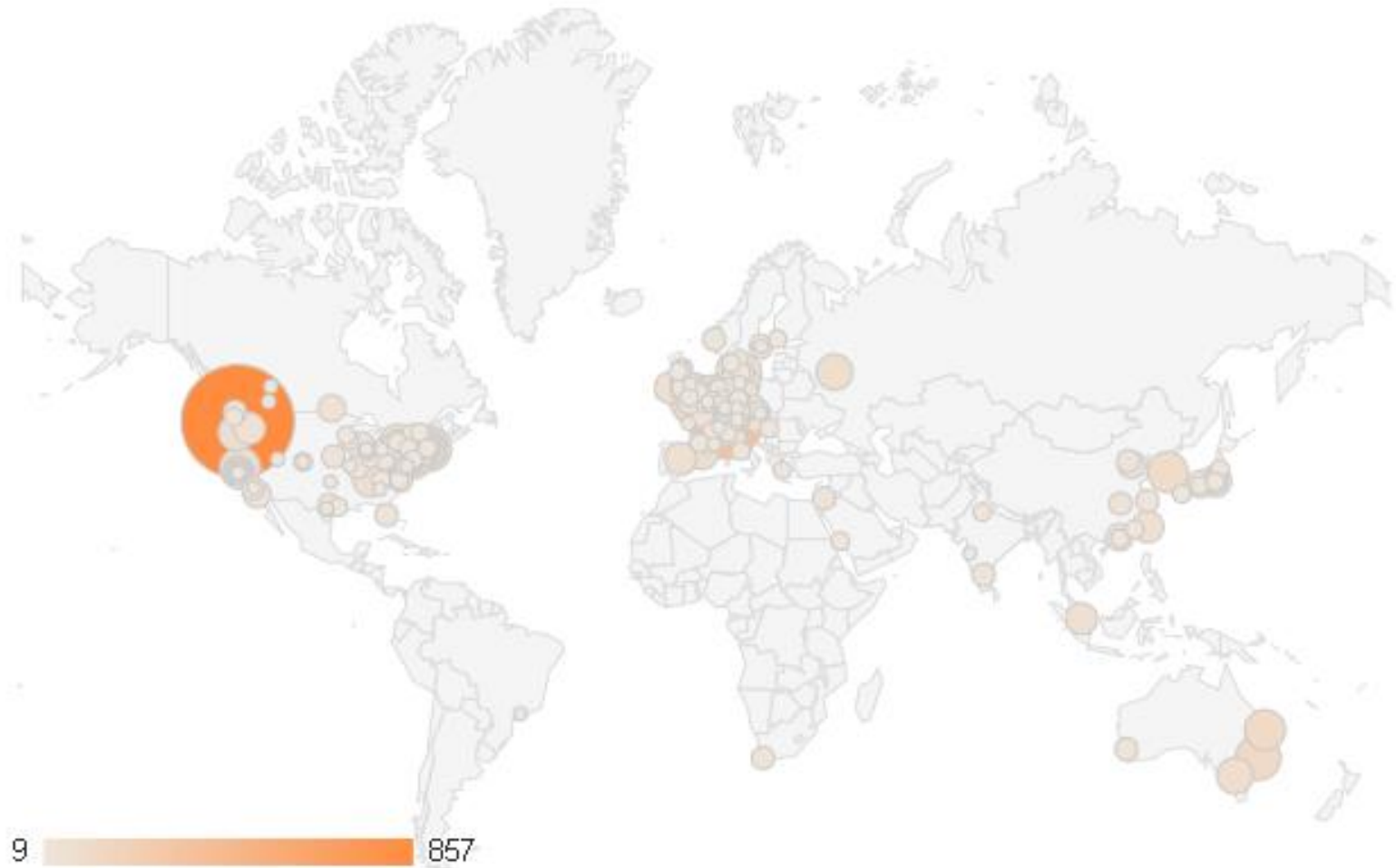


Skyline Instances Started Trailing 7 Days



# Skyline Web Site Visits (past 3 months)

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# Learning More

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- ▶ Au PS Workshop, Melbourne (February)
- ▶ US HUPO Workshop, Baltimore (March)
- ▶ Skyline User Group Meeting! here (now)
- ▶ SRM Course, Zurich (July)
- ▶ Proteomics Course, Cold Springs Harbor (July)
  
- ▶ **Targeted Quant. Course, Seattle**
- ▶ **September 9-14**



# Instrument Vendor Partnerships

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# Panorama Partnership Program

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- ▶ 2 – 4 labs
- ▶ Seeking leading edge targeted proteomics infrastructure
- ▶ Local Panorama server installation
- ▶ Direct collaboration with Skyline/Panorama team
- ▶ Full support for 1 year





# Prior Knowledge and Consistency

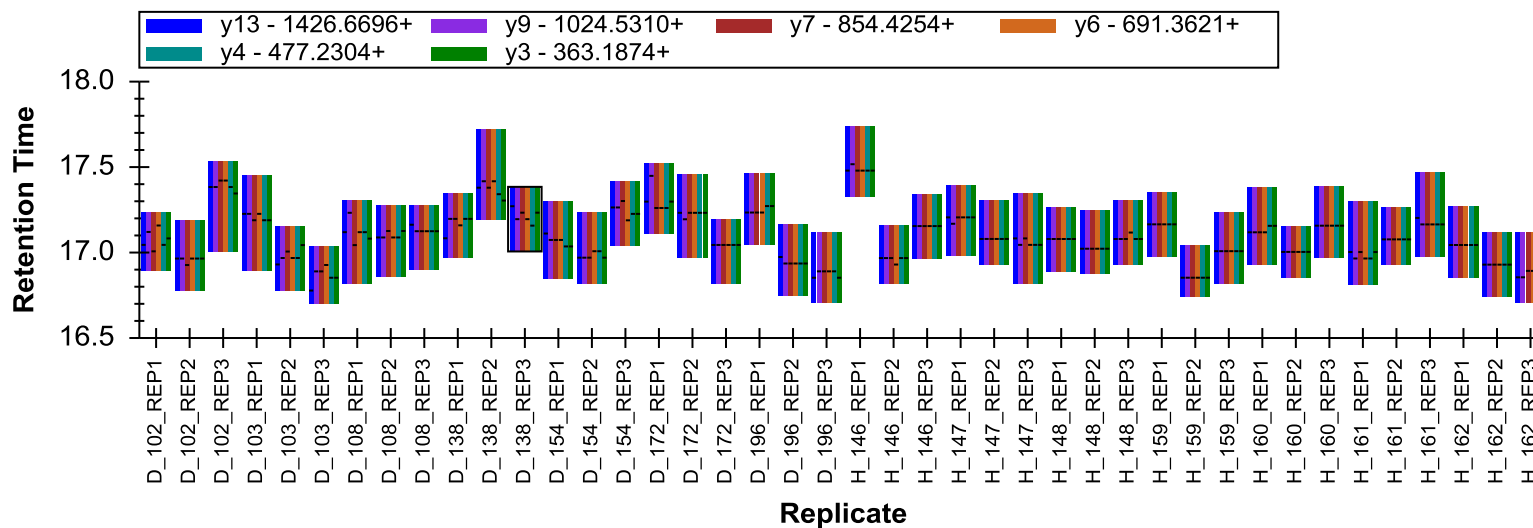
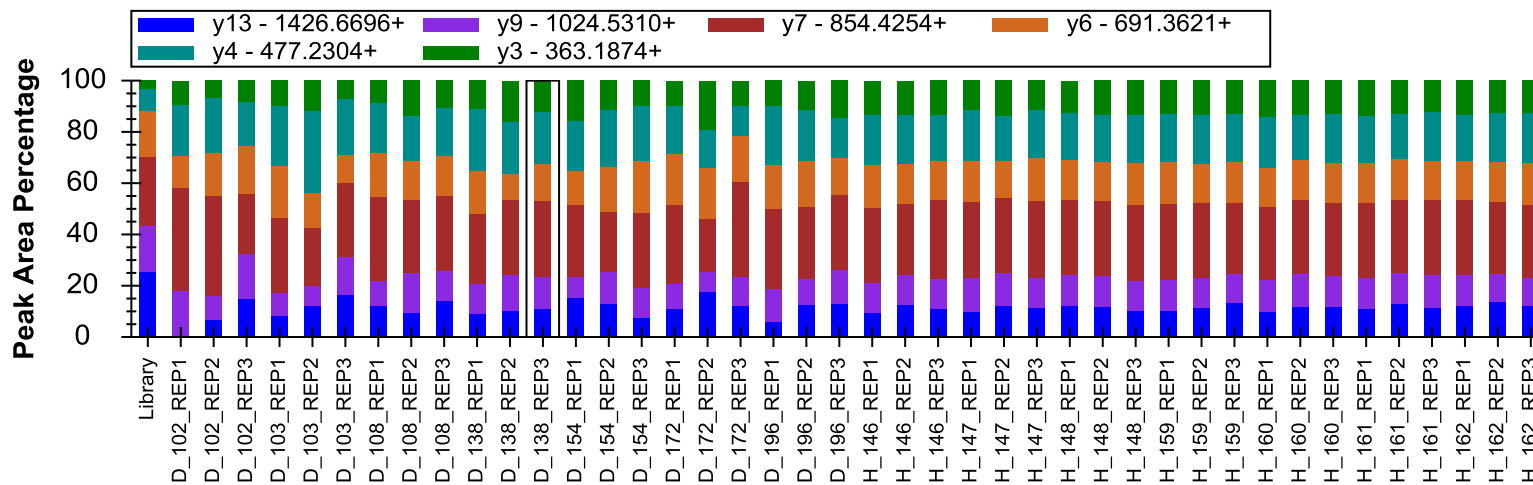
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- ▶ Powerful enough to be used cross-lab / cross experiment
- ▶ More powerful run-to-run
  
- ▶ Relative ion abundance
  - ▶ Spectral and chromatogram libraries
- ▶ Retention time
  - ▶ iRT
  
- ▶ Does ensuring comparable measurements require ID?



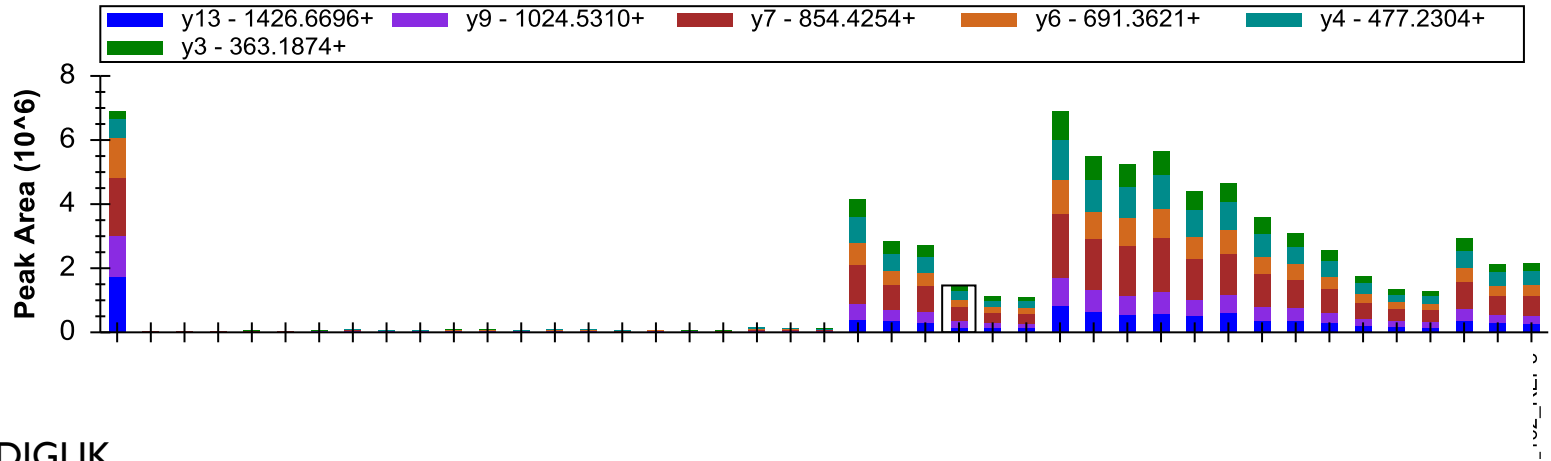
# Haptoglobin

LQTEGDGIYTLNSEK

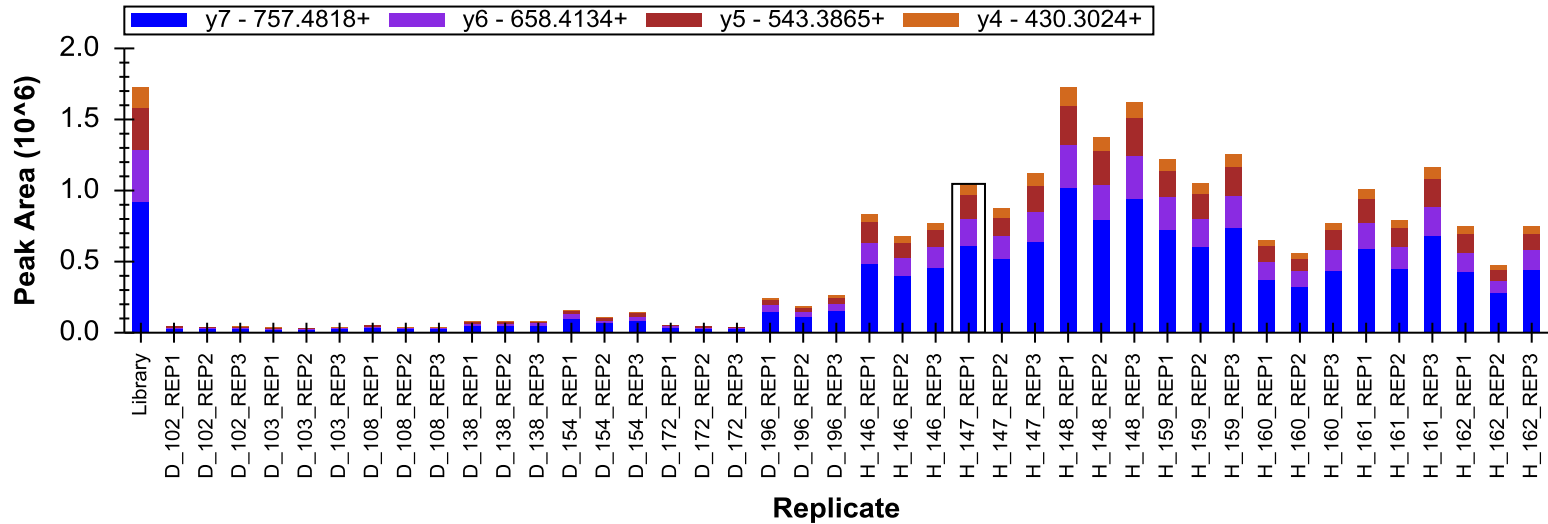


# Haptoglobin

LQTEGDGIYTLNSEK

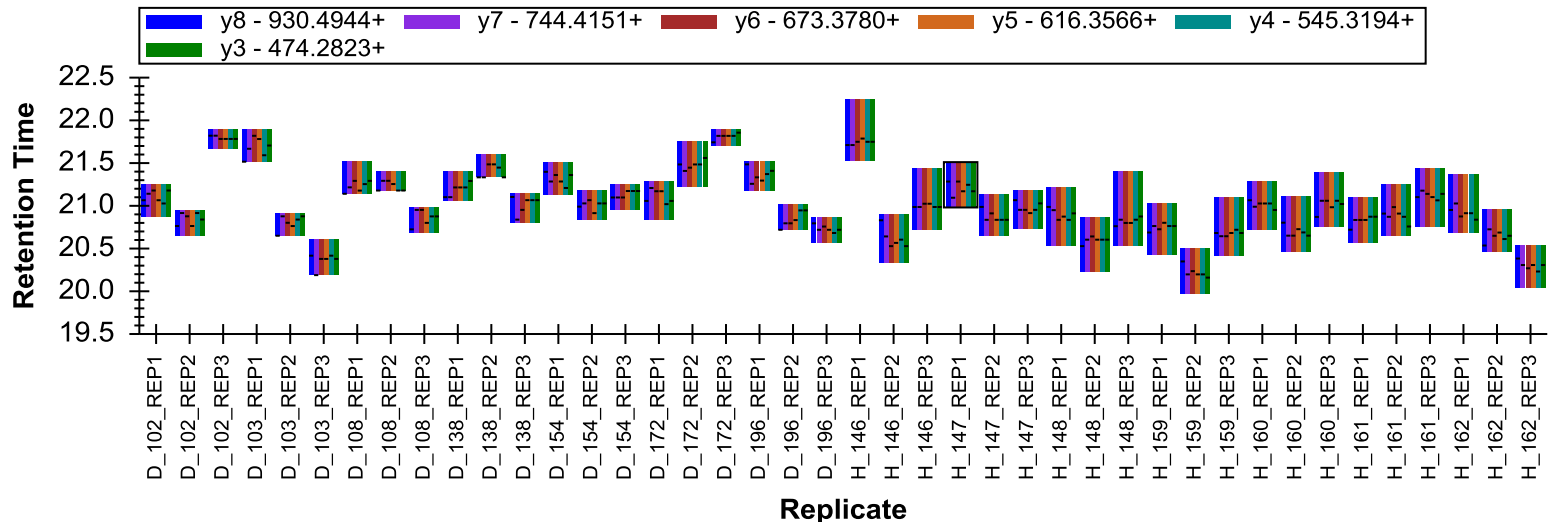
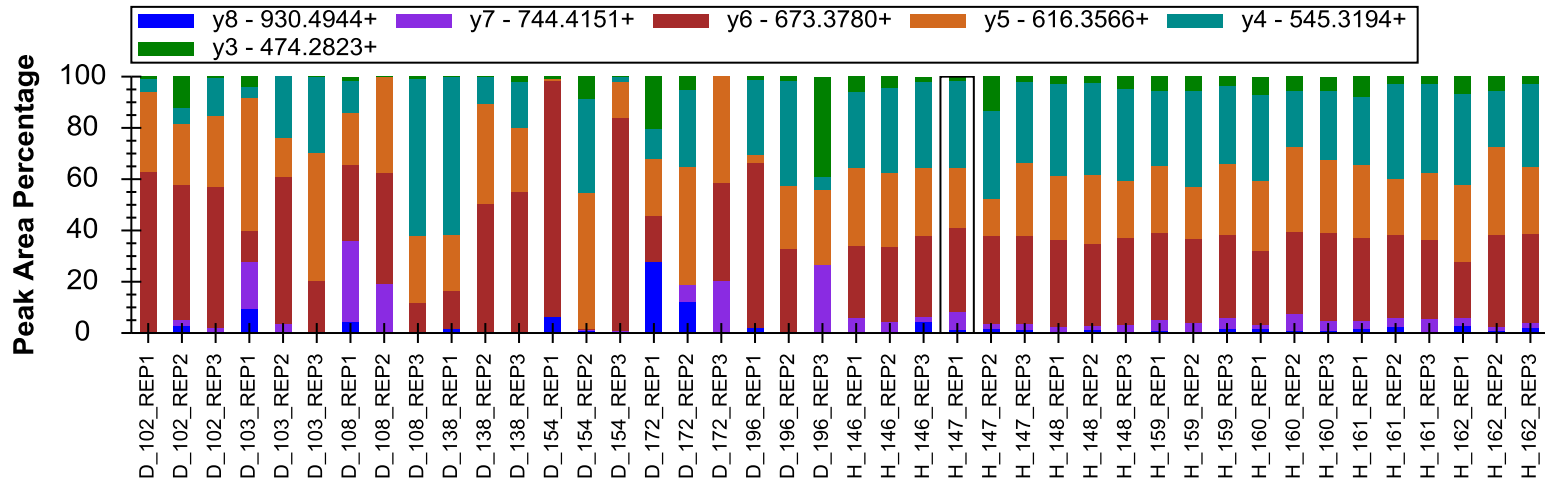


SVVDIGLIK



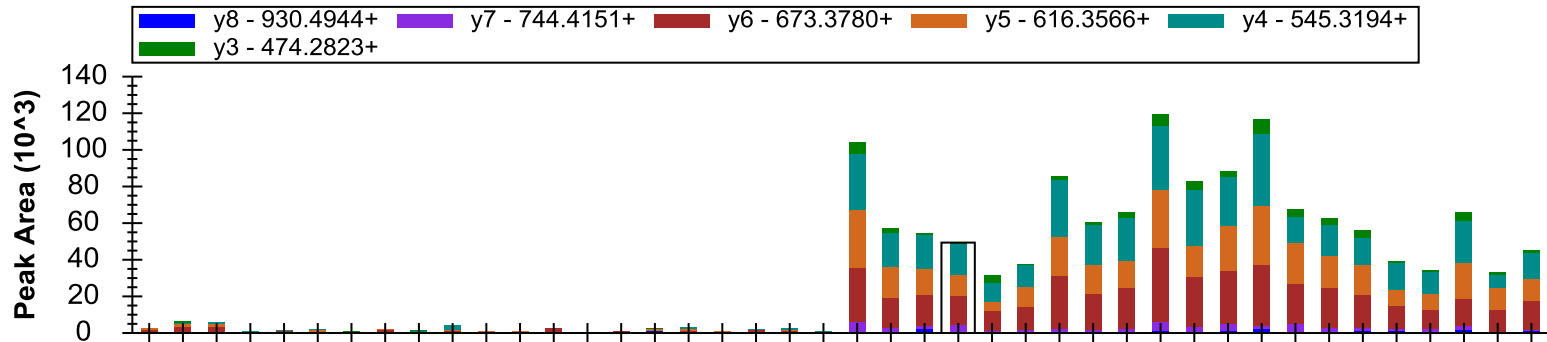
# Mitochondrial 39S ribosomal protein L9

CSSLWAGAAWLR

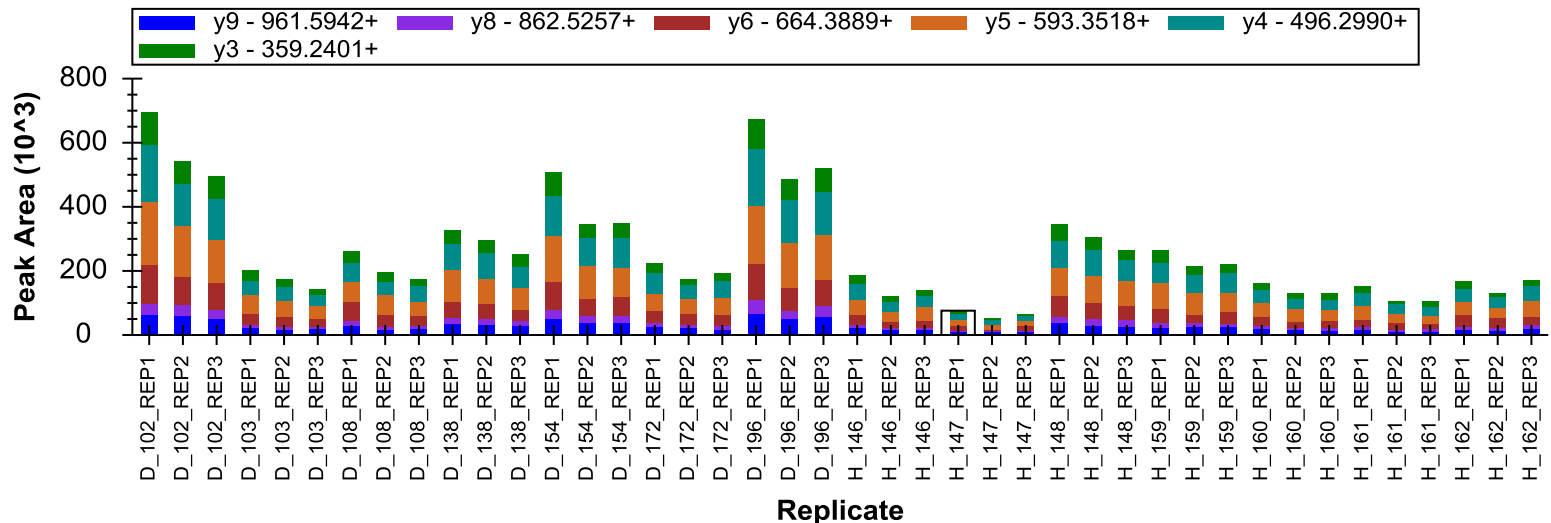


# Mitochondrial 39S ribosomal protein L9

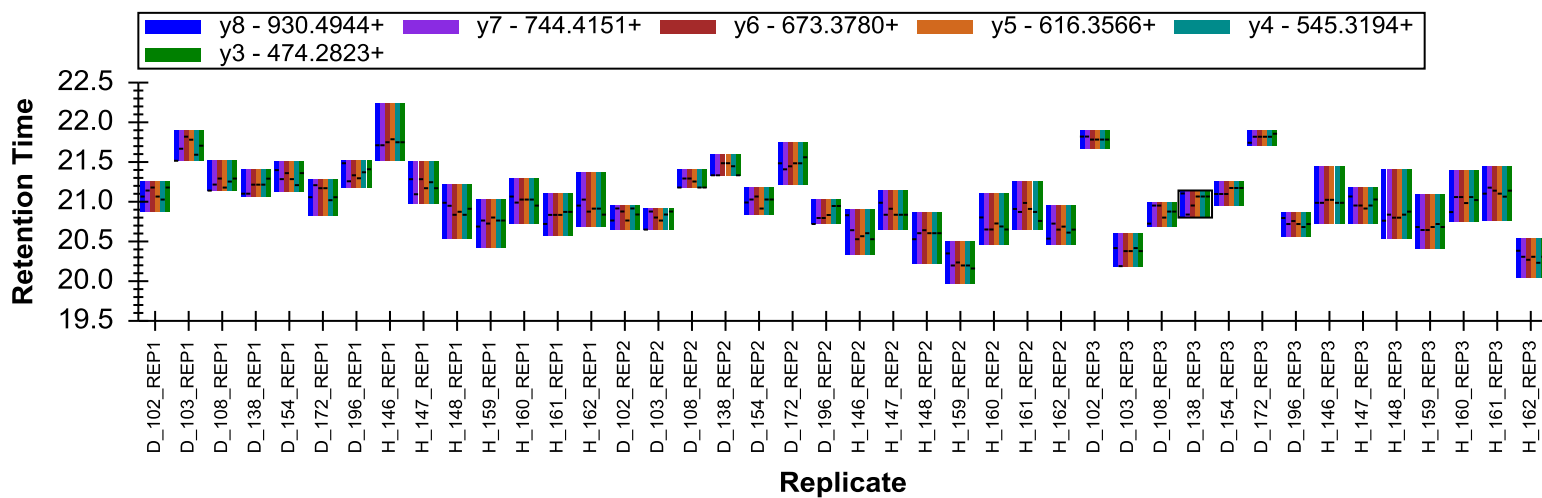
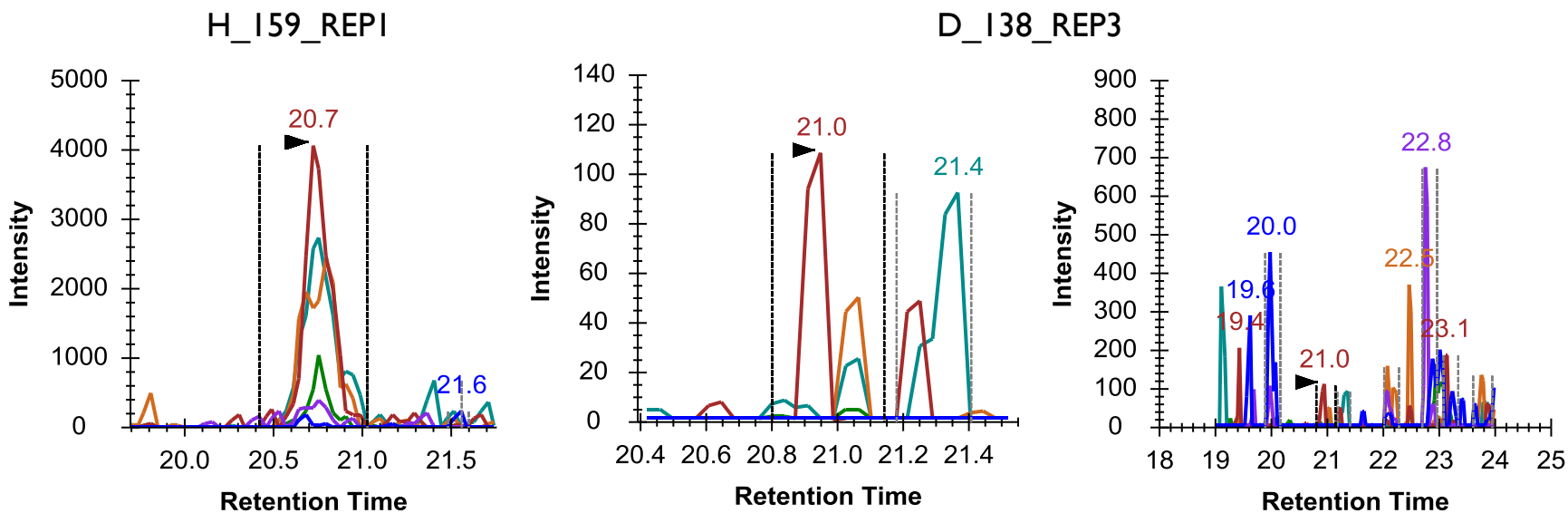
CSSLLWAGAAWLR



SVVDIGLIK

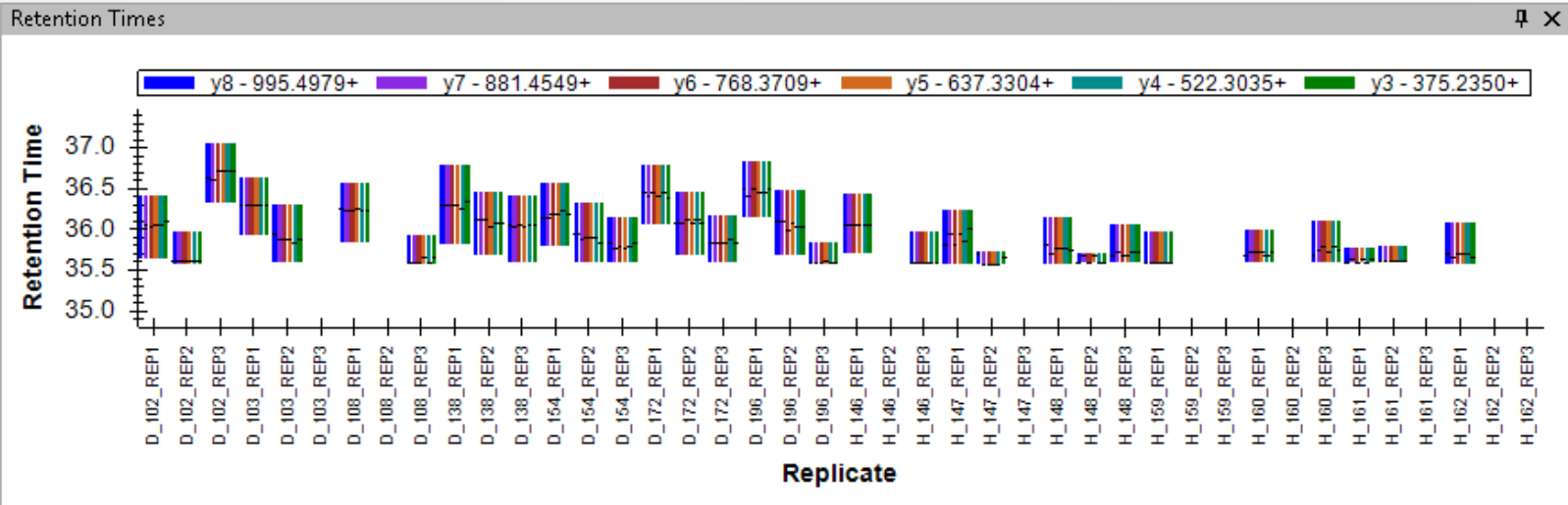
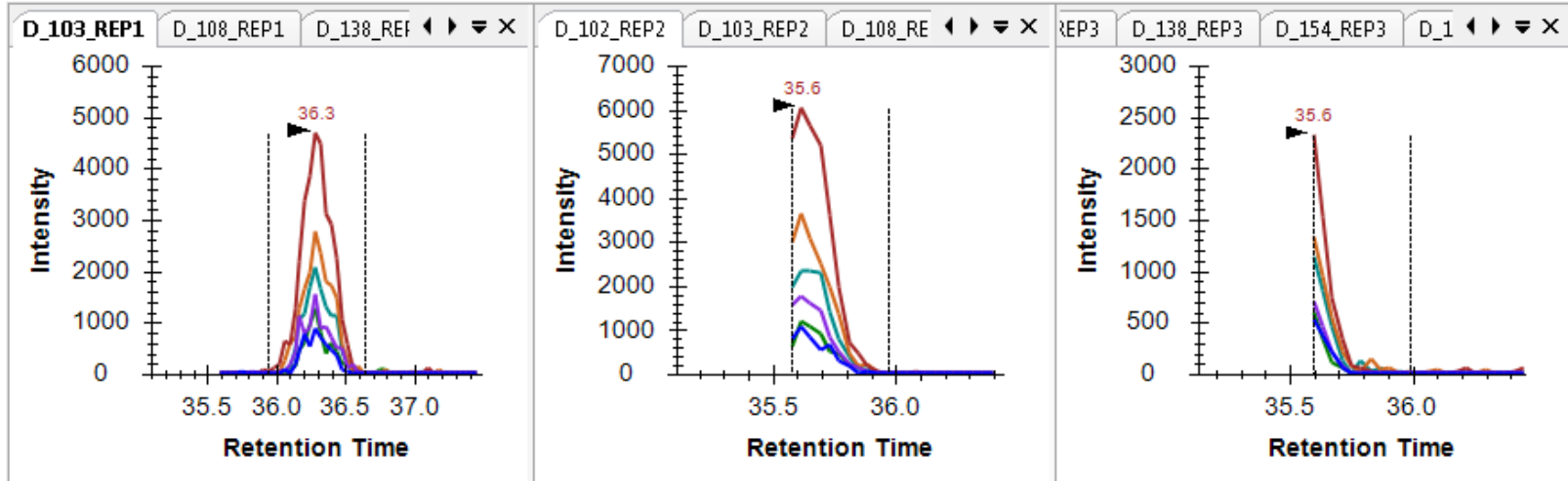


# CSSLWAGAAWLR

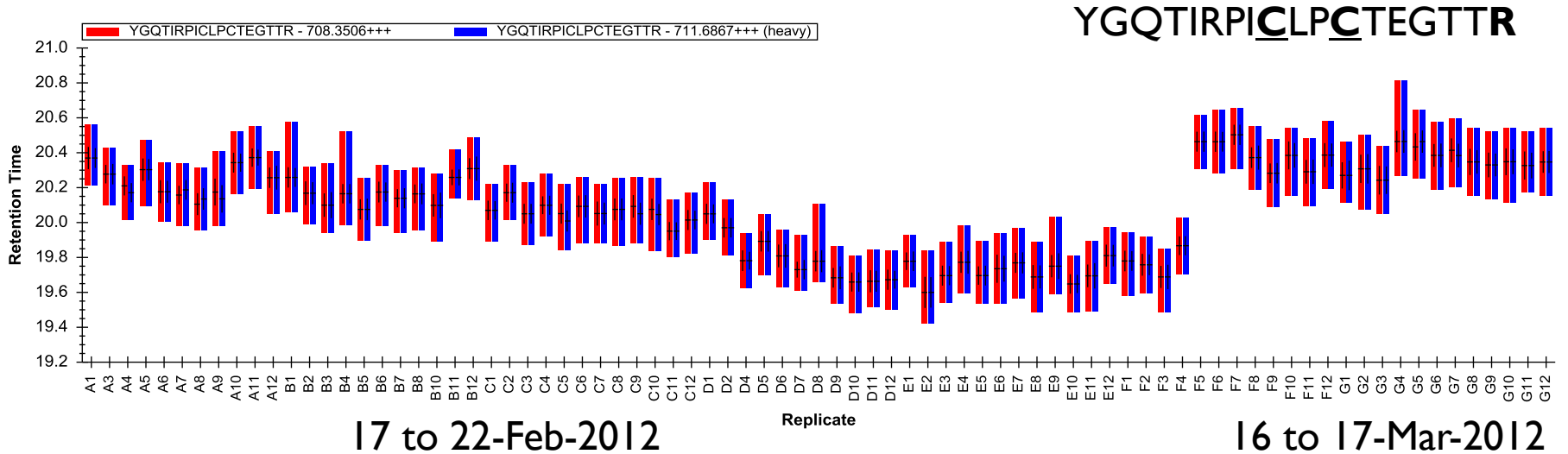


# Truncated and Missing Peaks

TGTLNLMDFLSR



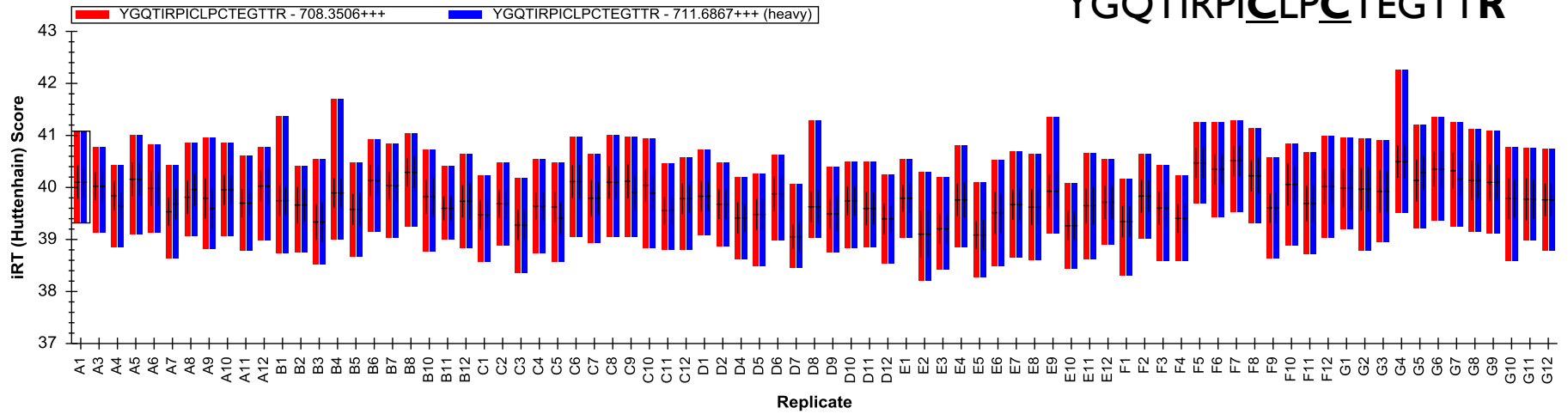
# Deciphering the Unexpected



Replicate Name	Acquired Time	Peptide Peak Found Ratio	Peptide Retention Time	Ratio To Standard	BioReplicate	Run	Condition
F2	2/21/2012 9:02:23 PM	0.83	19.46	0.7895	59	59	Disease
F3	2/21/2012 10:15:23 PM	0.83	19.39	1.1252	60	60	Disease
F4	2/22/2012 12:41:17 AM	1	19.66	1.2937	61	61	Disease
F5	3/16/2012 6:47:27 AM	1	20.13	1.2389	62	62	Healthy
F6	3/16/2012 8:00:25 AM	1	20.18	0.9268	63	63	Healthy
F7	3/16/2012 9:13:23 AM	1	20.17	1.3614	64	64	Healthy



# Aligned by iRT



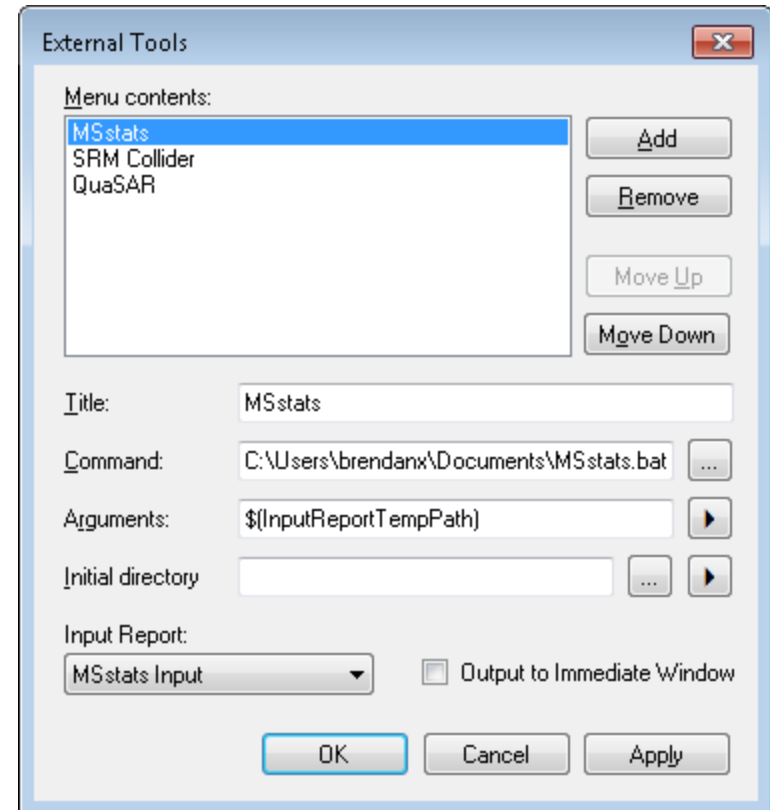
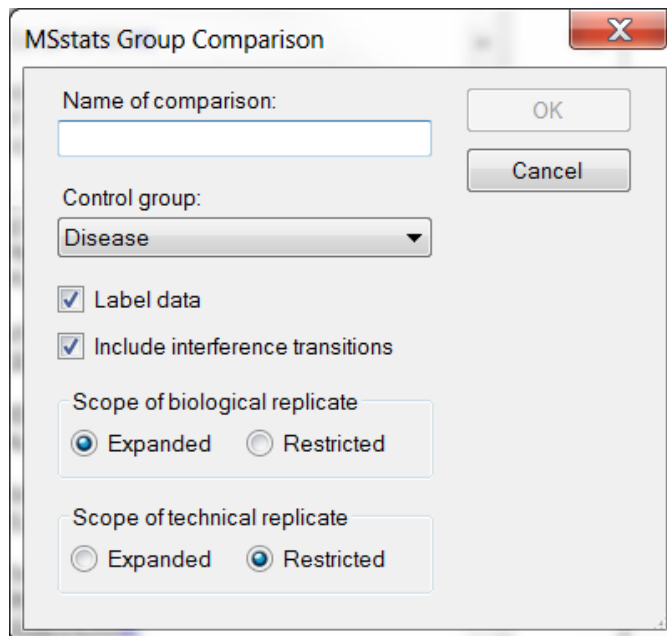
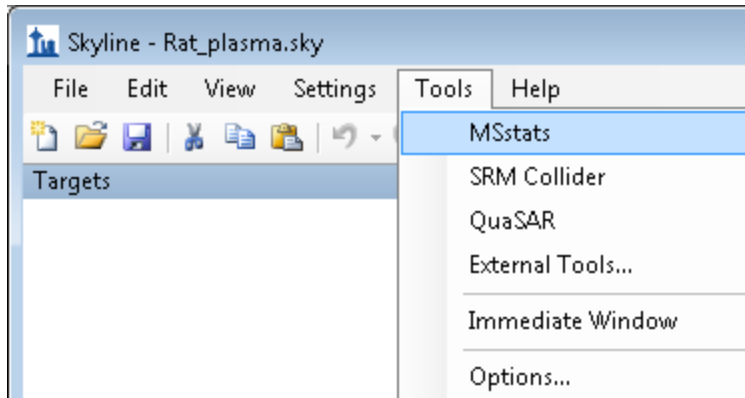
# Integrating with External Tools

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- ▶ GeneSpring & Skyline Automation Tool (Agilent)
- ▶ MSstats (Purdue) – Grouped study analysis
- ▶ QuaSAR (Broad Institute) – Response curves, LOD, LOQ
- ▶ MSI Probe (Buck Institute) – MSI quant. statistics
  - ▶ TP28 – Alexandria D'Souza
- ▶ SRM Collider (IMSB) – Interference probability calculator



# External Tool MSstats



# Replicate Annotations

**Define Annotation**

Name:

Type:

Values:

Applies To:

- Proteins
- Peptides
- Precursors
- Transitions
- Replicates
- Precursor Results
- Transition Results

**Results Grid**

Replicate Name	SubjectId	BioReplicate	Run	Condition
D_172_REP1	D172	6	6	Disease
D_172_REP2	D172	6	20	Disease
D_172_REP3	D172	6	34	Disease
D_196_REP1	D196	7	7	Disease
D_196_REP2	D196	7	21	Disease
D_196_REP3	D196	7	35	Disease
H_146_REP1	H146	8	8	Healthy
H_146_REP2	H146	8	22	Healthy
H_146_REP3	H146	8	36	Healthy
		9	9	Healthy
		9	23	Healthy
		9	37	Healthy
		10	10	Healthy
		10	24	Healthy
		10	38	Healthy
		11	11	Healthy
		11	25	Healthy
		11	39	Healthy

Filter: > >|

**Annotation Settings**

Annotations are extra pieces of data which you can attach to elements in a Skyline document. Use this dialog to control which annotations are available in this document, as well as to define new annotations.

- SubjectId
- BioReplicate
- Run
- Condition
- Concentration

# Custom Reports

ProteinName	PeptideSequence	Precurs	Frag	Produ	IsotopeLa	Condition	Bio	Run	Area
NP_036629	CSLPRPWALTF SYGR	2	y10	1	light	Disease	1	1	14516
NP_036629	CSLPRPWALTF SYGR	2	y10	1	light	Disease	1	15	9607
NP_036629	CSLPRPWALTF SYGR	2	y10	1	light	Disease	1	29	7480
NP_036629	CSLPRPWALTF SYGR	2	y10	1	light	Disease	2	2	5692
NP_036629	CSLPRPWALTF SYGR	2	y10	1	light	Disease	2	16	5953
NP_036629	CSLPRPWALTF SYGR	2	y10	1	light	Disease	2	30	649

**Edit Report** ✕

Report Name:  Preview...

- Peptides
- Results
  - ProteinName
  - ProteinDescription
  - ProteinSequence
  - ProteinNote

Add >

ProteinName

PeptideSequence

PrecursorCharge

FragmentIon

ProductCharge

IsotopeLabel Type

Condition

BioReplicate

Run

Area

Pivot Replicate Name
  Pivot Isotope Label
 OK
Cancel

FSYGR	2	y10	1	light	Disease	3	3	10476
FSYGR	2	y10	1	light	Disease	3	17	3952
FSYGR	2	y10	1	light	Disease	3	31	3165
FSYGR	2	y10	1	light	Disease	4	4	9830
FSYGR	2	y10	1	light	Disease	4	18	10671
FSYGR	2	y10	1	light	Disease	4	32	6369
FSYGR	2	y10	1	light	Disease	5	5	15037
FSYGR	2	y10	1	light	Disease	5	19	9128
FSYGR	2	y10	1	light	Disease	5	33	6918
FSYGR	2	y10	1	light	Disease	6	6	11991
FSYGR	2	y10	1	light	Disease	6	20	8630
FSYGR	2	y10	1	light	Disease	6	34	6896
FSYGR	2	y10	1	light	Disease	7	7	13061
FSYGR	2	y10	1	light	Disease	7	21	12258
FSYGR	2	y10	1	light	Disease	7	35	9037
FSYGR	2	y10	1	light	Healthy	8	8	7891
FSYGR	2	y10	1	light	Healthy	8	22	3362
FSYGR	2	y10	1	light	Healthy	8	36	4448





# Skyline Automation with SkylineRunner

The screenshot displays the Skyline Automation software interface. The window title is "Skyline Automation - D:\SkylineData\199bug\200CEOpt.sky". The interface is divided into several sections:

- Project setting:** Includes fields for "Template method" (D:\MassHunter\Methods\sky1.m), "Study folder" (D:\MassHunter\Data), and "Study name" (AutoTest1). There are "Browse..." buttons for the first two fields and a "Timestamp" checkbox.
- Action selections:** A list of checkboxes for "Step-A (Update Retention Times)", "Step-B (Optimize Collision Energy)", "Step-C (Export method, create worklist)", and "Execute worklist", all of which are checked.
- Step-A configuration:** A sub-panel for "Step-A" with tabs for "Step-A", "Step-B", and "Step-C". It includes an "Export method name:" field (MethodA), radio buttons for "Single method", "One method per protein", and "Multiple methods" (selected), and a checkbox for "Ignore proteins". It also has a "Max transitions per sample injection:" field (200), an "Optimizing:" dropdown (None), and "Method type:" (Standard) and "Dwell time (ms):" (10) fields.
- Workflow diagram:** A visual representation of the automation process showing three "Skyline" steps (Step-A, Step-B, Step-C) connected by arrows, with sample vials and data files.
- Sample configuration tables:** Three tables, one for each step, showing "Sample prefix" (Sample), "Start position" (P1-A1), and "All samples in same position" (checkbox). Each table has an "Edit" button and columns for "Sample Name", "Sample Position", "Method", and "Data File".
- Buttons:** "Create Project", "Submit to Study Manager", and "Close" buttons are located at the bottom.
- Status bar:** Displays "Project D:\MassHunter\Data\Test4.s has been submitted to Study Manager successfully! (5/29/2013 7:02:10 PM)" and "Total samples: 1".

# MS/MS Spectral Library Sources

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- ▶ Global Proteome Machine
- ▶ NIST
- ▶ Peptide Atlas
  
- ▶ Build your own from peptide search results
  - ▶ *Mascot*
  - ▶ **MaxQuant Andromeda**
  - ▶ **Morpheus**
  - ▶ Myrimatch / IDPicker
  - ▶ OMSSA
  - ▶ **PRIDE XML**
  - ▶ *Protein Pilot*
  - ▶ Protein Prospector
  - ▶ **Proteome Discoverer (MSF)**
  - ▶ *Scaffold – mzIdentML / MGF*
  - ▶ Spectrum Mill
  - ▶ TPP – pepXML / mzXML files – Peptide Atlas
  - ▶ Waters Mse
  - ▶ *X! Tandem*





# Integrating Great Ideas (iRT)

The screenshot displays the Skyline software interface for peptide analysis. The main window shows a list of targets on the left, with 'DLATVYVDV LK' highlighted. The central plot shows the chromatogram for this peptide, with two peaks labeled 'DLATVYVDV LK - 618.3477++' (red) and 'DLATVYVDV LK - 622.3548++ (heavy)' (blue). The predicted retention time is 28.5, and the measured retention time is 28.4. The bottom plot shows the relationship between measured and predicted retention times for various peptides, with regression statistics: slope = 0.20, intercept = 11.96, window = 1.5, r = 0.9985. The right panel shows the 'Edit iRT Calculator' dialog, which lists standard peptides and their iRT values, and measured peptides and their iRT values.

**Targets**

- Replicates: D8
- A2MG
  - SSSNEEVMFLTVQVK
- AACT
  - EIGELYLPK
- APOA
  - NPDAAAPYCYTR
- APOA1
  - DLATVYVDV LK
  - DYVSQFEQSALGK
  - VSFLSALEEYTK
- APOB
  - FSVPAGIMPSFQALTAR
- APOE
  - AATVGLAGQPLQER
  - SELEEQLTPVAEETR
  - SWFEPLVEDMQR
- B2MG
  - VEHSDLSFSK
  - VNHVTLSQPK
- C1QA
  - SLGFCDDTNK
- C1QB
  - GNLCVNLMR
  - LEQGENVFLQATDK
- C1QC
  - FQSVFTVTR
  - QTHOPPAPNSI IR

**Chromatogram**

Intensity ( $10^6$ ) vs Retention Time

DLATVYVDV LK - 618.3477++ (red)  
DLATVYVDV LK - 622.3548++ (heavy) (blue)

Predicted 28.5  
28.4

**Retention Times**

Measured Time vs Human\_plasma

Regression: slope = 0.20, intercept = 11.96, window = 1.5, r = 0.9985  
Predictor: slope = 0.20, intercept = 12.20, window = 3.0, r = 0.9985

**Edit iRT Calculator**

Name: Human\_plasma

iRT\_database: C:\Users\Brendan\Downloads\20130315\_USHUPC

Standard peptides:

Modified Sequence	iRT Value
ADVTPADFSEWSK	54.97
DGLDAASYAPVR	43.28
GAGSSEPVTGLDAK	0.23
GTFIDPAVIR	86.72
GTFIDPGVIR	71.38
LELQFGAQQSPFLK	98.09

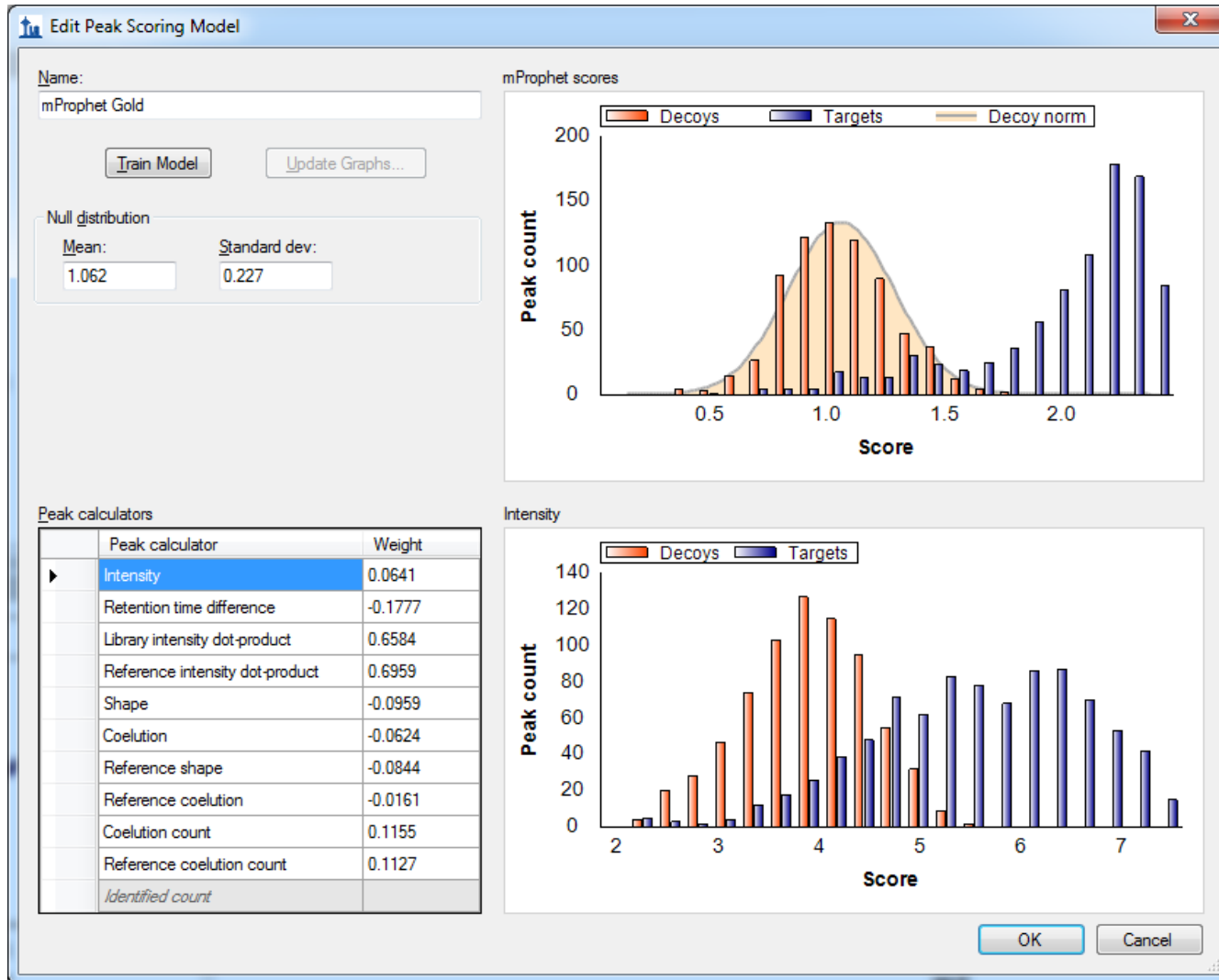
Measured peptides:

Modified Sequence	iRT Value
SSSNEEVMFLTVQVK	71.68
EIGELYLPK	46.27
NPDAAAPYC[+57.0]YTR	20.24
DLATVYVDV LK	83.56
DYVSQFEQSALGK	58.88
VSFLSALEEYTK	105.87

71 Peptides

4/37 prot 4/82 pep 7/153 prec 19/459 tran

# Integrating Great Ideas (mProphet)



# Skyline Team

---

▶ Nick Shulman



▶ Don Marsh



▶ Kaipo Tamura



▶ Danny Broudy

▶ Trevor Killeen

▶ Vagish Sharma



▶ Josh Eckels



▶ Greg Taylor



▶ Jarrett Egertson

▶ Dario Amodei



# Collaborators:

---

## ▶ U. of Wa.

- ▶ Eva Baker
- ▶ Jarrett Egertson
- ▶ Jimmy Eng
- ▶ Andrew Stergachis

## ▶ Biognosys

- ▶ Lukas Reiter
- ▶ Oliver Rinner
- ▶ Claudia Escher

## ▶ Broad Institute

- ▶ Sue Abbatiello
- ▶ Steve Carr
- ▶ Jake Jaffe
- ▶ D. R. Mani

## ▶ Buck Institute

- ▶ Birgit Schilling
- ▶ Matthew Rardin
- ▶ Brad Gibson

## ▶ Duke

- ▶ Will Thompson
- ▶ Arthur Moseley

## ▶ IMSB

- ▶ Rudolph Aebersold
- ▶ Christina Ludwig
- ▶ Olga Schubert
- ▶ Hannes Röst
- ▶ Lucia Espona Pernas

## ▶ Purdue

- ▶ Veavi Chang
- ▶ Meena Choi
- ▶ Olga Vitek

## ▶ Stanford

- ▶ Dario Amodei
- ▶ Parag Mallick

## ▶ Vanderbilt

- ▶ Matthew Chambers
- ▶ Daniel Liebler
- ▶ David Tabb



# Instrument Vendor Support

---

## ▶ Agilent Technologies

- ▶ Christine Miller
- ▶ Juli Salcedo
- ▶ Shripad Torvi
- ▶ Yinghang Yang



**Agilent Technologies**

## ▶ Bruker

- ▶ Carsten Baessmann
- ▶ Marius Kallhardt
- ▶ Stephanie Kaspar



## ▶ AB Sciex

- ▶ David Cox
- ▶ Christie Hunter
- ▶ Brent Lefebvre



## ▶ Thermo-Scientific

- ▶ Markus Kellmann
- ▶ Andreas Kuehn
- ▶ Vlad Zabrouskov



## ▶ Waters

- ▶ Laurence Firth
- ▶ James Langridge
- ▶ Roy Martin
- ▶ Kieran Neeson
- ▶ Keith Richards

