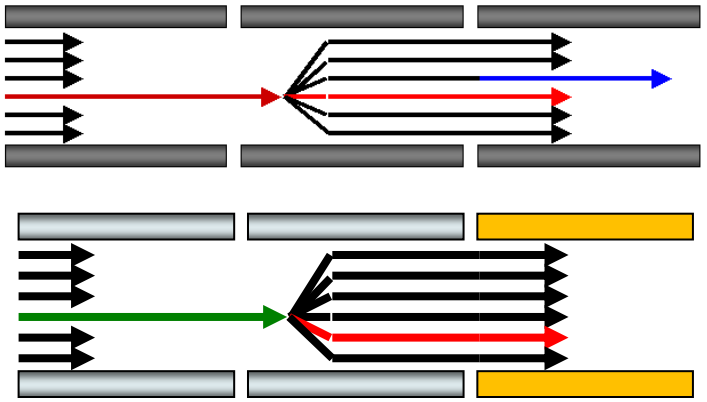
Skyline

Targeted Proteomics Environment

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

Brendan MacLean

UNIVERSITY of WASHINGTON



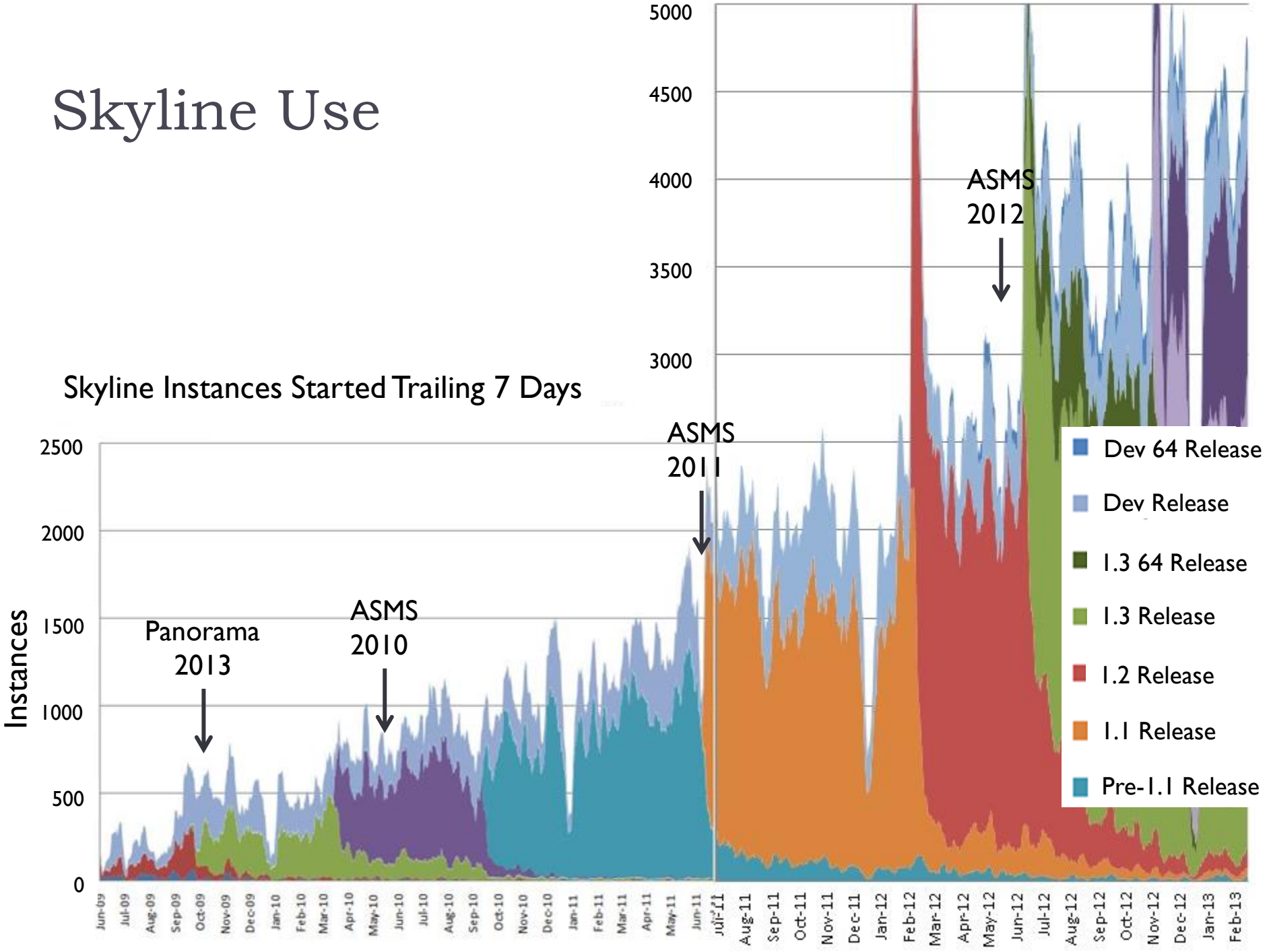
User Community After 4 Years

- 560 registered users
- 150 registered for this meeting
- 4 new papers in May
  - **Platform Independent and Label-free Quantitation of Proteomic Data using MS1 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

- 1000+ registered users
- 250 registered for this meeting
- More papers and press
  - **Targeted proteomics Nature Methods Method of the Year in 2012**
  - **MS1 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
Instances
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Panorama 2013
ASMS 2010
ASMS 2011
ASMS 2012
Dev 64 Release
Dev Release
1.3 64 Release
1.3 Release
1.2 Release
1.1 Release
Pre-1.1 Release
Jun-09
Jul-09
Aug-09
Sep-09
Oct-09
Nov-09
Dec-09
Jan-10
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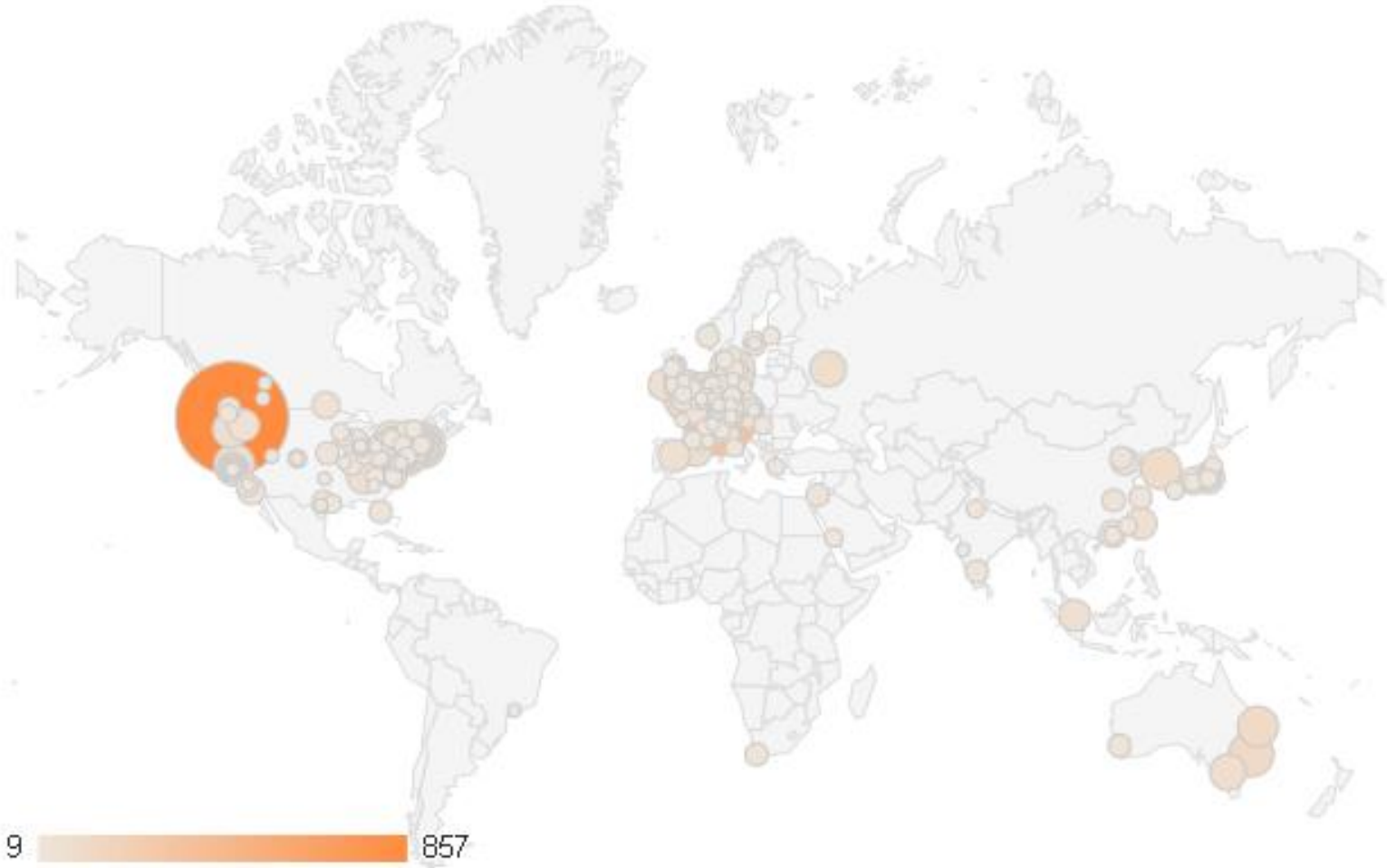
Feb-13

Skyline Web Site Visits (past 3 months)



Learning More

- 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

Agilent Technologies

BRUKER

Thermo
SCIENTIFIC

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Panorama Partnership Program

- 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

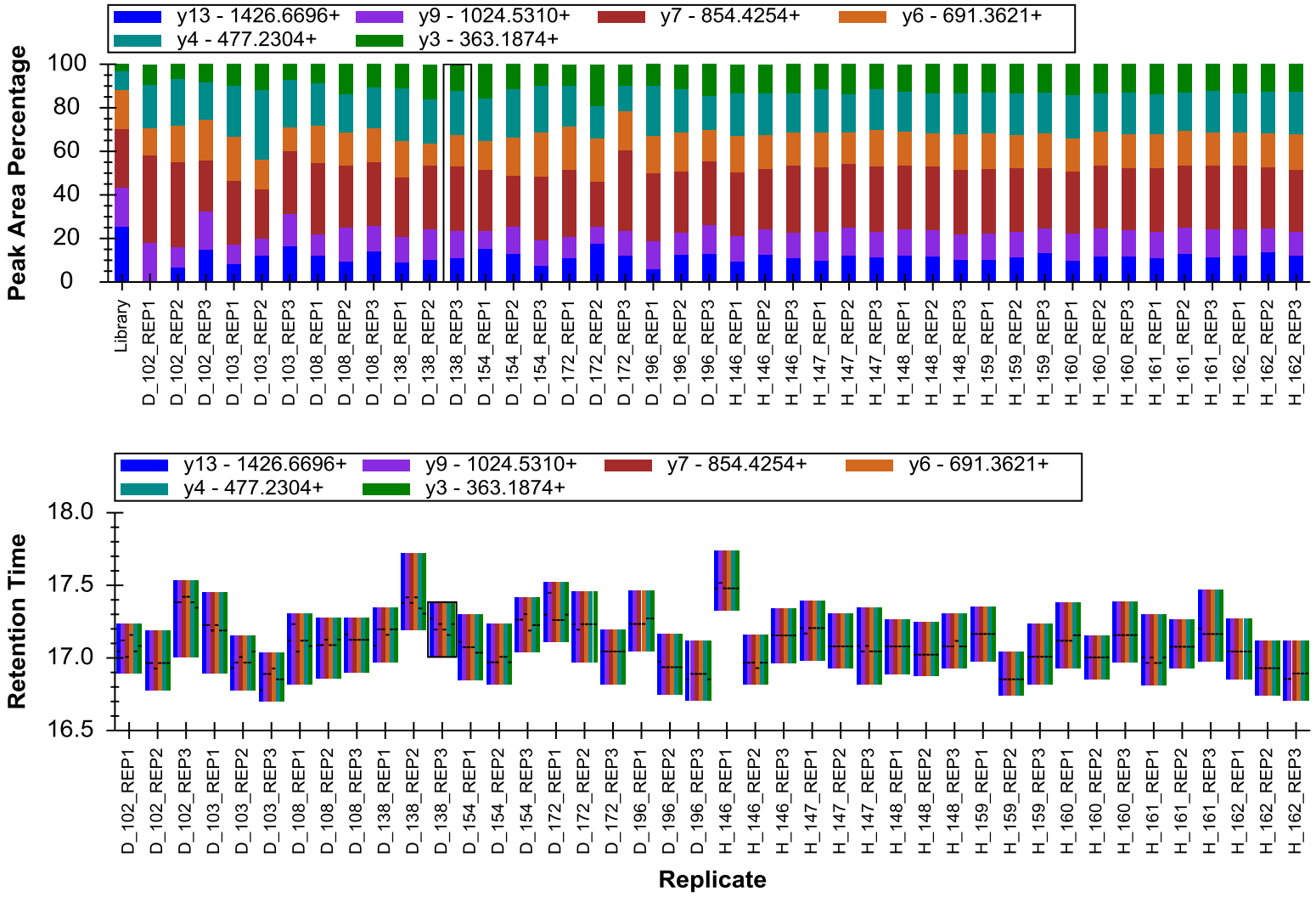
- Powerful enough to be used cross-lab / cross experiment
- More powerful run-to-run

- Relative ion abundance
  - Spectral and chromatogram libraries
- Retention time
  - iRT

- Does ensuring comparable measurements require ID?

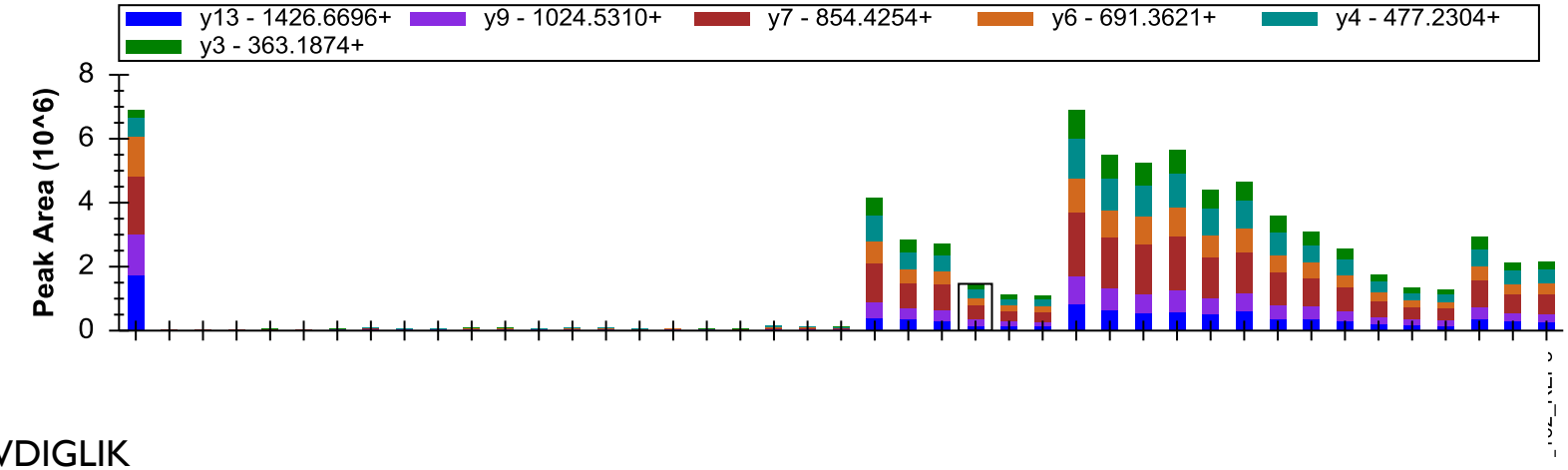
Haptoglobin

LQTEGDGIYTLNSEK

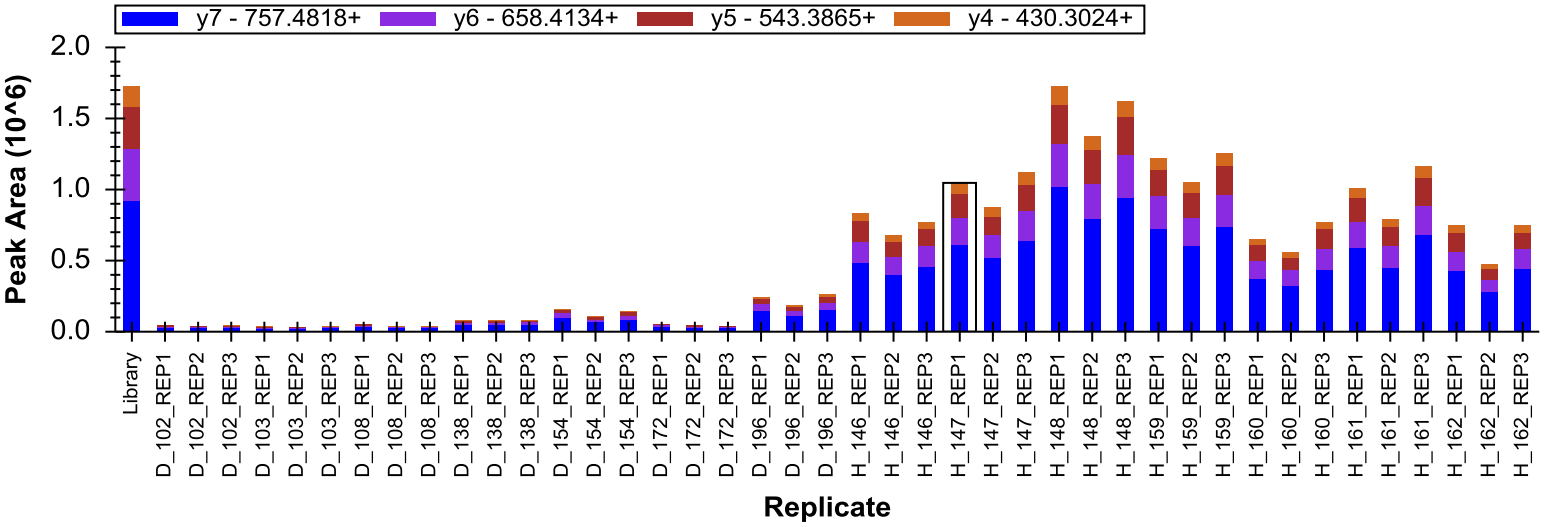


Haptoglobin

LQTEGDGIYTLNSEK

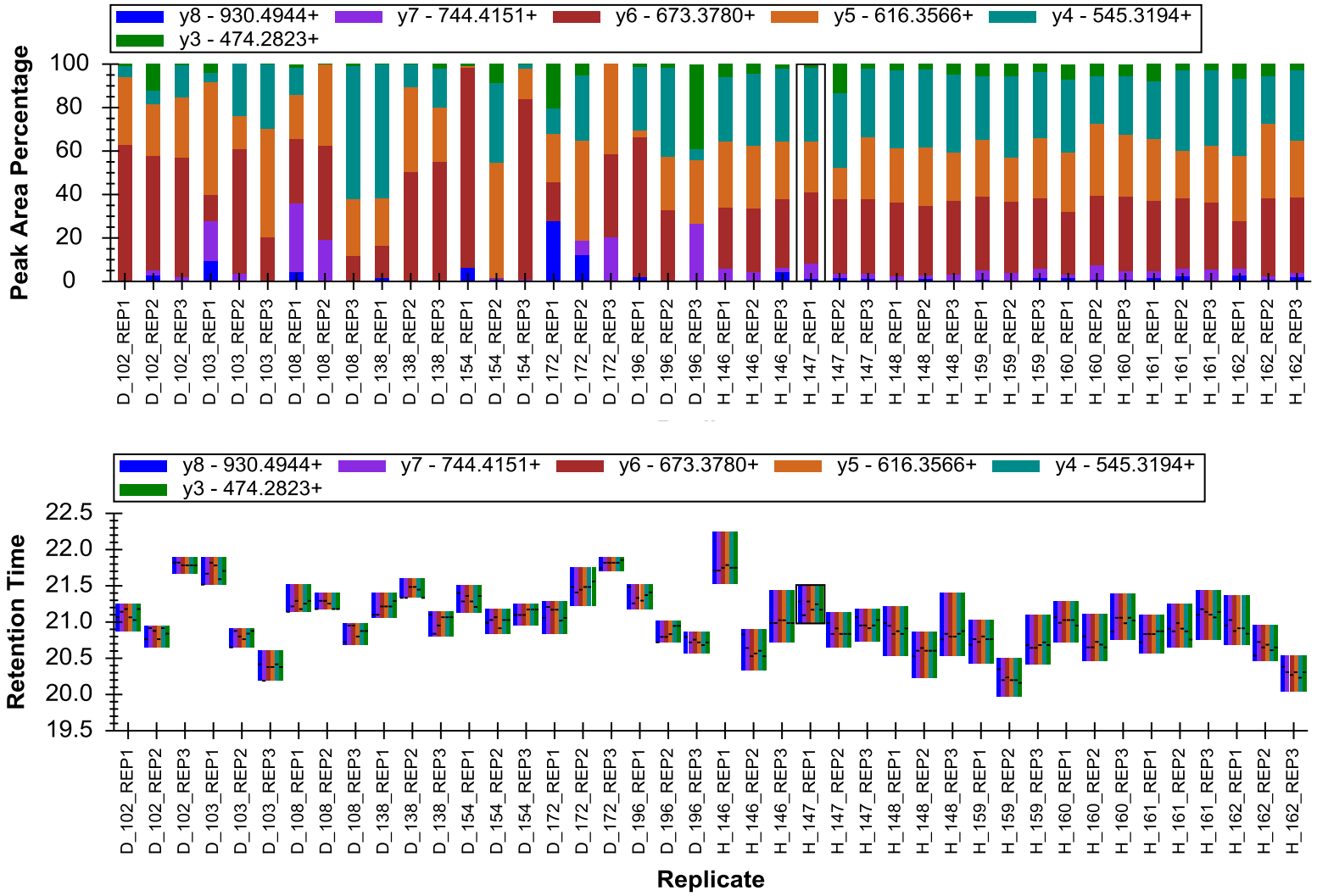


SVVDIGLIK



Mitochondrial 39S ribosomal protein L9

CSSLLWAGAAWLR



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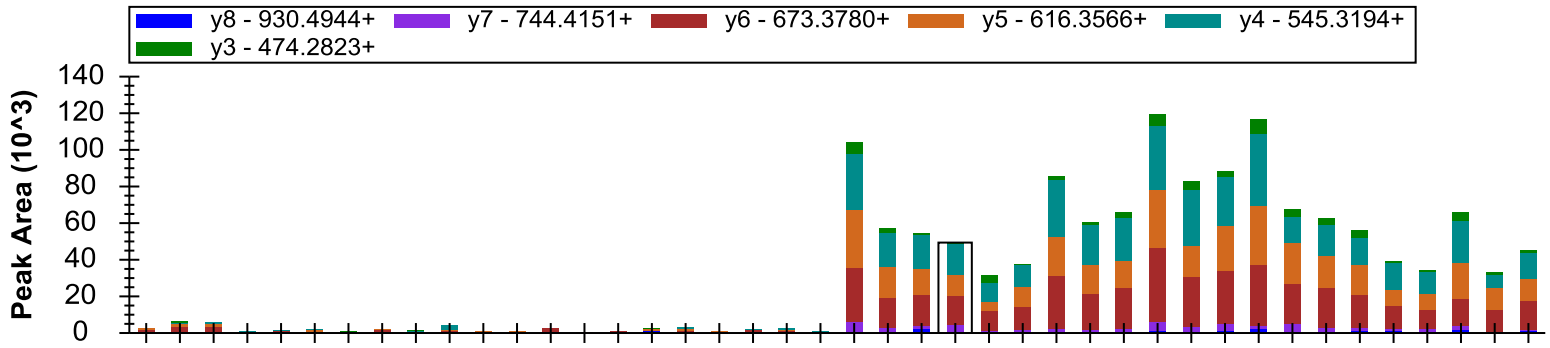

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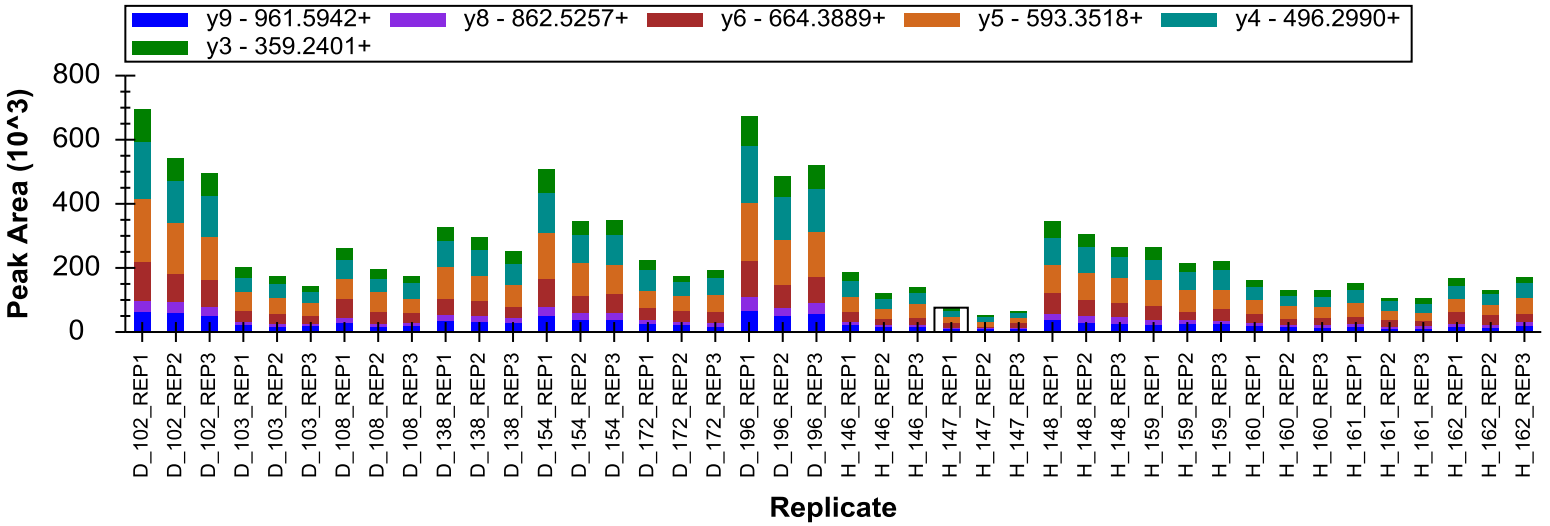

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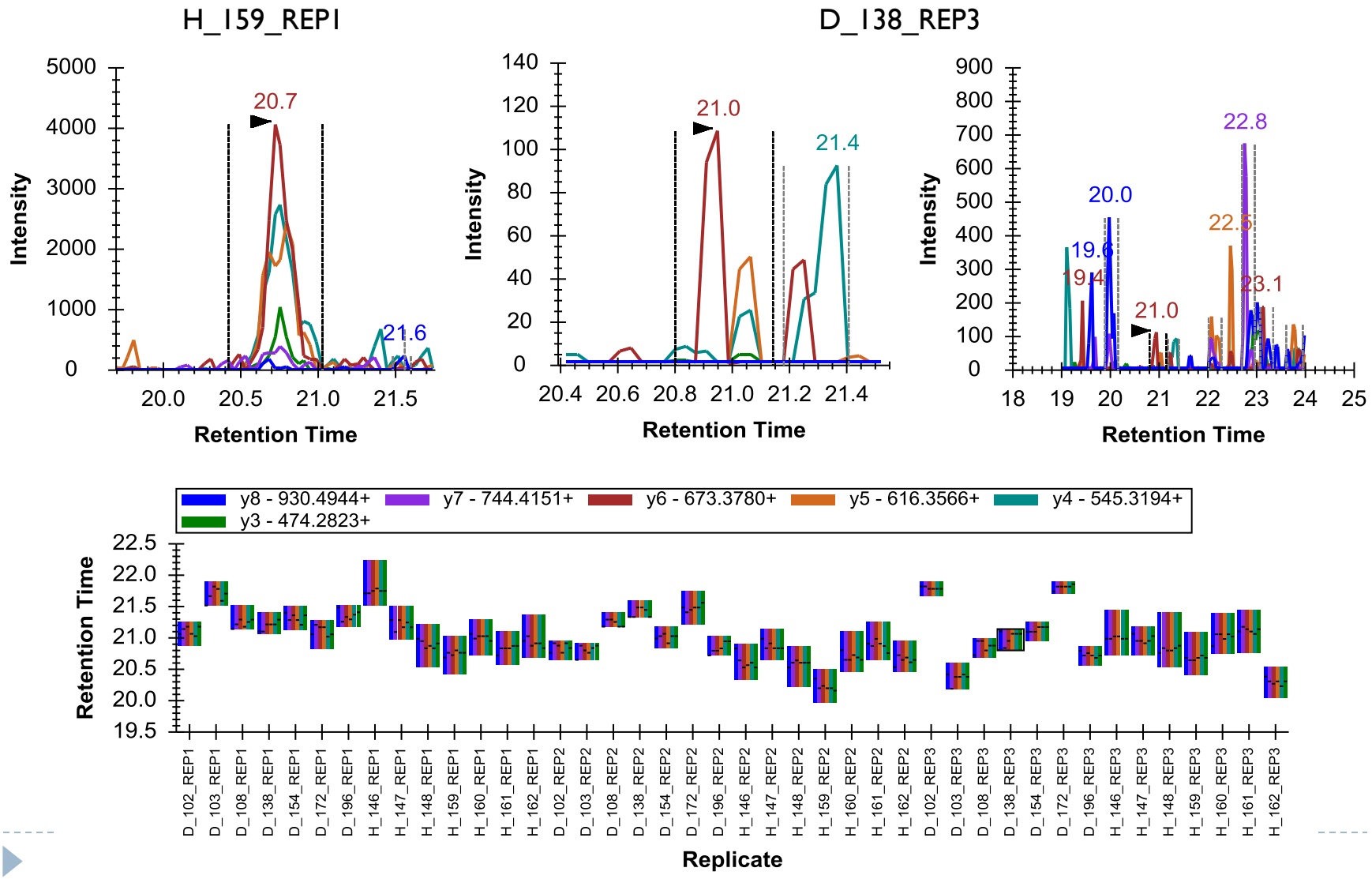
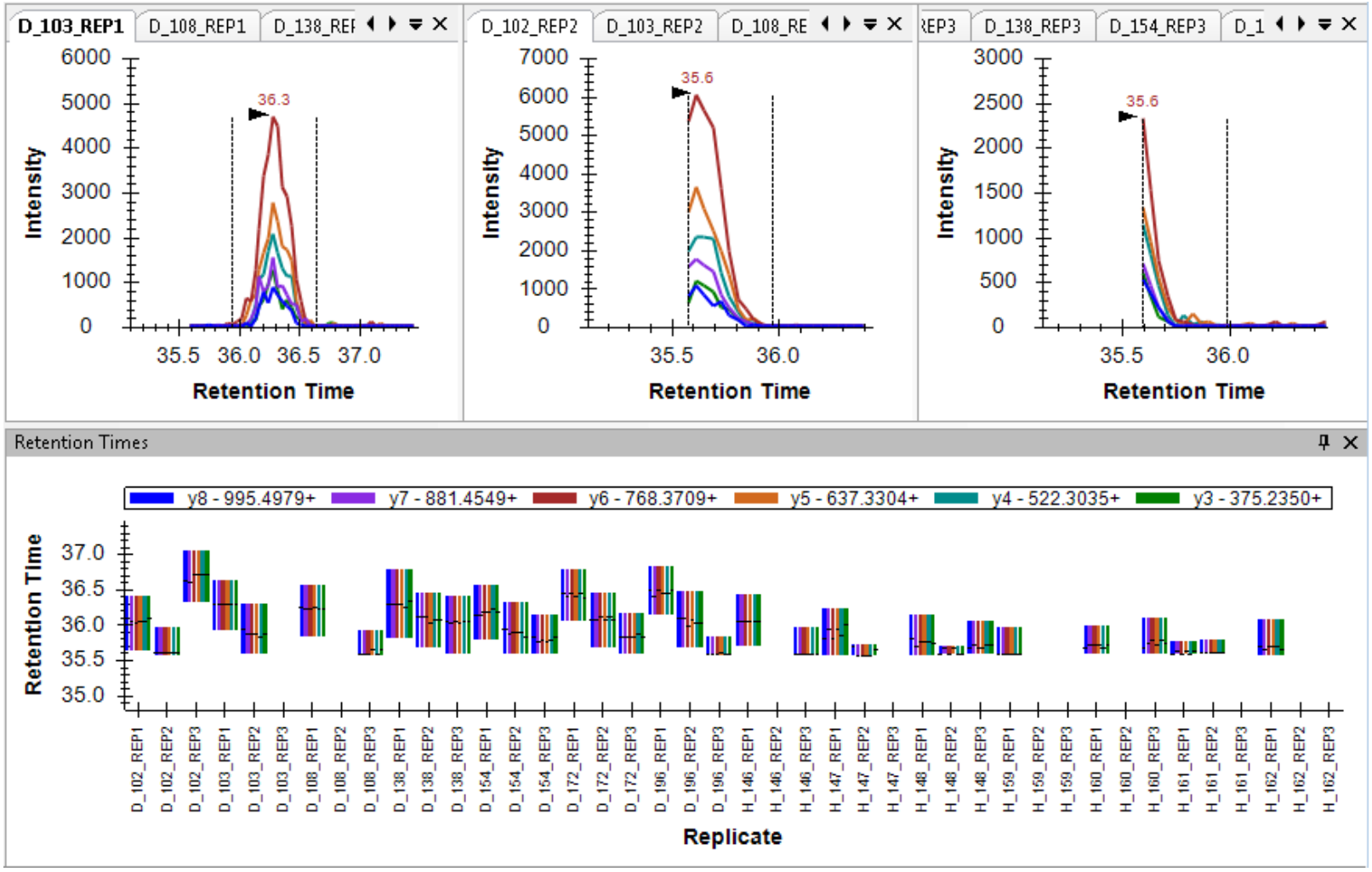
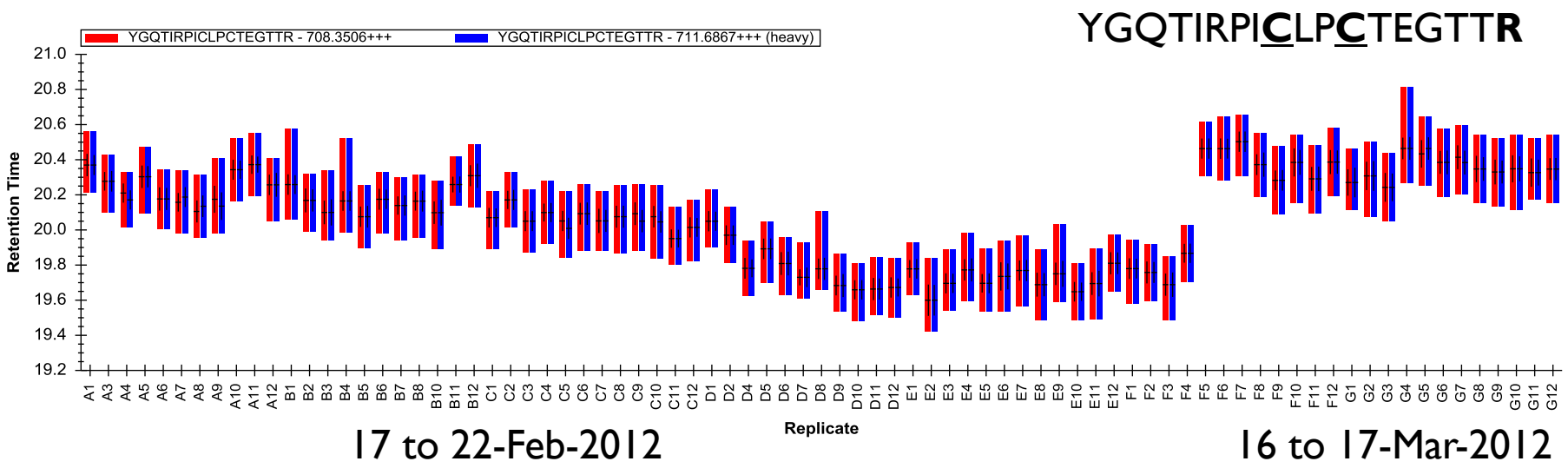


Truncated and Missing Peaks

TGTNLMDFLSR



Deciphering the Unexpected



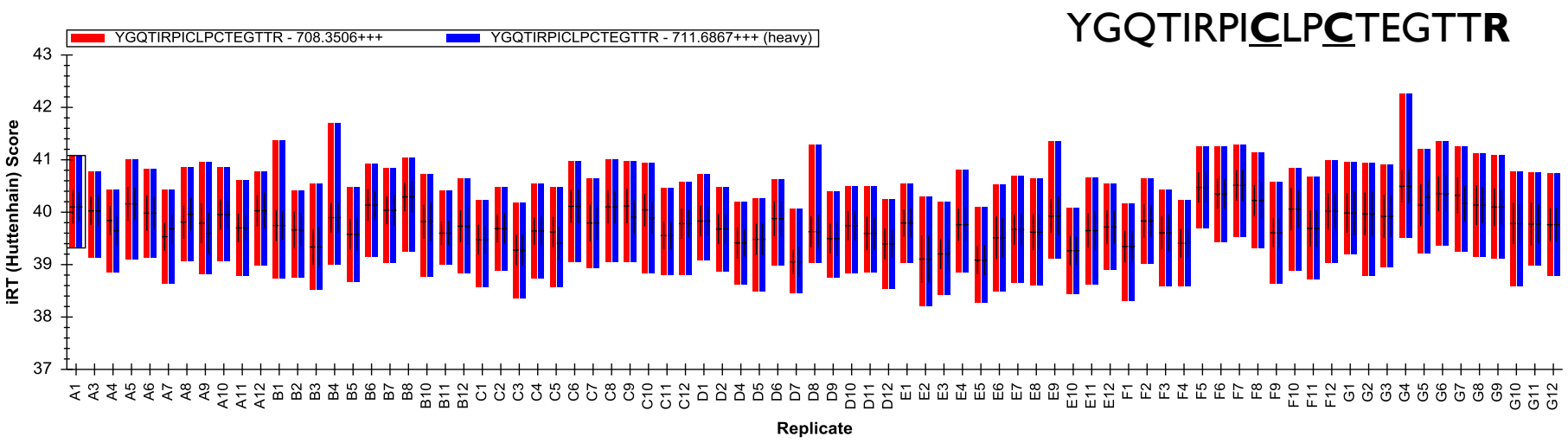
17 to 22-Feb-2012

16 to 17-Mar-2012

Results Grid

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

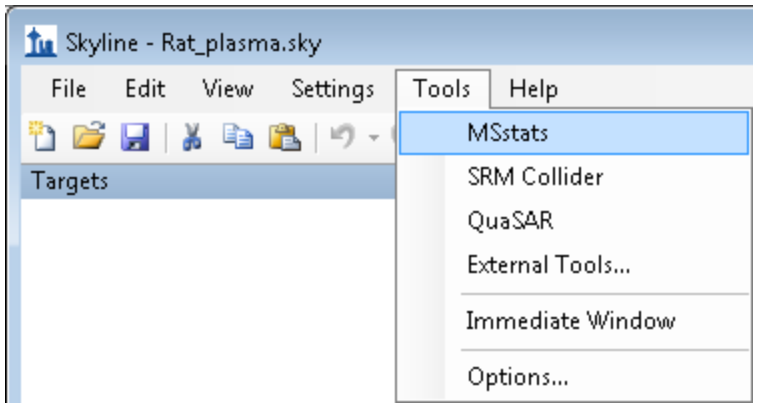
Aligned by iRT

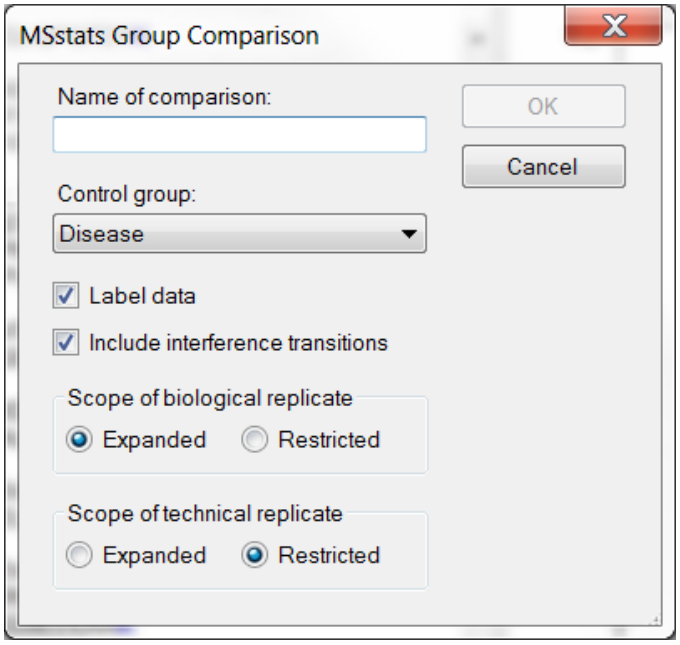


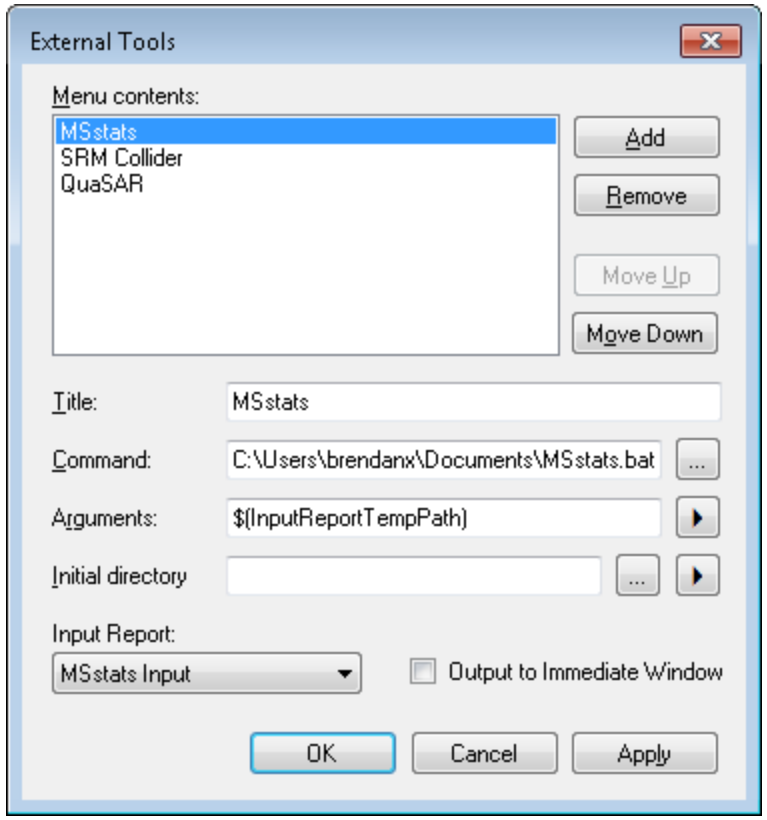
Integrating with External Tools

- GeneSpring & Skyline Automation Tool (Agilent)
- MSstats (Purdue) – Grouped study analysis
- QuaSAR (Broad Institute) – Response curves, LOD, LOQ
- MS1 Probe (Buck Institute) – MS1 quant. statistics
  - TP28 – Alexandria D'Souza
- SRM Collider (IMSB) – Interference probability calculator

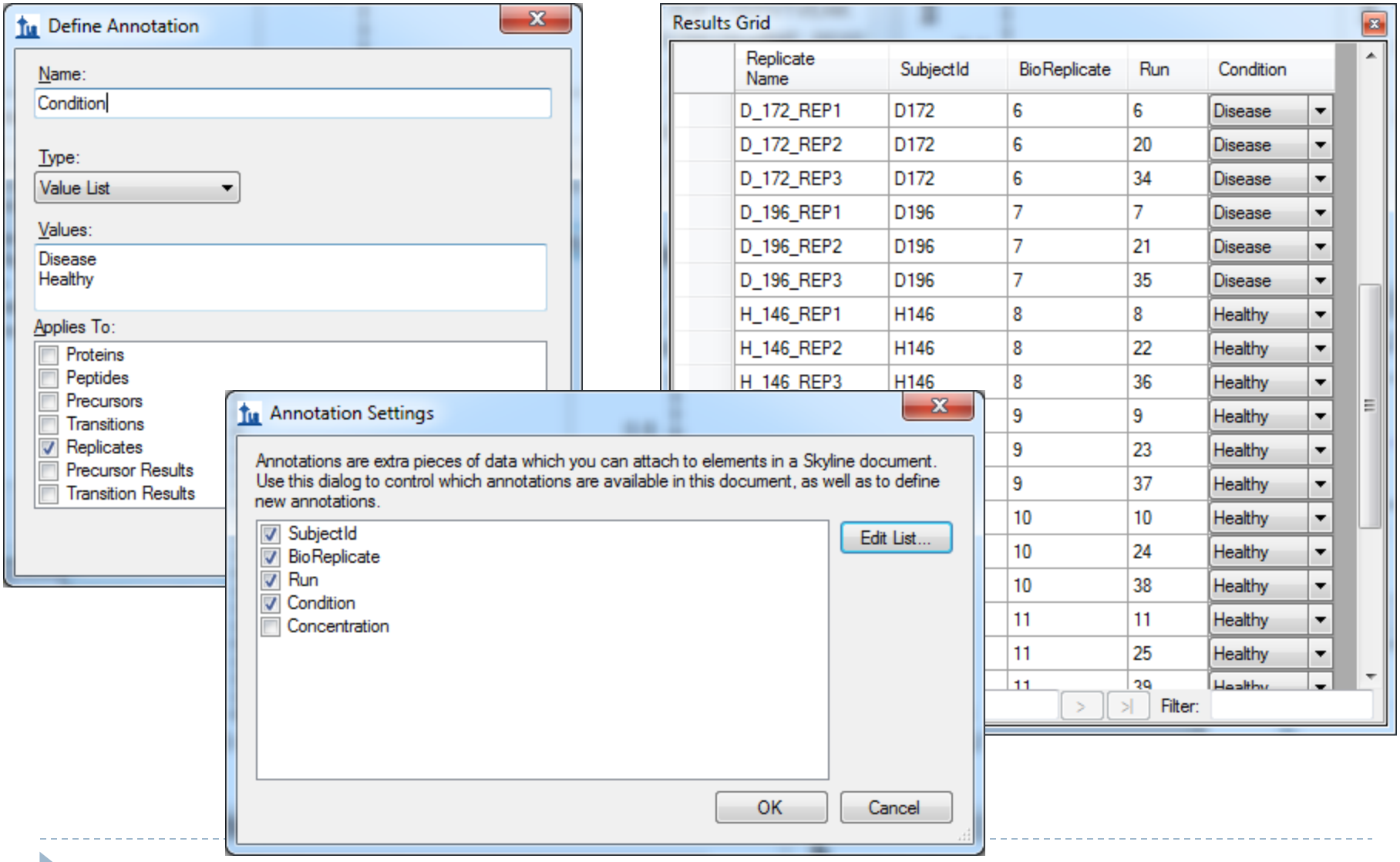
External Tool MSstats





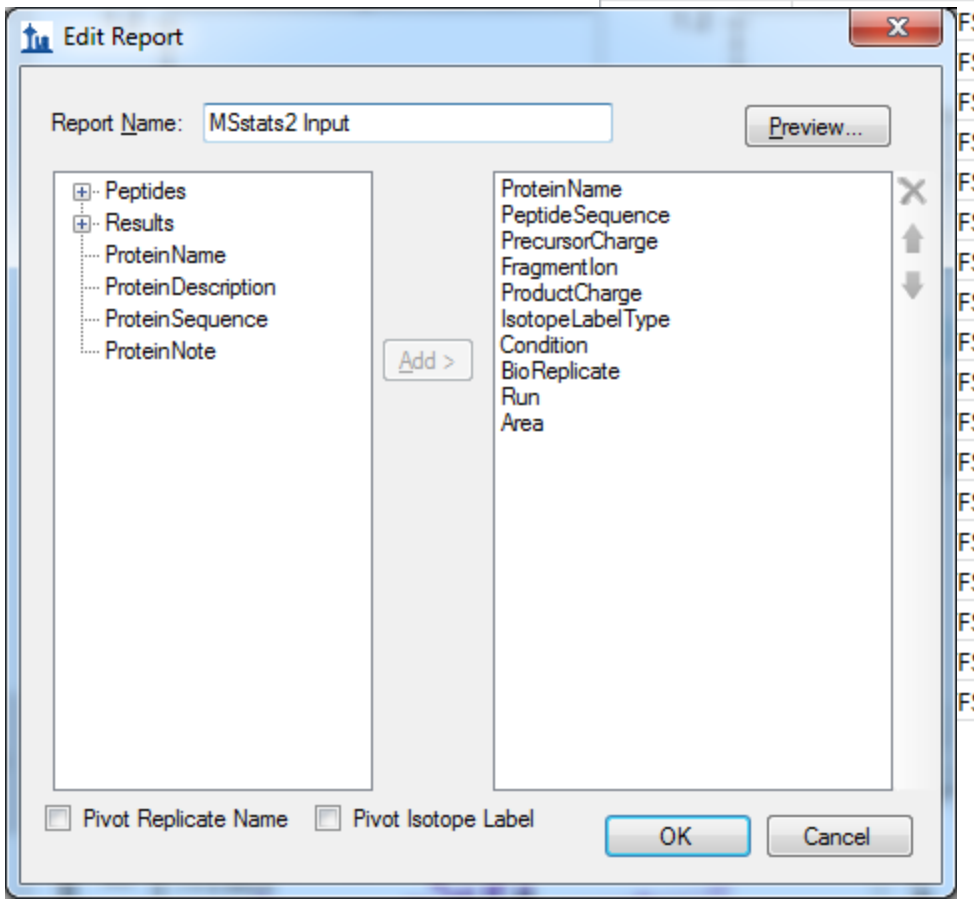


Replicate Annotations



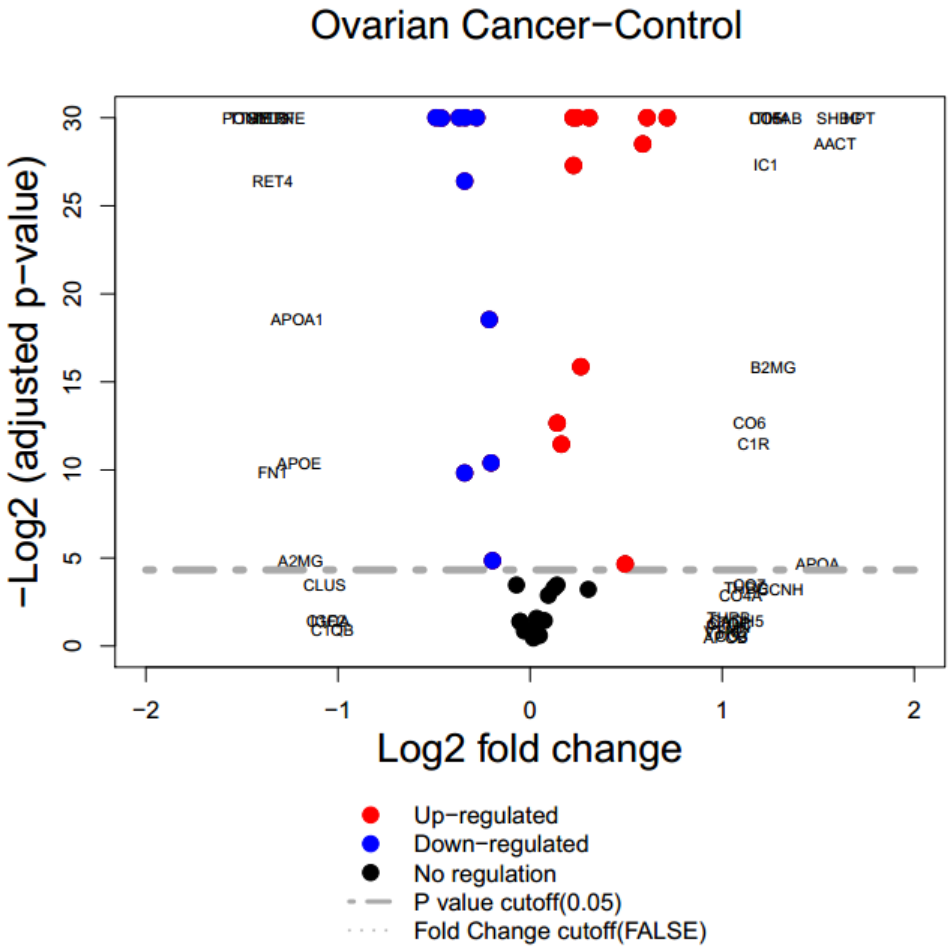
Custom Reports

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

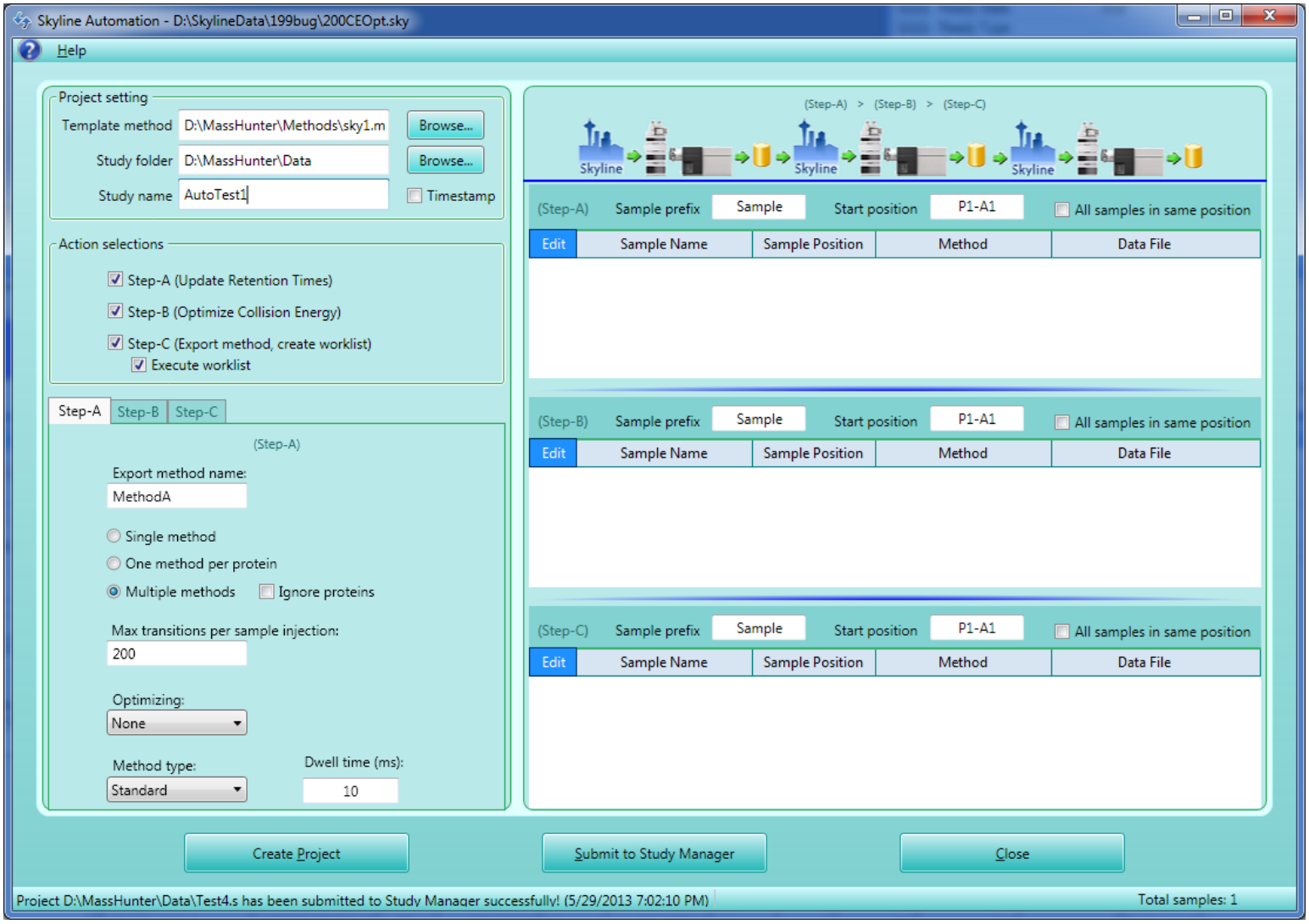


Downstream Analysis with Statistical Tools

- Analysis of reports with R – MSstats



Skyline Automation with SkylineRunner

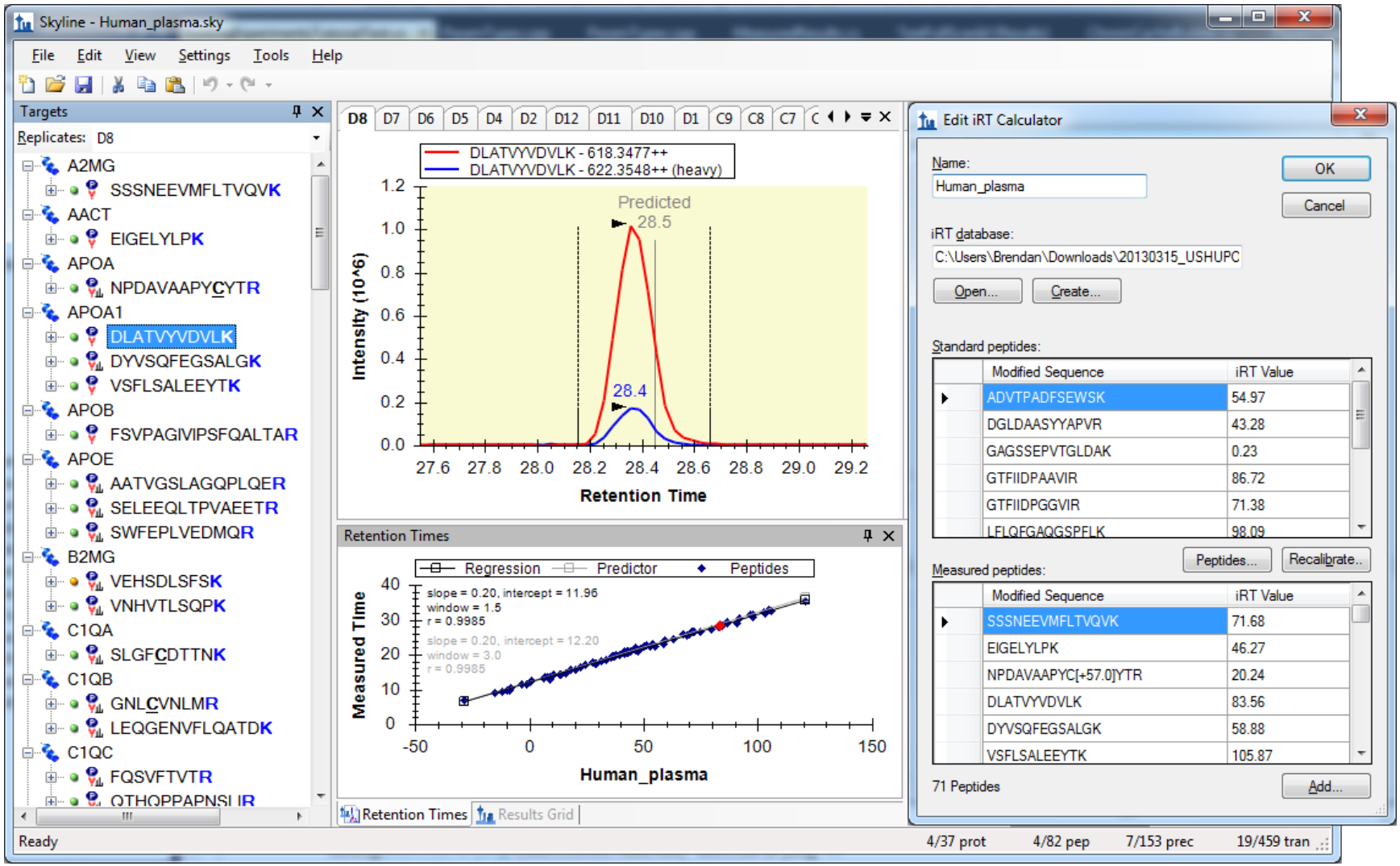


MS/MS Spectral Library Sources

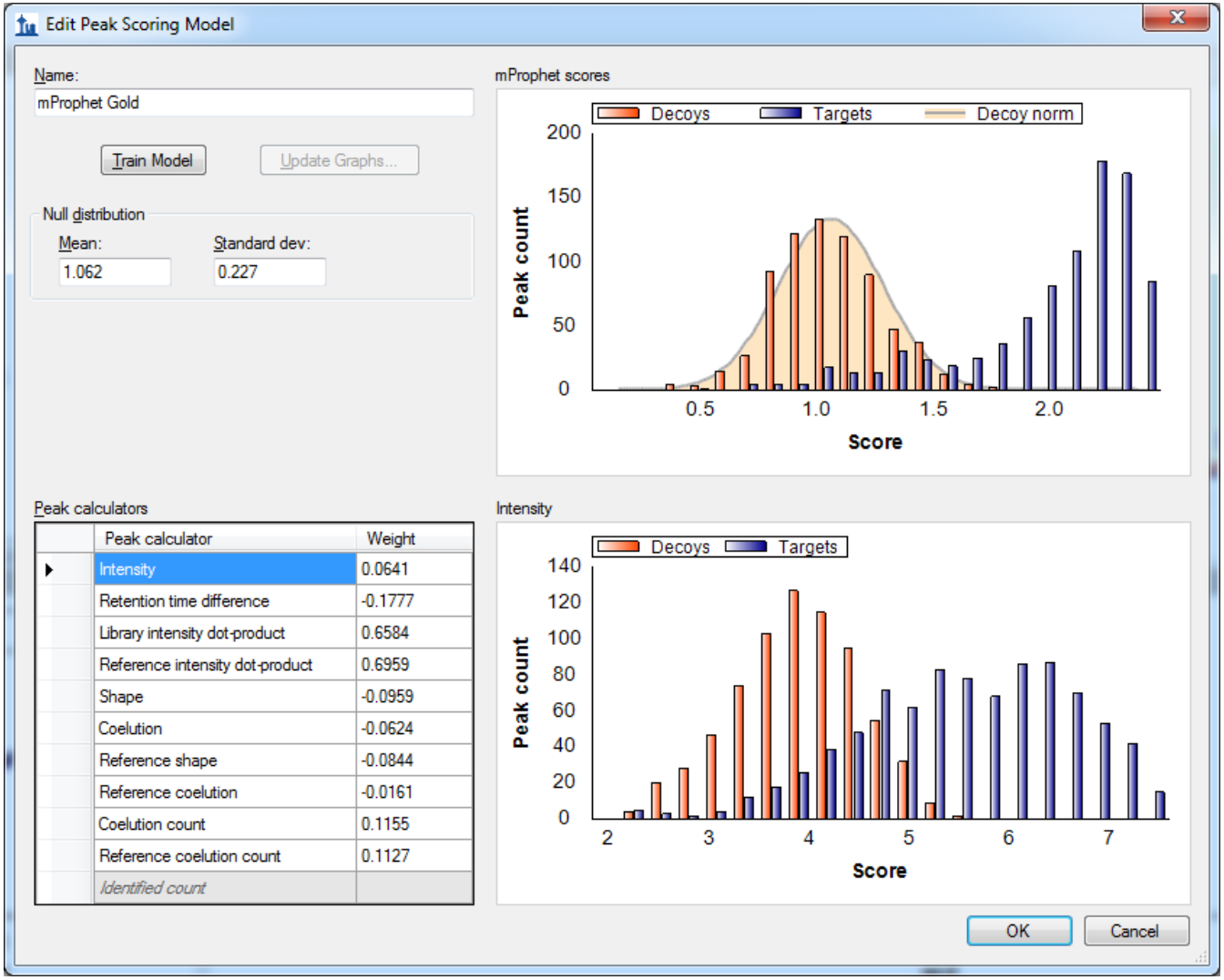
- Global Proteome Machine
- NIST
- Peptide Atlas

- 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)



Integrating Great Ideas (mProphet)



- TP 499 - MacLean

Skyline Team

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- Vagish Sharma

- Don Marsh

- Josh Eckels

- Kaipo Tamura

- Greg Taylor

- Danny Broudy
- Trevor Killeen
- Jarrett Egertson
- Dario Amodei

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  - 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

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  - Carsten Baessmann
  - Marius Kallhardt
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  - Andreas Kuehn
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  - Keith Richards