



*Proteomics Standards
Research Group (sPRG)*

Proteomics Standards Research Group (sPRG)

www.abrf.org/sprg

The Integration of Skyline, Panorama, and LabKey Server Interface for R to Analyze the 2013-2014 ABRF sPRG Research Group Study

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Proteomics Standards Research Group (sPRG) 2013-2014

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Proteome Software (ASMS Booth 78)

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Standard Design

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- Sequences of synthetic peptides were derived from approximately 552 proteins, conserved across proteomes of commonly analyzed species: *Homo sapiens*, *Mus musculus* and *Rattus norvegicus*.
- 1,000 isotope-labeled peptides which were spot-synthesized by JPT (www.jpt.com).
- Peptides represent a wide range of hydrophobicities and isoelectric points typical of complex proteomics samples.
- Individual peptides were reconstituted, combined and desalted by solid-phase extraction.



Study Timeline

www.abrf.org/sprg

Two Year Study

- **Year 1:** Peptide synthesis and qualification by sPRG.
- **Year 2:** Initiate the study, collect and analyze the data

- The study was launched in August 2013
- Final study results presented at ABRF 2014
- Commercialization of Peptide Standard
- (Future) Publication of Manuscript



2013-2014 Study

- Focused on the ability of core facilities to determine relative quantitation of up to 1,000 heavy/light peptide pairs in a single sample.
- Study Launched on August 12, 2013
- 120 sample request in 45 days!
- 90 Participants were shipped one tube containing a lyophilized mixture of a tryptic digest of HEK293 cell lysate spiked with 1,000 stable isotope labeled (SIL) synthetic peptides.
- Participants had access to sPRG-generated spectral libraries, a Skyline tutorial , and two FASTA files.
- Over half the participants (47 laboratories) returned data analysis results via Panorama Web Repository and RAW data files to NIST fileserver.



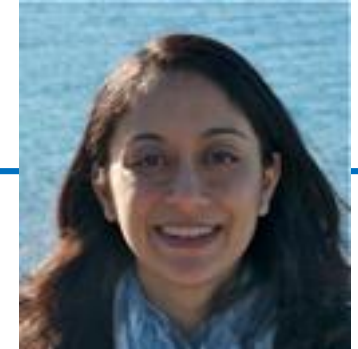
Instrument Platform	
Linear Ion Trap - Orbitrap	45.0%
Quadrupole - Orbitrap	20.0%
Triple TOF	15.0%
Quadrupole - TOF	7.5%
Linear Ion Trap - FT	5.0%
Triple Quadrupole	2.5%
no answer	5.0%



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Vagisha Sharma



- **Panorama**
 - Server software application for storing, sharing and analyzing results contained in Skyline documents
 - Integrated with Skyline
 - Facilitates public and private data sharing
 - Publish, explore and analyze large targeted datasets
 - Locally installable
- **PanoramaWeb (<https://panoramaweb.org>)**
 - Public Panorama server hosted by the MacCoss Lab at the University of Washington
 - Hosts projects for 39 labs.
 - Chosen as the repository for processed data submitted by labs participating in the ABRF sPRG 2013 study



Data on PanoramaWeb

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- Folders were created on PanoramaWeb for each participant
- Participants were assigned a username and password for accessing their folder
- Participants added PanoramaWeb as a Panorama server in Skyline
- Documents were published to PanoramaWeb by clicking on the 'Publish to Panorama' button in Skyline.

The image displays a screenshot of the Skyline software interface. The main window shows a list of protein targets and their associated mass spectrometry data. A red arrow points to the 'Publish Document' button in the Skyline menu. The 'Publish Document' dialog box is open, showing the file path and the Panorama Folders list. The 'Edit Server' dialog box is also visible, showing the URL for PanoramaWeb. The background shows the Skyline interface with a list of targets and two mass spectrometry plots showing retention time and intensity.

Options

Language Miscellaneous

Servers:

Edit list...

Edit Server

URL:

Email: (optional)

Password: (optional)

OK Cancel

Skyline-da - 1E356X_Try1.sky

File Edit View Settings Tools Help

Targets

Replicates: 1

ABRFsPRG2013

- GGASVWSEY [0, 9] (rank 642)
- YQGLCPPVPI [10, 19] (rank 641)
- RIGYSPQTLADQSSK [20, 34] (rank 640)
- KIGYSSP
- KALQSNH
- R.HSFFSG
- K.INPDHIG
- R.LLTFDEY
- R.NAGFT
- R.STYPPS
- R.IADFGW
- R.LSDSYS
- R.RGFPAR
- R.SNAQGI
- R.DNEDFG
- R.ELQDLA
- K.AALSSP
- K.ISNYGW
- K.DLTPEH
- R.EFTAQN
- K.DDFTEP
- K.IDIPSPD
- R.YIQHTY
- R.NQGIPLR [251, 257] (rank 649)
- R.GVGASGSRF [258, 266] (rank 648)
- R.FGLTPGSRH [267, 275] (rank 647)
- R.HSGPNSADSANDGFVRL [276, 291] (rank 646)

Publish Document

File: Browse...

Panorama Folders:

- https://panoramaweb.org/
- 18 CHR
- ABRF sPRG
- ABRF sPRG 2013 Study Results
- 1E356X
- Tutorials

OK Cancel

precursor - 510.2489++

precursor [M+1] - 510.7503++

precursor [M+2] - 511.2516++

Retention Time

41.8 D

42.1

43.8

or - 515.2530++ (heavy)

or [M+1] - 515.7545++ (heavy)

or [M+2] - 516.2558++ (heavy)

41.8 D

+2.1

43.4

Retention Time

1/1 prot 9/962 pep 19/2154 prec 55/6462 tran



Data on PanoramaWeb

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- Excel spreadsheets containing results from participants that did not use Skyline for data processing were added to PanoramaWeb by ABRF members.

The image shows two overlapping screenshots of the PanoramaWeb interface. The left screenshot shows the 'Create new List' page for study 56365A, with a red box highlighting the 'Import from TSV or Excel file.' section. The right screenshot shows the 'grid.view' page for the same study, displaying a table of protein data.

Import from TSV or Excel file.
Choose File | 56365A.csv

Showing first 5 rows (uncheck column checkboxes to ignore import)

LabID	Index	Peptide Sequence
Text (String)	Integer	Text (String)
56365A	1	GGASVWWSER
56365A	2	YQGLCPPVPR
56365A	3	IGYSSPQLADQSSK
56365A	4	IGYSSPLTSDQSSK
56365A	5	ALQSNHFELSLR

Table Data (from grid.view):

Lab ID	Index	Peptide Sequence	Protein Accession	Average Light/Heavy Ratio	CV	Charge State	Retention Time	Additional Information	
56365A	1	GGASVWWSER	A16A1_HUMAN						
56365A	2	YQGLCPPVPR	ACE_HUMAN						
56365A	3	IGYSSPQLADQSSK	ACSL3_HUMAN	0.1	5.96	2	38.53	3	
56365A	4	IGYSSPLTSDQSSK	ACSL4_HUMAN	0.14	4.33	2	51.65	3	
56365A	5	ALQSNHFELSLR	AGRIN_HUMAN	0.18	2.6	3	53.01	2	
56365A	6	HSFFSGVNWQDVYDK	AKT3_HUMAN			3	71.39	1	
56365A	7	INPDHIGFYR	AMPE_HUMAN		0.46	1.3	2,3	44.39	5
56365A	8	LTFDEYR	ANXA3_HUMAN						
56365A	9	NAGFTPQER	APEX1_HUMAN	0.35	1.58	2	22.22	3	
56365A	10	STYPPSGPTYR	ASAH1_HUMAN	1.0	0.5	2	29.03	2	
56365A	11	IADFGWSVHAPSLR	AURKB_HUMAN						
56365A	12	LSDYSNTLPVR	BAIP2_HUMAN	0.27	2.1	2	44.09	3	
56365A	13	FGPPAFSGISR	BIEA_HUMAN	0.05	12.84	2	74.31	2	
56365A	14	SNAQGIDLNR	CBPE_HUMAN						
56365A	15	DNEDFQESNR	CERU_HUMAN	0.04	19.08	2	17.08	3	
56365A	16	ELQDLALQGAK	CMGA_HUMAN	0.1	6.27	2	44.16	3	
56365A	17	AALSSFQK	CSN2_HUMAN	0.17	2.93	2	28.39	2	
56365A	18	ISNYGWDQSDK	CYBP_HUMAN	1.28	0.49	2	35.89	3	



Data Analysis

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- Data for all the participants was downloaded from PanoramaWeb
 - Panorama provides an interface to explore the underlying database schema and design custom views on tables, including foreign-key based lookups to other tables
 - Panorama also provides a SQL query interface for writing custom queries
 - Data can be downloaded as Excel spreadsheets or as tab-delimited text files

Folder	Replicate	Peptide Sequence	Modified Peptide Sequence	Precursor Isotope Label Type	Precursor Charge
ABRF sPRG 2013	5600TT13-1322	YQLCPVPVPR	YQLCL(+57)PPVPR	593.8055	light

```
SELECT precursorchrominfo.SampleFileId,ReplicateId,Name AS Replicate, precursorchrominfo.PrecursorId,PeptideId,Sequence AS PeptideSequence, precursorchrominfo.PrecursorId,PeptideId,PeptideModificationSequence AS ModifiedPeptideSequence, precursorchrominfo.PrecursorId,Charge AS PrecursorCharge, precursorchrominfo.PrecursorId,IsotopeLabelId,Name AS IsotopeLabelType, precursorchrominfo.TotalArea, precursorchrominfo.TotalAreaNormalized, precursorchrominfo.TotalBackground, precursorchrominfo.BestRT, precursorchrominfo.MinStart, precursorchrominfo.MaxEnd, precursorchrominfo.TotalAreaNormalized, precursorchrominfo.TotalBackground, precursorchrominfo.MaxFWHM, precursorchrominfo.PeakCountRatio AS Ratio, peDotProduct, d.peptidegroupid.runid.container.name AS SampleId, d.peptidegroupid.runid.filename AS fileName, precursorchrominfo.id =
```

Custom View Designer

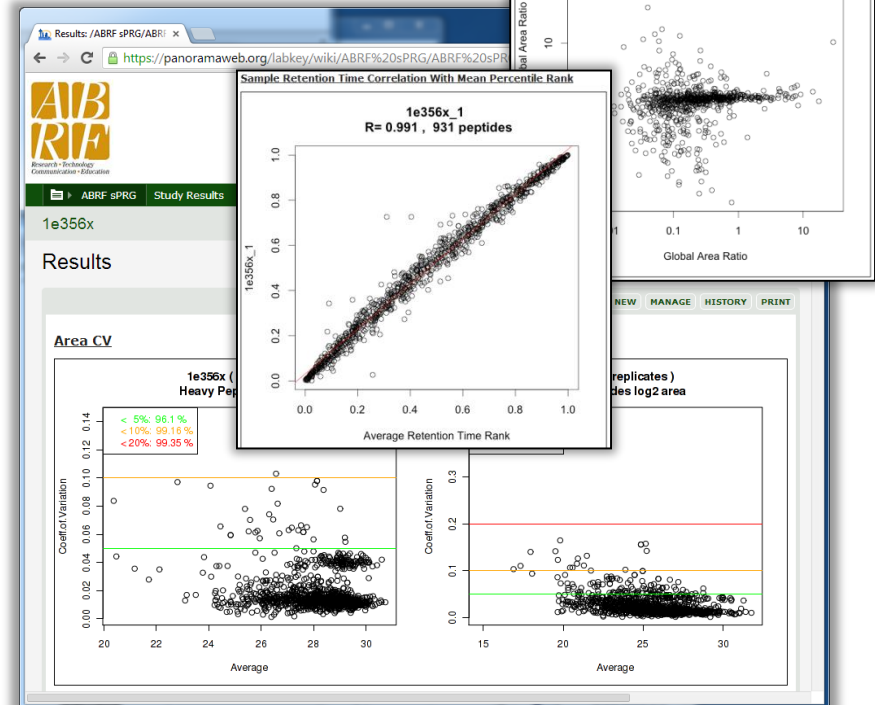
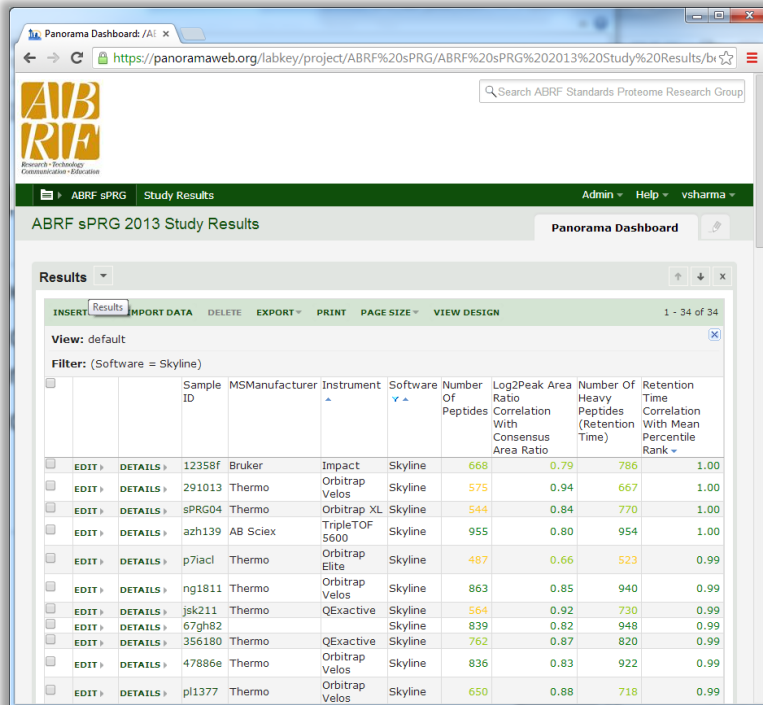
SQL Query Interface



Data Analysis

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- R scripts were developed by ABRF members to assess data quality
- Analysis was done offline using the data downloaded from PanoramaWeb and Excel spreadsheets provided by participants
- Summary results as well as results for each participant were uploaded to PanoramaWeb and added to wiki pages

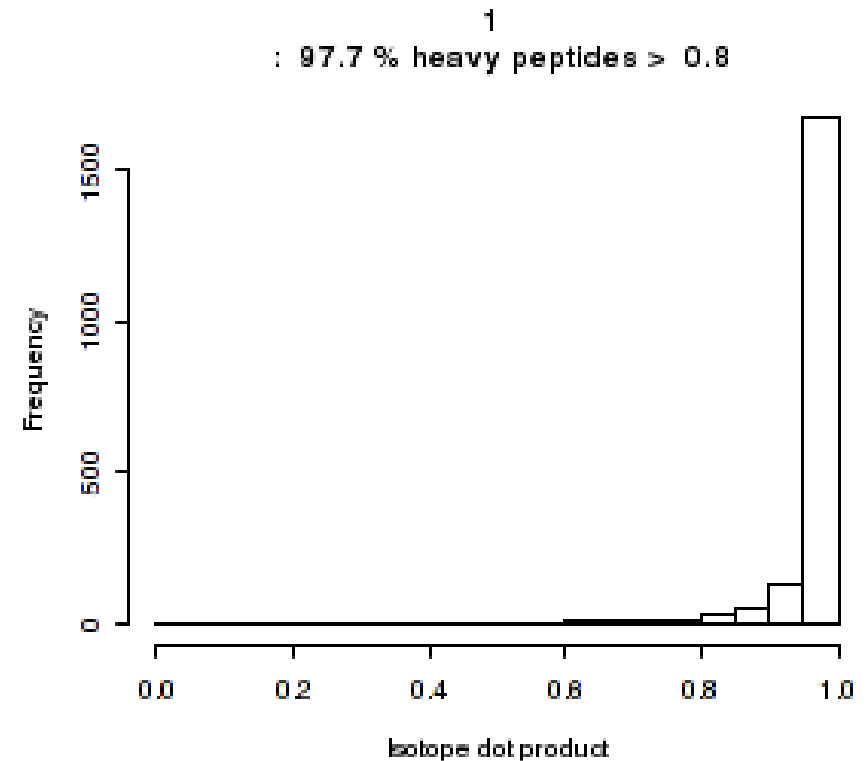
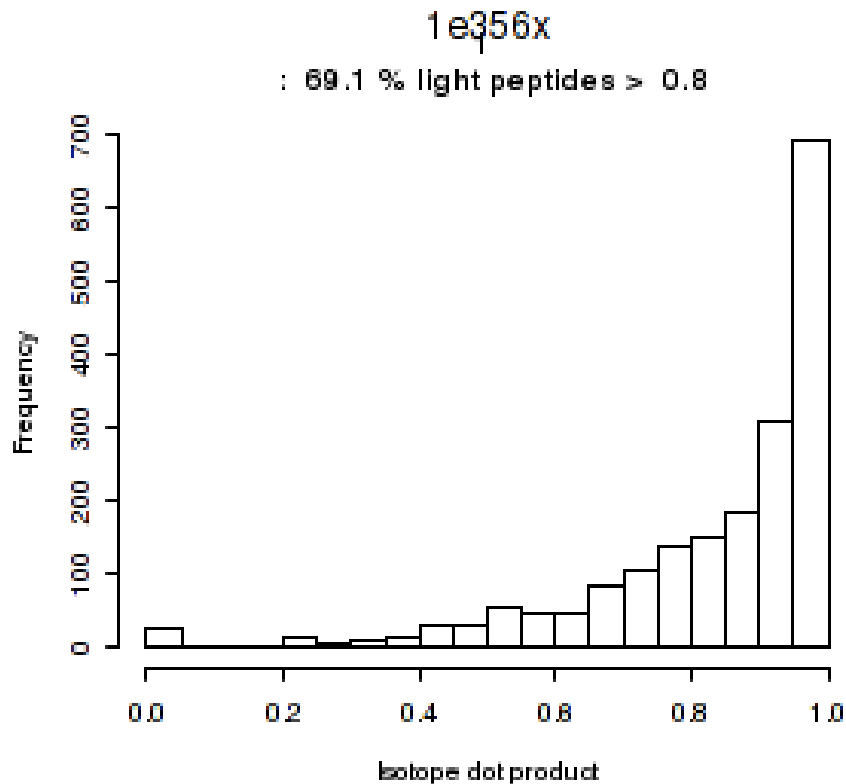


<http://tinyurl.com/ABRF-sPRG>



Summary Results Example

Histogram of Isotope Dot Product



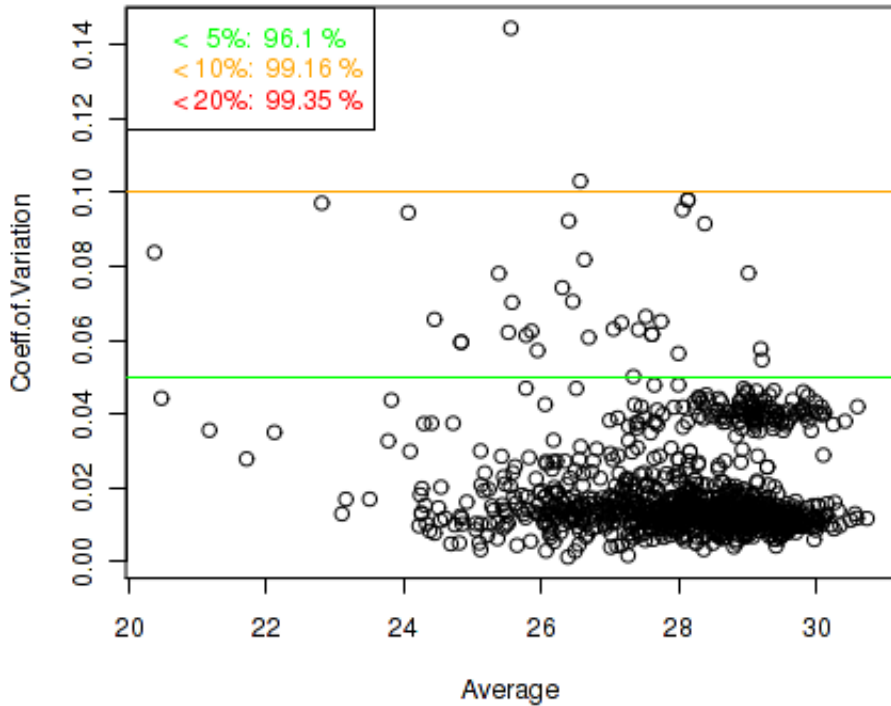
Individual participant data in the same formatted charts can be found
at <http://tinyurl.com/ABRF-sPRG>



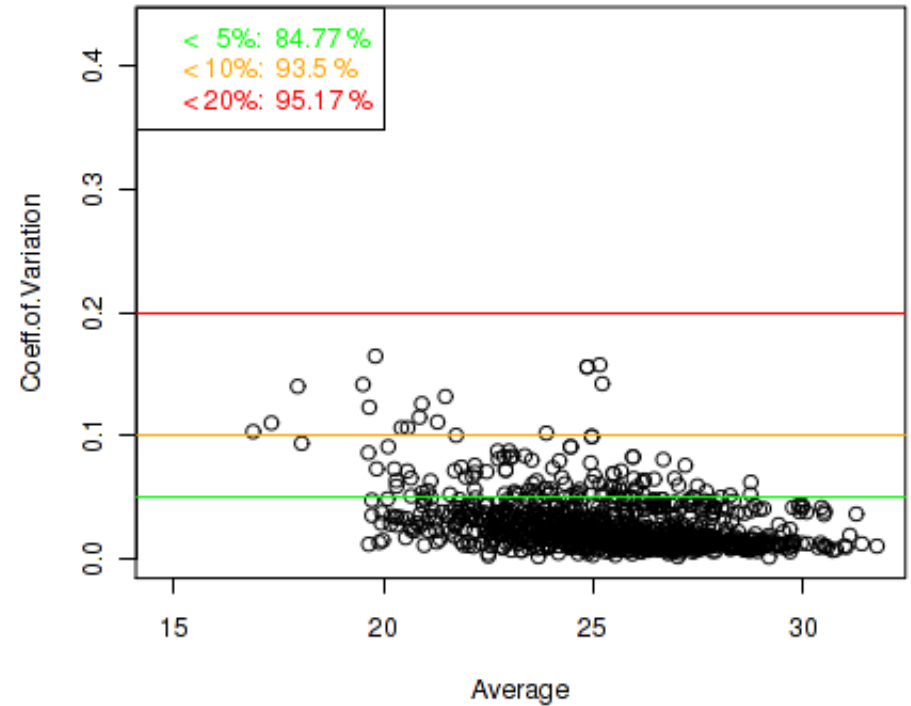
Peak Area CV

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1e356x (3 replicates)
Heavy Peptides log2 area



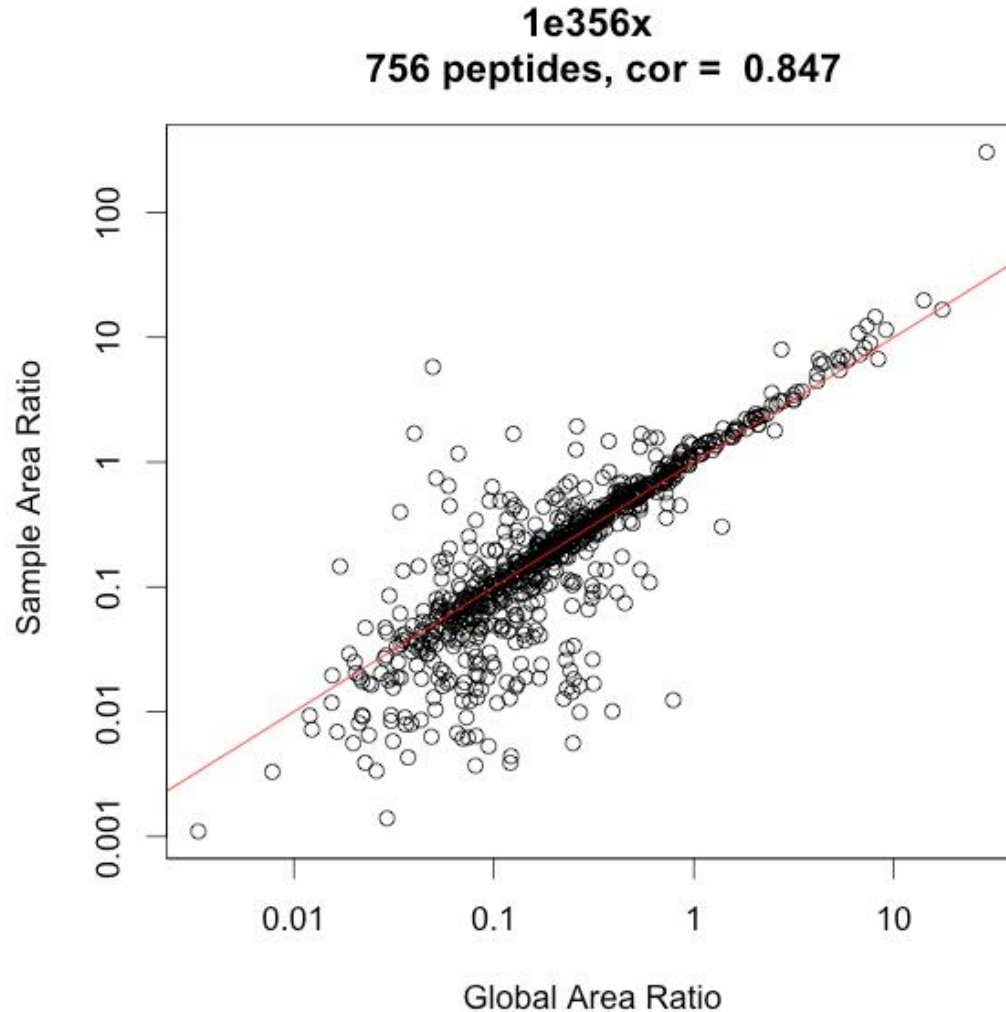
1e356x (3 replicates)
Light Peptides log2 area





Example of Participant Peak Area Ratio vs. Global Consensus

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The advertisement graphic features a 3D ribbon diagram of a protein structure on the left, overlaid with a mass spectrometry chromatogram showing several peaks. To the right, the text 'SpikeMix™' is displayed in a large, red, serif font, preceded by a small cluster of red dots. Below this, the text 'Ready-to-use proteotypic heavy-labeled peptide pools for mass spectrometry' is written in a smaller, red, sans-serif font. A prominent red starburst graphic with the word 'NEW' in white capital letters is positioned to the right of the main text. The background of the graphic is a light green and white gradient with faint circular patterns.

SpikeMix™ ABRF (cross-species standard)

10pmol - \$ 495.00

1pmol - \$ 149.00

Pool of 1000 stable isotope-labeled proteotypic peptides from conserved human, mouse and rat proteins to be used as standard across the three species. All SpikeMixes™ are stable isotope-labeled at their C-terminus by means of heavy arginine (U-13C6; U-15N4) or lysine (U-13C6; U-15N2).



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THANK YOU!!!

PARTICIPANTS

Please visit our poster number 216 (Monday)

Please Visit Our Website

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Click on Research Groups then sPRG

Questions, Ideas or Interested in Joining Us?

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