R Fundamentals and Best Practices for Mass Spectrometry Data Analysis

Sunday, November 15 (12:00-3:15pm Eastern)

Meena Choi, Genentech
Olga Vitek, Northeastern University

Module #7: Analysis of proteomic data with MSstats

Introduction to MSSTATS

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Genentech

Ting Huang
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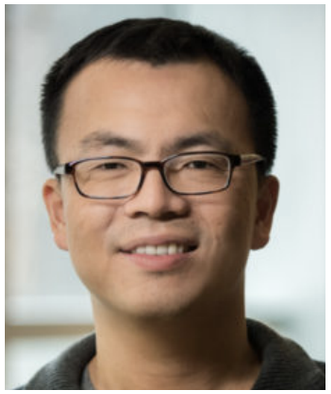
Mateusz Staniak
University of Wrocław



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Kent State

and

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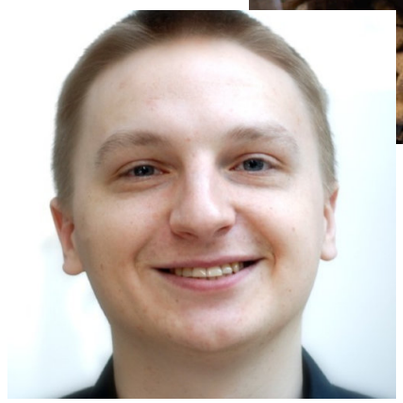
MSSTATS

www.msstats.org

R-based open-source tool for quantitative proteomics

- Which proteins change in abundance?
  - Complex designs: factorial experiments, paired designs, time course
  - Abundance of each subject on a relative scale
- Label-free or label-based
  - Shotgun DDA, data independent DIA/SWATH, PRM, targeted SRM
  - TMT; soon: differential PTMs
- Data processing
  - Free, open-source and inter-operable with other tools
  - Converteres to Skyline, MaxQuant, OpenMS, Spectronaut, ProteomeDiscoverer…
- Statistical analysis
  - Flexible models, account for complex designs, missing values and outliers
  - Stable parameter estimation
- Method validation, system suitability, experimental design
  - System suitability/QC, assay characterization
  - Sample size for testing and classification

Relative protein quantification in MS-based proteomics



Meena Choi
Ting Huang
Mateusz Staniak
Tsung-Heng Tsai

- Analytical method validation
- System suitability testing
- Experimental design
- Data acquisition
  Quality control
- Data processing
- Statistical analysis
- Data for future designs

Assay characterization
LOB/LOD

MSstatsQC
Software package
System suitability monitoring

Experimental design
Sample size for testing and classification

R software package	*Open source software*	*Commercial software*
MSnbase	**Skyline, MaxQuant**	**Proteome Discoverer**
RforProteomics	**OpenMS, OpenSWATH**	**Spectronaut, SpectroMine**
	DIA-Umpire	**Progenesis**

MSstats
Software package
Significance analysis for DDA, SRM, DIA

MSstatsTMT
Software package
Significance analysis for TMT

MSstatsBioData
Experiment package
Published studies with DDA or SRM

42 datasets, > 178 reanalyses

MassIVE
Mass Spectrometry
Interactive **V**irtual **E**nvironment

INTRODUCTION TO MSSTATS

- Overview
- Statistical modeling
- Community resources

MS EXPERIMENT: DESIGN

Analytical method validation

System suitability testing

Experimental design

Data acquisition
Quality control

Study goals

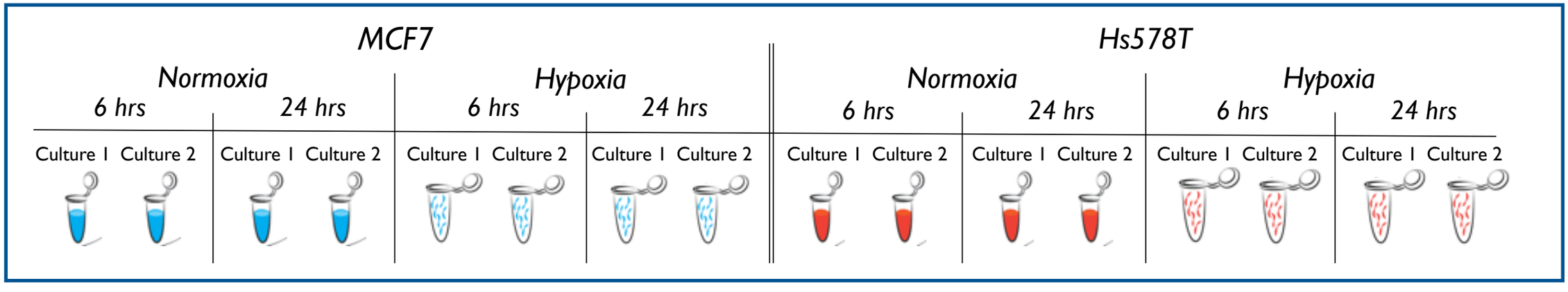
- Exploratory analysis
- Differential analysis
- Biomarker discovery

Biological aspects

- Selection of conditions
- Selection of replicates

Technological aspects

- Sample prep
- Data acquisition
- Randomization



T. Clough et al. *BMC Bioinformatics., 2012*

MS EXPERIMENT: DESIGN

Analytical method validation

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System suitability testing

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Experimental design

↓

Data acquisition
Quality control

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Biological aspects

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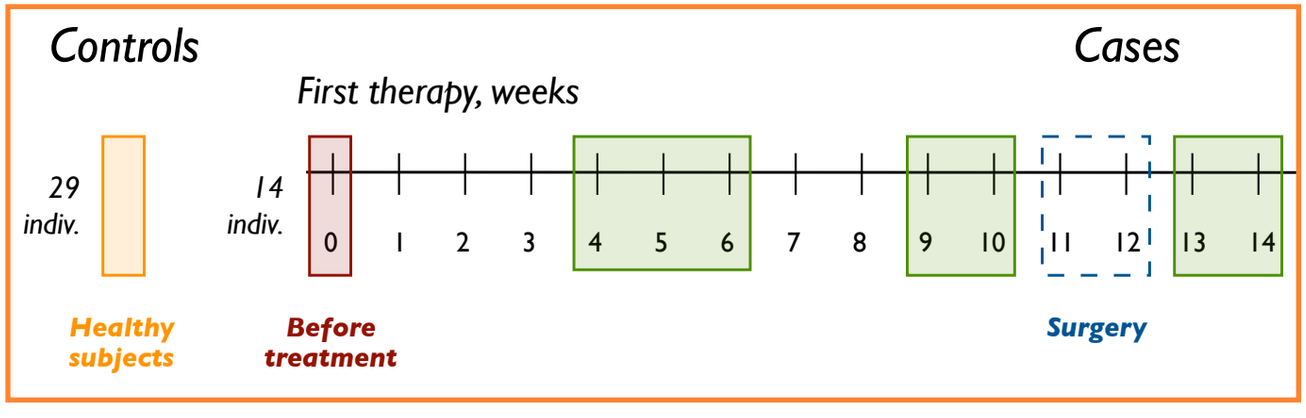
- Randomization



T. Clough et al. *BMC Bioinformatics., 2012*

MS EXPERIMENT: DATA PROCESSING

Analytical method validation

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System suitability testing

↓

Experimental design

↓

Data acquisition
Quality control

↓

Data processing

↓

Statistical analysis

↓

Experimental design

Input: list of identifies and quantified peaks

- MaxQuant
- OpenMS
- Progenesis
- Skyline
- Spectrnaut
- DIAUmpire
- …

Steps

- Data viz & QC
- Normalization
- Missing & outlying peaks
- Quantify protein in a run
- …

www.msstats.org

INPUT DATA FORMAT

	A	B	C	D	E	F	G	H	I	J
1	ProteinName	PeptideSequence	PrecursorCharge	FragmentIon	ProductCharge	IsotopeLabelType	Condition	BioReplicate	Run	Intensity
2	ACEA	EILGHEIFFDWELP	3	y3	0	H	1	ReplA	1	66472.3847
3	ACEA	EILGHEIFFDWELP	3	y3	0	L	1	ReplA	1	5764.16228
4	ACEA	EILGHEIFFDWELP	3	y4	0	H	1	ReplA	1	101005.166
5	ACEA	EILGHEIFFDWELP	3	y4	0	L	1	ReplA	1	61.65238
6	ACEA	EILGHEIFFDWELP	3	y5	0	H	1	ReplA	1	90055.4993
7	ACEA	EILGHEIFFDWELP	3	y5	0	L	1	ReplA	1	472.691803
8	ACEA	TDSEAATLISSTID	2	y10	0	H	1	ReplA	1	43506.5425
9	ACEA	TDSEAATLISSTID	2	y10	0	L	1	ReplA	1	217.203553
10	ACEA	TDSEAATLISSTID	2	y7	0	H	1	ReplA	1	68023.0377
11	ACEA	TDSEAATLISSTID	2	y7	0	L	1	ReplA	1	725.284308
12	ACEA	TDSEAATLISSTID	2	y8	0	H	1	ReplA	1	68276.0489
13	ACEA	TDSEAATLISSTID	2	y8	0	L	1	ReplA	1	243.658527

Reports produced by (or converted from) data processing tools

MS EXPERIMENT: NORMALIZATION

Analytical method validation

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System suitability testing

↓

Experimental design

↓

Data acquisition
Quality control

↓

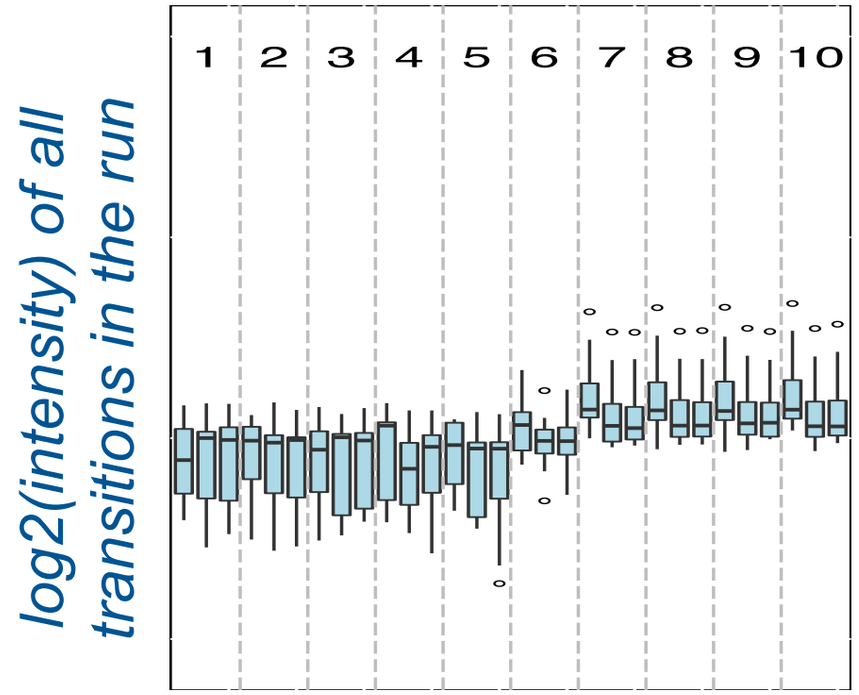
Data processing

↓

Statistical analysis

↓

Experimental design



C.-Y. Chang et al. *MCP*, 2012

MS EXPERIMENT: NORMALIZATION

Analytical method validation

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System suitability testing

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Experimental design

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Data acquisition
Quality control

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Data processing

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Statistical analysis

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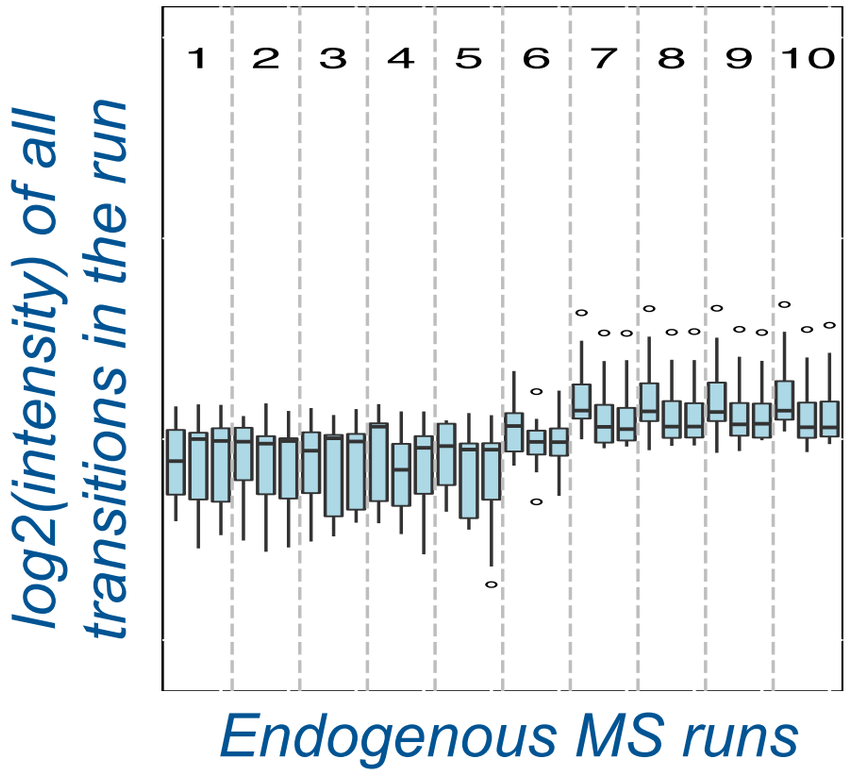
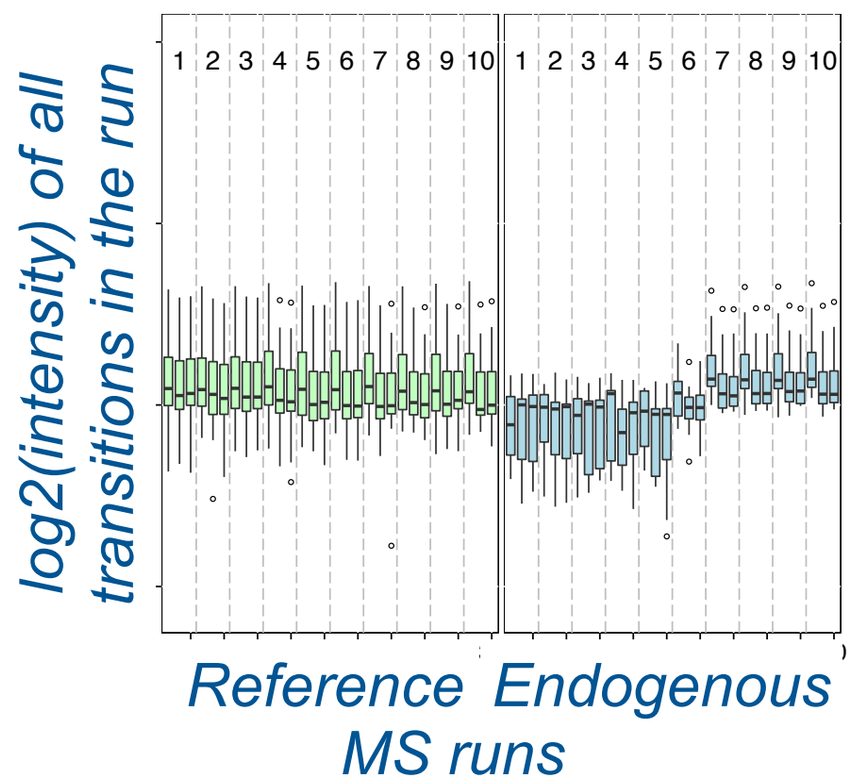
Experimental design

NORMALIZATION





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MS EXPERIMENT: NORMALIZATION

Analytical method validation

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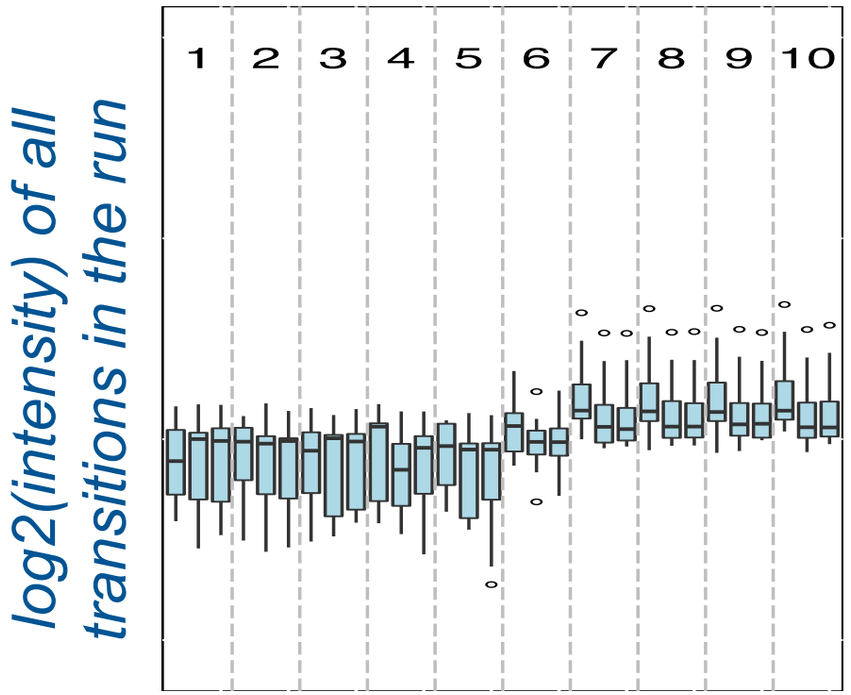
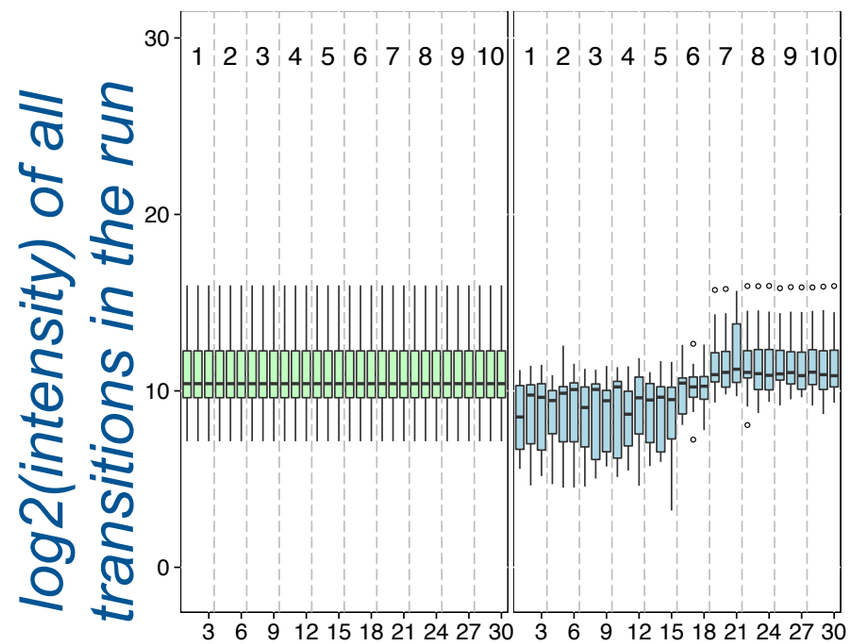
Experimental design

NORMALIZATION



Endogenous MS runs



Reference Endogenous MS runs

C.-Y. Chang et al. *MCP*, 2012

MS EXPERIMENT: INFERENCE

Analytical method validation

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System suitability testing

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Experimental design

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Data acquisition
Quality control

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Data processing

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Statistical analysis

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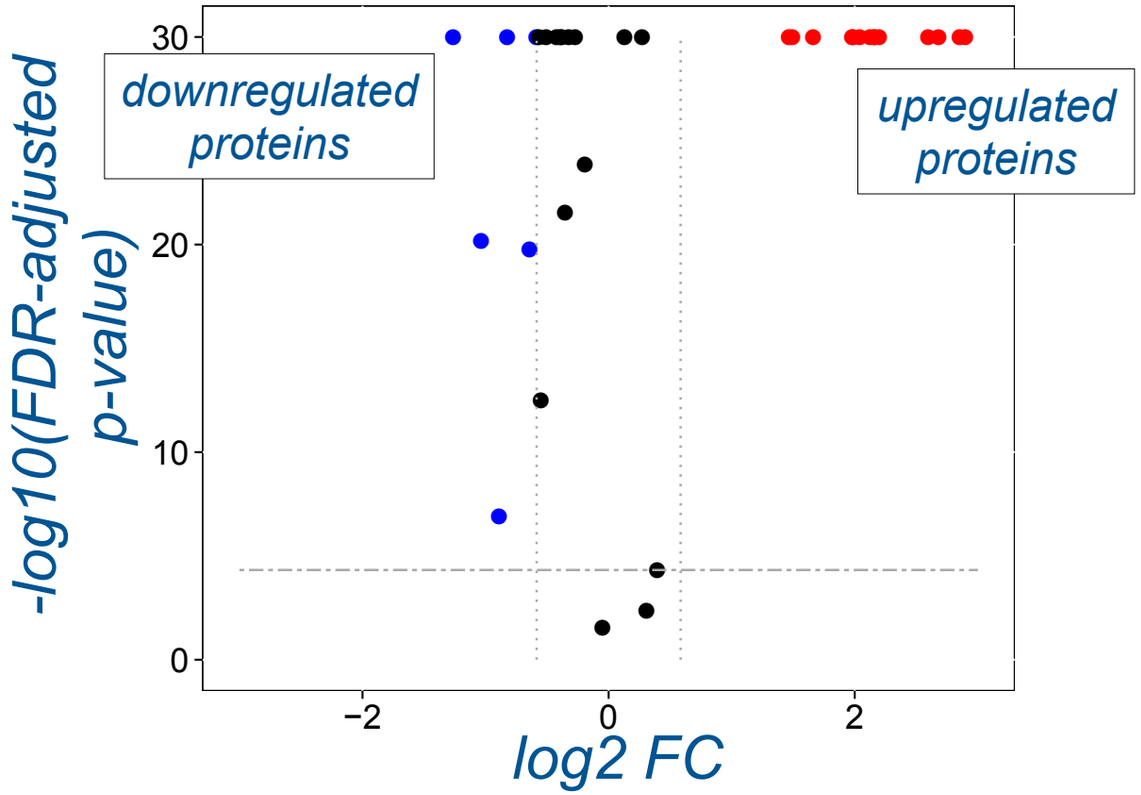
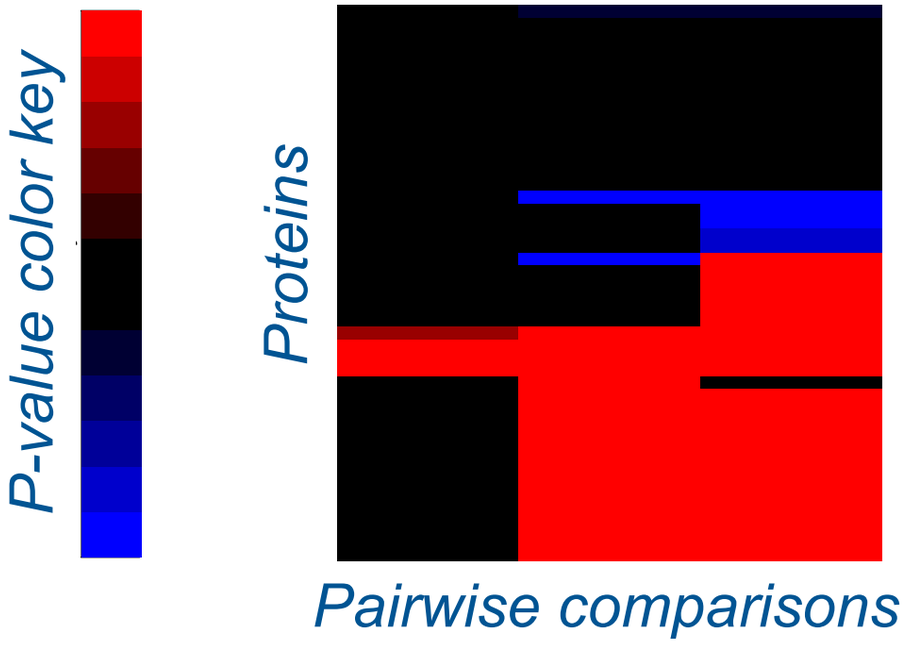
Experimental design





C.-Y. Chang et al. *MCP*, 2012

MS EXPERIMENT: DESIGN

Analytical method validation

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System suitability testing

↓

Experimental design

↓

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Quality control

↓

Data processing

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Statistical analysis

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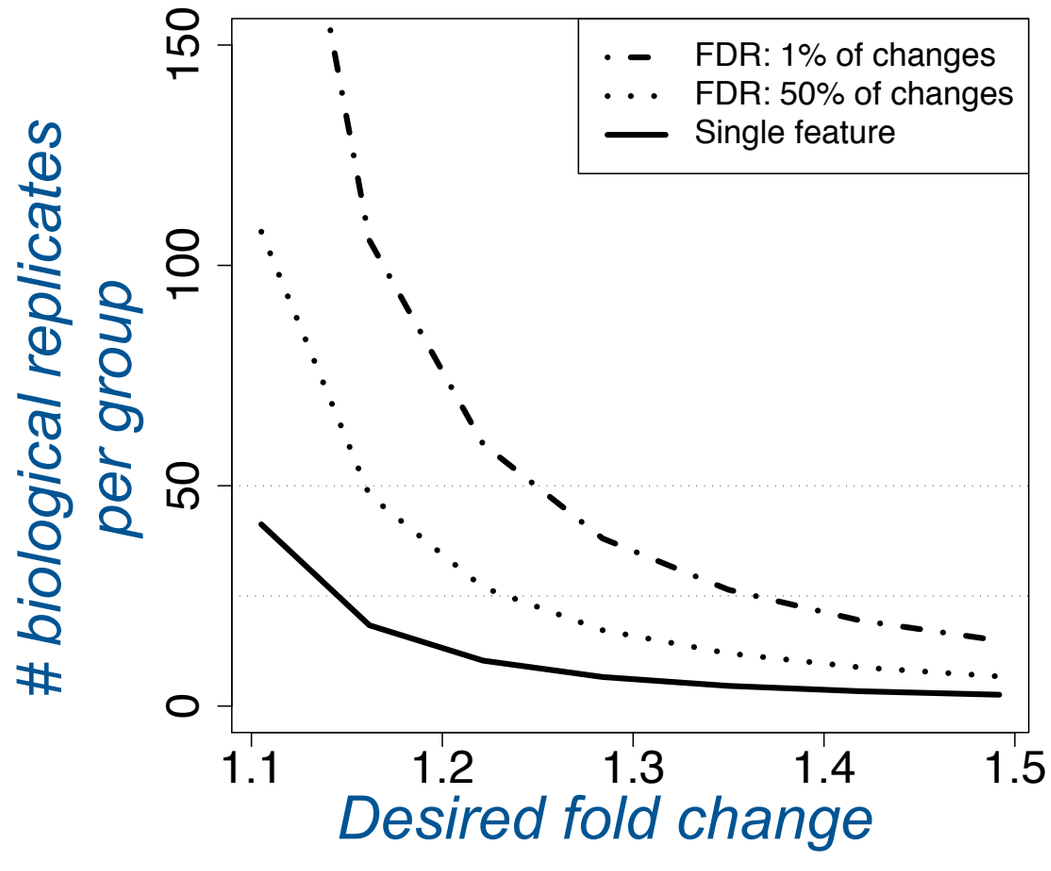
Experimental design

Use the dataset to improve:

- Subject selection: matching
- Resource allocation: blocking
- Calculation of sample size



INTRODUCTION TO MSSTATS

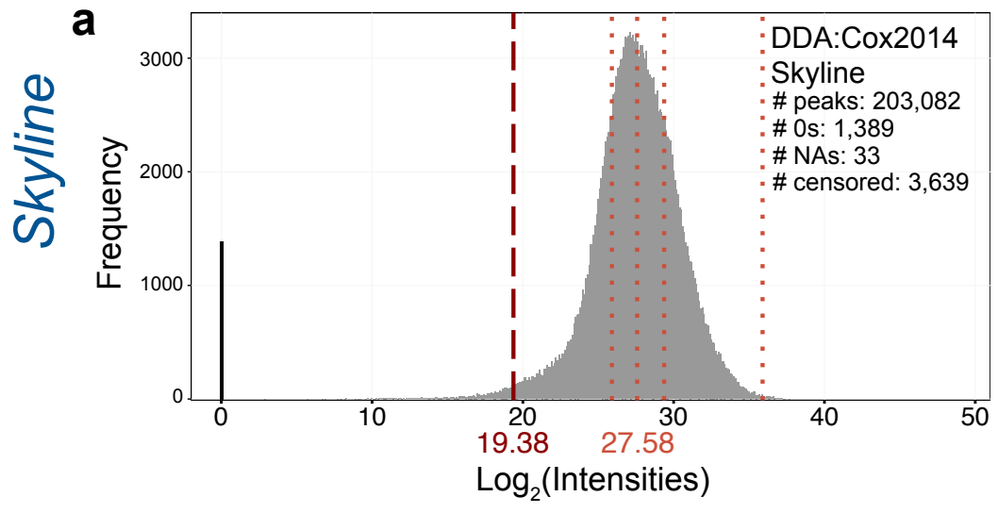
- Overview
- Statistical modeling
- Community resources

PROPERTIES OF PEAK INTENSITIES VARY BETWEEN DATA PROCESSING TOOLS

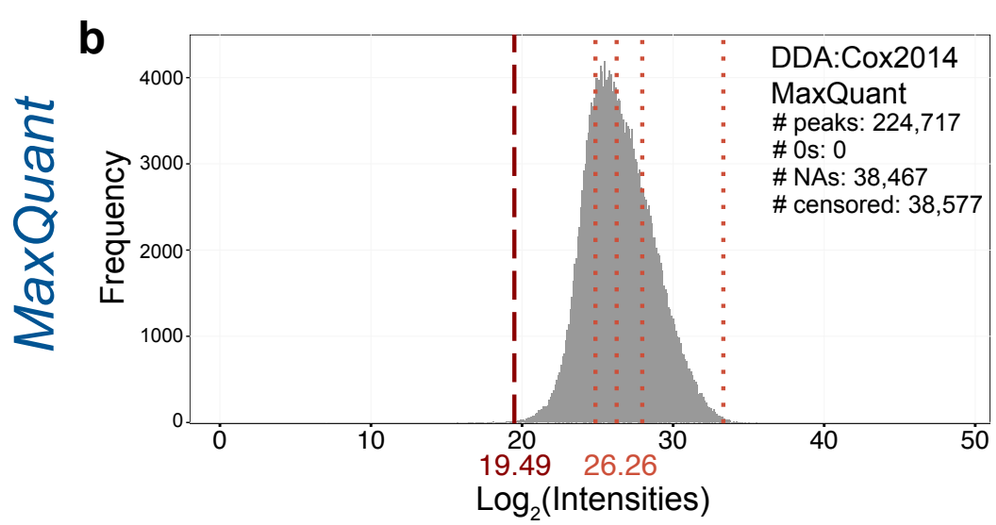
DDA: Cox 2014

DIA: Bruderer 2015

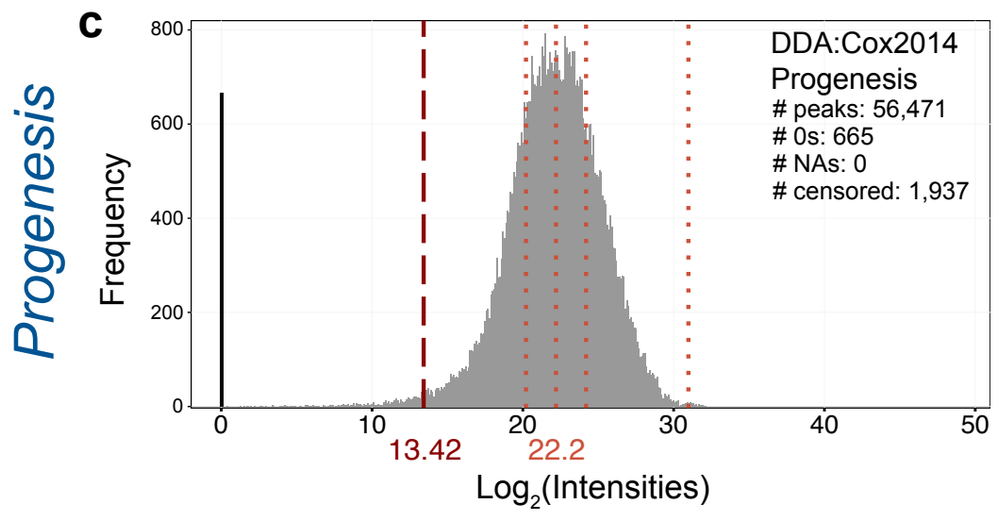
Skyline



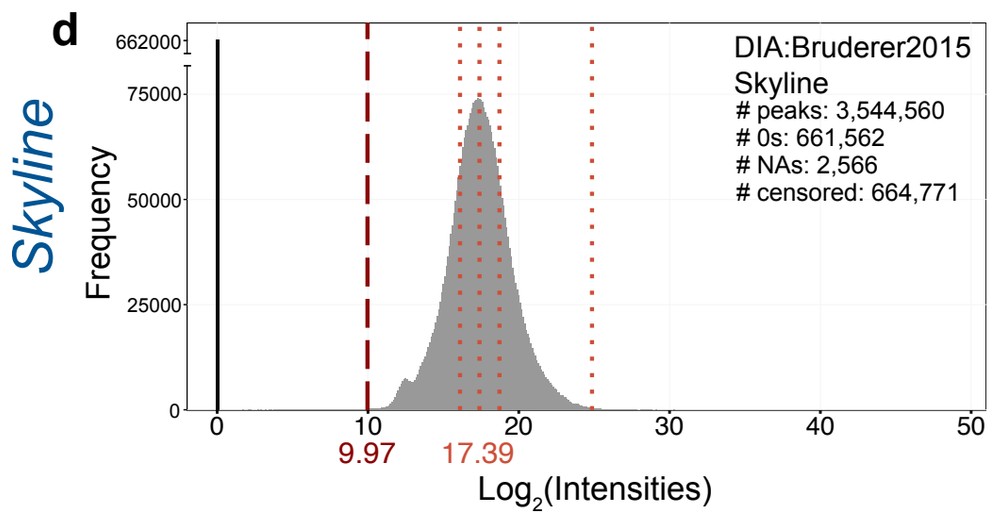
MaxQuant



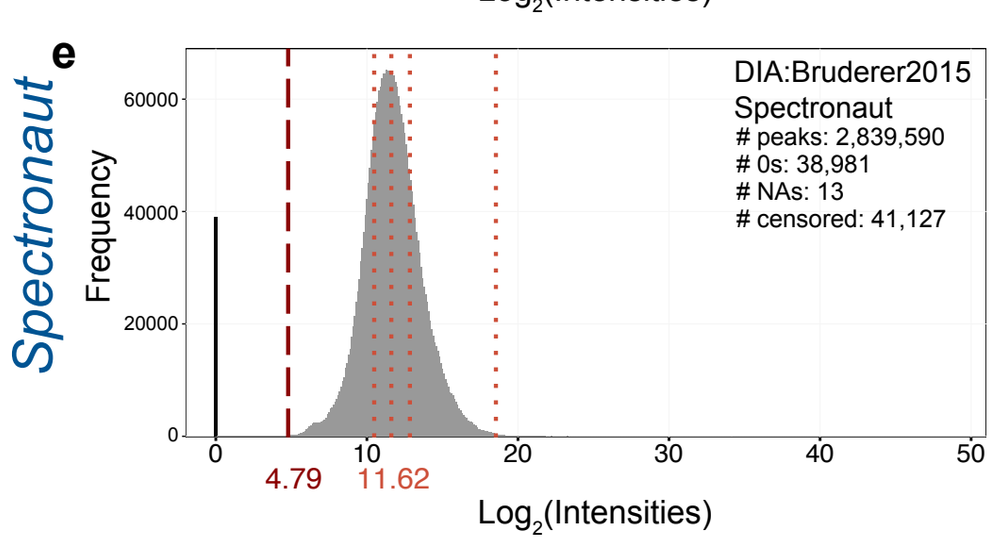
Progenesis

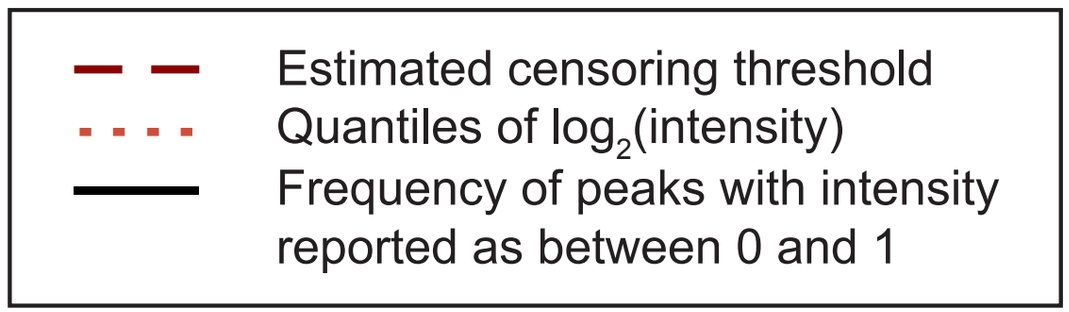


Skyline



Spectronaut





SCHEMATIC DATA REPRESENTATION

Repeat for every protein

Whole plot

Subplot

	Condition$_1$										. . .	Condition$_I$									
	Subject$_1$			Subject$_2$			. . .	Subject$_J$			. . .	Subject$_{(I-1)J+1}$			Subject$_{(I-1)J+2}$			. . .	Subject$_{IJ}$		
	Run$_1$	Run$_2$	Run$_3$	Run$_4$	Run$_5$	Run$_6$	. . .	Run$_{JK-2}$	Run$_{JK-1}$	Run$_{JK}$	. . .	Run$_{(I-1)JK+1}$	Run$_{(I-1)JK+2}$	Run$_{(I-1)JK+3}$	Run$_{(I-1)JK+4}$	Run$_{(I-1)JK+5}$	Run$_{(I-1)JK+6}$	. . .	Run$_{IJK-2}$	Run$_{IJK-1}$	Run$_{IJK}$
Feature$_1$	y	y	y	y	y	y	. . .	y	y	y	. . .	y	y	y	y	y	y	. . .	y	y	y
Feature$_2$	y	y	y	y	y	**NA**	. . .	y	y	y	. . .	y	y	y	y	y	y	. . .	y	y	y
. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
Feature$_L$	y	**Cens**	y	**Cens**	**Cens**	y	. . .	**Cens**	y	y	. . .	**NA**	y	y	y	y	y	. . .	y	**Cens**	y

Spectral features: technological aspects of the experiment

Missing values

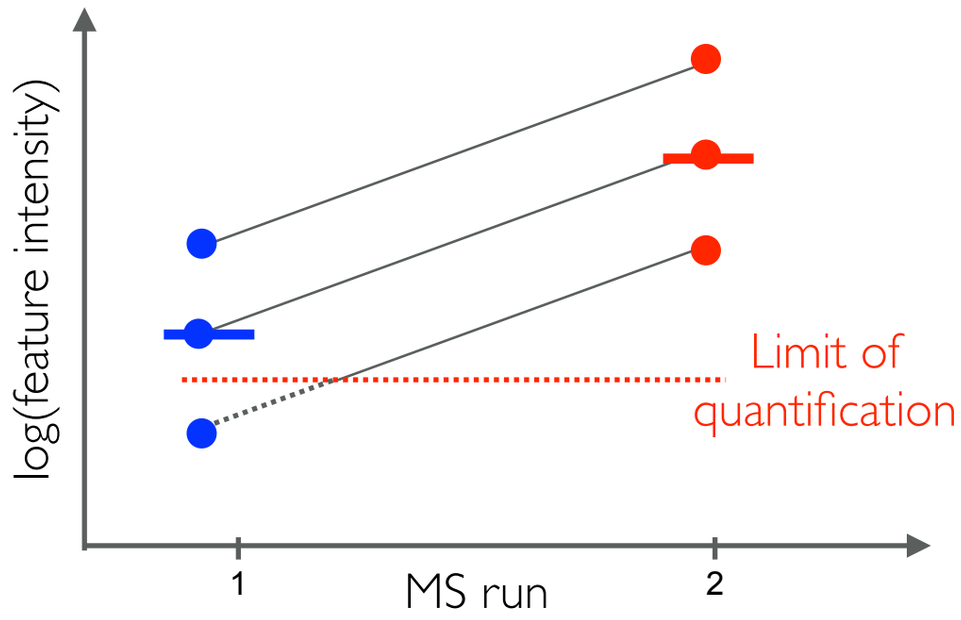
View feature intensities below the threshold as censored

Log(feature intensities)

Challenges

- Represent experimental design
- Represent peak intensities
- Estimate variance components & inference

IMPUTATION OF MISSING VALUES

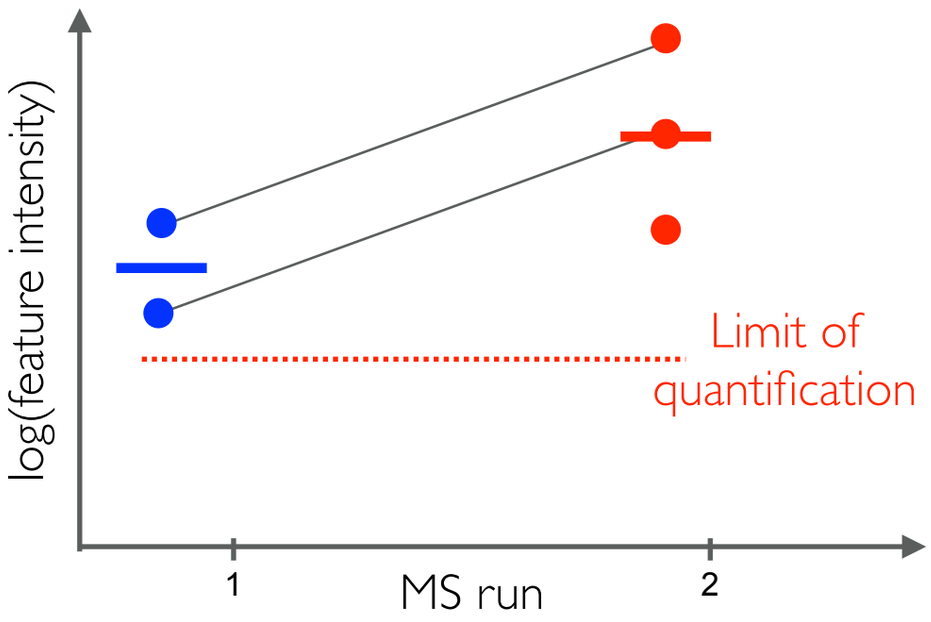


MSstats

Relies on information from other features and runs

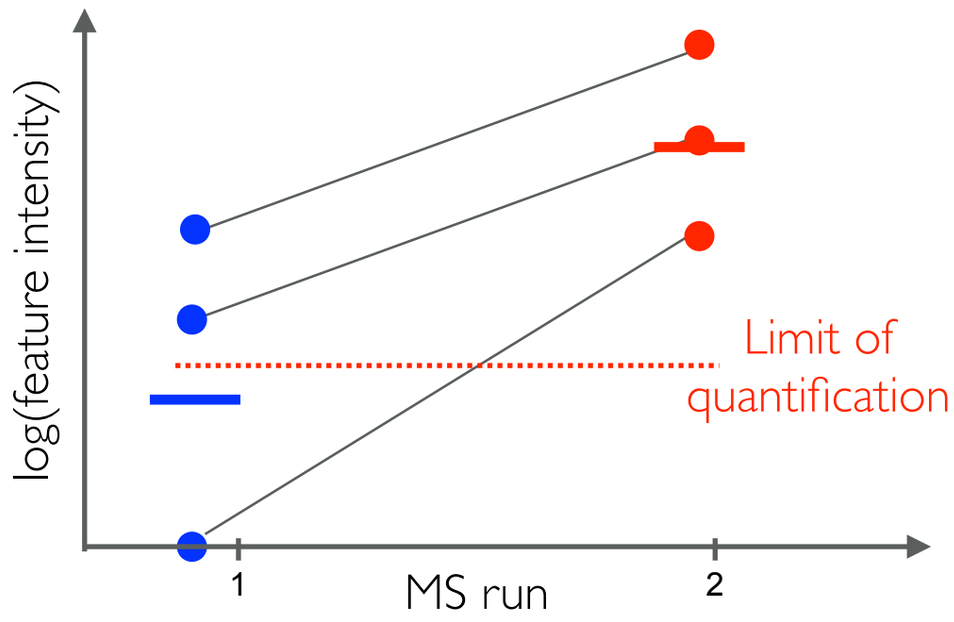
No imputation

Underestimates the regulation



Imputation with 0

Overestimates the regulation



MSSTATS: MODEL AND INFERENCE

A

	Run_1	Run_2	Run_3	Run_4	Run_5	Run_6	· · ·	Run_{JK-2}	Run_{JK-1}	Run_{JK}	· · ·	$Run_{(I-1)JK+1}$	$Run_{(I-1)JK+2}$	$Run_{(I-1)JK+3}$	$Run_{(I-1)JK+4}$	$Run_{(I-1)JK+5}$	$Run_{(I-1)JK+6}$	· · ·	Run_{IJK-2}	Run_{IJK-1}	Run_{IJK}
$Feature_1$	y	y	y	y	y	y	· · ·	y	y	y	· · ·	y	y	y	y	y	y	· · ·	y	y	y
$Feature_2$	y	y	y	y	y	NA_{rand}	· · ·	y	y	y	· · ·	y	y	y	y	y	y	· · ·	y	y	y
· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·
$Feature_L$	y	NA_{cen}	y	NA_{cen}	NA_{cen}	y	· · ·	NA_{cen}	y	y	· · ·	NA_{cen}	y	y	y	y	y	· · ·	y	NA_{cen}	y

↓

B

Step 1 : Run-level subplot summarization

AFT model : Impute censored missing values by accelated failure model

$$y_{ijkl} = \mu + Run_{ijk} + Feature_l + \epsilon_{ijkl} \text{ , where } \sum_{ijk} Run_{ijk} = 0,\ \sum_{l} Feature_l = 0, \epsilon_{ijkl} \overset{iid}{\sim} \mathcal{N}(0, \sigma^2_\epsilon)$$

	Run_1	Run_2	Run_3	Run_4	Run_5	Run_6	· · ·	Run_{JK-2}	Run_{JK-1}	Run_{JK}	· · ·	$Run_{(I-1)JK+1}$	$Run_{(I-1)JK+2}$	$Run_{(I-1)JK+3}$	$Run_{(I-1)JK+4}$	$Run_{(I-1)JK+5}$	$Run_{(I-1)JK+6}$	· · ·	Run_{IJK-2}	Run_{IJK-1}	Run_{IJK}
$Feature_1$	y	y	y	y	y	y	· · ·	y	y	y	· · ·	y	y	y	y	y	y	· · ·	y	y	y
$Feature_2$	y	y	y	y	y		· · ·	y	y	y	· · ·	y	y	y	y	y	y	· · ·	y	y	y
· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·	· · ·
$Feature_L$	y	y_{imp}	y	y_{imp}	y_{imp}	y	· · ·	y_{imp}	y	y	· · ·	y_{imp}	y	y	y	y	y	· · ·	y	y_{imp}	y

↓

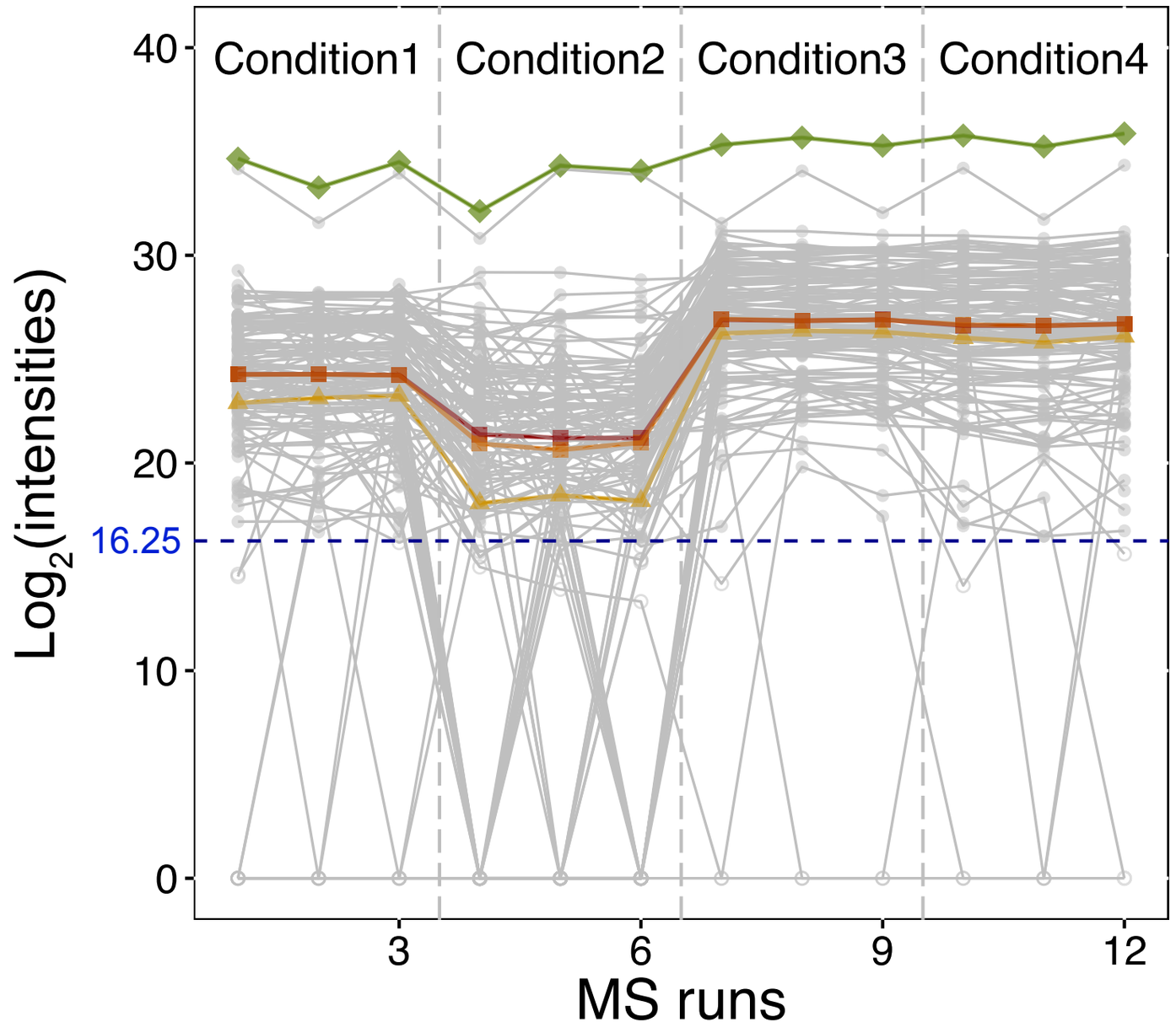
TMP : Parameter estimation by robust method

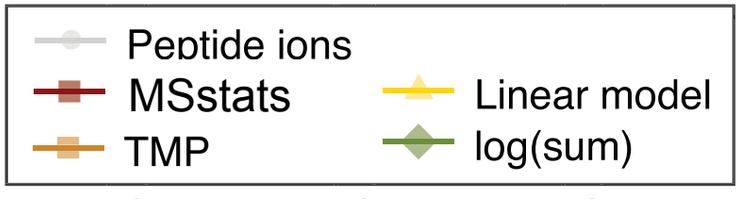
Whole plot model

	$Condition_1$										· · ·	$Condition_I$									
	$Subject_1$			$Subject_2$			· · ·	$Subject_J$			· · ·	$Subject_{(I-1)J+1}$			$Subject_{(I-1)+2}$			· · ·	$Subject_{IJ}$		
	Run_1	Run_2	Run_3	Run_4	Run_5	Run_6	· · ·	Run_{JK-2}	Run_{JK-1}	Run_{JK}	· · ·	$Run_{(I-1)JK+1}$	$Run_{(I-1)JK+2}$	$Run_{(I-1)JK+3}$	$Run_{(I-1)JK+4}$	$Run_{(I-1)JK+5}$	$Run_{(I-1)JK+6}$	· · ·	Run_{IJK-2}	Run_{IJK-1}	Run_{IJK}
Summarized	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	· · ·	$\hat{y}$	$\hat{y}$	$\hat{y}$	· · ·	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	$\hat{y}$	· · ·	$\hat{y}$	$\hat{y}$	$\hat{y}$

ROBUSTNESS TO OUTLIERS

Outliers in both high and low intensities: TMP improves upon linear model and log(sum)





Condition1-Condition2 : True fold change=7.5

	EstimatedFC	Adj.pvalue
MSstats	8.015	< 0.001
TMP	10.605	< 0.001
Linear model	29.106	< 0.001
log(sum)	1.552	0.999

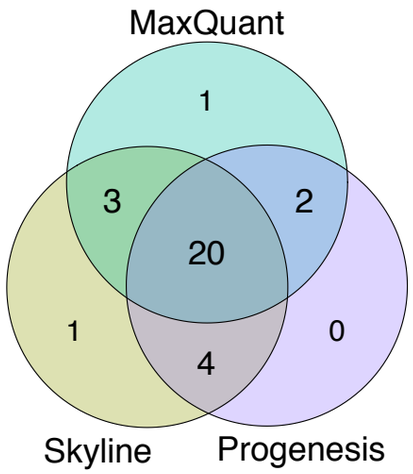
STATISTICAL METHODS ENHANCE REPRODUCIBLE RESEARCH

Better agreement in correct detection of differentially abundant proteins between tools

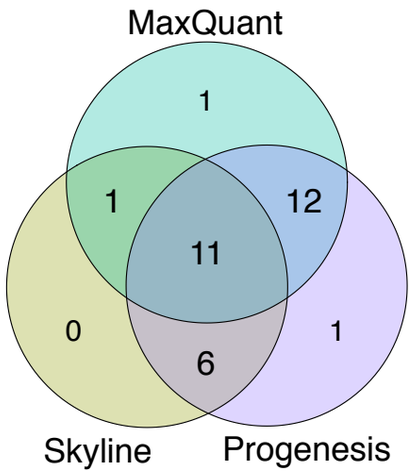
Meena Choi

DDA: iPRG2015

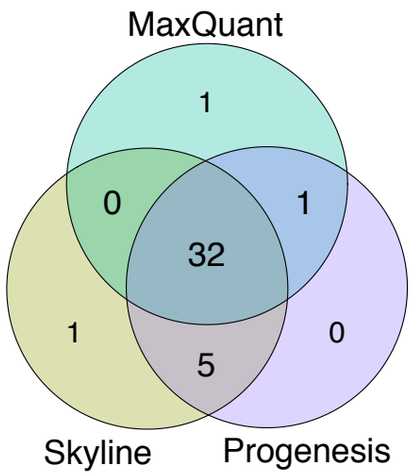
MSstats

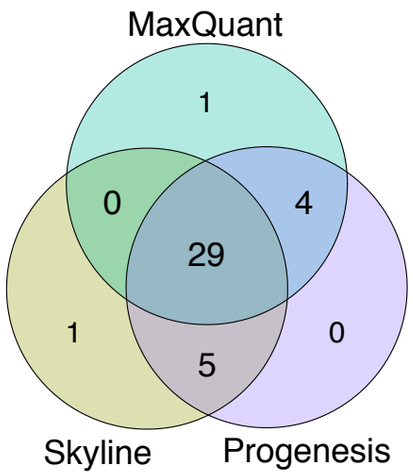


Log(sum)

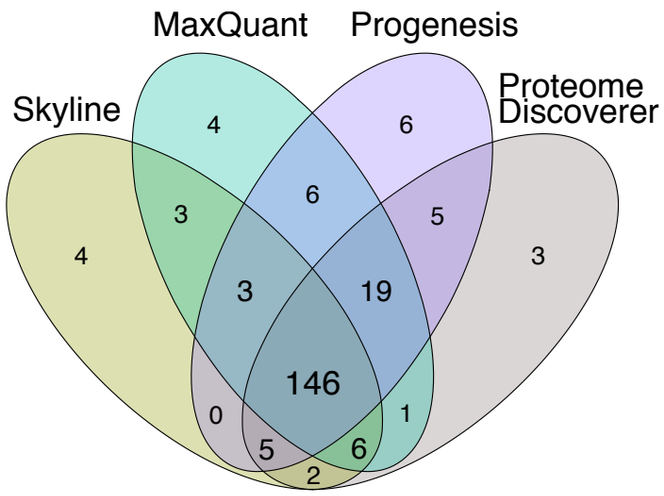


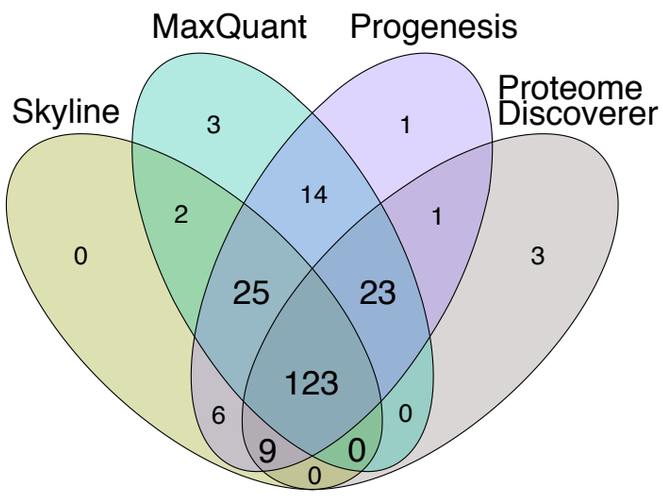
DDA: Cox 2014





DDA: Spike-in





INTRODUCTION TO MSSTATS

- Overview
- Statistical modeling
- Community resources

DATA PROCESSING IMPACTS STATISTICAL ANALYSIS

Publicly available data are key for method development and evaluation

https://massive.ucsd.edu/ProteoSAFe/static/massive.jsp

MassIVE

Mass Spectrometry
Interactive **V**irtual **E**nvironment

MassIVE.quant Statistics

Public Datasets:	46
Quant Reanalyses:	188
Number of Datasets by Species:	
Homo sapiens	23
Mus musculus	12
Saccharomyces cerevisiae	5
Human immunodeficiency virus 1	4
Others	14

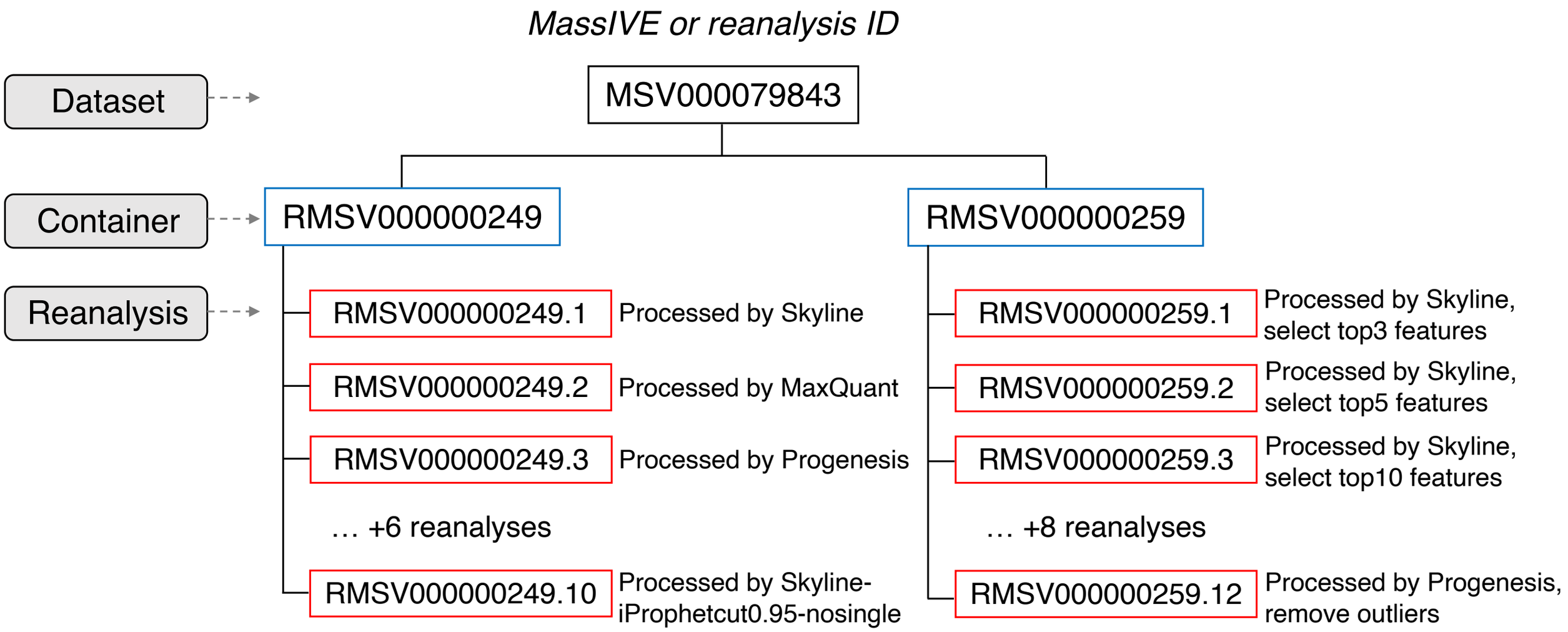
nature methods
BRIEF COMMUNICATION
https://doi.org/10.1038/s41592-020-0955-0

MassIVE.quant: a community resource of quantitative mass spectrometry–based proteomics datasets

Meena Choi[1], Jeremy Carver[2], Cristina Chiva[3,4], Manuel Tzouros[5], Ting Huang[1], Tsung-Heng Tsai[1], Benjamin Pullman[2], Oliver M. Bernhardt[6], Ruth Hüttenhain[7], Guo Ci Teo[8], Yasset Perez-Riverol[9], Jan Muntel[6], Maik Müller[10], Sandra Goetze[10,11], Maria Pavlou[10], Erik Verschueren[7], Bernd Wollscheid[10,11], Alexey I. Nesvizhskii[8], Lukas Reiter[6], Tom Dunkley[5], Eduard Sabidó[3,4], Nuno Bandeira[2]✉ and Olga Vitek[1]✉

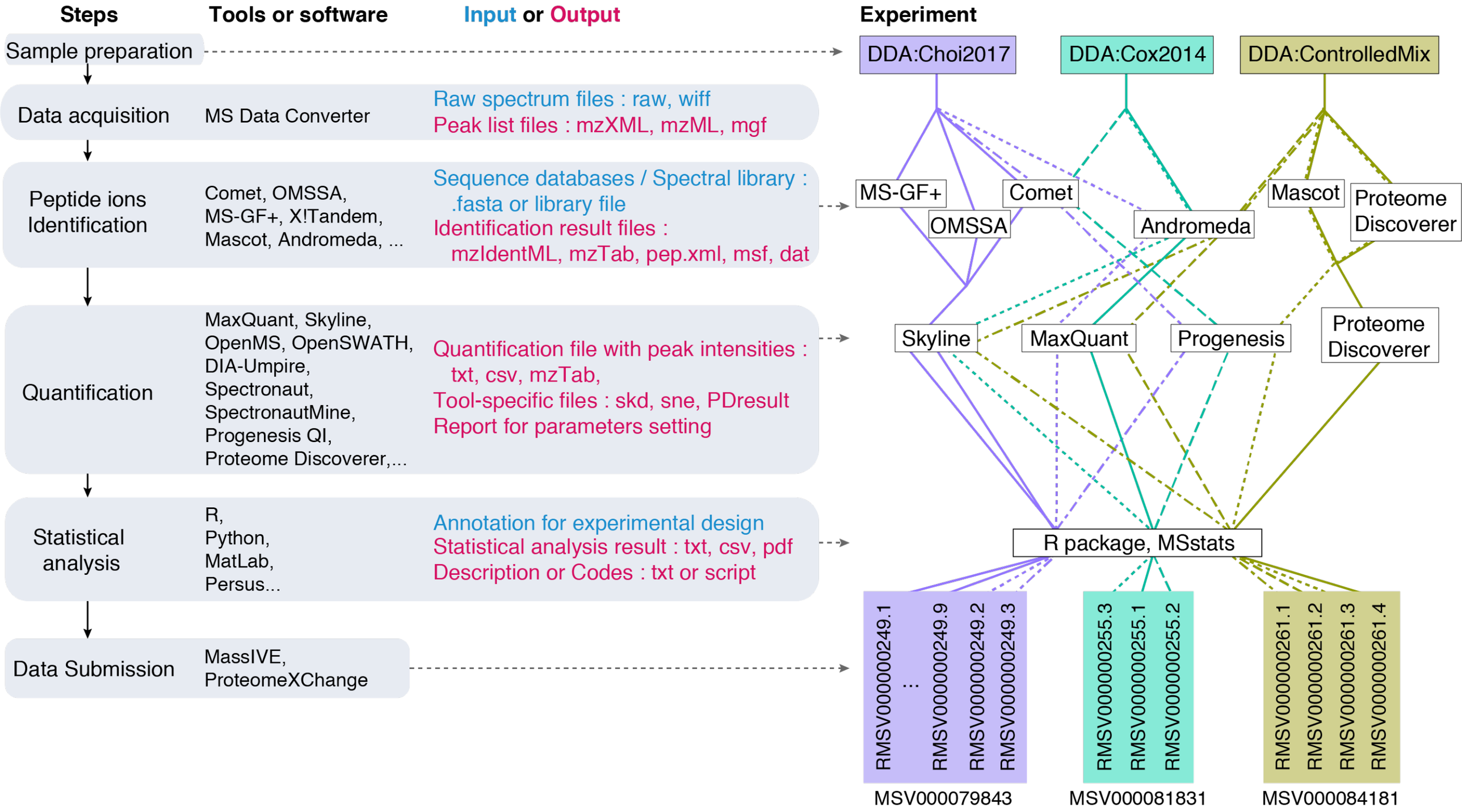
MASSIVE.QUANT

Repository for quantitative data and reanalysis



MASSIVE.QUANT

Repository for quantitative data and reanalysis



https://computationalproteomics.khoury.northeastern.edu/

May Institute goes ONLINE!
Computation and statistics for mass spectrometry and proteomics

April 27 – May 8, 2020, Northeastern University, Boston MA
Organizers : Meena Choi and Olga Vitek

Week 1:

April 27, 11:00am-1:00pm	**Alicia Williams:** Scientific writing workshop (live)-registration closed	Docs Video
April 28, 11:00am-12:30pm	**Lindsay Pino:** Targeted analysis with Skyline, a PRM perspective (live)-registration closed	Docs Video
April 28, 12:45pm-2:15pm	**Brian Searle:** Introduction to DIA (live)-registration closed	Docs Video
April 29, 11:00am-12:30pm	**Bernhard Kuster:** Large-scale proteomics with TMT (live)-registration closed	Docs Video
April 29, 12:45pm-2:15pm	**Ruedi Aebersold:** Modern technology for modern biology (live)-registration closed	Docs Video
April 30, 11:00am-1:00pm	**Brendan MacLean:** Skyline for DIA: Intro with a controlled mixture and exploring Prosit spectrum and retention time prediction (video+live)-registration closed	Docs Video
May 1, 11:00am-1:00pm	**Brendan MacLean:** Comparing DIA with a deep fractionation library versus a refined subset in Skyline (video+live)-registration closed	Docs Video


Sue Abbatiello

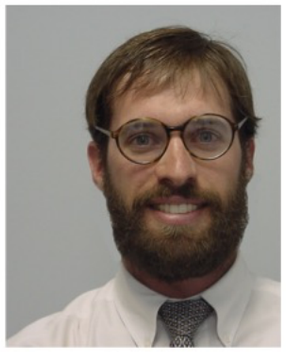

Ruedi Aebersold


Nuno Bandeira


Kylie Bemis


Meena Choi


Laurent Gatto


Andy Hoofnagle


Oliver Kohlbacher


Bernhard Kuster


Brendan MacLean


Brian Searle


Olga Vitek

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Anuska Tak
Derrie Susan Varghese
Amrutha Vempati

Video of the presentation: https://www.youtube.com/channel/UCnbUMFlIRLaY7fwfSintWuQ/