TWO-DAY SHORT COURSE, Saturday and Sunday
14 Case Studies in Quantitative Proteomics

Instructors

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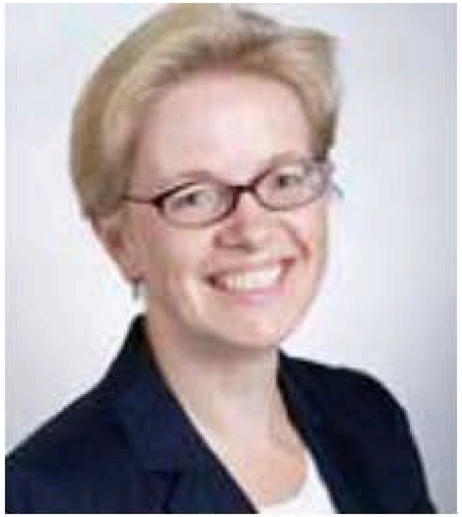
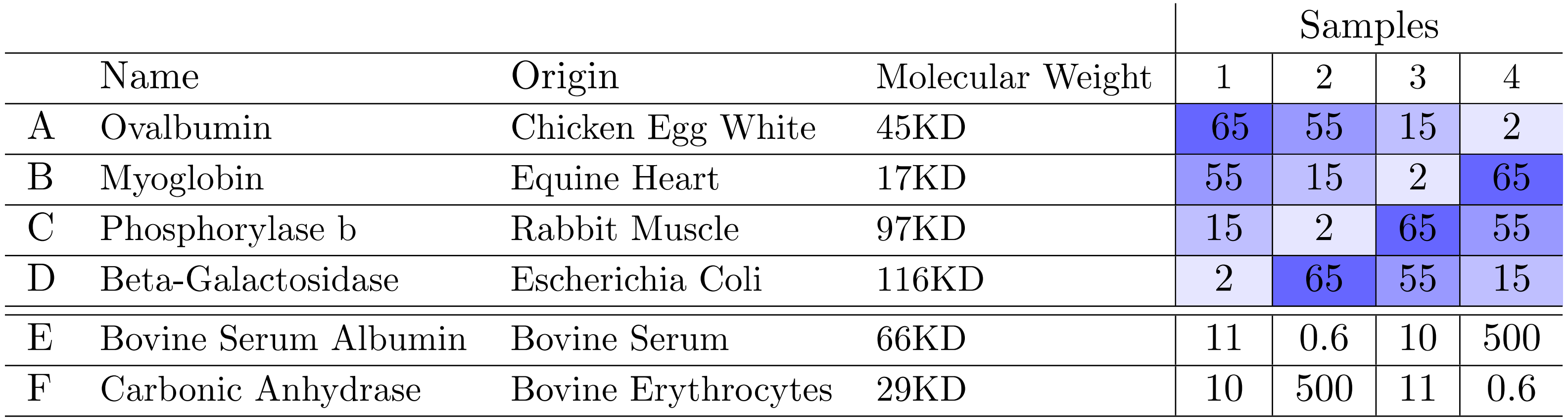
Buck Institute



Olga Vitek
Northeastern University

ABRF iPRG Study 2015

Detection of differentially abundant proteins in controlled mixture



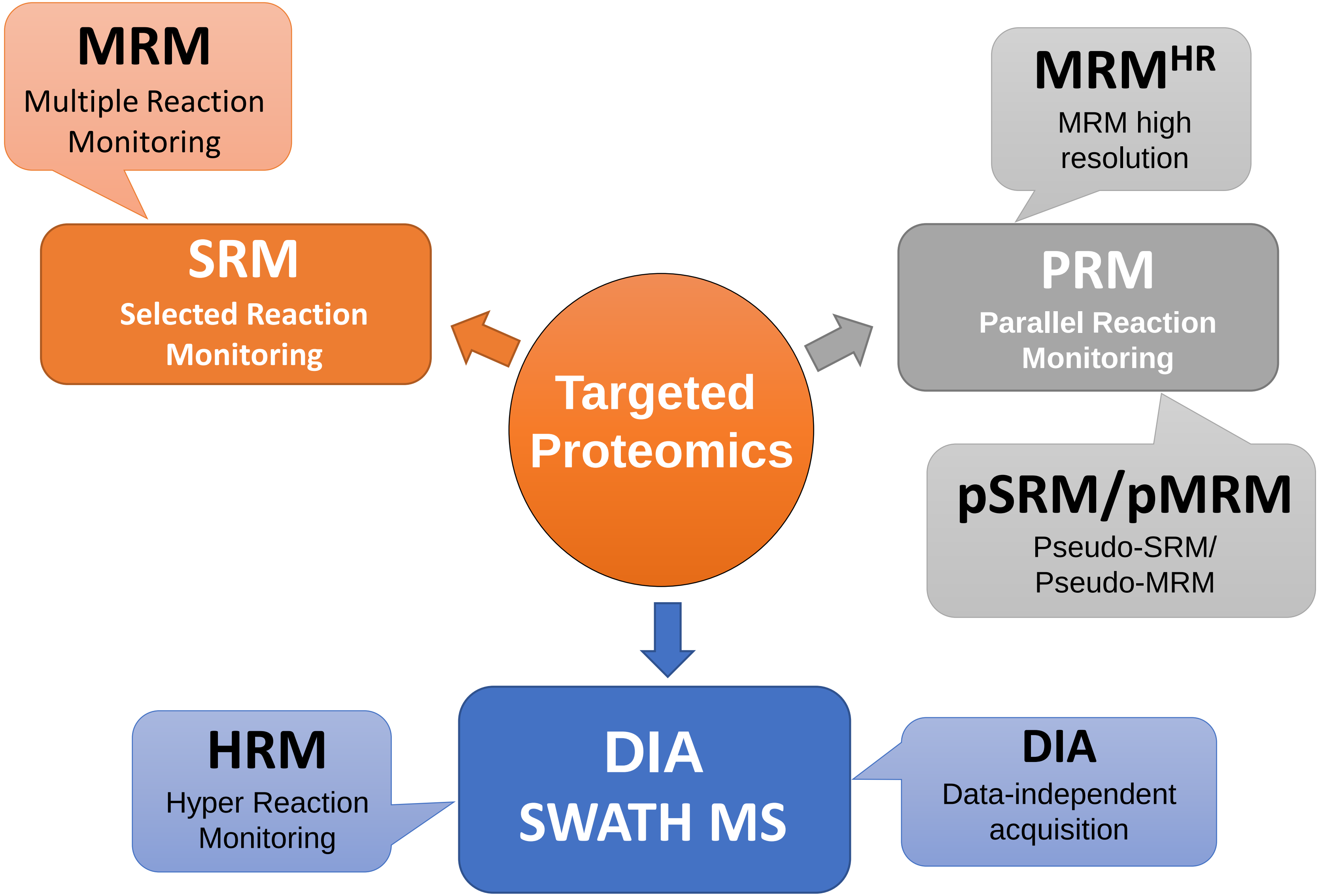
				Samples			
	Name	Origin	Molecular Weight	1	2	3	4
A	Ovalbumin	Chicken Egg White	45KD	65	55	15	2
B	Myoglobin	Equine Heart	17KD	55	15	2	65
C	Phosphorylase b	Rabbit Muscle	97KD	15	2	65	55
D	Beta-Galactosidase	Escherichia Coli	116KD	2	65	55	15
E	Bovine Serum Albumin	Bovine Serum	66KD	11	0.6	10	500
F	Carbonic Anhydrase	Bovine Erythrocytes	29KD	10	500	11	0.6

Spiked into a constant background: tryptic digests of S. cerevisiae

- Three technical replicates per sample
    - Thermo nLC 1000 system
    - 110-min linear gradient
- DDA profile mode in Orbitrap
- Data processing with Skyline

Choi et al., *Journal of Proteome Research,* 2017.

Targeted proteomics – various approaches



MRM
Multiple Reaction Monitoring

SRM
Selected Reaction Monitoring

Targeted Proteomics

PRM
Parallel Reaction Monitoring

MRM^{HR}
MRM high resolution

pSRM/pMRM
Pseudo-SRM/
Pseudo-MRM

HRM
Hyper Reaction Monitoring

DIA
SWATH MS

DIA
Data-independent acquisition

Discovery to Targeted with Skyline

- DDA Acquisition
- Spectrum Matching
- Skyline Import
- Group Comparisons
- Discover Candidates
- Validate

Skyline

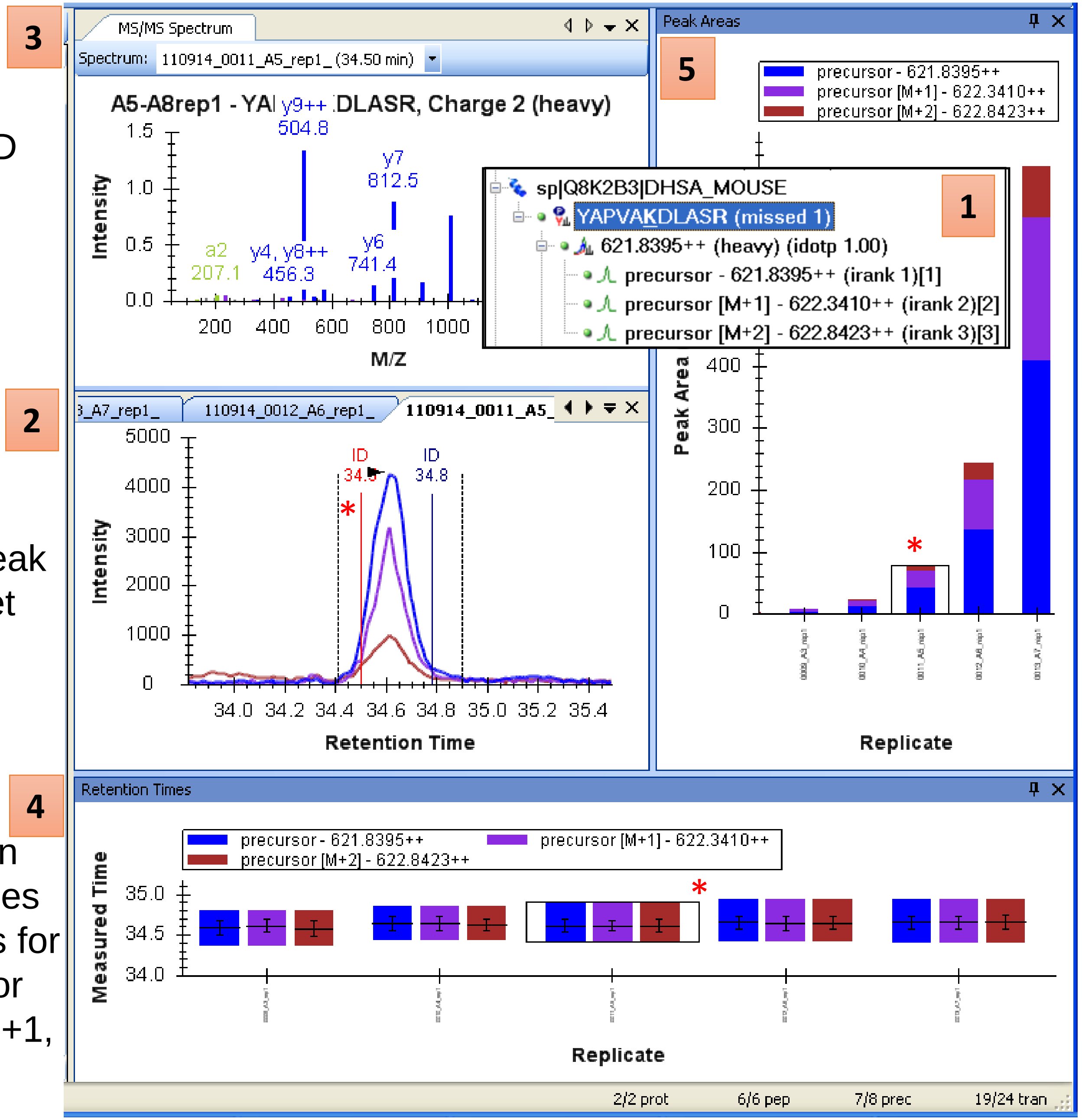
Got HYPOTHESIS!!

Skyline interface for MS1 Filtering data

3) MS/MS spectra and ID

2) RT and ID correlation; peak boundaries set for integration

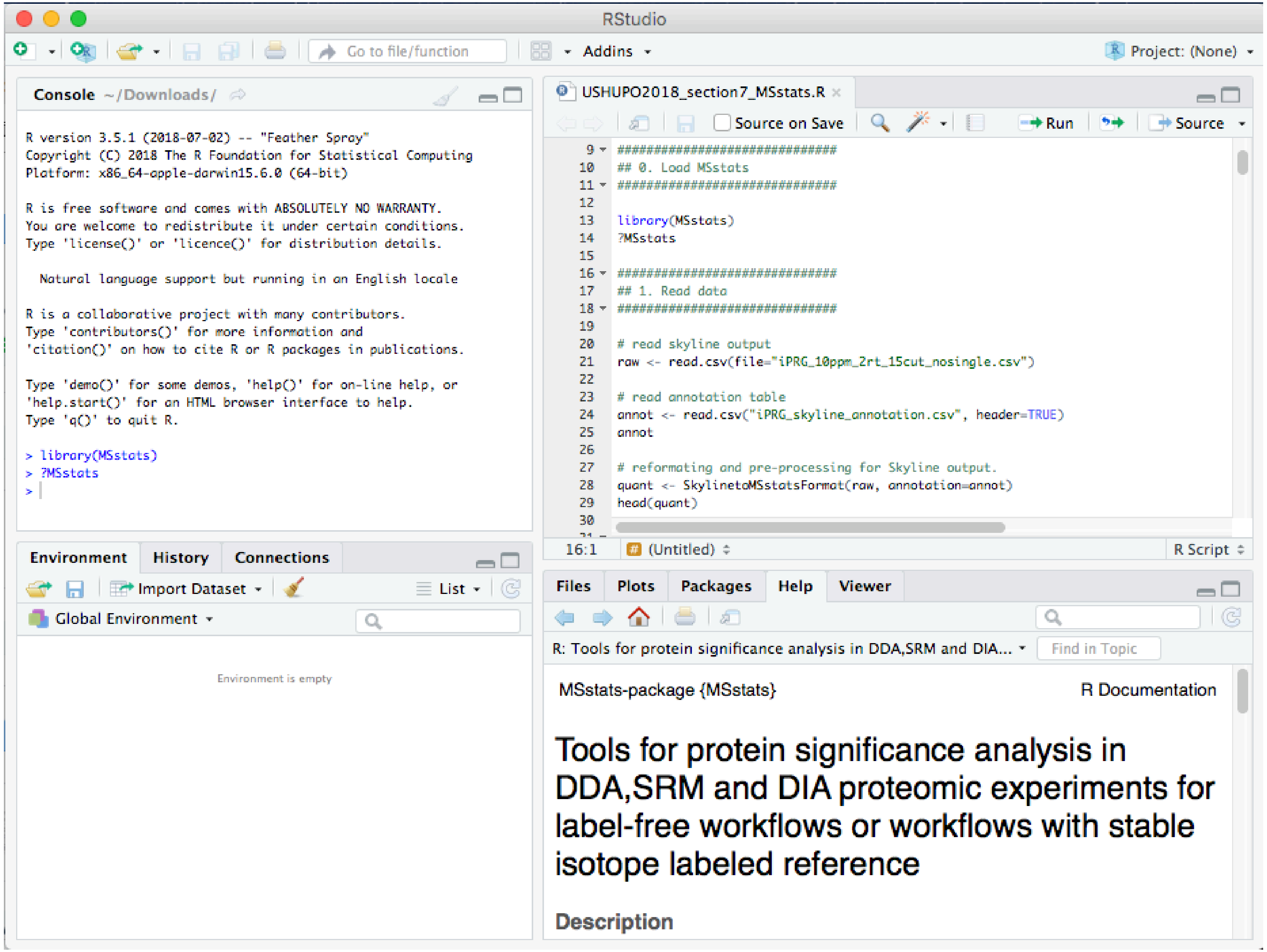
4) RT variation among peptides and replicates for each precursor isotope (M, M+1, M+2)



5) M, M+1,M+2 precursor peak areas

1) Peptide ‘tree’ with precursors
- irank
- idotp

Statistical analysis by MSstats in R



```
R version 3.5.1 (2018-07-02) -- "Feather Spray"
Copyright (C) 2018 The R Foundation for Statistical Computing
Platform: x86_64-apple-darwin15.6.0 (64-bit)

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  Natural language support but running in an English locale

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Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

> library(MSstats)
> ?MSstats
> 
```
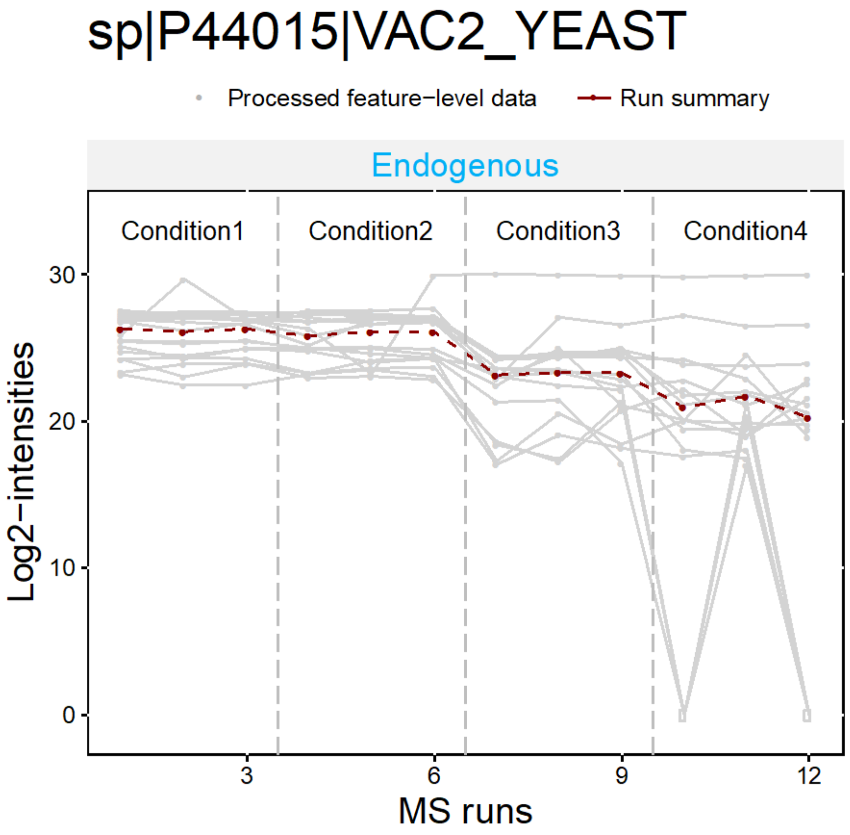

```r
##############################
## 0. Load MSstats
##############################

library(MSstats)
?MSstats

##############################
## 1. Read data
##############################

# read skyline output
raw <- read.csv(file="iPRG_10ppm_2rt_15cut_nosingle.csv")

# read annotation table
annot <- read.csv("iPRG_skyline_annotation.csv", header=TRUE)
annot

# reformating and pre-processing for Skyline output.
quant <- SkylinetoMSstatsFormat(raw, annotation=annot)
head(quant)
```

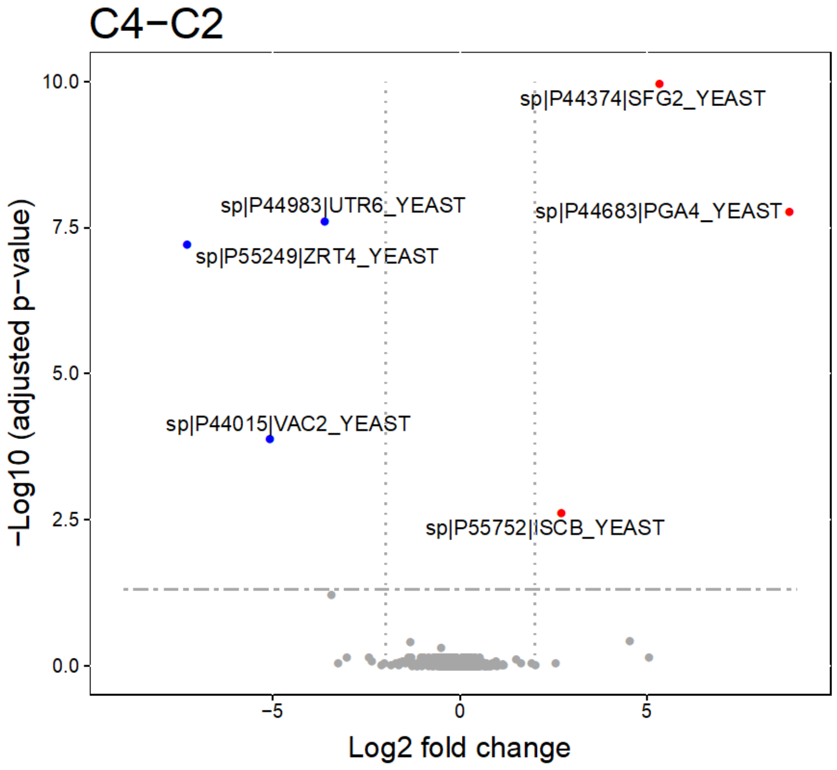
MSstats-package {MSstats} R Documentation

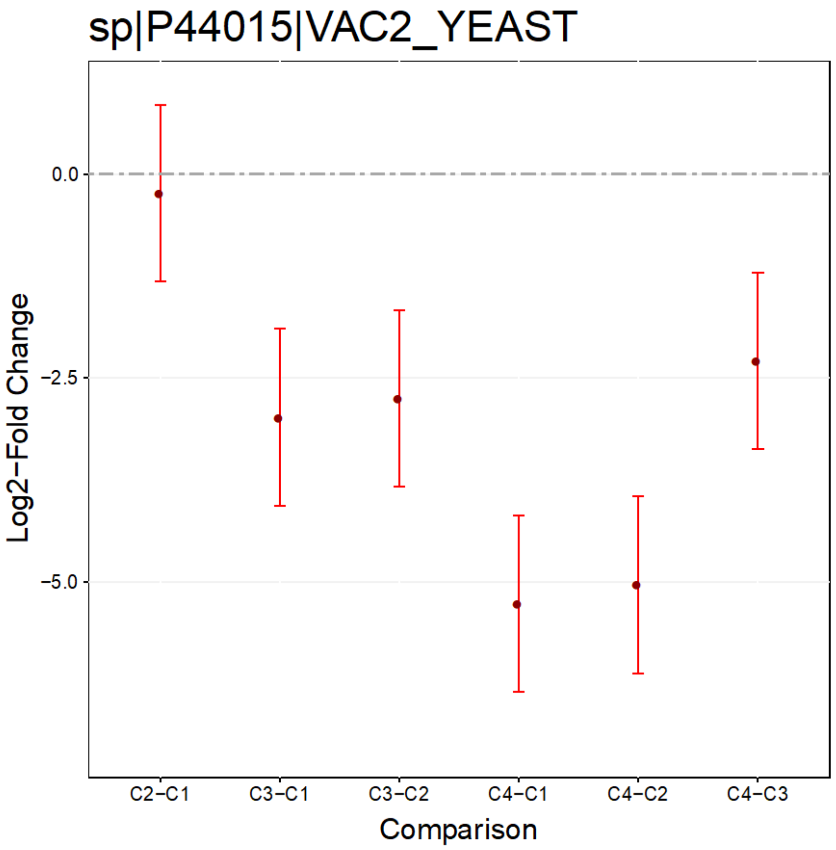
Tools for protein significance analysis in DDA,SRM and DIA proteomic experiments for label-free workflows or workflows with stable isotope labeled reference

Description

Differentially abundant proteins across conditions


sp|P44015|VAC2_YEAST


C4−C2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