

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

Instructors



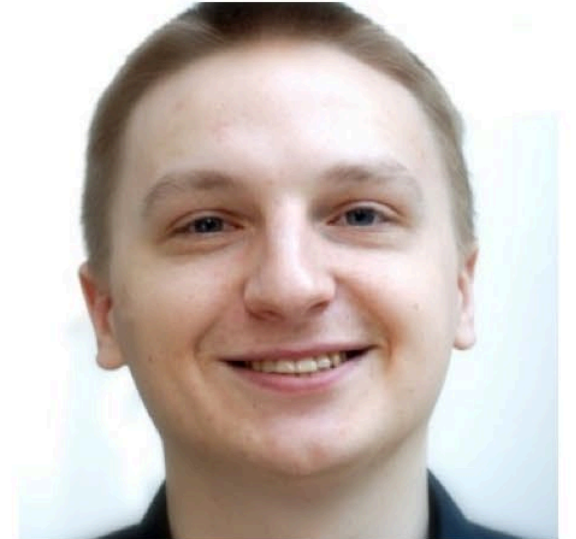
Meena Choi
Northeastern University



Brendan MacLean
University of Washington



Olga Vitek
Northeastern University



Mateusz Staniak
University of Wrocław

ABRF iPRG Study 2015

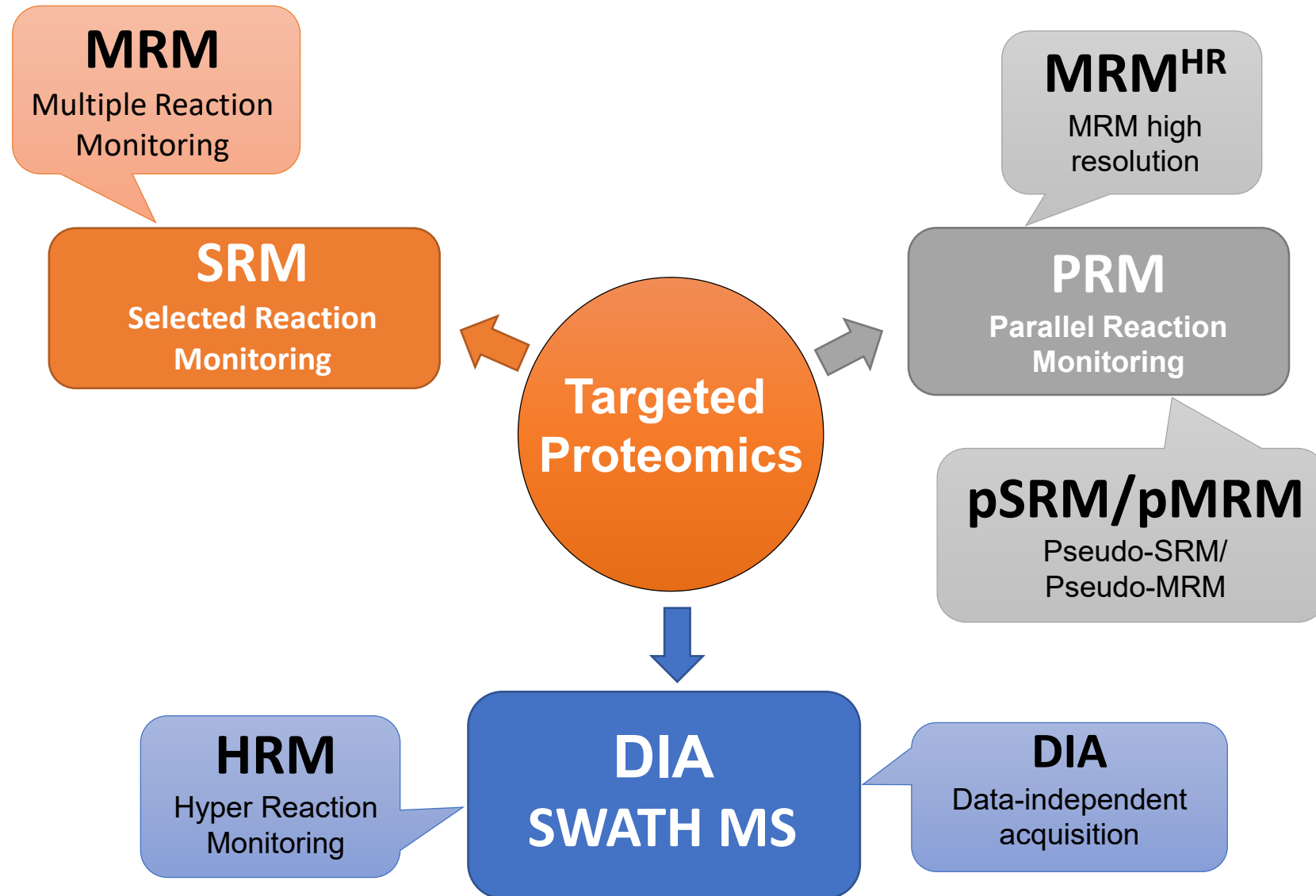
Detection of differentially abundant proteins in controlled mixture

				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	

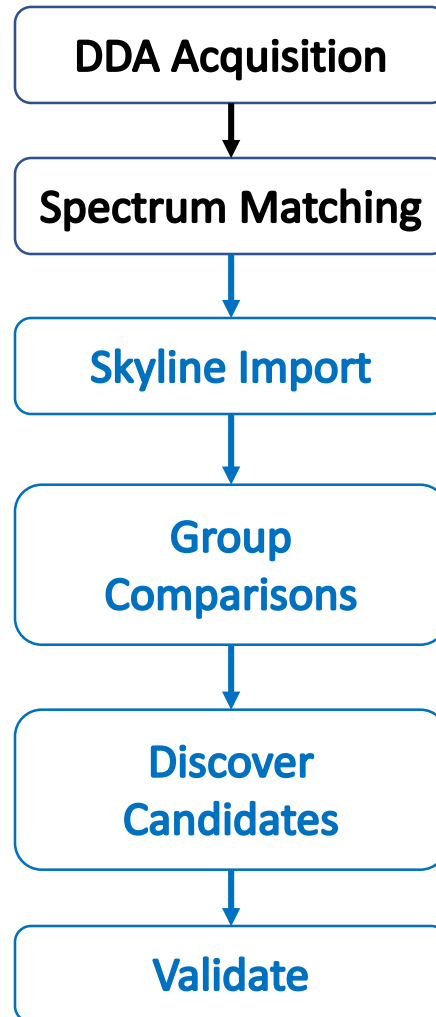
Spiked into a constant background: tryptic digests of S. cerevisiae

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

Targeted proteomics – various approaches



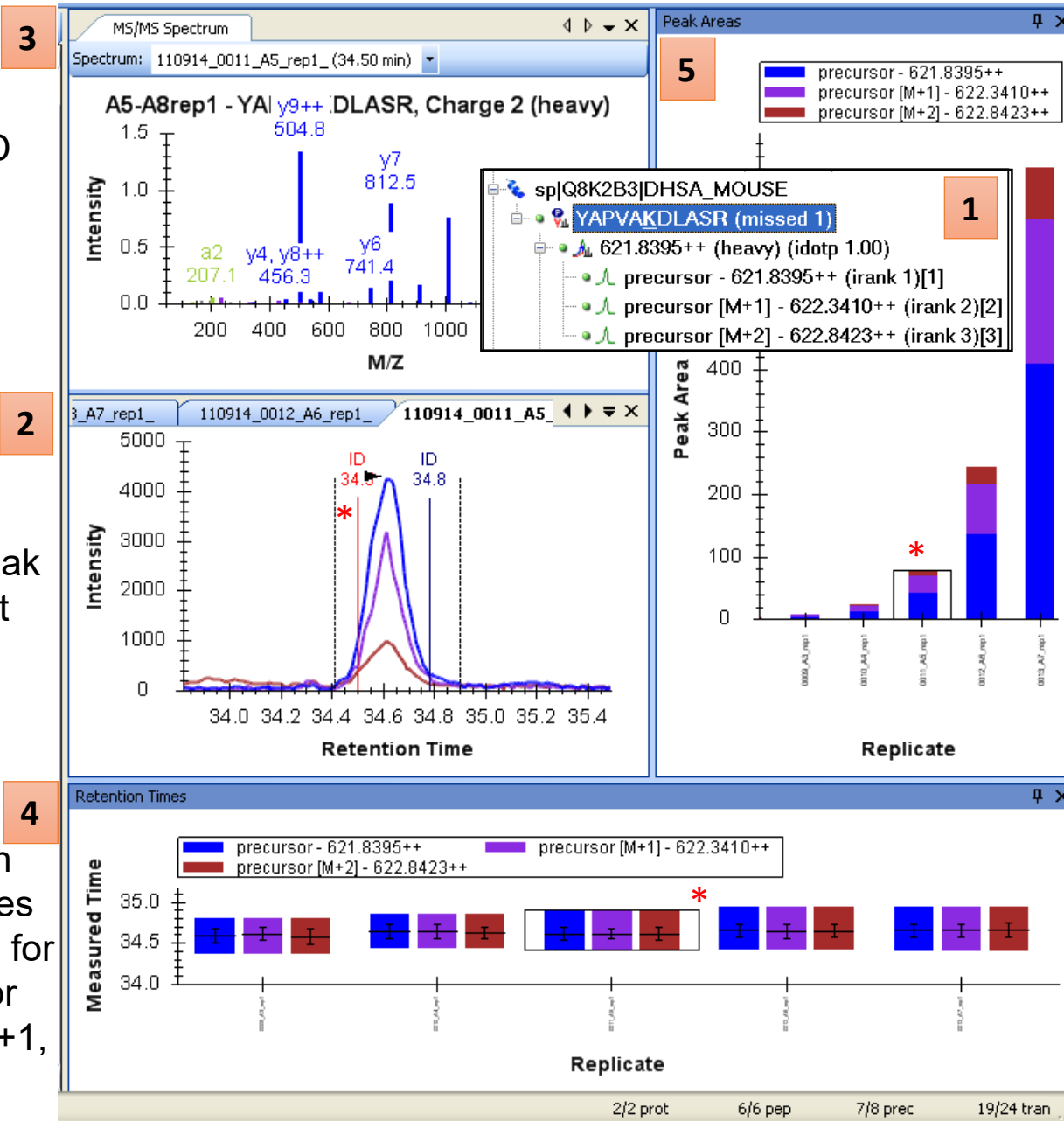
Discovery to Targeted with Skyline



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



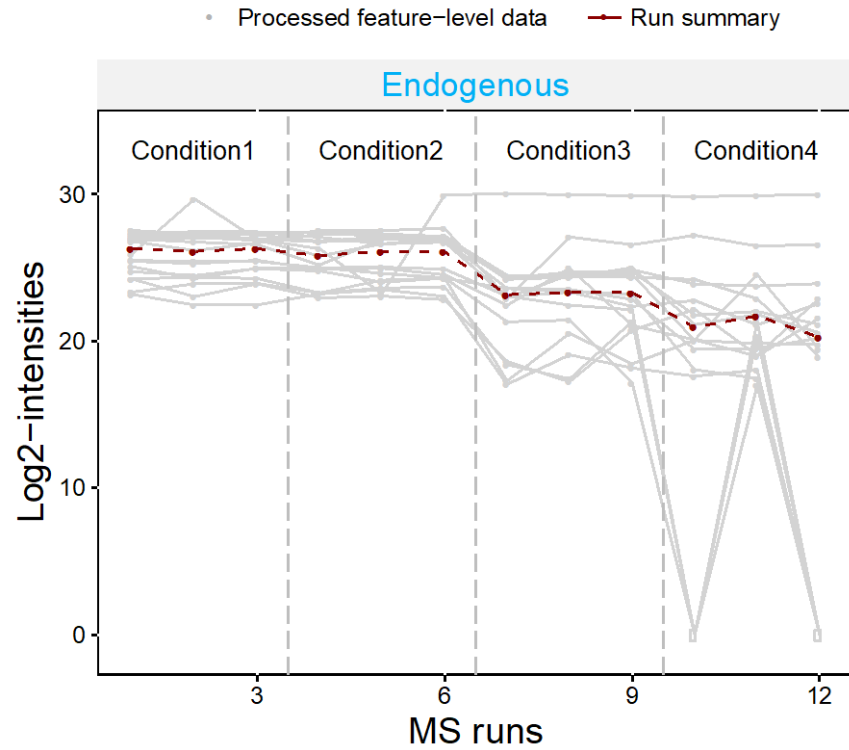
The screenshot shows the RStudio interface with the following components:

- Console:** Displays the R version (3.5.1) and the execution of `library(MSstats)` and `?MSstats`.
- Source Editor:** Contains the R script `USHUPO2018_section7_MSstats.R` with the following code:

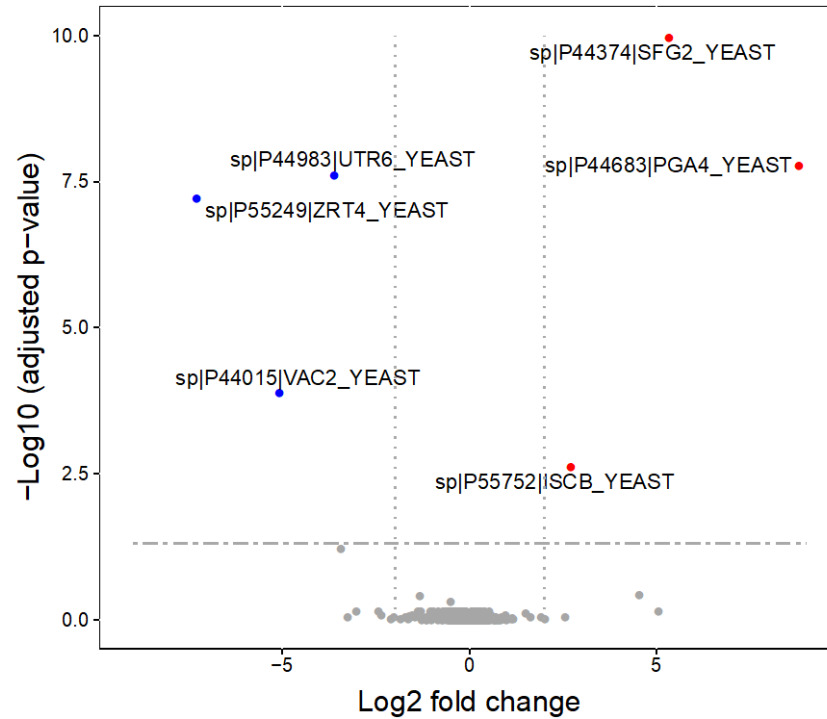
```
#####  
## 0. Load MSstats  
#####  
library(MSstats)  
?MSstats  
#####  
## 1. Read data  
#####  
# read skyline output  
raw <- read.csv(file="iPRG_10ppm_2rt_15cut_nosingle.csv")  
# read annotation table  
annot <- read.csv("iPRG_skyline_annotation.csv", header=TRUE)  
annot  
# reformatting and pre-processing for Skyline output.  
quant <- SkylineToMSstatsFormat(raw, annotation=annot)  
head(quant)
```
- Environment:** Shows the Global Environment with an empty environment.
- Viewer:** Displays the documentation for the `MSstats` package, titled "Tools for protein significance analysis in DDA,SRM and DIA proteomic experiments for label-free workflows or workflows with stable isotope labeled reference".

Differentially abundant proteins across conditions

sp|P44015|VAC2_YEAST



C4-C2



sp|P44015|VAC2_YEAST

