

**TWO-DAY SHORT COURSE, Saturday and Sunday
08 Quantitative Proteomics: Case Studies**

Instructors



Brendan MacLean
U. of Washington



Devon Kohler
Northeastern U.



Deanne Plubell
U. of Washington

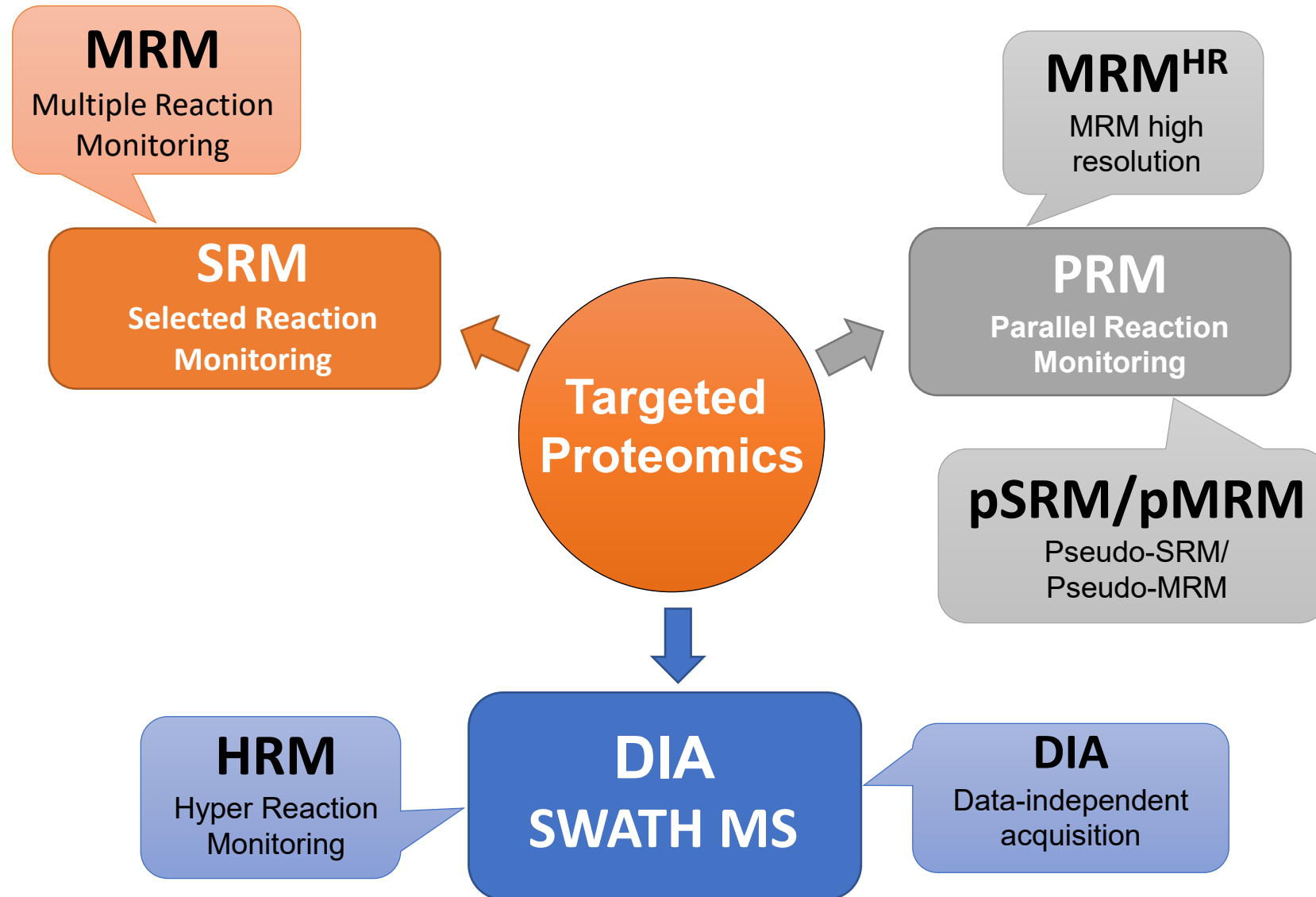


Olga Vitek
Northeastern U.

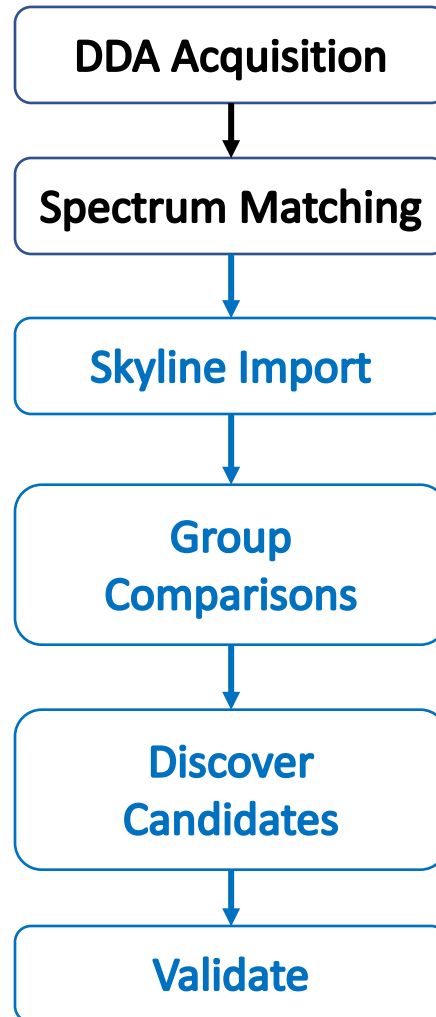
	Day1: 06/01/24 - Introduction	Day2: 06/02/24 - Advanced
Time		
9:00am-10:00am	Lecture : Fundamental concepts of statistical experimental design	Case study 2: Refining targets with DIA data in Skyline. Highlights: Preparing targets for a DIA experiment with many replicates
10:00am-10:30am	break	break
10:30am-12:00pm	Case study 1 : Two-group group comparison design; SRM data in Skyline. Highlights: (1) Importance of experimental design, distinction between bio and tech replicates, and randomization. (2) Data exploration with chromatograms, signal interference, types of missing values). (3) Normalization with global standards.	Case study 2: Multi-group comparison design with many bio replicates; DIA data in Skyline. Highlights: (1) Batch analysis of subsets of replicates. (2) Use of controls. (3) Between- and within-batch normalization. (3) Types of missing values. (4) Filtering with respect to quality of quantitative signals
12:00pm-1:00pm	Lunch	Lunch
1pm-2pm	Case study 1, continued.	Case study 2, continued.
2pm-2:30pm	break	break
2:30pm-3:15pm	Lecture : Statistical inference, multiple testing, MSstats package	Case study 2 : Statistical inference for multi-group experimental design in MSstatsShiny. Highlights: (1) Annotations of unbalanced experimental design with multiple groups and age confounder. (2) Impact of adjustment for age on statistical conclusions. (3) Impact of various choices of normalization, filtering, and handling missing values on statistical conclusions.
3:15 - 4 pm	Case study 1 : Statistical inference for two-group comparison designs in MSstatsShiny. Highlights: (1) Annotations of experimental design in experiment with bio and tech reps. (2) Use and interpretation of statistical models. (3) Visualization of the results.	
Extra time	Extra practice and Q&A: Skyline and MSstats based on participants' feedback	Extra practice and Q&A: Skyline and MSstats based on participants' feedback

Brendan
Olga
Devon
Deanna

Targeted proteomics – various approaches



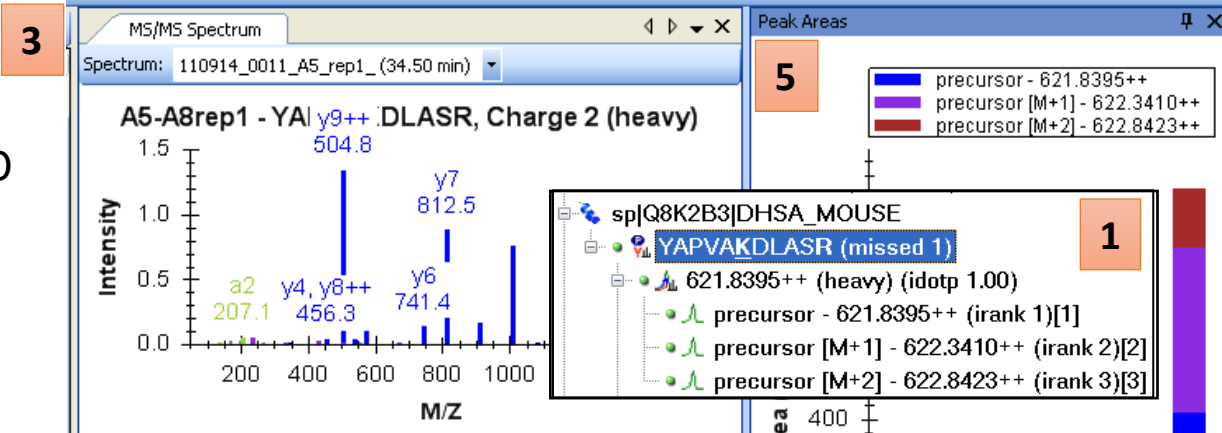
Discovery to Targeted with Skyline



Got HYPOTHESIS!!

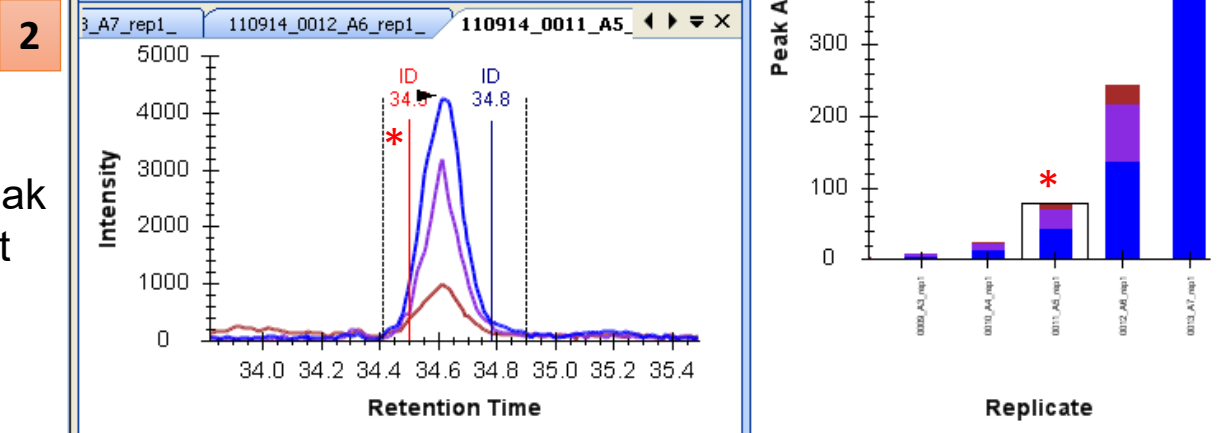
Skyline interface for MS1 Filtering data

3) MS/MS spectra and ID



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

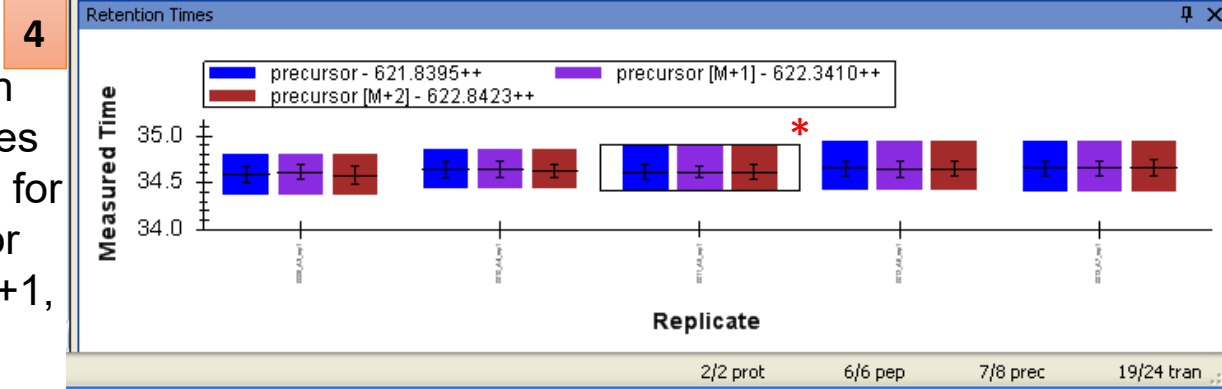
2) RT and ID correlation; peak boundaries set for integration



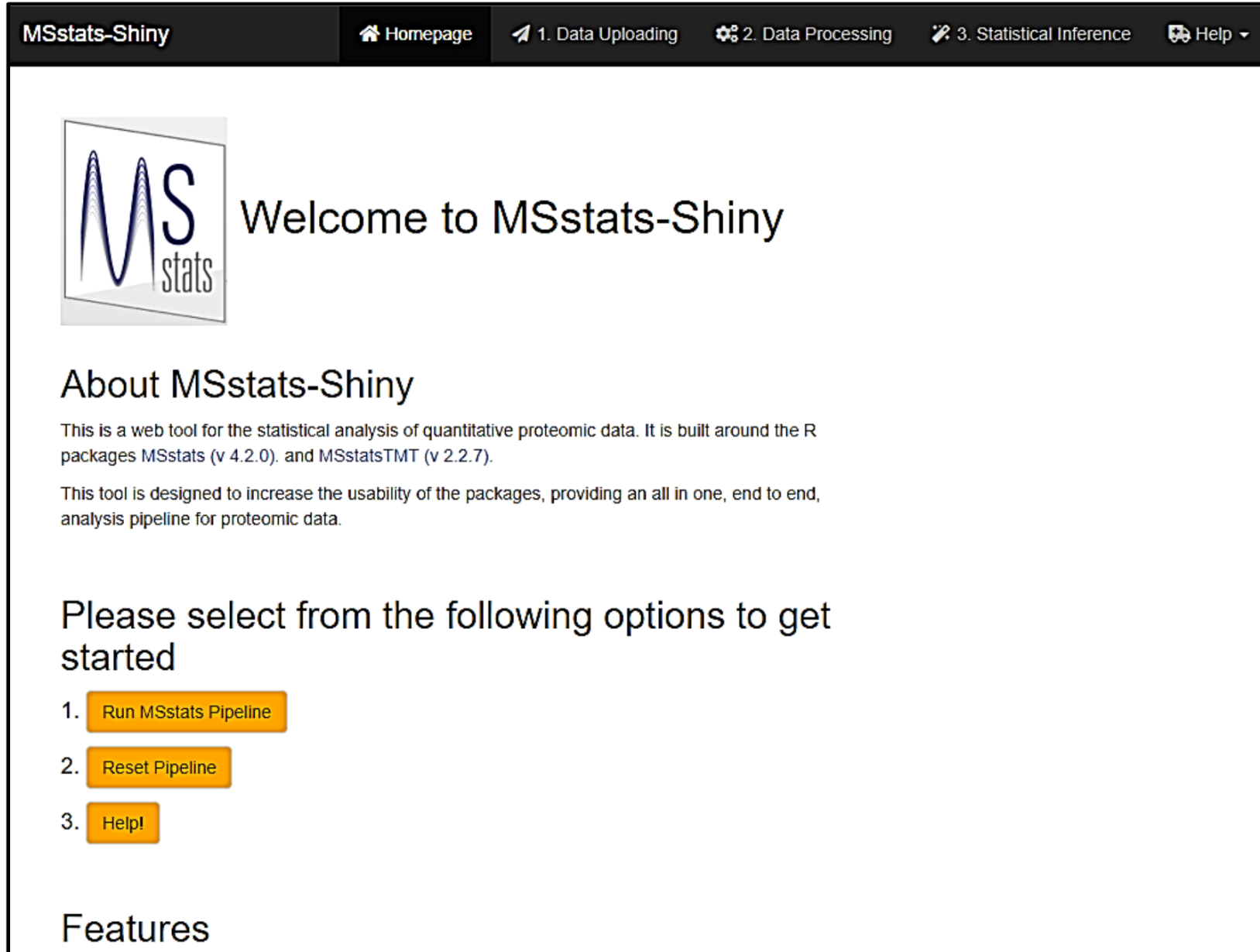
1) Peptide 'tree' with precursors

- irank
- idotp

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



Statistical analysis with MSstatsShiny



The screenshot shows the MSstats-Shiny web application interface. At the top, there is a navigation bar with the following items: "MSstats-Shiny", "Homepage", "1. Data Uploading", "2. Data Processing", "3. Statistical Inference", and "Help". Below the navigation bar, there is a logo for MSstats (a stylized 'MS' with 'stats' underneath) and the text "Welcome to MSstats-Shiny".

About MSstats-Shiny

This is a web tool for the statistical analysis of quantitative proteomic data. It is built around the R packages MSstats (v 4.2.0) and MSstatsTMT (v 2.2.7).

This tool is designed to increase the usability of the packages, providing an all in one, end to end, analysis pipeline for proteomic data.

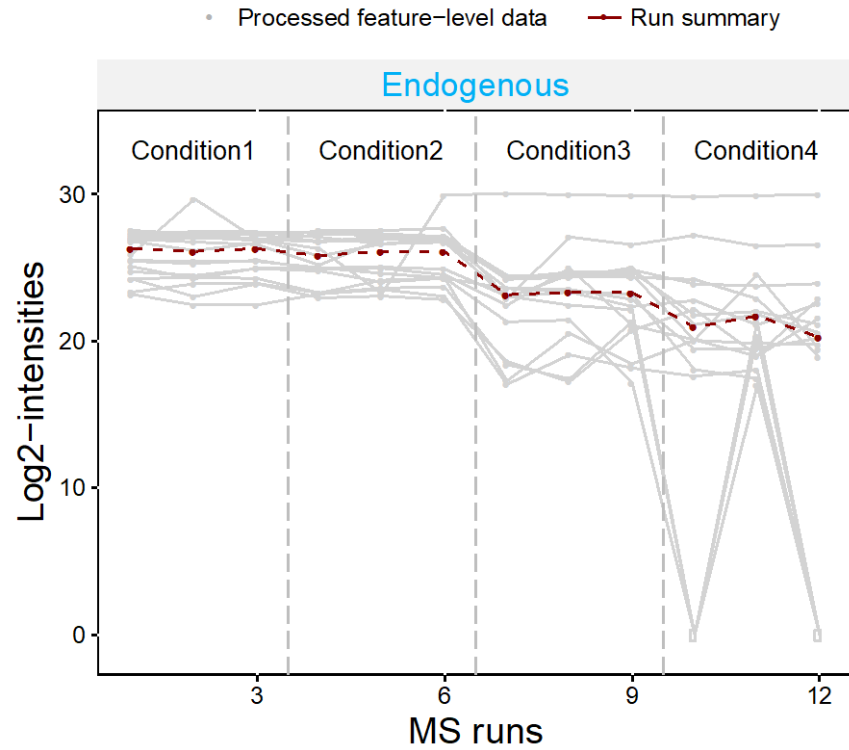
Please select from the following options to get started

1. [Run MSstats Pipeline](#)
2. [Reset Pipeline](#)
3. [Help!](#)

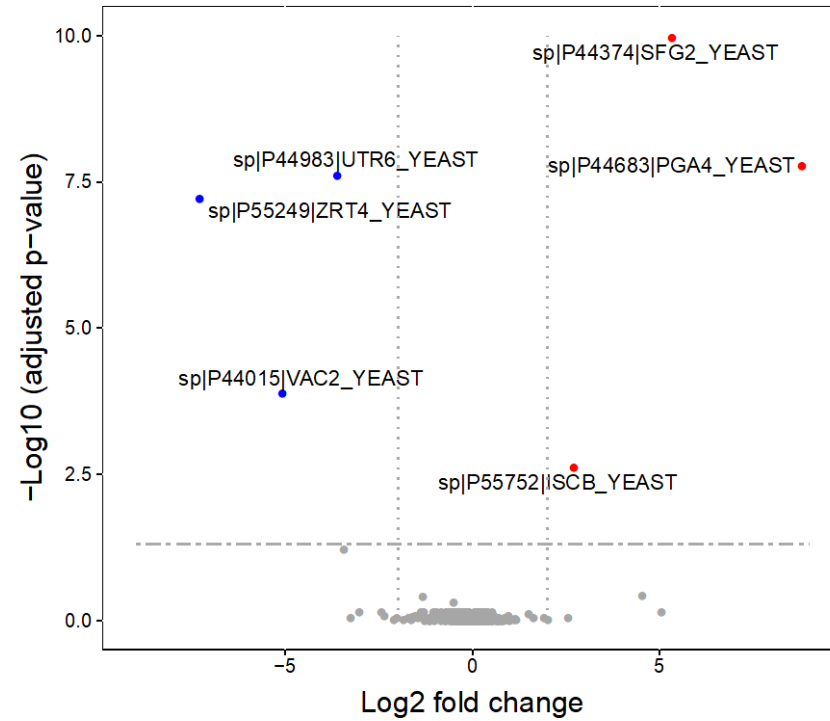
Features

Differentially abundant proteins across conditions

sp|P44015|VAC2_YEAST



C4-C2



sp|P44015|VAC2_YEAST

