

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

Instructors



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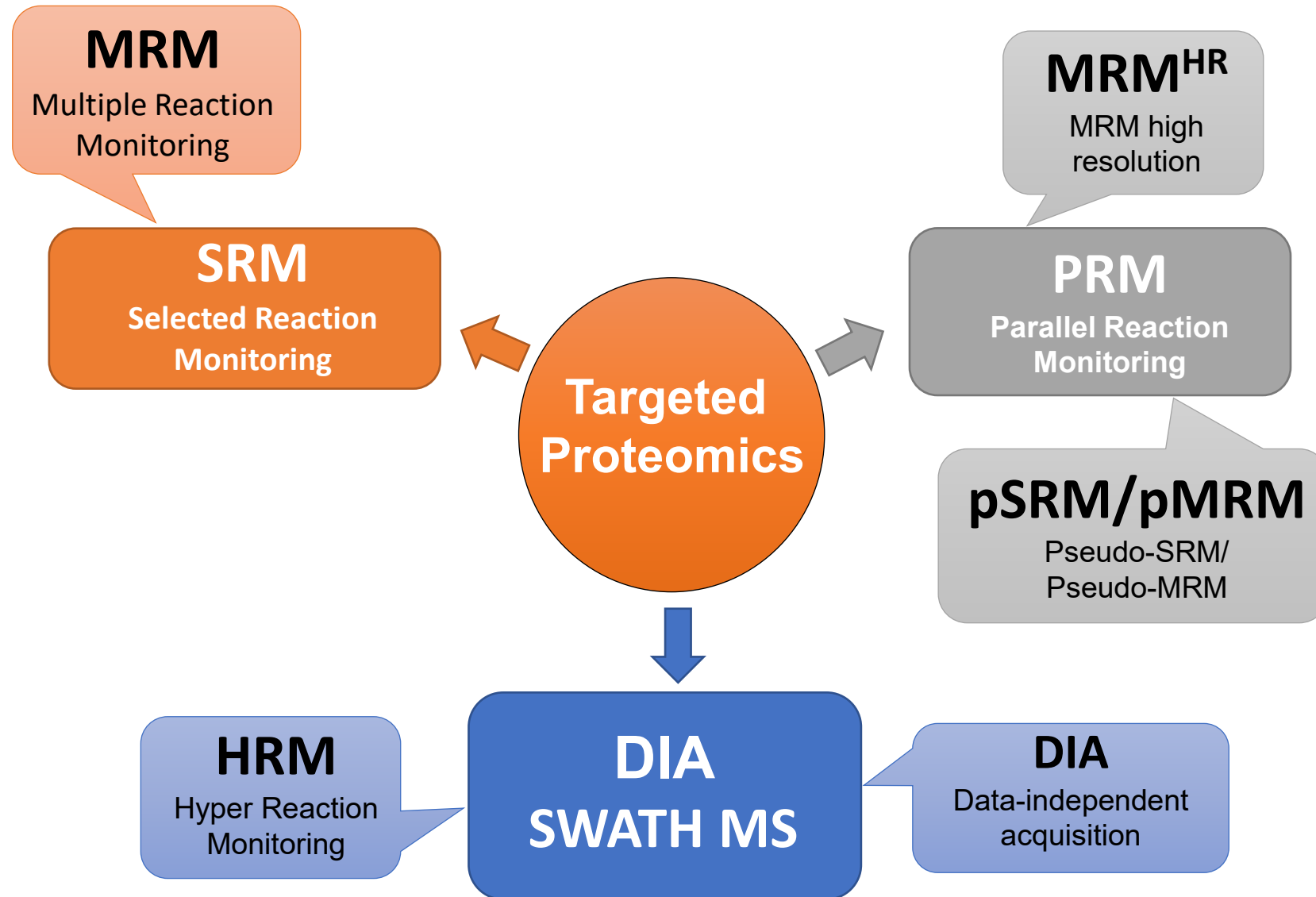


Olga Vitek
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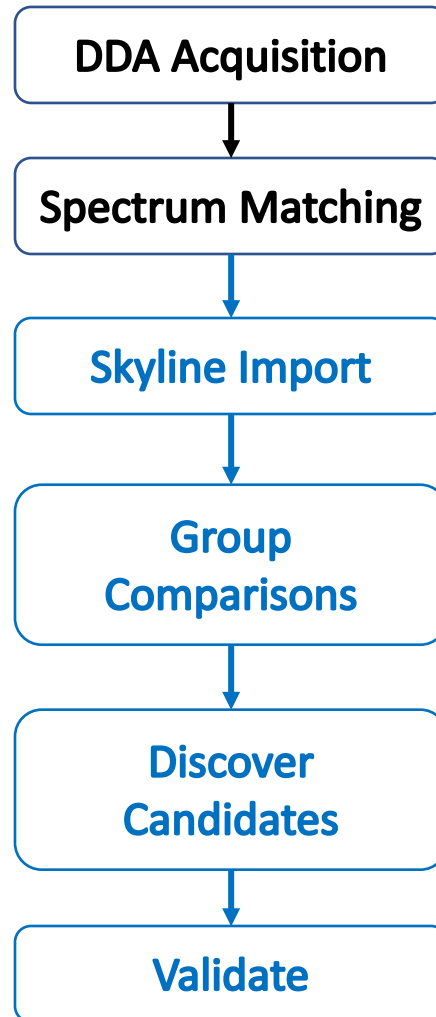
| | Day1: 05/31/25 - Introduction | Day2: 06/01/25 - Advanced |
|-----------------|---|---|
| Time | | |
| 9:00am-10:00am | Lecture : Fundamental concepts of statistical experimental design | Case study 2: Refining targets with DIA data in Skyline. <i>Highlights:</i> Preparing targets for a DIA experiment 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. <i>Highlights:</i> (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. <i>Highlights:</i> (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. <i>Highlights:</i> (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. <i>Highlights:</i> (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



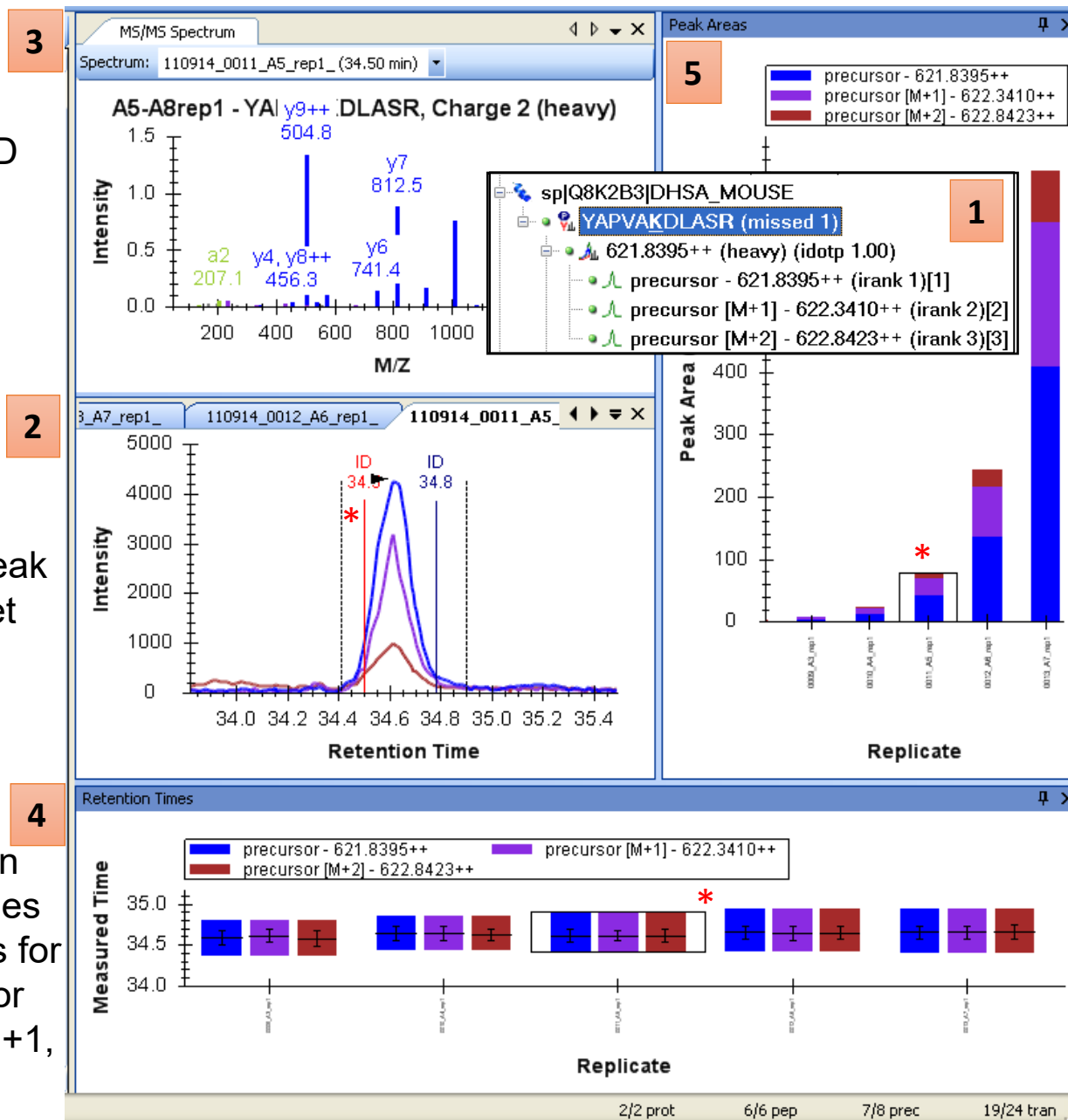
Discovery to Targeted with Skyline



Got HYPOTHESIS!!

Skyline interface for MS1 Filtering data

3) MS/MS spectra and ID



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 with MSstatsShiny

MSstats-Shiny [Homepage](#) [1. Data Uploading](#) [2. Data Processing](#) [3. Statistical Inference](#) [Help](#)



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.

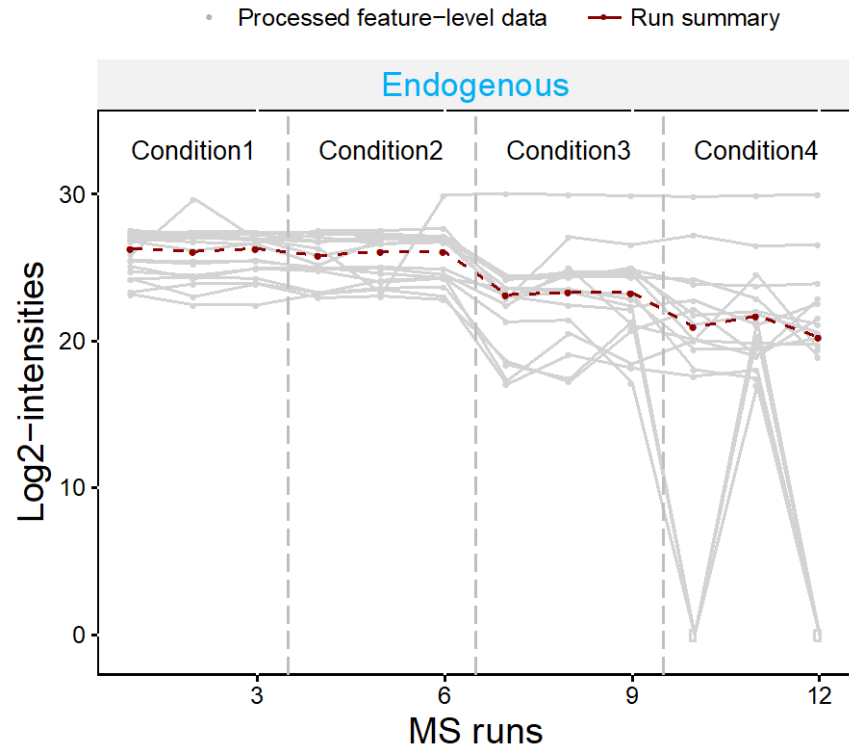
Please select from the following options to get started

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

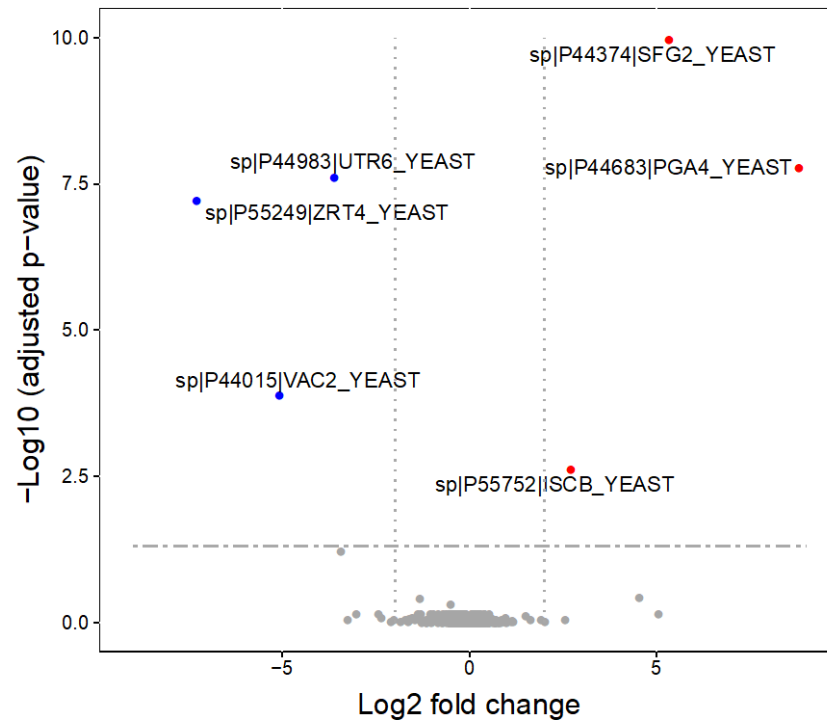
Features

Differentially abundant proteins across conditions

sp|P44015|VAC2_YEAST



C4-C2



sp|P44015|VAC2_YEAST

