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

Instructors

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Targeted proteomics – various approaches

- **MRM** Multiple Reaction Monitoring
- **SRM** Selected Reaction Monitoring
- **DIA** Data-independent acquisition
- **HRM** Hyper Reaction Monitoring
- **SWATH MS**
- **PRM** Parallel Reaction Monitoring
- **pSRM/pMRM** Pseudo-SRM/ Pseudo-MRM
- **MRM^HR** MRM high resolution
Discovery to Targeted with Skyline

DDA Acquisition
\[\rightarrow\]
Spectrum Matching
\[\rightarrow\]
Skyline Import
\[\rightarrow\]
Group Comparisons
\[\rightarrow\]
Discover Candidates
\[\rightarrow\]
Validate

Got HYPOTHESIS!!
Skyline interface for MS1 Filtering data

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

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

3) MS/MS spectra and ID

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
Statistical analysis by MSstats in R
Differentially abundant proteins across conditions

sp|P44015|VAC2_YEAST

Endogenous

Condition 1  Condition 2  Condition 3  Condition 4

MS runs

Log2-intensities

-Log10 (adjusted p-value)

C4–C2

sp|P44015|VAC2_YEAST

sp|P44983|UTR6_YEAST

sp|P55249|ZRT4_YEAST

sp|P44683|PGA4_YEAST

sp|P44015|VAC2_YEAST

sp|P55752|SGC6_YEAST

Log2-fold change

Log2-Fold Change

Comparison

C2–C1  C3–C1  C3–C2  C4–C1  C4–C2  C4–C3