

Summary

Aim: Demonstrate the utility of leveraging Protein Metrics Protein Centric Analysis with GraphPad Prism. Allowing for robust statistical analysis and complex visualizations.

This workflow will help scientists in every phase of biopharmaceutical discovery and development from early cell line development to final drug purity verification.

This workflow produces auto-curated analyses for quantification of thousands of proteins from sample replicates, quickly processing gigabytes of input data.

The Byosphere® cloud platform rapidly compares data across samples, projects, and geographic sites leveraging Deep Query and Dashboard capabilities in a GxP compliance-ready environment.

Introduction

The Top-N method is a widely used strategy in label-free quantitative proteomics for determining protein abundance. Since its introduction, the Top-N method has been extended to various applications, including relative quantification in comparative proteomics and biomarker discovery. Notably, Top-N is extensively utilized for analyzing host cell proteins in therapeutic protein development.

Protein Metrics has introduced a tailored software solution for the Top-N method, named Multi-Protein Quantitation (MPQ). This workflow streamlines the application of the Top-N method, enhancing its utility and efficiency. Here, we demonstrate the capability of this workflow using samples from a study investigating heart proteome changes in a diet-induced obesity model. The visualization of the results is performed using GraphPad Prism.

Methods

The previously published tryptic peptide datasets (*Vileigas et al.*) were acquired using a Q-Exactive HF Orbitrap mass spectrometer (Thermo Fisher Scientific) coupled to a Dionex Ultimate 3000 RSLC nano-liquid chromatography (Thermo Fisher Scientific). The data was downloaded from the public repository and analyzed using the Multi-protein Quantitation workflow (Protein Metrics, LLC).

The search allowed for trypsin cleavage sites and up to 2 missed cleavages. A match between runs was automatically preformed on the search results and the results were then filtered to the top 3 peptides of each protein. The results were then manually validated and exported to GraphPad Prism for visualization.

Data Processing

Automated Data Filtering

- Rapid analysis that immediately shows you the relative abundance of a complex mixture of proteins
- Match Between Runs
- Automatic PSM filtering of length and score
- Top-N Specification
- Homology Controls
- Dynamic Filtering allows analyst control over peptide quantitation during and after project creation

PSM filtering

Minimum peptide length	6
Maximum peptide length	32
Maximum missed cleavage count	2
Minimum peptide score	80.00
Minimum peptide replicate count	1
Keep homologous sequences	✓ Yes
Minimum peptide matching a protein count	2
Include modifications	None

Match between runs

Enable match between runs	✓ Yes
Mode	Between all samples
Enable sample-to-sample XIC alignment	✓ Yes

Reported peptide filtering

Retain only primary MS2 peptide ID	✓ Yes
Use only top charge state for quantification	✓ Yes
Use the same top N peptides for quantification	✓ Yes
Top N count	3
Peptide prioritization	Unique prioritized over Shared
Apply RSD limit for replicates	— No
Maximum acceptable %RSD for replicates	30.00

Protein Centric View

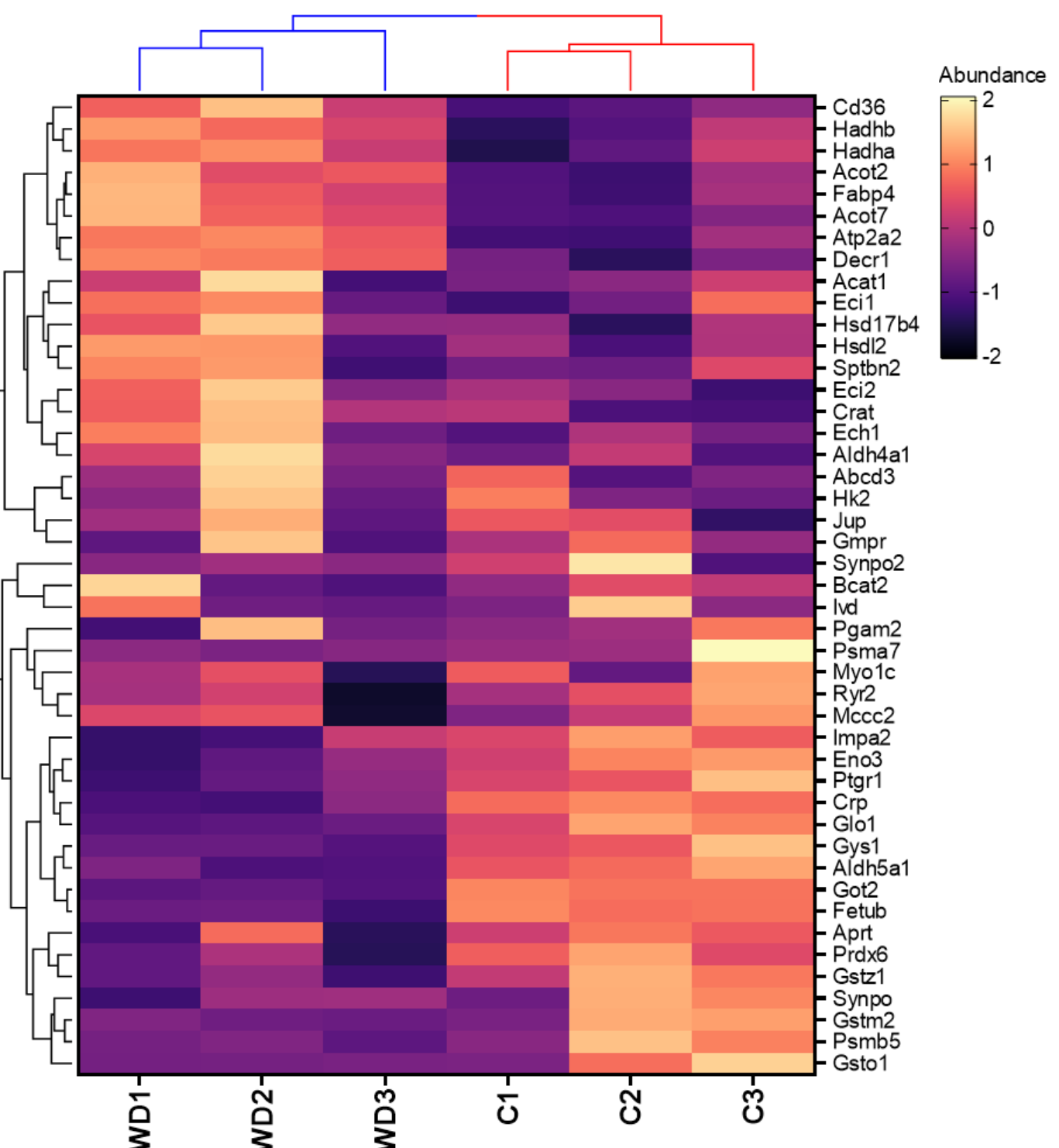
- Detailed inspection view
- Post Processing peptide filtering
- XIC Adjustments
- Single protein selection with a single click

Automated Reporting

- Automatically generated Top-N Report
- Built in statistical analysis
- Easily exportable CSV for Prism visualizations

		Condition	AssayMAP-10ug					
		Replicates	1	2	3	[Avg]	[RSD]	[StdDev]
		MS Alias name	AssayMAP-10ug-001 (%)	AssayMAP-10ug-002 (%)	AssayMAP-10ug-003 (%)	value (%)	value (%)	value (%)
Protein name	Protein Accession							
Brown Choline (Anti-BL-8 mAb)	Brown Choline (Anti-BL-8 mAb)		100.00	100.00	100.00	100.00	0.00	0.00
Light Choline (Anti-BL-8 mAb)	Light Choline (Anti-BL-8 mAb)		93.11	93.91	94.77	93.93	1.28	1.84
ipP006630B2_BOVIN Glutamate dehydrogenase 1, mitochondrial OX-Bio source (NCBI) (P01151)		P00666	0.42	0.47	0.50	0.46	0.28	0.04
ipP00021_CAB1_BOVIN Carbamate sulfotransferase 2 OX-Bio source GS-CAL PE4 SV-2		P00021	0.46	0.51	0.52	0.50	0.40	0.03
ipP00633AAL_BOVIN Alpha-51-casein OX-Bio source GS-CAL PE4 SV-2		P00662	1.02	1.03	0.99	1.02	0.07	0.03
ipP00633AAL_BOVIN Alpha-52-casein OX-Bio source GS-CAL PE4 SV-2		P00663	0.17	0.18	0.20	0.18	0.09	0.02
ipP0276-LACB_BOVIN Beta-tropomyosin OX-Bio source GS-CAL PE4 SV-2		P02764	1.13	1.28	1.15	1.19	0.33	0.06
ipP0276ALBU_BOVIN Serum albumin OX-Bio source GS-CAL PE4 SV-2		P02769	1.71	1.96	1.92	1.87	0.10	0.13
ipP04380FBL_BOVIN Fibrinogen heavy chain OX-Bio source GS-CAL PE4 SV-2		P04389	0.09	0.10	0.10	0.09	0.09	0.01
ipP00022_FBL_BOVIN Latiperoxidase OX-Bio source GS-CAL PE4 SV-2		P00022	0.33	0.36	0.42	0.37	0.44	0.04
ipP00022_FBL_BOVIN Latiperoxidase OX-Bio source GS-CAL PE4 SV-2		P00022	0.07	0.08	0.08	0.08	0.44	0.01

Conclusion



- Clear identification of diet-induced proteomic changes, with clustering separating the two diet groups.
- Discovery of co-regulated protein clusters, highlighting potential pathway-level dependencies for further investigation.

Byos provides robust, streamlined workflows for efficient data processing, and GraphPad Prism complements these by offering comprehensive visualization and clustering capabilities. Together, they form a powerful toolkit for advanced proteomic studies in both academic and industrial settings.

Conflict of Interest Statement

The authors are employees and stake holders of Protein Metrics LLC

References

Vileigas, Danielle F et al. "Landscape of heart proteome changes in a diet-induced obesity model." *Scientific reports* vol. 9,1 18050. 2 Dec. 2019, doi:10.1038/s41598-019-54522-2