Protein Metrics

Streamlined High-Confidence Plasma Proteomics Reveals Condition-Specific Apolipoprotein E and Fibrinogen Dynamics

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Summary

Novel: Protein Metrics' Multi-Protein Quantitation workflow enables streamlined, automated discovery of condition-specific Apolipoprotein E and fibrinogen isoform dynamics in Seer-enriched plasma proteomics with exceptional efficiency and reproducibility.

Applied to Seer Proteograph XT-enriched plasma (~4,500 proteins detected), the Protein Metrics Multi-Protein Quantitation workflow delivered robust label-free quantification using Top-3 peptide LFQ, with unique peptides prioritized to resolve biologically distinct isoforms across NPA and NPB fractions.

Stringent automated filtering within the Protein Metrics workflow — requiring ≥3 supporting peptides per protein and detection across ≥3/5 replicates — combined with %RSD variability controls ensured high-confidence, reproducible quantification with minimal manual intervention.

Interactive visualizations generated through the Protein Metrics Multi-Protein Quantitation workflow rapidly revealed pronounced differential patterns, including consistent ApoE upregulation in NPA and fibrinogen isoform elevation in NPB, demonstrating a clear path from raw plasma data to actionable biological insight.

Introduction

Vendor-Neutral, Application-Specific Workflows Identify and Quantify Thousands of Proteins from Challenging Plasma Proteomic Data:

Multi-Protein Quantitation

Identification and Quantification of Protein Mixtures

Multi-Protein Quan-DIA

Identification and Quantification of Protein Mixtures from DIA data

Multi-Protein Identification

Proteomics ID, characterization and annotation of PTMs

Multi-Protein Preview

Preview of MS2 Data Quality and Search Parameters

Enhanced Performance, Time Saving Solutions:

- **Multi-Protein Quantitation:**
  - Rapid analysis that immediately shows you the relative abundance of a complex mixture of proteins
  - Label-free Quantitative analysis of thousands of proteins at a time
  - Protein-centric analysis
  - Dynamic Filtering allows analyst control over peptide quantitation during and after project creation
- **Multi-Protein Preview to automatically assess sample status:**
  - Mass spectrometer calibration/performance
  - Enzymatic digestion specificity
  - Alkylation efficiency
  - Sample quality/unexpected artifacts
- **Multi-Protein search and identification of thousands of proteins:**
  - Protein-centric layout
  - Focused review
  - Quicker result verification
  - Built by the same team that created the ground-breaking Byonic algorithm
- **MPQ-DIA**
  - New Multi-Protein Quantitation-DIA workflow now available
  - Improved reproducibility and deeper proteome coverage
  - Less prone to missing low-abundance peptides than DDA

Identification and Quantitation Dynamic Inspection

The Protein Abundance Stacked XIC Bar Charts Quickly Identify the Differentially Expressed Proteins:

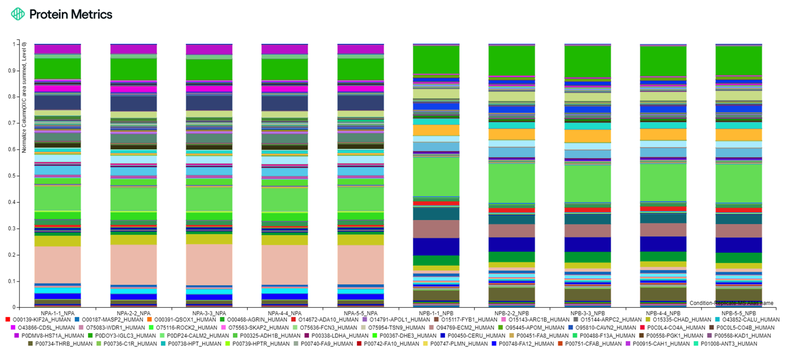

Figure 1 (above).

Protein Metrics Multi-Protein Quantitation workflow stacked XIC bar chart displaying peptide-level relative protein abundance across five NPA and five NPB replicate samples. Each colored band represents an individual peptide contribution to total protein quantification, enabling rapid visual assessment of both isoform-level composition and cross-replicate consistency.

The high degree of visual similarity within NPA replicates (samples 1–5) and within NPB replicates (samples 6–10) demonstrates the exceptional reproducibility achieved through automated %RSD filtering and match-between-runs integration.

Condition-specific differences in band composition between NPA and NPB fractions, including the pronounced presence of Apolipoprotein E peptides in NPA and fibrinogen isoform peptides in NPB are immediately apparent, illustrating how the Protein Metrics interactive visualization tools translate complex quantitative data into clear, actionable biological insights with minimal manual effort.

Heatmaps Showcase Condition-Specific Differential Expression Patterns

Protein Metrics Multi-Protein Quantitation workflow peptide-level relative abundance heatmaps showingthe two most prominentcondition-specific differential expression patterns between NPA and NPB enrichment fractions.

		Condition	NPA							NPB							
		Replicate	1	2	3	4	5	[Avg]	[RSD]	[StdDev]	1	2	3	4	5	[Avg]	[RSD]	[StdDev]
		MS Alias name	1_NPA (%)	2_NPA (%)	3_NPA (%)	4_NPA (%)	5_NPA (%)	value (%)	value (%)	value (%)	1_NPB (%)	2_NPB (%)	3_NPB (%)	4_NPB (%)	5_NPB (%)	value (%)	value (%)	value (%)
Protein Accession	Protein Entry Name	Sequence (unformatted)																
P02649	APOE_HUMAN	ELAQAQAR	93.59	86.34	90.88	93.27	100.00	92.22	6.41	5.85	7.07	7.41	7.42	7.90	7.22	7.40	4.21	0.31
		LAVYQAGAR	77.12	85.54	89.67	93.15	89.24	86.94	7.04	6.12	5.57	5.60	5.50	5.28	5.38	5.46	2.48	0.14
		LGPLVEQGR	100.00	100.00	100.00	100.00	95.40	99.08	2.08	2.06	5.95	5.99	6.78	6.11	5.94	6.15	5.82	0.36

Figure 2a (above).

Apolipoprotein E was the most highly expressed protein across all five NPA replicates (blue), with uniform downregulation across all NPB replicates: a robust fraction-specific finding confirmed by Protein Metrics automated %RSD filtering.

		Condition	NPA							NPB							
		Replicate	1	2	3	4	5	[Avg]	[RSD]	[StdDev]	1	2	3	4	5	[Avg]	[RSD]	[StdDev]
		MS Alias name	1_NPA (%)	2_NPA (%)	3_NPA (%)	4_NPA (%)	5_NPA (%)	value (%)	value (%)	value (%)	1_NPB (%)	2_NPB (%)	3_NPB (%)	4_NPB (%)	5_NPB (%)	value (%)	value (%)	value (%)
Protein Accession	Protein Entry Name	Sequence (unformatted)																
		AQAVQGDLEK	0.64	0.70	0.80	0.77	0.70	0.72	9.00	0.06	0.02	0.01	0.02	0.03	0.02	0.02	19.29	0.00
P35858	MOES_HUMAN	ESEAWVWQQK	0.54	0.53	0.51	0.59	0.58	0.55	6.18	0.03	0.01	0.01	0.01	0.02	0.01	0.01	13.38	0.00
		TANDMIHAENMR	0.66	0.67	0.65	0.76	0.62	0.67	7.54	0.05	0.03	0.05	0.05	0.06	0.04	0.04	26.77	0.01
		ECEEHR	1.74	1.65	1.47	1.68	1.84	1.68	8.15	0.14	33.19	21.99	26.37	23.53	21.80	25.38	18.66	4.73
P02675	FIBB_HUMAN	QGFGNVATNTDGK	1.76	1.35	1.18	1.32	1.54	1.43	15.69	0.22	35.72	23.92	20.70	24.76	21.53	25.32	23.87	6.04
		VQSVNK	2.55	2.20	2.04	2.12	2.52	2.29	10.25	0.23	44.29	32.33	32.73	33.61	31.29	34.85	15.33	5.34
		APIAAYR	0.79	0.84	0.80	0.85	0.86	0.83	3.71	0.03	0.05	0.04	0.05	0.05	0.05	0.05	7.47	0.00
P14618	KPYM_HUMAN	GDVPLEAVR	0.35	0.38	0.30	0.29	0.35	0.31	10.10	0.03	0.27	0.22	0.44	0.25	0.27	0.29	28.58	0.09
		GSGTAEVELK	0.52	0.53	0.52	0.55	0.51	0.53	2.30	0.01	0.04	0.05	0.05	0.05	0.05	0.05	11.53	0.01
		GSGDSQVTQVSPQR	0.62	0.56	0.55	0.63	0.63	0.60	6.64	0.04	0.09	0.10	0.11	0.11	0.10	0.10	10.53	0.01
P05106	ITB3_HUMAN	NEDDCVYR	0.89	0.92	0.83	0.87	0.86	0.88	4.02	0.04	0.11	0.13	0.20	0.16	0.15	0.15	23.22	0.03
		VLEDRPLSDK	1.55	1.38	1.50	1.35	1.43	1.44	5.67	0.08	0.22	0.24	0.23	0.23	0.19	0.22	8.32	0.02
		AIQELYNPDESSK	1.07	0.78	0.77	0.79	1.07	0.90	17.61	0.16	23.36	15.48	17.81	17.59	15.11	17.87	18.48	3.30
P02679	FIBG_HUMAN	RLDGNVDFK	1.70	1.29	1.31	1.40	1.71	1.48	14.22	0.21	23.89	17.38	18.13	17.35	17.20	18.79	15.30	2.88
		YEAHLTHDDSSR	1.63	1.11	1.13	1.13	1.62	1.33	20.40	0.27	28.06	18.84	21.15	22.05	17.00	21.42	19.63	4.21

Figure 2b (above).

Fibrinogen isoforms (alpha, beta, and gamma chains) showed consistently elevated peptide abundance across NPB replicates relative to NPA. Concordance across multiple isoforms and supporting peptides reflects the isoform-resolving power of Protein Metrics Top-3 unique peptide LFQ quantification.

Together, these heatmaps demonstrate how the Protein Metrics workflow rapidly surfaces clear, reproducible, condition-specific biology from Seer-enriched plasma proteomics data.

Acknowledgements

The authors wish to thank Lucas Calestini from Real Retina Analytics for his much appreciated help and advice.

Conflict of Interest Statement

Some of the authors are employees and/or shareholders of Protein Metrics, LLC, which has commercialized the software described here.

Citation of data used

Beimers WF, Overmyer KA, Sinitcyn P, Lancaster NM, Quarmby ST, Coon JJ. Technical evaluation of plasma proteomics technologies. J. Proteome Res. 2025, 24, 3074–3087. doi:10.1021/acs.jproteome.5c00221 · Data: PRIDE PXD060573