PROTEOME DYNAMICS

Quantitative Phosphoproteomics

IQ PROTEOMICS

Phosphorylation is a key regulator of many cellular processes, and aberrant signaling in phosphorylation pathways is a hallmark of many diseases. Targeted enrichment of phosphorylation events, combined with multiplexed LC-MS, enables the quantification of thousands of unique phosphosites. IQ Proteomics offers unbiased, phosphosite-specific, quantitative mapping of proteome-wide cellular signaling dynamics.

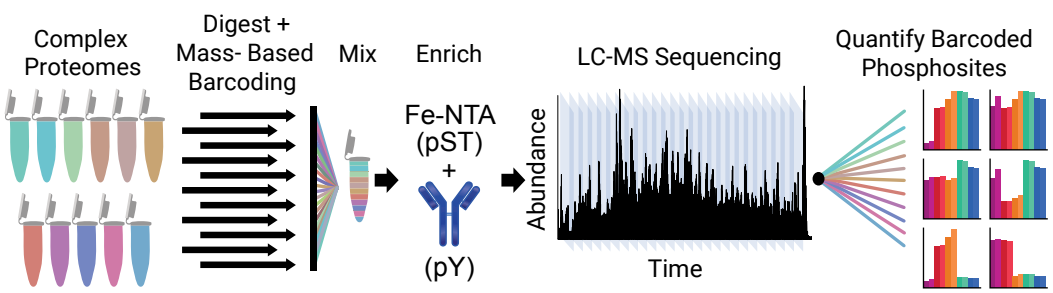
Features

- Thousands of Phosphosites Quantified
- High Affinity Enrichment:
  - Fe-NTA (pST)
  - PTMScan® Technology (pY)
- High Throughput Proteomics:
  - TMT™ 11-Plex Technology
  - Orbitrap Lumos (SPS-MS3)

Applications

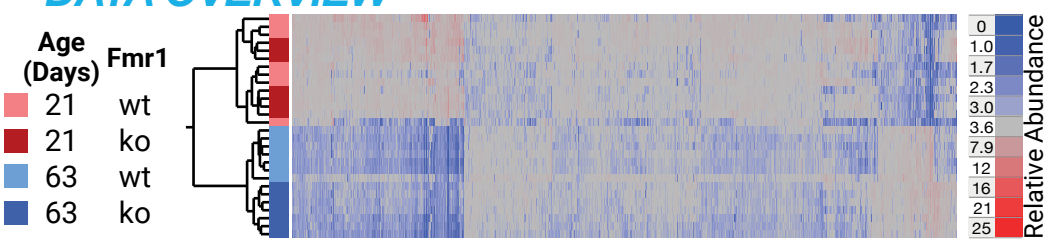
- Kinase Inhibitor Profiling
  - Selectivity Screening
  - Dose/Time-Response
- Pathway Dissection
- Disease Driver Discovery
- Cell Lines/Tissues

ASSAY OVERVIEW



Protein extracts from up to 11 samples are digested and labeled with isobaric reagents for mass-based barcoding. Barcoded samples are mixed, and enriched for phosphopeptides (pST: Fe-NTA, pY: Anti-pY). The phosphopeptide mixture is analyzed by LC-MS using an SPS-MS3 method on an Orbitrap Fusion Lumos mass spectrometer to ensure maximum sensitivity and quantitative accuracy.

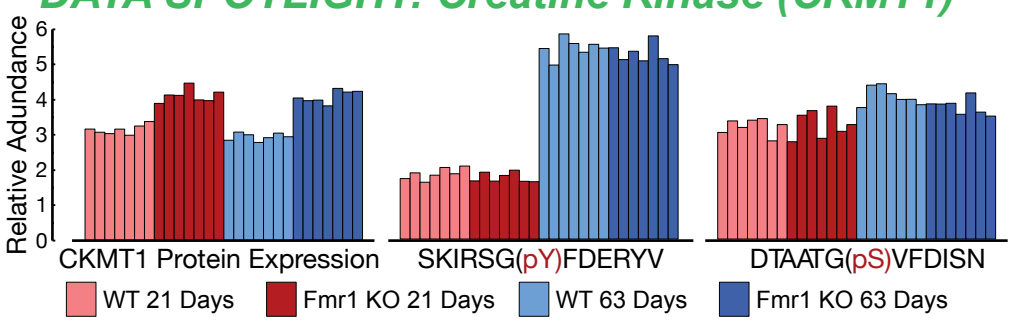
DATA OVERVIEW



Unique Phosphosites Quantified	Plex-1			Plex-2			Plex-3			Total		
	pS	pT	pY	pS	pT	pY	pS	pT	pY	pS	pT	pY
	10741	1272	367	8619	1039	319	11090	1262	434	14612	1777	657

The phosphoproteomes from brain cortex of wild type and Fmr1 (Fragile X Mental Retardation Protein - 1) knockout mice were quantitatively compared across young (21 Day) and old (63 days) cohorts. Quantification of 7 replicates for each condition (28 total) was achieved by arraying the samples across three multiplexed LC-MS experiments, with an identical reference sample included within each plex. Distinct phosphoproteome signatures for both age and strain are readily apparent.

DATA SPOTLIGHT: Creatine Kinase (CKMT1)



Evaluation of individual phosphosites within Creatine Kinase (CKMT1) reveals differential regulation of Tyr154. CKMT1 protein expression is dictated exclusively by strain (Left), while tyrosine phosphorylation is differentially regulated by age (Center). Notably, a distinct CKMT1 phosphosite (S366) does not exhibit differential signaling by either age or strain (Right).

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