Data Independent Acquisition DIA & Library Strategies — Hands-on

Learn how to design, acquire and analyze data independent acquisition (DIA) proteomics experiments. You will configure DIA window schemes, build and curate spectral or chromatogram libraries, run library based and library free DIA pipelines, and implement robust QC and quantitation strategies suitable for high coverage discovery and longitudinal studies.

Data Independent Acquisition DIA & Library Strategies

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Session Index

Session 1

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DIA Concepts & Acquisition Design

DIA vs DDA and targeted acquisition

concept of multiplexed MS2 **coverage vs specificity** **common DIA use cases**

Window schemes and mass ranges

fixed vs variable windows **m/z coverage planning** **cycle time and points across peak**

Instrument and LC settings for DIA

resolution and AGC targets **HCD energy ranges** **gradient length vs throughput**

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Spectral / Chromatogram Library Generation

Building spectral libraries from DDA

high quality DDA sets **FDR controlled IDs** **export to library formats**

Library formats and content curation

.blib / .tsv / TraML concepts **peptide uniqueness and PTMs** **RT normalization with iRT**

External resources and chromatogram libraries

using public libraries **pan human and tissue specific sets** **chromatogram libraries overview**

Session 3
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Library Free DIA & Quant Workflows

Overview of DIA analysis toolchains

Skyline, DIA NN and related tools **pipeline inputs and outputs** **computational requirements**

Library based vs library free DIA

pseudo library generation **direct DIA identification ideas** **FDR handling in DIA context**

Quant tables and data structures for DIA

precursor and peptide level matrices **protein**

inference approaches export for downstream statistics

Session 4

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QC, Normalization & Biological Readouts

DIA specific QC and performance tracking

ID rates and peak counts RT stability and window utilization reference sample monitoring

Normalization and batch handling in DIA data

global and reference based scaling use of pooled QCs batch correction ideas

From DIA quant to biology

differential abundance analysis pathway and network level views figures and tables for manuscripts