Data-Independent Acquisition (DIA) and Spectral Libraries — Hands-on

Learn how to design and interpret data independent acquisition (DIA) proteomics workflows. This module focuses on DIA concepts, spectral library generation and curation, window schemes, DIA method planning, and library based data interpretation for deep and reproducible quantitative proteome profiling.

Data-Independent Acquisition (DIA) and Spectral Libraries

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Session Index

Session 1

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DIA Concepts and Rationale

DIA vs DDA: motivation and key differences

comprehensive MS MS sampling **improved reproducibility** **large cohort suitability**

Basic DIA acquisition principle (concept level)

isolation windows across m/z range **mixed fragment spectra** **cycle time thinking**

DIA use cases and study types

quantitative discovery | longitudinal and clinical cohorts | panel like quantitation without direct targeting

Session 2

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Spectral Libraries: Generation and Curation

What is a spectral library and why it matters in DIA

reference fragment patterns | link to peptide sequences | retention time and intensity profiles

DDA based library generation concepts

project specific libraries idea | fractionation to boost coverage | library quality filters (concepts)

Library formats and curation principles (overview)

precursor and fragment m/z fields | normalized retention times (iRT concept) | removal of low quality entries

Session 3

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DIA Acquisition Strategies and Window Schemes

Fixed, variable and overlapping window concepts

uniform window approaches | variable width based on precursor density | trade offs between coverage and clarity

DIA method planning basics (concept level)

m/z range selection | cycle time and number of windows | integration with LC gradient length

Library based vs library free DIA concepts (overview only)

using prior spectral libraries | direct deconvolution style approaches | choice based on project context

Session 4

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DIA Data Interpretation, QC and Mini Capstone

DIA signal extraction and scoring ideas

Theory plus planning style practical

QC and performance checks for DIA runs (concept level)

library coverage view | retention time and intensity stability | missing value patterns and drift

Mini capstone: design sheet for a small DIA experiment and library plan

window scheme sketch | library generation and or selection plan | QC and reporting checklist outline