

Isobaric Labeling — TMT, iTRAQ and SILAC — Hands-on

Learn how to design and execute multiplexed quantitative proteomics using isobaric tagging and metabolic labeling. This module focuses on TMT and iTRAQ chemistry, workflow design, channel setup, basic SILAC concepts, and acquisition considerations that enable robust relative protein quantitation across multiple conditions.

Isobaric Labeling — TMT, iTRAQ and SILAC

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Session 1

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Isobaric Labeling Concepts & Experimental Design

Why isobaric labeling for multiplexed proteomics

relative quant across many samples **batch compression** **comparison to label free**

TMT and iTRAQ tag principles (reporter, balance, reactive group)

isobaric mass in MS1 **reporter ions in MS2 or MS3** **lysine and N terminus labeling**

Experimental design and channel assignment strategy

biological groups vs tags **reference channel**

concepts **balancing loads across channels**

Session 2

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TMT / iTRAQ Chemistry, Sample Prep & Workflows

Labeling chemistry and reaction conditions (concepts)

amine reactive NHS esters **organic solvents and buffers** **pH and temperature ranges**

Peptide level labeling workflow (from digest to quench)

peptide desalting before labeling **tag reconstitution and addition** **quenching unreacted label**

Pooling, cleanup and fractionation options (overview)

mixing labeled channels **high pH fractionation idea** **storage and stability notes**

Session 3

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SILAC & Hybrid Quant Strategies (Concepts)

Stable isotope labeling by amino acids in cell culture (SILAC)

light, medium and heavy channels **label incorporation considerations** **mixing cultures before lysis**

Conceptual comparison: SILAC vs TMT / iTRAQ vs label free

advantages and limitations **instrument and sample constraints** **when to choose which approach**

Hybrid designs and reference channel ideas (conceptual)

TMT with bridge reference sample **linking multiple TMT sets** **integrating with LFQ cohorts**

Session 4

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Acquisition, Ratio Handling & Mini Capstone

Acquisition considerations for TMT / iTRAQ data (overview)

Theory + Practical (planning exercise)

Reporter ion based ratios and ratio compression concepts

co isolation and co fragmentation ideas **MS3 and SPS**
concepts (high level) **basic ratio QC thinking**

Mini capstone: design sheet for a simple TMT or iTRAQ experiment

channel layout and sample map **workflow and QC**
checklist **notes for downstream analysis team**