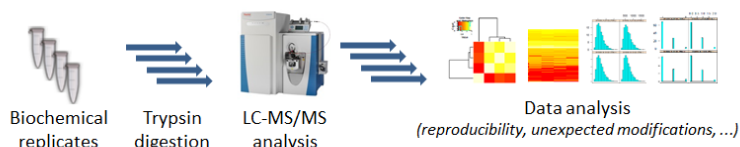# Info Sheet: **Label-free protein quantification**

General description:

The field of proteomics enables the identification and quantification of large numbers of proteins in a biological specimen, allowing to obtain key information about chemical or biologically induced perturbations and pinpoint pathways affected over time or across conditions. Multiple approaches can provide proteome-wide quantitative information, all with their benefits and caveats. Among them, label-free proteomics quantification (LFQ) became an established approach to relatively quantify proteins on large dataset in a rapid, reproducible, flexible and affordable manner.
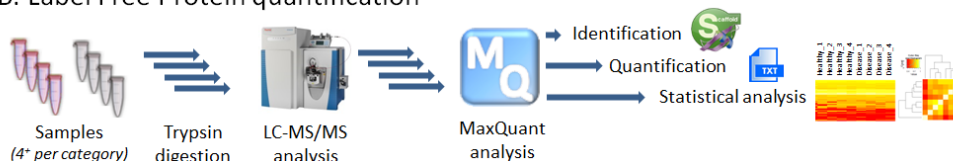
FGCZ offers a two-step experiment:
- Preliminary pilot (QC): 2-4 biochemical replicates, processed in parallel
- Main Label Free Quantification (LFQ)

A. Quality control step

Biochemical replicates → Trypsin digestion → LC-MS/MS analysis → Data analysis *(reproducibility, unexpected modifications, ...)*

B. Label Free Protein quantification

Samples *(4+ per category)* → Trypsin digestion → LC-MS/MS analysis → MaxQuant analysis → Identification / Quantification / Statistical analysis

The workflow consists of:
- Normalization of protein amounts (Qubit)
- Protein digestion (Lys C/Trypsin)
- Randomized LC-MS analysis of each samples
- Bioinformatics
    o Protein identification and quantification (e.g MaxQuant)
    o Visualization of identified protein and peptides (Scaffold)
    o Statistical analysis (t-test/ANOVA/Linear-Modelling)
    o Report (with plots)
    o (optional): pathway analyses, customized scripts
- Expected turnaround time (after physical arrival of samples):
    o Pilot (QC): 1-2 weeks
    o LFQ: 4-6 weeks

Requirements and considerations:
- First QC step is mandatory. Do not prepare the samples for the main LFQ until these results are available.
- Ideal protein amount: 25-50 µg (minimum 1 µg)
- Replicates: 4+ for in-vitro experiments, 5/10+ for animal/human experiments
- Buffer composition: every experiment requires some optimization, please contact us at proteomics@fgcz.ethz.ch