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Proteomics and Metabolomics

Metabolomics Service Guidelines

Metabolomics Service

Instrumental Analysis	Output
Semi Targeted	Quantitative analysis of requested specific metabolites
Untargeted	Quantitative analysis of **unspecific** metabolites
Stable Isotope Tracing	Quantitative analysis of the enrichment from a labeled metabolite in specific metabolic pathways
Analyzed Matrix	**Specifications**
Cells	Intracellular and extracellular components
Yeast	Intracellular components
Tissues	

Metabolomics Study Design

Data Analysis	Output
Raw data Matrix	Qualitative and quantitative data matrix with metabolite annotations and intensities (MultiQuant/Xcalibur)
Normalized Data Matrix	Metabolite intensities will be normalized (technical normalization and/or biological normalization according to the type of matrix)
Multivariate Statistical analysis	Normalized data are plotted as PCA (principal component analysis) for a general insight of the model (Metaboanalyst)
Univariate Statistical analysis	t-Test/ANOVA (or similar) are applied to the normalized data (statistical tests can be discussed according to the model) (Metaboanalyst)
Data graphs	Normalized data can be plotted as: • Box Plot/Bar Graphs • Unsupervised Heatmap • Volcano Plot
Stable Isotope tracing Data Analysis	Tracing data are corrected for the Natural abundance (R script) and expressed as: • Single isotopologue intensities • Fractional enrichment • Total pools

Sample labelling and submission

Samples should be shipped with dry ice and the user should send by email the sample submission form with the following information:

The shipping address is:
Laura Tronci
IFOM, Via Adamello 16 - 20139, Milan (MI) - Italy

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Proteomics and Metabolomics

Lipidomics Service Guidelines

▪ Lipidomics Service

Instrumental Analysis	Output
Shotgun Lipidomics Analysis* (NanoLCMS)	Quantitative and qualitative analysis of lipid species

**Method published:* *Cattaneo A, Martano G, Restuccia U, Tronci L, Bianchi M, Bachi A, Matafora V. Opti-nQL: An Optimized, Versatile and Sensitive Nano-LC Method for MS-Based Lipidomics Analysis. Metabolites. 2021 Oct 21;11(11):720. doi: 10.3390/metabo11110720. PMID: 34822378; PMCID: PMC8623082.*

▪ Lipidomics Study Design

Type of Matrix	Specifications
Type of Matrix	Sample type should be specified prior the analysis (ex. cells, tissue, biofluids…) Cell Compartments (mitochondria, nuclei, droplets…)
Number of replicates	Minimum 3 technical replicates per biological conditions
Sample preparation	Samples should be shipped as dried and clean pellets. The sample amount should be discussed (example: for cells a minimum of $1x10^6$cell pellet is required)

▪ Lipidomics Data Analysis

Data Analysis	Output
Raw data Matrix	Qualitative and quantitative data matrix with lipid species annotations and intensities *(**data analysis done with MSdial)*
Normalized Data Matrix	Lipid intensities will be normalized by sum
Multivariate Statistical analysis	Normalized data are plotted as PCA (principal component analysis) for a general insight of the model *(**https://www.metaboanalyst.ca/)*
Univariate Statistical analysis	t-Test/ANOVA (or similar) are applied to the normalized data (statistical tests can be discussed according to the model) *(**https://www.metaboanalyst.ca/)*
Data graphs	Normalized data can be plotted as: • Box Plot/Bar Graphs • Unsupervised Heatmap • Volcano Plot *(**https://www.metaboanalyst.ca/)*
Extra Data Analysis	Lipid classes and unsaturation levels analysis can be performed (to be discussed)

***Software:*
Raw data analysis with MS dial: http://prime.psc.riken.jp/compms/msdial/main.html
Post Data analysis with Metaboanalyst: https://www.metaboanalyst.ca/

▪ Sample labelling and submission

Samples should be shipped with dry ice and the user should send by email the sample submission form with the following information:

The shipping address is:
Angela Cattaneo or Laura Tronci
IFOM, Via Adamello 16 - 20139, Milan (MI) - Italy

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Proteomics and Metabolomics

Proteomics Service Guidelines

Proteomics Service

Instrumental Analysis	Output
Proteomics Untarget	Proteome Qualitative and Quantitative analysis (Label Free e SILAC) of simple and complex samples; phospho-proteome profiling.
Post-Translation Modification analysis	Qualitative PTMs analysis.
Protein Intact Molecular Weight	Determination of monoisotopic and average Mw of intact protein by applying a deconvolution algorithm to the MS spectra
Analyzed Matrix	**Specifications**
Immunoprecipitate	IP elutions loaded on gel SDS-PAGE or in solution.
Cells	Total cell lysate, secretome, matrisome, isolated organelles
Tissues	Homogenate tissue lysate

Proteomics Study Design

Information/Steps	Specifications
Type of Matrix	Sample type should be specified prior the analysis (e.g. cells, tissue, biofluids...). In the case of recombinant proteins or with mutations, the amino acid sequence is required to be entered in the Data Base. For proteins with unusual taxonomy, the Database with the amino acid sequences of proteins is required in .fasta format.
Number of replicate	Minimum 3 biological replicates per every condition.
Sample concentration	Samples concentration should be discussed according to the biological question of the study (es.: for global proteome profiling at least 25µg of proteins / sample).
Sample preparation	The protein extraction protocol depends on the sample proteome of interest and will be provided once the experiment has been discussed.

Proteomics Data Analysis

Data Analysis	Output
Raw data Matrix	Data matrix with proteins identified (Scaffold) and quantified (MaxQuant/Spectronaut/Perseus) with annotations and intensities. If required, output .raw files of the mass spectrometer can be provided.
Normalized Data Matrix	Protein intensities will be normalized (technical normalization and/or biological normalization according to the type of matrix).
Statistical analysis	t-test/ANOVA or similar are applied to the normalized data; statistical tests can be discussed according to the model (Perseus).
Data graphs	Data can be plotted as: - PCA - Box Plot/Grafici a barre - Heatmap - Volcano Plot - Scatter Plot

Sample labelling and submission

SDS-Page gels should be shipped at room temperature in container that maintain gel hydration.
In solution samples, in eppendorf tubes, should be shipped with dry ice.

For SDS-Page gels, the image with the annotation of the loaded samples and the marker ladder MW should be provided.
Samples in eppendorf tubes should be labelled in a simple way (es. with letters and/or numbers) and a legend with the information on the composition buffer, proteins concentration, biological class, etc. should also be provided.

The shipping address is:
Angela Cattaneo e Giorgia Parodi
IFOM, Via Adamello 16 - 20139, Milano (MI) - Italia

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Proteomics and Metabolomics

Metabolomics and Lipidomics Service

Sample Submission Form

Please email the sample submission form to proteomicsfacility-desk@ifom.eu

Contact Details	
PI name:	
Researcher Name:	
Contact email:	
Lab/institution:	
Address:	
Submission Date:	

Project Description	
Project name:	
Sample matrix: *(cells, tissue, media, etc)*	
Number of samples:	
Metabolites of interest: *(glycolysis, TCA, amino acids, etc)*	
Type of analysis required: *(Target, Unitarget, Stable Isotope Tracing, Consumption release)*	

Sample Details

Vial Identifier *(Researcher initials+Experimental number + Sample number):*	**Sample Group** *(cell lines, genotype, treatment, time point, control, etc)*	**Sample Matrix**	**mg**	**Total Protein**	**Volume of extraction buffer uL**

Please label the samples with letters and/or numbers and add the biological classes in the form (example: vial identifier=AB01, AB02, AB03…Sample group= WT, KO…)

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