Request Form: External user
Proteomics and Metabolomics Facility: ProMeFa

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User informations:

Name:……Phone:……e-mail:……

Address:…….……VAT Number:……..…..…..

Requested analysis:

Protein characterization
Protein/peptide analysis by HPLC/MS and MS/MS
Sample purification (zip tip or similar) and intact protein MW determination by nanospray off line MS

Protein identification: PMF and sequencing
PTM-characterization: validation by Western Blotting and 2DE +/- removal of the PTM
Phosphorylation characterization by TiO_2 or IMAC enrichment and mass spectrometry
Identification of a spot or band by in gel digestion and LC-MS/MS

Biomarker profiling in biological samples by 2DE, image analysis and statistical evaluation
Differential protein display in control *vs* patient biological samples: plasma, serum, urine, tissue
Six samples: control *vs* treated/patient in triplicates

Quantitative protein profiling by mass spectrometry and statistical analysis
Differential protein display in two or more diverse conditions using:
iTRAQ or TMT labelling of peptides (run in technical duplicate)
SILAC methodology (run in technical duplicate)
Label-free approach (run in technical triplicate)

Metabolomics/Lipidomics by mass spectrometry and statistical analysis
Untargeted workflow for differential small molecules/lipids display in: plasma, serum, urine, tissues

Quantitative Metabolomics/Lipidomics by mass spectrometry and statistical analysis
Targeted workflow for specific small molecules/lipids display in: plasma, serum, urine, tissues

Data analysis

Sample informations (according to the wanted activity, fill in the fields):

Sample name or gel ID number:
Buffer/solvent composition:
Quantity and volume:
Expected mass (for peptides, indicate the protecting groups):
Organism or source of protein sample:
Number of bands/spots to be identified:

FACILITY USE ONLY:

Date of receipt: Analysis Performed:

N° of samples analysed: Cost (€):

Frequently Asked Questions

1. Where are you located?
2. I would like to submit a sample for analysis. What do I need to do?
3. How do I arrange to drop off a sample? Do I need to make an appointment?
4. In what buffer can I submit my sample?
5. How much protein do I need to submit for protein identification or quantification?
6. I have an immunoprecipitated and/or tagged protein for which I would like to qualitatively map unknown phosphorylation sites. How much material do I need and how pure does the sample have to be? Can I work directly from a SDS-PAGE gel or does the sample need to be in-solution?
7. Does the facility recommend a specific SDS-PAGE gel system for downstream LC-MS analysis?
8. For phosphorylation analysis via mass spectrometry, will you give me just the protein that is phosphorylated, or the actual phosphorylated residue?
9. What is SILAC and how would I utilize it?
10. What cells are amenable to SILAC and how do I prepare media?

1. Question: Where are you located?
Answer: Dibit 2, C1, 4Th floor, OSR

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2. Question: I would like to submit a sample for analysis. What do I need to do?
Answer: Instructions for this can be found here (link to protocols). Please schedule a meeting with Dr. Annapaola Andolfo to discuss your experimental goals and sample preparation before beginning your experiments for analysis by mass spectrometry. Please print out the request form to bring with you when you drop off samples.

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3. Question: How do I arrange to drop off a sample? Do I need to make an appointment?
Answer: While there is usually someone in the lab to accept samples throughout the day, there may be times where we are unavailable. To ensure that someone will be here to accept your samples or if you would like to speak with someone regarding your samples or our workflow, please email one of us to schedule a sample drop-off time.

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4. Question: In what buffer can I submit my sample?
Answer: We prefer to receive samples stored at 4°C in either 50 mM Ammonium Hydrogen carbonate (AmBic) or water, in a 1.5 mL eppendorf tube. If this is not possible, please give us as many details as

possible on the request form. We need to know how to properly clean up the sample if it is in any other buffer, so it is critical that we know ALL OF THE BUFFER COMPONENTS. We have protocols available for the removal of many MS incompatible buffer components (some are less compatible than others), including the following:

- Buffers: PBS, Tris-HCl, HEPES, MES, MOPS,etc
- Detergents: Triton X-100, NP-40, SDS

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5. Question: How much protein do I need to submit for protein identification or quantification?
Answer: For an in-solution digestion and analysis, we ask for 10 ug for LC-MS/MS if possible, based on a BSA-calibrated Bradford assay. We can many times complete an analysis with a bit less; we aim at loading 1-2 ug total protein onto our system for each run but we can require the overage due to sample losses during preparation. For gel bands, we have over 95% success rate for positive identification if the band is visible on a Coomassie-stained gel. We have about a 75% success rate with Silver- or Sypro-stained gels because of the higher sensitivity and sample losses that occur with these staining techniques.

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6. Question: I have an immunoprecipitated and/or tagged protein for which I would like to qualitatively map unknown phosphorylation sites. How much material do I need and how pure does the sample have to be? Can I work directly from a SDS-PAGE gel or does the sample need to be in-solution?
Answer: In nearly all circumstances, phosphorylated peptides exist in a much lower stoichiometry than non-phosphorylated peptides and, as such, require an enrichment procedure prior to LC-MS analysis. To perform our TiO_2 and or IMAC-based phosphopeptide enrichment protocol and be left with enough material for downstream LC-MS analysis, we ask that users provide a minimum of 10-15 ug total protein of a more pure (≥ 80% based on a Coomassie stained SDS-PAGE) sample or 20-30 ug total protein of a less pure (≥ 50% based on Coomassie stained SDS-PAGE) sample to maximize the chances of success. Although samples can be analyzed directly from an SDS-PAGE band if needed, it is most often preferred to work directly from solution as digestion efficiency is higher and peptides do not need to be extracted from the gel band. Note: phosphatase inhibitors such as NaF and sodium orthovanadate are compatible with the enrichment-LC-MS protocol and should be included during sample preparation.

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7. Question: For phosphorylation analysis via mass spectrometry, will you give me just the protein that is phosphorylated, or the actual phosphorylated residue?

Answer: The analysis of phosphorylation via mass spectrometry is done on proteins which have undergone proteolytic digestion, commonly via trypsinization, so the sequence information (and thus the phosphorylation status) is obtained at the peptide level. Therefore, at the very least, we can localize the phosphate group within a few residues. If there is only one phosphorylatable residue on the peptide (i.e. only one Ser, Thr, or Tyr) or the number of phosphates equals the number of phosphorylatable residues, then the localization is unambiguous. If there is more than one S, T or Y, we utilize the MS/MS spectrum and a scoring algorithm to provide a statistical confidence for the localization of the phosphate on the peptide.

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8. Question: What is SILAC and how would I utilize it?
Answer: SILAC stands for stable isotope labeling with amino acids in cell culture. It can be used to perform relative quantification across several samples generated from cultured cells. In a typical experiment, two populations of cells are grown in light or heavy media, where the latter contains 15N/13C-containing Arg and Lys. After cells are treated under two conditions (e.g. test and control), the samples are mixed, and this mixture is subjected to MS analysis.

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9. Question: What cells are amenable to SILAC and how do I prepare media?
Answer: Many transformed cell lines are amenable to SILAC. Several companies sell Arg- and Lys-free media, including DMEM and RPMI. Custom formulations are also available from Invitrogen. We can provide detailed instructions for preparing SILAC media before purchasing SILAC reagents.

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