# User guide for Proteomic Core Facility

## Mode of operation

We operate as a full-service core facility (CF) and our service is free-of-charge for users from the DKFZ. We foster a collaborative approach when working with our users to tailor the available methods towards the specific needs of each project. Hence, do not hesitate to reach out to us already at an early stage of your project.

## Sample requirements

- **Protein amount:**
  - Full-proteome: 5-10 µg (min. 0.25 µg/µl for 10 µg input)
  - Phospho-proteome: 60 µg (min. 1.5 µg/µl)
- **Protein concentration:**
  - Range of 1-2 µg/µl.
  - We rely on the concentration information provided by our users. We do not remeasure it in house.
  - We kindly ask that all submitted samples have the same protein concentration, which makes downstream processing significantly easier for us.
- **Replicates (biological):**
  - Cell culture experiments: min. 4
  - Animal/human studies: 5-6 (the more the better)
  - Affinity purifications/Immunoprecipitations: min. 4
  - We do not accept technical replicates.
- **Lysis conditions/Buffer composition:**
  - Protocols for cell culture samples is available upon request.
  - Conditions for fresh frozen tissue/FFPE material need to be adapted and tested per project/sample type.
  - Other elution/lysis buffers can be used upon discussion on the project design meeting.
- **Biological safety level S1:** Samples need to comply with the S1 safety level at the moment of submission.
- **Project size/number of samples:** Projects can consist of up 100-300 individual samples.

Please do not hesitate to reach out to us in case your samples/set-up differ from some of the above-mentioned requirements.

## Results discussion/Statistical analysis

We can provide you with a statistical analysis and you will not be left alone with the data. Especially, users new to the field of proteomics will not be left alone. Initially we send you the results with a short commentary about the overall performance of the experiment. We ask you to have a first look into the data by yourself. Afterwards, arising questions can be taken care of in a follow-up discussion, where we also layout the details of the statistical analysis.

## Block randomization – before online project request

To enhance data quality, we recommend adapting the sample order before submitting the online project request. We will perform this step for you according to the guidelines presented in 'Importance of Block Randomization When Designing Proteomics Experiments' (Journal of Proteome Research 2021 20 (1), 122-128; DOI: 10.1021/acs.jproteome.0c00536).

## Online project request via PPMS

After the project was discussed please make an online request via our PPMS website. If you are a first-time user, we might need to activate your account first. Deactivated accounts can be re-activated in time. In both cases, please contact us.

## Selection of database for protein identification

- For peptide/protein identification, we use reference proteome databases from uniport.org.
- Proteins that are not listed in these databases will not appear in your results.
- *Homemade* sequences (genomes/transcriptomoes) can be used but need to be provided as an amino-acid sequence (fasta-format) comparable to the uniport databases. We do not accept genomic/transcriptomic sequences.
- If your project requires adjustments to the protein database, please inform the CF team.

## Selection of post-translational modifications for analysis

- Do not select multiple or all modifications in the submission sheet out of curiosity, as this can significantly impact data analysis time and the results are not likely to be useful/verifiable.
- PTMs are of low abundance, and LC-MS/MS analysis requires an enrichment step.
- An enrichment protocol for global Phosphorylation (STY) analysis is available at the CF.
- This may vary for AP-MS/IP samples.
- If you are interested in any other PTM, please feel free to contact us.

## Mycoplasma contamination

We strongly recommend testing all samples for potential contamination before submitting them for proteomic analysis. Mycoplasma contamination can have a dramatic impact on the proteome, rendering results unusable. Testing services are available at Multiplexion, a DKFZ associated laboratory in Heidelberg.

## Sample hand-over

After we accepted your request, you will receive a mail with a project ID (e.g. DH0123). This number is not identical with the request number from PPMS. The message will contain all necessary instructions for you to deliver your samples to us. Please label the sample vials with numbers (1, 2, …, n). Please mark six and nine with a dot: 6 = 9 => 6. ≠ 9. Once you are done you can bring the samples Monday to Friday between 10:00 and 11:00 to our lab in the TP3, room 3.210 or 3.201.

## Turnaround time

The time it takes us to process your project depends on multiple factors, including project size, sample complexity, and the current workload of the CF at the time of submission.

## Sample storage

Please retrieve any remaining lysate 2 months after receiving the data, as the lysate will be discarded thereafter. Please contact us to coordinate the retrival.

## Acknowledgment

The minimum requirement is to acknowledge the PCF in any publication containing data generated using our services. This is a mandatory condition outlined in our 'Terms of Use,' and it serves as a crucial key performance indicator during our evaluation. In case our users judge that we have given significant scientific input beyond our services co-authorships are also possible and highly appreciated.