Information needed for a successful experiment

There are several pieces of general information we'll need to perform a successful proteomic analysis on your samples. Because the sample preparation and data analysis procedures vary so much between the different types of samples and experiments we do, it's important for us to obtain as much information as possible about the nature of the experiment, and the samples you're submitting. Below is an overview of some of the questions on the form, and why the information is important to us. We strongly recommend consulting with members of PSR before starting your experiment to maximize your chance of success. Consultation is free.

Basic Information

If you're unfamiliar with preparing samples for Proteomics experiments it's important to note that there are some unique problems to be addressed compared to other analyses. The Sample Preparation page (<u>https://www.ohsu.edu/proteomics-shared-resource/sample-preparation</u>) on our website works through some of these concerns, and is a recommended read before preparing your samples.

The affiliations listed are all important for tracking hours and usage from different institutes. Please indicate if you are part of any of the institutes listed.

Analysis Type

The requested analysis is, of course, important for us so we can be sure we're treating your samples the right way. If you're uncertain of how to classify your samples, you can always check "other" and fill in the details in the project overview section at the end of the form.

Genus and Species is important to make sure we have the correct database for the data analysis. You can learn more about our data analysis methods through several of the links on our Educational Links and Publications page (<u>https://www.ohsu.edu/proteomics-shared-resource/educational-links-and-publications</u>).

However for most Proteomics data analysis there is an overarching theme that **we can't find what we aren't looking for!** So it's important to know which proteins and Post-translational modifications we will be expecting to see, so we can configure the software to look for them. If protein sequences come from different species, or have been altered in some way from what is present in the common public data repositories, we'll likely need this information to find your protein in the samples.

Information about the number of samples and their names is important to help us keep your project straight. It's also helpful to have the names typed out, as handwritten labels can become smeared or be hard to read. The run order is important for the mass spectrometer. We make efforts to minimize and track carry-over between runs, but it can still happen. If you want to be certain a particular protein isn't present in a sample it can often be useful to run that sample first. It is best to run controls first followed by the experimental samples.

Solution samples

If your samples consist of liquid solutions, or were taken to dryness from liquid samples, it's important to note all of the reagents in the recent solutions that still may be present in the sample, and their approximate concentrations (if applicable). There are a number of common reagents (detergents, salts, polymers) used in protein purification and extraction that are harmful to the mass spectrometer. If these reagents are not removed prior to injection they can do thousands of dollars in damage and result in no useful data being generated.

Gel samples

If you are submitting samples in an SDS-page gel it is important to note the stain used and include an image of the gel. This helps us ensure we're using the correct method to remove the stain from the gel, and that the protein amount present in the gel matches with the results we get from the instrument.

MSMS Analysis

There are options to choose the length of the MS/MS analysis, and the instrument that is used. Longer runs on the instruments will usually yield more protein identifications, but the cost will go up as well. The shorter times (60&90min) are usually sufficient for simple samples such as gel bands or IP experiments, and the longer times (120,140,240min) are better suited for complex mixtures. The 2D-LC experiments are usually for large-scale quantitative experiments.

For instrument choice the QExactive and Orbitrap Fusion are largely equivalent instruments. However there are some experiments better suited for one or the other. It is also useful to run the samples on the same instrument if an experiment is stretched out over several months and multiple submissions.

In either case, if you're not certain of what would be the best choice, feel free to leave these option blank, and a PSR member will choose the options that are most appropriate for your samples.

Overview

For the project overview a paragraph or two outlining the project and what you hope to accomplish with this set of samples is useful for helping us put the experiment and results in context. If you have had other sample submissions that are related it can be helpful to know what those are as well. That way we can reference previous sample prep details and other related information. For quantitative analysis using TMT method please indicate samples to be compared for data analysis.

There are several pieces of information that we might need included as attachments if applicable. This includes (but is not limited to):

- The sequences of proteins you're hoping to identify, but that aren't in the database of the species given in the field above.
- Images of a gel, or other related information that shows the presence of protein, like protein or peptide assay results.
- Protocols used in the preparation of the samples, especially if they're more unusual or may have buffers that aren't common.
- Sample keys indicating control and experimental samples (especially when a quantitative comparison is being done).