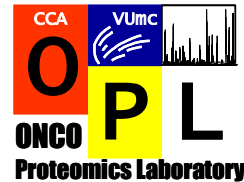


## Planning nanoLC-MS/MS experiments

Scheduling many label-free nanoLC-MS/MS experiments on a limited number of instruments (2) is similar to making pieces of a puzzle fit, where we apply some sort of fuzzy logic to decide on the best order of the experiments.



### Starting points:

- Optimal quantitative performance: Label-free discovery experiments are analyzed in one series (so they are not interrupted by other measurements)
- The instruments are running 24/7 to accommodate the experiments in the queue in the most efficient manner
  - Due to the high-tech nature of the instrument, which is actually an in-line series of two instruments (nanoLC, and QE (tandem) MS), technical issues may cause down-time from time to time
- Cancer projects have higher priority than non-cancer projects

### General considerations that we take into account for the planning:

- Size of the experiment
- Urgency
  - An experiment is considered to be urgent eg., when the investigator is stuck without the dataset or when the experiment is needed as a final proof for a manuscript. We also give priority to an experiment when a commercial fee is paid (this happens only occasionally).
- Sample submission date
- Available time-slots

### What does this mean in practice?

In the current situation, with many projects, a two discovery instruments and a single operator, the above means that:

- Small experiments (measurements of a few days) have shorter waiting times (typically in the order of weeks) than large-scale discoveries that keep the instrument booked for several weeks (typical waiting time of several months).
- At the time of sample submission, we can not tell you exactly when we will run the samples. We will contact you a few days- 1 week before we plan to run the samples, so that the final concentration of the samples can be planned just prior to analysis.
- The indication of waiting time that you get at time of sample submission may change due to:
  - technical problems per se (eg., spraying/ stability problems)
  - technical problems may to altered time-slots (eg before a holiday) and thereby rescheduling of experiments to make them fit into the available slots
  - unforeseen circumstances (eg., newly submitted urgent experiments that are run with high(er) priority, unavailable operator)

## **Planning data processing, database searching and data summary**

After data acquisition, the raw LC-MS/MS dataset needs to be processed and searched to identify proteins. This requires transfer of a multitude of nanoLC-MSMS files to a separate hardware-and-software pipeline. Currently the file transfer is a manual process and the subsequent processing/ searching takes a few days to a week (depending on the size of the dataset).

## **Planning state-of-the-art biostatistics**

We are in the privileged situation to have an informatician in our team (Dr. Thang Pham), who performs dedicated (state-of-the-art) statistics and (cluster) analyses on your dataset.

Thang has to accommodate such analysis requests into his own research activities and therefore it may take some time (days or weeks) before he can perform a biostatistical analysis of your dataset.

### **Guidelines for optimal planning and streamlining of biostatistics and further data analysis:**

- At the time of sample submission, clearly indicate on the PROteomics Experiment File (PROEF): the aim of the experiment, the sample labels, the groups to be compared and whether this should be a paired or non-paired analysis
- In case of a complex experiment (eg., multi-group, or multiple questions), by the time the experiment is actually being measured, we advice you to plan a meeting with Thang and Connie to discuss the required analyses. In this way, all comparisons and analyses can be performed in one analysis session. This will ensure efficient use of precious time and a relative fast delivery of results (for standard requests, typically days to a week).