Experimental Procedure Tips for quantitative LCMS

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Content:

1.Co-IP for identifying specific bait interaction partners

Short protocol for filtering contaminant proteins from specific interaction partners in a co-IP experiment

General remarks:

- The cleaner the co-IP, the better are the results. Large amounts of contaminant peptide will mask low abundance features and will lead to lower coverage of interesting peptides in a random sequencing (DDA) mode.
- The control sample is as important as the bait sample. Filtering works only if the control is similar to the bait except for the bait interaction partners.
- Quantification is difficult below a certain intensity level. Make sure that the peptides from interaction partners are not too close to detection limit.

Procedure:

1. Perform co-IPs of bait and control samples in parallel. Adjust protein levels in the lysis buffer to about equal concentrations.
2. Run a small amount of the pure peptide samples on a LC/MS to estimate the relative intensity of both samples. This can be determined by manual inspection of prominent peaks, which are common in both samples, or by calculating the median of feature intensities in both samples. The control sample should be at least as intense as the bait sample otherwise dilution profiling becomes less discriminative.
3. The same is true for comparing two bait samples. Dilution profiling works best, if the two samples are similar in sample amount.
4. Create a dilution series by mixing bait sample with control sample. The basic idea is to quantify a strong signal against a weak signal to get confidence for bait specific samples that a signal is present, but only to a low amount in the mixed samples. Therefore a signal must be strong enough to be detected also in diluted samples.
    - If interaction partners are intense enough a dilution series of 0%, 10%, 20%, and 100% bait sample discriminates well between constant background and enriched peptides.
    - If signals are already in the pure bait sample close to detection limit a dilution series of 0%, 30%, 60%, and 100% may give more quantifiable profiles.