

- Pre-processing steps:

1. Shared peptides between protein-groups removed -> unique peptide for each protein.
2. PSM which has missing measurements within each run removed.  
(each PSM should have 6 measurements -number of channels- in each run, i.e.: no NA per Run per PSM)
3. Peptide ions overlapped among multiple fractions of same biological mixture removed.
4. When there are multiple measurements for certain feature and certain run, the maximum intensities is selected.
5. Proteins which have only 1 peptide removed. — —> no Single-Shot protein
6. Fractions belonging to same mixture are combined.

- Summarizing PSM level quantification to protein level quantification —> method: Median Polish

1. Log2 transformation
2. Median Polish summarization (similar to averaging)
3. Reversing Log2 transformation
4. Apply VSN normalisation between Runs and Channels.  
VSN automatically transforms intensities to log scale, similar to log2. That's why I reversed the Log2 transformation before VSN normalisation.  
There might be better ways. The standard way of doing BETWEEN run normalization for TMT is to have a reference channel (Norm) for each Run. We don't have!  
Imp!!! —> Normalization is performed AFTER protein-level summarization

- **Finding differentially abundant proteins across conditions in TMT experiment**

1. **Using moderated t-test from Limma**
2. **Adjusted p-value —> benjamini-hochberg correction**
3. **Pairwise comparison between All conditions**
4. **Pairwise comparison between WT and CBLB**

- **Plots**

1. **Correlation plots between conditions and Bioreplicates.**
2. **Profile plots of every protein. The pdf file contains all ~2500 proteins, showing the intensities of every single Peptide of the protein for each condition.**
3. **Two pairwise volcano plot: one between all possible conditions, and one between WT and CBLB KO.**

- **Lists**

1. **List of top 1% significantly regulated Proteins for each pairwise comparison.**
2. **List of top 1% significantly regulated Proteins for combined comparison between WT and CBLB KO.**  
(1% is arbitrary, can be changed as desired.)

