PRECURSOR ION INTENSITY OR SPECTRAL COUNTING?

LC-MS/MS data processing and statistical assessment for relative protein-level quantification in label-free proteomics

ANANDASHANKAR ANIL, ANGELIKI MARAKI, JAMES ERICSON

aaníl@kth.se, aggmara@gmail.com, jameser@kth.se

Precursor Peak Intensity Method

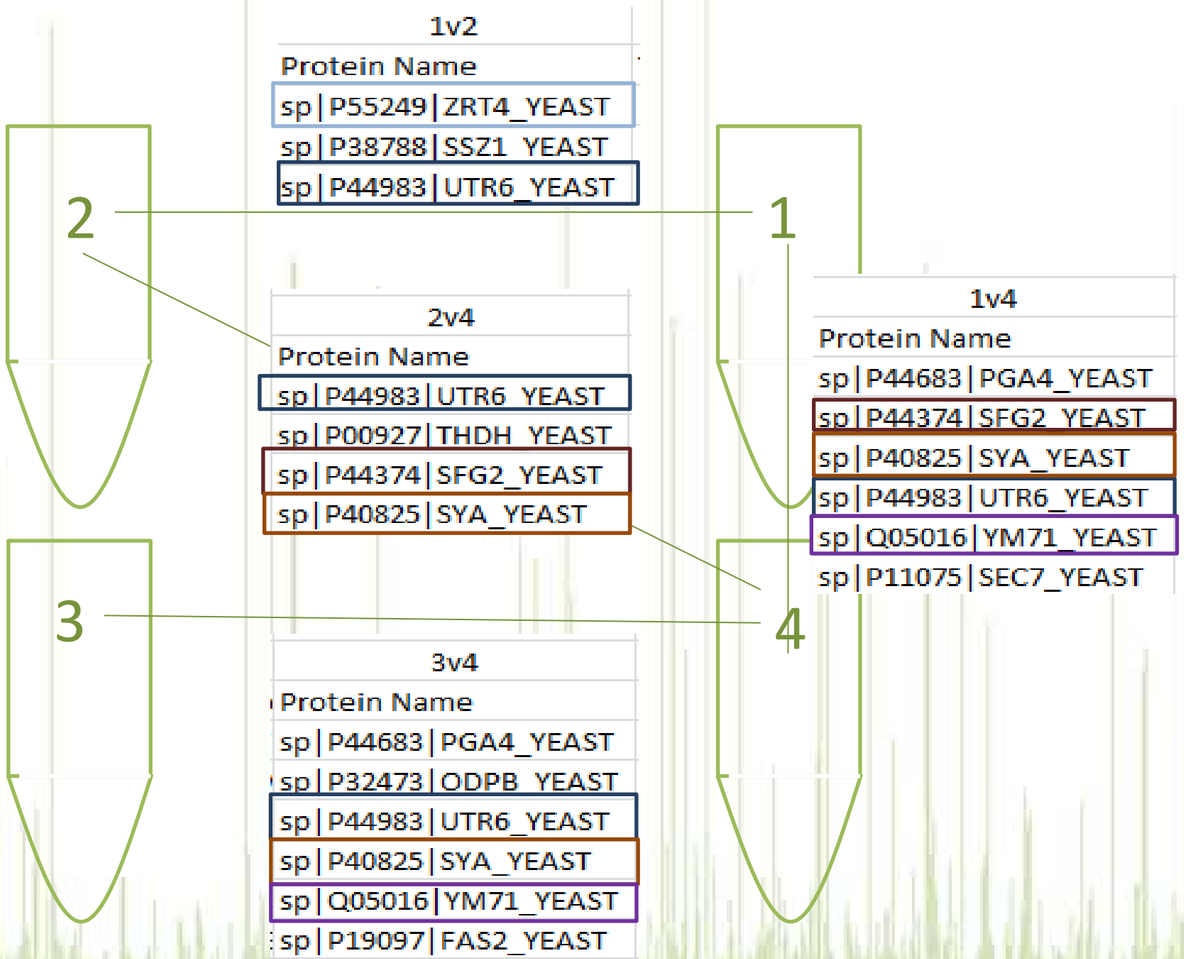
- For each sample:
  - ❖ Normalization of the Intensity Area of each peptide against the total Intensity of the sample.
  - ❖ Sum normalized intensity for all identical peptides identifying a protein.

⟶ Unique peptides (summed intensity area) identifying a single protein

⟶ Removal of peptides that do not occur in all three replicates

- Comparison between two samples:
  - ❖ Create a single list containing all peptides that occur in both samples
  - ❖ Perform **t.test** on log(sum(intensities))
  - ❖ Sort ascending by p-value from t.test
  - ❖ Obtain **q-value**

Significantly Differentially Abundant Proteins



Spectral Counting Method

- NSAF(Normalized Spectral Abundance Factor)
  - ❖ Filter: get only the peptide measurements for which the q value was less than 0.01.
  - ❖ NSAF calculated as the number of spectral counts(SpC) identifying a protein, divided by the protein's length(L), divided by the sum of SpC/L for all proteins in the replicate
  - ❖ Merged the NSAF scores for all replicates in two specific runs and performed a t.test.
  - ❖ Obtained q-value

Significantly Differentially Abundant Proteins

1v3

Protein Name
sp|P44374|SFG2_YEAST

2v3

Protein Name
sp|P55249|ZRT4_YEAST

- emPAI(Exponentially modified protein abundance index)
  - ❖ Filter: get only the peptide measurements for which the q value was less than 0.01.
  - ❖ Removed [0-9] modification sites in peptides for comparison against digested peptides
  - ❖ Removed peptide duplicates and count Observed peptides
  - ❖ Calculated normalized emPAI by dividing individual emPAI score with sum of all emPAI scores from a replicate in a sample
  - ❖ Merged the emPAI scores for all replicates in two specific runs and performed a t.test.
  - ❖ Obtained q-value

No Significantly Differentially Abundant Proteins Found

Conclusion

- ❖ The Precursor ion peak intensity method identified more differentially abundant proteins than the Spectral Counting methods.
- ❖ Out of the two Spectral Counting approaches, the NSAF method identified only two significantly differentially abundant proteins, while emPAI none.