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Approaching absolute quantification using label-free shotgun proteomics coupled with external protein standards spiked in yeast extracts

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Background

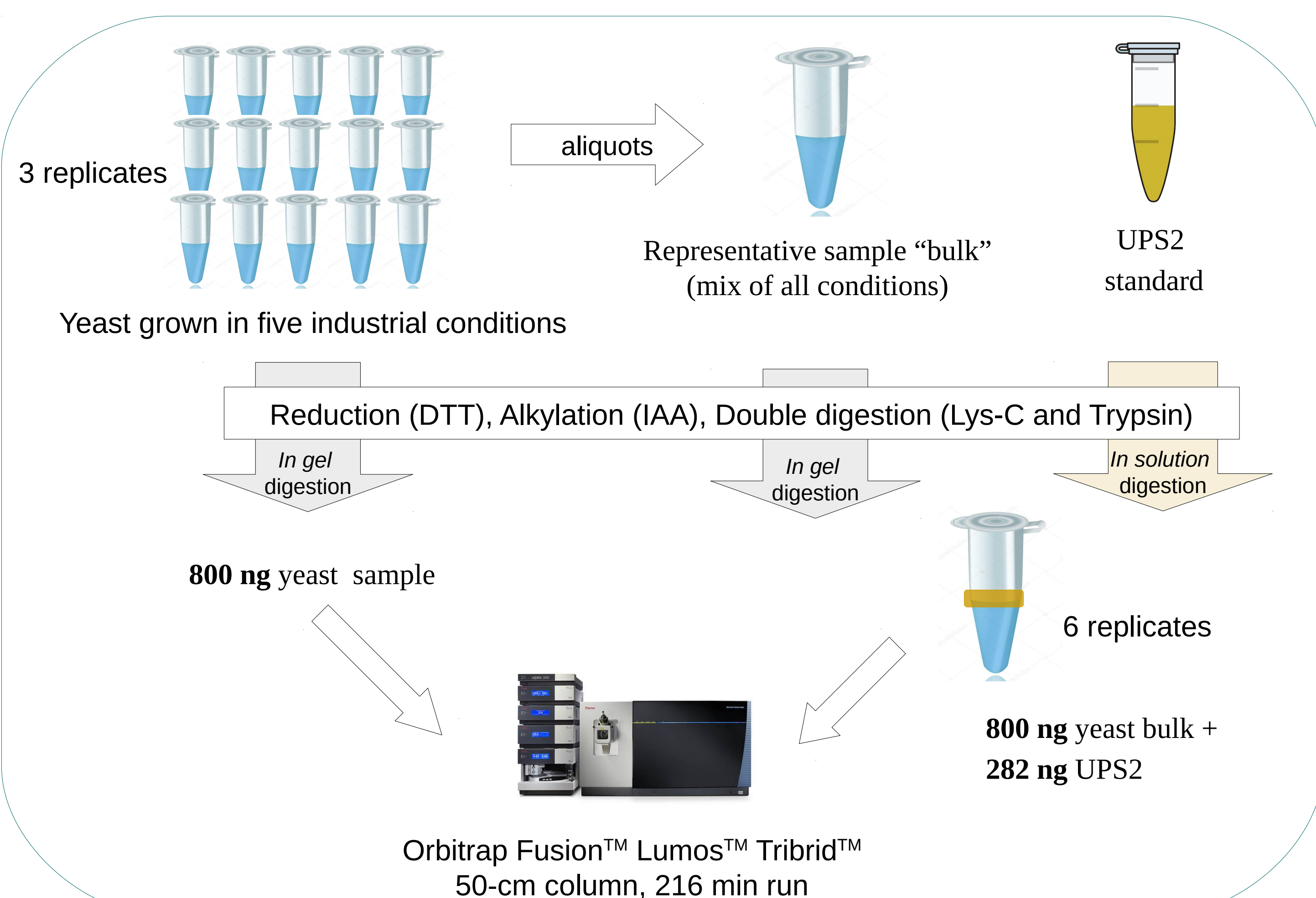
Label-free quantitative proteomics remains the “gold standard” strategy to easily handle a large number of samples to compare the abundance of thousands of proteins and to portray a global view in biological samples. Nonetheless, in the framework of Systems Biology, the **need of absolute quantification** in proteomics is fundamental to understand the dynamic state of cells as well as to integrate high-throughput biological datasets into genome-scale models.

To obtain absolute values from label-free experiments, several efforts have been carried out by spiking the Universal Protein Standard (UPS, Sigma) into different biological samples [1, 2]. However, these studies were limited by the huge amounts of standard protein required, the limited number of samples analyzed, and the limited orders of magnitude detected (4/6). The state of the art in the domain highlights the urgent need to develop robust methods to perform absolute protein quantification using label-free methods with reliable accuracy.

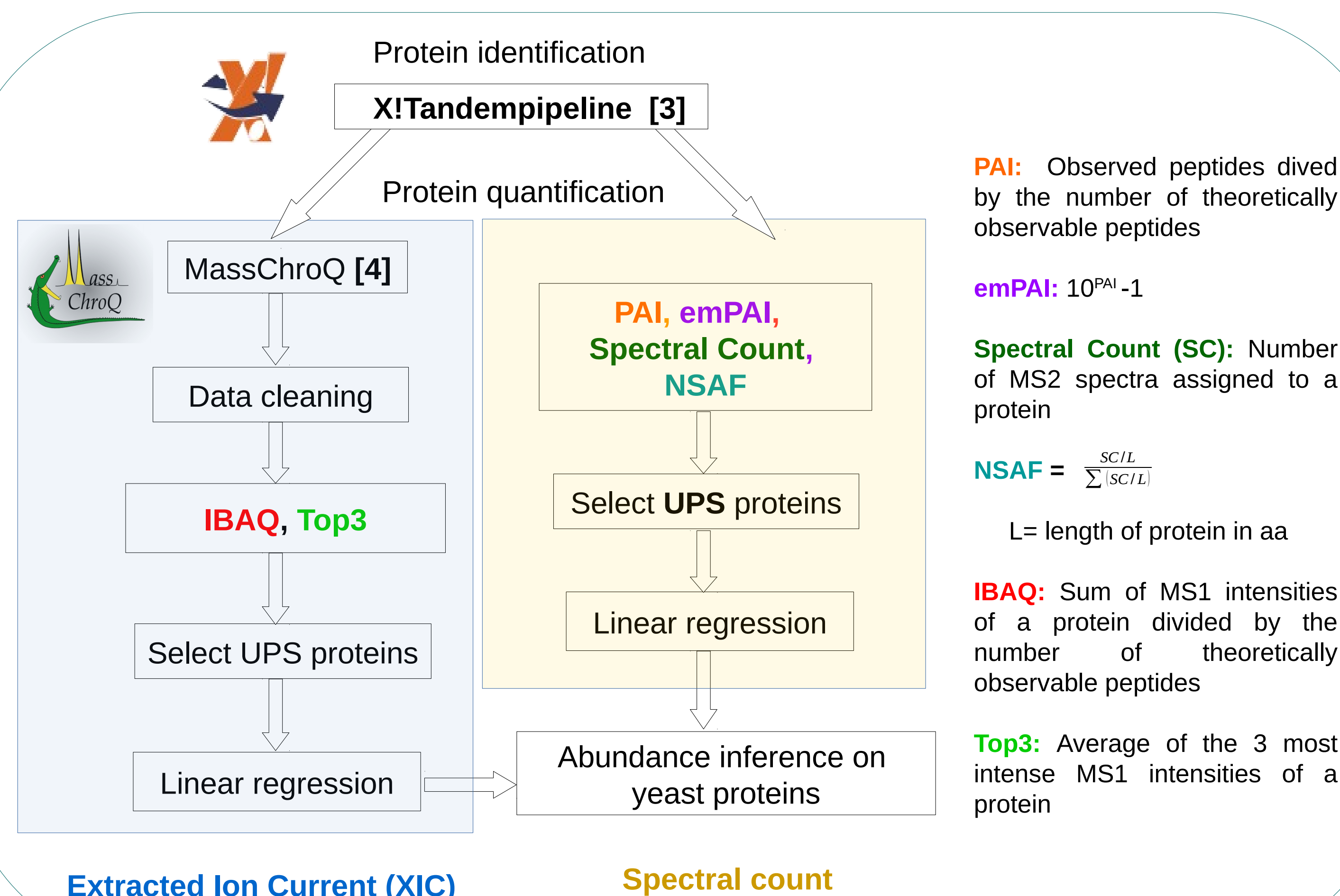
Aim

To develop a strategy to approach **absolute quantification using label-free** proteomics by spiking the external protein standard UPS2 (Sigma), a mixture of 48 proteins at 6 different molar concentrations, in protein extracts of the model yeast *Saccharomyces cerevisiae* cultivated in five industrial conditions (chemostats). To do so, we tested six different methods of protein abundance quantification based on eXtracted Ion Current (IBAQ, Top3) and Spectral count (PAI, emPAI, Spectral count, NSAF).

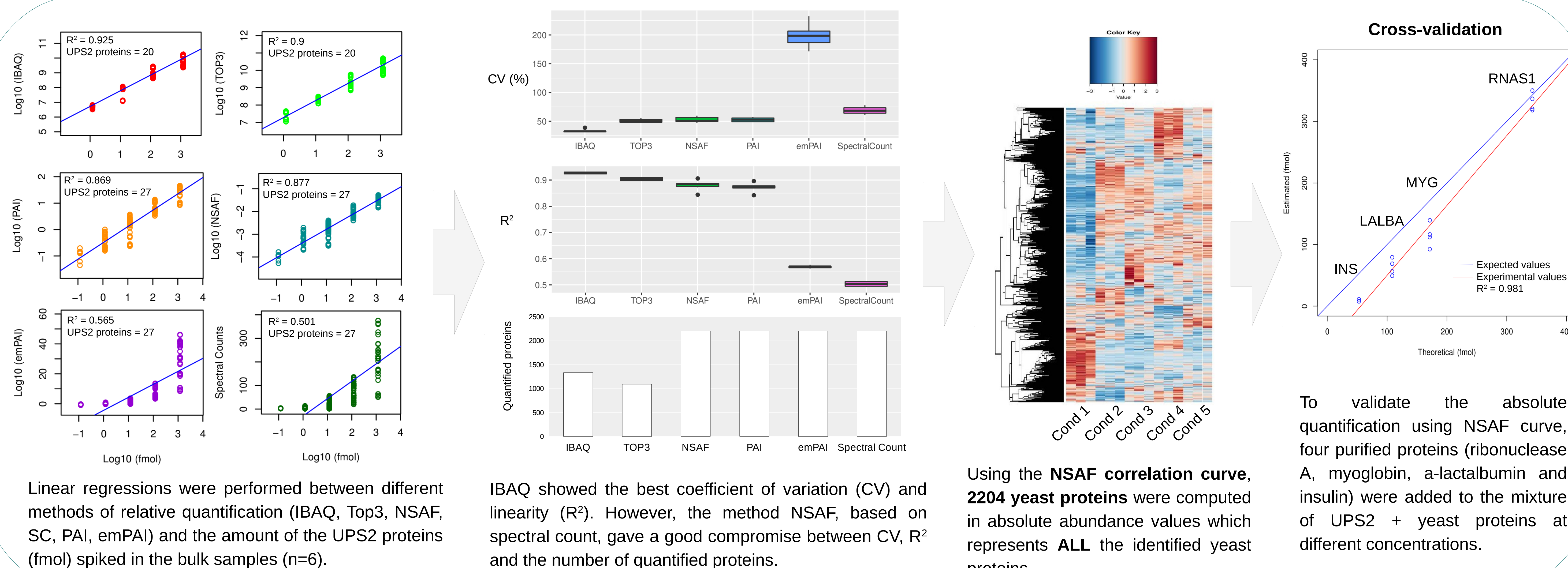
Sample preparation



Bioinformatic workflow



Results



Conclusion

- Considering the performance of the six methods of protein quantification we propose the **use of NSAF** since it offers good balance between linearity, CV and the number proteins quantified.
- The analysis of bulk samples to establish a linear correlation between relative protein abundances and absolute values represents a solution to the high costs of UPS2 standard.
- Approaching absolute quantification from label-free proteomics using the UPS2 standard is a suitable option in the context of modeling at genome-scale level (interval of 5 orders of magnitude, 27/48 UPS proteins in a complex mixture). Despite the fact that analyzing 400 ng of UPS2 alone resulted in a maximum of 34/48 UPS2 proteins identified.

References

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