Evaluation of cell factory performance through determination of intracellular metabolites using LC-MS/MS

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might be viewed as a predisposing factor towards stress-induced pathologies.

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Poster 4.2.16

Evaluation of cell factory performance through determination of intracellular metabolites using LC-MS/MS

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A major objective in biotechnology is the improvement of the efficiency of host microorganisms used as cell factories. Engineering a strain capable of producing high amounts of a desired biochemical is a multi-step process consisting of design, construction, and analysis of the constructed cell factory. In order to address the function or disfunction of the engineered cells, systems biology tools are employed by using the multi "omics" approach (genomics, transcriptomics, proteomics, metabolomics and fluxomics). Metabolomics is a tool aimed at a quantitative understanding of metabolism. By quantification of intracellular metabolite changes over time, under given genetic and environmental perturbations, key regulatory nodes may be identified in the cellular metabolic network. In target metabolome analysis, the quantification is applied to only one or a small group of metabolites and is very useful for studying the effect of a genetic modification. The approach of targeted metabolomics was applied for the analysis of metabolite extracts from *Saccharomyces cerevisiae* and *Lacctococus lactis*. The analytical technique employed was ion-pair liquid chromatography tandem mass spectrometry (LC-MS/MS). The unique combination of sensitivity and specificity of this technique allowed qualitative and quantitative analysis of low metabolite concentrations. The mains steps involved were: (i) metabolite sample preparation by quenching, extraction, sample purification using dispersive Solid Phase Extraction; (ii) quantitative analysis, (iii) data analysis and interpretation. The established analytical method covers analysis of sixty metabolites from glycolysis, cofactors, coenzymes and nucleotides. Implementation of this method provides a powerful new tool in future cell factory design and characterization.

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Poster 4.2.17

Evaluating the effects of clustering methods in co-expression-based functional inference in *Arabidopsis thaliana*

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Gene co-expression analysis has been widely used for hypothesizing gene functional relations. This is because genes with similar expression patterns are more likely to have similar regulatory mechanisms and, therefore, similar functions. In the model plant *Arabidopsis thaliana* genome, there are still genes that have no experimentally verified functions. Thus functional inference based on co-expression can be particularly useful for predicting unknown gene functions. In this study, our first goal is to assess how expression data clustering methods as well as input data may influence functional inference of *A. thaliana* genes based on co-expression. In addition, we would like to address the question of whether functions of genes in certain biological processes may be better predicted with the expression data. Different variables in clustering analysis are considered including input expression data, clustering algorithm, distance measure, number of clusters, and functional classification datasets for evaluating the performance in co-expression based functional predictions. Publicly available *A. thaliana* stress expression data is used in the analyses, along with the mostly used partitioning and hierarchical clustering algorithms. During the meeting, we will present how different variable combinations for clustering influence functional prediction accuracy. In addition, we will report whether the prediction performance differ between genes in different functional categories. This work will be useful for evaluating if expression data clustering methods can be optimized for functional prediction and for maximizing the benefits in obtaining biologically meaningful information from gene expression.

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Poster 4.2.18

A Comprehensive Protein Interaction Network of Calcium Triggered Mechanisms in S. cerevisiae

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Calcium, as a common second messenger in eukaryotic cells, plays a vital role in many signaling mechanisms by controlling an extensive variety of cellular processes. This work embodies the first attempt to discover protein interaction network for Calcium triggered mechanisms in S. cerevisiae by utilizing computational methods that integrate interactome data with Gene Ontology. Starting with 12 core proteins, a scale-free network mainly responsible for cell cycle and regulation of transcription is reconstructed. Besides investigating topological features of the network, for the proteins being present in the network but having no known/reported function, a function annotation strategy is applied which enabled to assign a functional role for some of these proteins. Moreover, the linear network decomposition analysis affords assistance to decipher the unknown components of documented pathways e.g. High Osmolarity Glycerol (HOG) and Cell Wall Integrity (CWI) and the crosstalks among them via calcium signaling. Eventually, this work aids to depict a global picture of calcium signaling and its ultimate effects in yeast which may serve as the basis for further studies in higher eukaryotes and lead to a better comprehension of the biological meanings behind them.

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