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Genome Scale Metabolic Models can be used to simulate the intracellular flux distributions under steady state conditions using different simulation tools such as Flux Balance Analysis (FBA)[1]. Lately, these models proved to be useful for predicting gene knock-outs that optimize the production of important industrial targets[2–4]. To obtain a good correlation between simulations and *in vivo* results, it is important to validate beforehand how well the model allows to predict the metabolic fluxes for the wild-type organism.

In this work, the accuracy of the simulated intracellular flux distributions in *Saccharomyces cerevisiae* was evaluated. The results revealed that steady-state simulations performed with FBA and the available genome-scale models [5–8] under fully aerobic conditions contained relevant mismatches in important areas of central metabolism, when compared with *in vivo* data[9, 10] and physiological knowledge, namely the absence of flux in the Pentose Phosphate Pathway. Since many of these mismatches are associated with reactions involving the cofactors $NADP_+$/NADPH and NAD_+/NADH, all the enzymatic reactions that included these cofactors were manually curated.

Because under fully aerobic conditions the ratios of NADPH/$NADP^+$ and NAD^+/NADH are high, it was assumed that the concentration of this cofactors would drive reactions near equilibrium in one direction. Therefore, if a reaction was found to be near equilibrium, its reversibility was constrained in the direction of NADPH consumption or NADH production.

To verify if the modifications applied had any effect on the predicted fluxes for the central carbon metabolism, the

models[5–8] were used for FBA simulations and the results were compared with experimental fluxes. The simulations performed with the curated models revealed several improvements in the Pentose Phosphate Pathway and other parts of NADPH metabolism, resulting in a flux distribution much closer to experimental values[9, 10]. The new flux distribution was then used as a reference for the MOMA[11] methodology in an *in silico* optimization of the production of two organic acids to evaluate its impact on quality the results. It was observed that the knock-out mutants obtained were consistent with experimental evidences in the literature and were only valid when the curated model was used.

In sum, it was shown that a careful curation of the wild-type network can improve the simulation accuracy, resulting in a better correlation with experimental data. Since *in vivo* strain design is very time consuming, these results can prove important to boost the reliability of *in silico* optimizations. However, since the proposed changes are only valid under specific conditions (full aerobiosis) it can also be concluded that the accuracy of flux distribution prediction in large-scale models might be dependent on condition-specific modifications.

References:

1. Edwards JS, Palsson BO: Metabolic flux balance analysis and the *in silico* analysis of *Escherichia coli* K-12 gene deletions. BMC Bioinformatics 2000, 1:1.

2. Brochado AR, Matos C, Møller BL, Hansen J, Mortensen UH, Patil KR: Improved vanillin production in baker's yeast through *in silico* design. Microb. Cell Fact. 2010, 9:84.

3. Fowler ZL, Gikandi WW, Koffas MAG: Increased malonyl coenzyme A biosynthesis by tuning the *Escherichia coli* metabolic network and its application to flavanone production. Appl. Environ. Microbiol. 2009, 75:5831–9.

4. Otero JM, Cimini D, Patil KR, Poulsen SG, Olsson L, Nielsen J: Industrial systems biology of *Saccharomyces cerevisiae* enables novel succinic acid cell factory. PLoS One 2013, 8:e54144.

5. Förster J, Famili I, Fu P, Palsson BØ, Nielsen J: Genome-scale reconstruction of the *Saccharomyces cerevisiae* metabolic network. Genome Res. 2003, 13:244–53.

6. Mo ML, Palsson BO, Herrgård MJ: Connecting extracellular metabolomic measurements to intracellular flux states in yeast. BMC Syst. Biol. 2009, 3:37.

7. Österlund T, Nookaew I, Bordel S, Nielsen J: Mapping condition-dependent regulation of metabolism in yeast through genome-scale modeling. BMC Syst. Biol. 2013, 7:36.

8. Heavner BD, Smallbone K, Price ND, Walker LP: Version 6 of the consensus yeast metabolic network refines biochemical coverage and improves model performance. Database (Oxford). 2013, 2013:bat059.

9. Jouhten P, Rintala E, Huuskonen A, Tamminen A, Toivari M, Wiebe M, Ruohonen L, Penttilä M, Maaheimo H: Oxygen dependence of metabolic fluxes and energy generation of *Saccharomyces cerevisiae* CEN.PK113-1A. BMC Syst. Biol. 2008, 2:60.

10. Gombert AK, Moreira dos Santos M, Christensen B, Nielsen J: Network identification and flux quantification in the central metabolism of *Saccharomyces cerevisiae* under different conditions of glucose repression. J. Bacteriol. 2001, 183:1441–51. 11. Segrè D, Vitkup D, Church GM: Analysis of optimality in natural and perturbed metabolic networks. Proc. Natl. Acad. Sci. U. S. A. 2002, 99:15112–7.

179. The Yeast Pathway Kit: A Method for Rational or Combinatorial Metabolic Pathways Design in *Saccharomyces cerevisiae*

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Metabolic engineering often requires multiple genetic modifications in order to alter the properties of a target organism, especially if an entirely new metabolic pathway has to be established. Pathway elements can be rationally or randomly assembled, the latter facilitates selection of the best performing pathway from a set of randomly generated pathways if a suitable screening or selection procedure is available. Methods described so far allow either rational or random assembly of metabolic pathways in *in vivo* or in *vitro*, but not both and usually providing few reusable genetic elements such as promoters and genes. We present here the Yeast Pathway Kit (YPK) that aids rational or random metabolic pathway assembly using the same genetic parts. The system is based on efficient and rapid cloning using positive selection vector in combination with hierarchical in-vivo gap repair. As a proof of principle, we assembled xylose metabolic pathways with up to eight genes producing a recombinant *S. cerevisiae* strain able to grow on xylose with a specific growth rate of 0.181 h-1. YPK relies on PCR reactions with short primers resulting in a relatively low cost of construction compared to other protocols such as Gibson assembly or DNA assembler.