Assessing glycolytic flux alterations resulting from genetic perturbations in E. coli using a biosensor

We describe the development of an optimized glycolytic flux biosensor and its application in detecting altered flux in a production strain and in a mutant library. The glycolytic flux biosensor is based on the Cra-regulated ppsA promoter of E. coli controlling fluorescent protein synthesis. We validated the glycolytic flux dependency of the biosensor in a range of different carbon sources in six different E. coli strains and during mevalonate production. Furthermore, we studied the flux-altering effects of genome-wide single gene knock-outs in E. coli in a multiplex FlowSeq experiment. From a library consisting of 2126 knock-out mutants, we identified 3 mutants with high-flux and 95 mutants with low-flux phenotypes that did not have severe growth defects. This approach can improve our understanding of glycolytic flux regulation improving metabolic models and engineering efforts.

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