Engineered Reversal of Function in Glycolytic Yeast Promoters

Promoters are key components of cell factory design, allowing precise expression of genes in a heterologous pathway. Several commonly used promoters in yeast cell factories belong to glycolytic genes, highly expressed in actively growing yeast when glucose is used as a carbon source. However, their expression can be suboptimal when alternate carbon sources are used, or if there is a need to decouple growth from production. Hence, there is a need for alternate promoters for different carbon sources and production schemes. In this work, we demonstrate a reversal of regulatory function in two glycolytic yeast promoters by replacing glycolytic regulatory elements with ones induced by the diauxic shift. We observe a shift in induction from glucose-rich to glucose-poor medium without loss of regulatory activity, and strong ethanol induction. Applications of these promoters were validated for expression of the vanillin biosynthetic pathway, reaching production of vanillin comparable to pathway designs using strong constitutive promoters.

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Corresponding author: Jensen, M. K.
Contributors: Rajkumar, A. S., Özdemir, E., Lis, A. V., Schneider, K., Qin, J., Jensen, M. K., Keasling, J. D.
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