Re-Factoring Glycolytic Genes for Targeted Engineering of Catabolism in Gram-Negative Bacteria

The Embden-Meyerhof-Parnas (EMP) pathway is widely accepted to be the biochemical standard of glucose catabolism. The well-characterized glycolytic route of Escherichia coli, based on the EMP catabolism, is an example of an intricate pathway in terms of genomic organization of the genes involved and patterns of gene expression and regulation. This intrinsic genetic and metabolic complexity renders it difficult to engineer glycolytic activities and transfer them onto other microbial cell factories, thus limiting the biotechnological potential of bacterial hosts that lack the route. Taking into account the potential applications of such a portable tool for targeted pathway engineering, in the present protocol we describe how the genes encoding all the enzymes of the linear EMP route have been individually recruited from the genome of E. coli K-12, edited in silico to remove their endogenous regulatory signals, and synthesized de novo following a standard (i.e., GlucoBrick) that facilitates their grouping in the form of functional modules that can be combined at the user's will. This novel genetic tool allows for the à la carte implementation or boosting of EMP pathway activities into different Gram-negative bacteria. The potential of the GlucoBrick platform is further illustrated by engineering novel glycolytic activities in the most representative members of the Pseudomonas genus (Pseudomonas putida and Pseudomonas aeruginosa).

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