Towards biosensor-assisted directed evolution of *myo*-inositol oxygenase

by
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Biosensors are powerful tools that leverage transcriptional regulation mechanisms to modulate gene expression in response to a variety of stimuli. Such behavior can allow for high-throughput screening (HTS), metabolic pathway regulation, and many other applications. In this work, we focus on their uses in HTS, the roadblocks that prevent their effective operation and the ways in which we overcame these challenges, and an example of the application of a biosensor applied to an optimized system. Our original intention was to apply the biosensor as a tool for HTS in the directed evolution of myo-inositol oxygenase (MIOX). This enzyme catalyzes the penultimate, rate-limiting step in the metabolic production of glucarate from glucose in *E. coli*. Here, we develop a biosensor that recognizes glucuronate, the direct product of MIOX, and optimize our system towards screening a library of MIOX genes for an improved enzyme variant.

Biosensors for fructuronate and glucuronate were developed and investigated for their ability to indirectly and directly detect glucuronate levels via the UxuR and ExuR transcription factors (TFs), respectively. Ultimately, due to its ability to directly detect glucuronate, the ExuR biosensor was selected for application to high throughput screening. This biosensor was characterized via exogenous glucuronate addition and endogenous glucuronate production from MI, the substrate of MIOX, and from glucose, the initial substrate of the glucarate pathway.

When characterizing the biosensor for its application to *in vivo* production we found that it was strongly impacted by experimental conditions. In particular, we observed varying growth and fluorescent output was when glucuronate was generating using differing MIOX homologs. It appeared that the capacity of the biosensor was limited when increased burden was imposed on the system. By adjusting the circuit architecture and culture conditions we were able to yield a system with a more consistent biosensor output. Finally, the optimal biosensor configuration and experimental setup was applied to successfully detect the difference between two homologs that differed in productivity.

This work investigated the challenges that prevent biosensors from successfully aiding high-throughput screening. Even with these challenges we were able to display the ability of the biosensor to separate a MIOX homolog with higher glucuronate output from a homolog with lower output, indicating its promise to effectively detect improved mutant library members should these barriers be fully overcome.

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