GENETICALLY ENCODED BIOSENSOR FOR ENGINEERING BRANCHED-CHAIN HIGHER ALCOHOL PRODUCTION PATHWAY IN *SACCHAROMYCES CEREVISIAE*

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Branched-chain higher alcohols (BCHAs) including isobutanol, isopentanol, and 2-methyl-1-butanol, are promising alternatives to the first-generation biofuel ethanol. These alcohols have better fuel properties than ethanol, such as higher energy density, ease of refining, and better compatibility with existing gasoline engines and infrastructures [1]. We have developed a genetically encoded biosensor to measure the metabolic activity of BCHA biosynthesis in *Saccharomyces cerevisiae*. This biosensor enables high-throughput screens to identify strains with higher metabolic flux to BCHA synthesis. The versatility of this tool has allowed us to use it in several applications, including *in vivo* BCHA metabolic pathway engineering/optimization and enzyme engineering. We have been able to screen for isobutanol hyper-producing stains with optimum combinations of genes from the mitochondrial isobutanol pathway (Mito-IbOH-pathway) [2]. The ability of this biosensor to monitor the activity of both the mitochondrial [2] and cytosolic isobutanol pathways [3], has allowed us to engineer several enzymes and regulatory proteins involved in the isobutanol pathways in either compartment, boosting enzymatic activity by as much as 400%. Thus, we have demonstrated the use of this new technology to accelerate the development of strains and enzymes to boost BCHA production in mitochondria and the cytosol. Future applications include combining the biosensor with optogenetic regulation of BCHA biosynthesis for closed-loop dynamic control of this pathway, and using the biosensor to empower systems biology studies for gene discovery, enzyme evolving, and enzyme engineering to boost BCHA production.

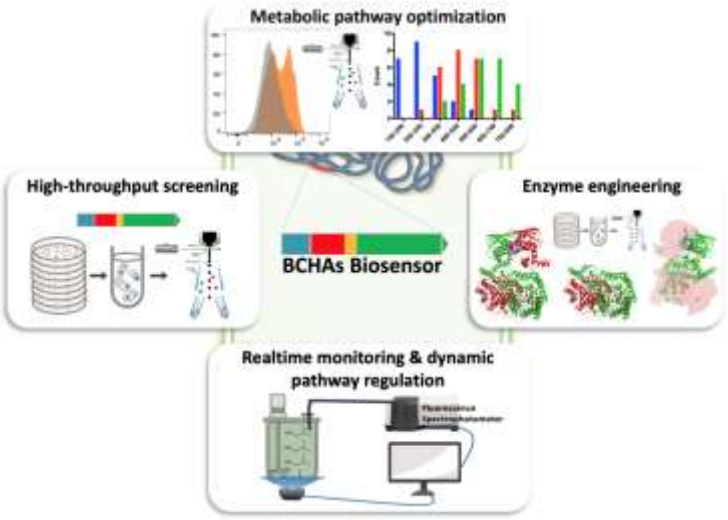

Figure 1 – The versatile applications of the branched-chain higher alcohols (BCHAs)

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