

Biochemical Production from Lignocellulose by CRISPR EnAbleD Trackable genome Engineering (CREATE)

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Project Goals: We are developing tools for rationally-based protein engineering which will allow multiple modifications from the single protein to the whole pathway levels. CRISPR EnAbleD Trackable genome Engineering (CREATE) is a new genome-scale engineering strategy that couples the high efficiency of CRISPR editing with the massively multiplexed rational design offered by parallel oligomer synthesis. Here we used CREATE to construct a 3HP producing strain which can utilize hemicellulose-based hydrolysate as carbon source.

Metabolic engineering has expanded from a focus on designs requiring a small number of genetic modifications to increasingly complex designs driven by advances in genome-scale engineering technologies. CRISPR EnAbleD Trackable genome Engineering (CREATE) is a new genome-scale engineering strategy that couples the high efficiency of CRISPR editing with the massively multiplexed rational design offered by parallel oligomer synthesis (1). Here we used CREATE to construct a 3HP producing strain which can utilize hemicellulose-based hydrolysate as carbon source.

After pretreatment of corn stover or corn stalk, glucose and xylose are the main carbon sources in these lignocellulose hydrolysate. However, *Escherichia coli* can not co-utilize glucose and xylose at the same time. To solve the problem, we used CRISPR editing method to delete the *ptsHI* genes. As a result, the strain *E. coli* BG_{3HP}, Δ *ptsHI* can utilize glucose and xylose simultaneously, but the glucose consumption rate was also decreased by the deletion of *ptsHI*. To enhance the glucose consumption rate, we used RBS calculator to design a RBS library for *glk* gene that is important gene in another glucose transport system (2). Furthermore, we used CREATE strategy to construct this library and did the selection. As a result, one of the library strains, BG_{3HP}, Δ *ptsHI*, *glk*^{up-7}, can grow faster than others.

During the pretreatment of corn stover or corn stalk, side-reaction products (furfural, 5-hydroxymethylfurfural, formate, acetate, and soluble lignin products) are formed. Furfural (dehydration product of pentose sugars) is widely regarded as one of the most important inhibitors. To enhance the tolerance of furfural, we used CREATE strategy to construct the library targeting on 7 transcription and translation genes and 10 high level regulators, and did the selection for furfural tolerance. After sequencing of positive mutant, several mutant of *rpoD*, *crp*, and *nusA* can grow faster than the control strain with 2 g/L furfural. We used one of the positive mutant of *rpoD* strain as host strain, and introduced 3HP pathway into this positive mutant. As a result, the strain BG_{3HP}, $\Delta ptsHI$, *glkUp-7*, *rpoD-2*, psmart-J23119-Camcr-ydfG, can generate 5 g/L 3HP in the flask fermentation with sugar mixture media containing 2 g/L furfural, while the control strain can only synthesis 0.5 g/L 3HP under same conditions.

Reference

- 1 Andrew Garst et al., Nature Biotechnology (2016), under review.
- 2 Iman Farasat et al., Molecular Systems Biology (2014) 10: 731.

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