

Global Catalog of the Transcriptional Response to Lignocellulosic Biomass-derived Inhibitors in *Escherichia coli* Identifies Promoters for Synthetic Engineering of Biofuel Microbes

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**Project Goals: Knowledge of the mechanisms by which microbes respond to lignocellulosic biomass-derived inhibitors will inform approaches to engineer biocatalysts that can efficiently convert biomass to biofuels. In this study, we identified *Escherichia coli* promoters responsive to inhibitors present in ammonia-pretreated corn stover hydrolysate in order to exploit their regulatory properties for native-signal expression programming in microbes. In addition to increased knowledge of the global effects of lignocellulosic inhibitors on microbial transcription, the data obtained expands the catalog of available synthetic biology parts available for rational engineering of microbes.**

A major challenge to efficient biological conversion of lignocellulosic hydrolysates to biofuels is the presence of toxic inhibitors (referred to here as lignotoxins) derived from biomass pretreatment. Knowledge of the mechanism by which microbes respond to lignotoxins (LTs) is crucial in order to engineer tolerant strains and increase biofuel yields. This study expands upon previous analysis of the global transcriptional response to LTs in *E. coli*<sup>1</sup> by identifying LT-specific promoters. Chromatin immunoprecipitation-sequencing (ChIPseq) assays, using antibodies directed against RNA polymerase subunits  $\sigma^{70}$ ,  $\sigma^S$ , or  $\sigma^N$ , were performed on cultures grown in the absence or presence of LTs, from the exponential, transition, and stationary growth phases. Differentially bound ChIP sites were identified and mapped to the nearest novel or published transcription start site (TSS). Direct effects on transcription were found by comparing to the RNAseq data<sup>1</sup> of the associated condition. Moreover, examination of promoter occupancy by housekeeping and alternative  $\sigma$  factors across the genome provide insight into the effect of LTs on RNA polymerase composition and global effects on transcription. Identification of LT-specific promoters can enable engineering of microbial gene-induction systems that naturally respond to hydrolysate components and expand the microbial promoter catalog and associated regulatory elements for use in synthetic biology.

## References

1. Keating, D.H., et al., *Aromatic inhibitors derived from ammonia-pretreated lignocellulose hinder bacterial ethanologensis by activating regulatory circuits controlling inhibitor efflux and detoxification*. *Frontiers in Microbiology*, 2014. **5**:402, DOI: 10.3389/fmicb.2014.00402.

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