Quantitative-trait loci (QTL) mapping: a novel method for dissecting the genetic basis of complex phenotypes in bacteria

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Project Goals: The Center for Bioenergy Innovation (CBI) vision is *to accelerate domestication of bioenergy-relevant, non-model plants and microbes to enable high-impact innovations at multiple points in the bioenergy supply chain.* CBI addresses strategic barriers to the current bioeconomy in the areas of 1) high-yielding, robust feedstocks, 2) lower capital and processing costs via consolidated bioprocessing (CBP) to specialty biofuels, and 3) methods to create valuable byproducts from the lignin. CBI will identify and utilize key plant genes for growth, composition, and sustainability phenotypes as a means of achieving lower feedstock costs, focusing on poplar and switchgrass. We will convert these feedstocks to biofuels using CBP with cotreatment at high rates, titers and yield in combination with catalytic upgrading into drop-in hydrocarbon fuel blendstocks.

Most phenotypes in bacteria are dictated by multiple loci spread across the entire chromosome which have varying magnitudes of effects. However, despite rapid increase in the number of sequenced bacterial genomes, our ability to decipher the genetic architecture of such complex traits has lagged far behind the ability to gather genomic data. Assigning genotypes to phenotypes is of great importance in order to achieve a highly complete picture of metabolic, regulatory and signalling networks that would accelerate rational engineering of cell functions in nonmodel microbes.

QTL mapping is a powerful technique in eukaryotic genetics for identifying genetic loci that affect a phenotype of interest. However, the potential of this method for gene discovery in bacteria has remained unexplored because it traditionally relies on sexual recombination to break linkages between genetic variants. In this study, our approach uses genome shuffling by protoplast fusion to mimic the effects of sexual recombination in bacteria. Using various *Bacillus subtilis* strains as parents, we showed that genome shuffling produces multiple random recombination events throughout the chromosome creating mosaic genomes with unique recombination landscapes suitable for QTL mapping. We developed an integrated computational workflow for bacterial QTL mapping and through recursive protoplast fusion we constructed a QTL population of highly recombinant progeny that can be phenotypically screened in a high-throughput manner to identify causal genetic variants. Using several phenotypic assays, we demonstrated the power of bacterial QTL mapping to link phenotypes to genotypes at the level of biosynthetic pathways and even subgene regions. Furthermore, we developed experimental methods for protoplast fusion in *Clostridium thermocellum* and showed that genome shuffling between *C. thermocellum* strains yields genome-wide recombination patterns. Our work sets a

platform for studying the genetic architecture of complex phenotypes in model and non-model bacteria providing knowledge that will facilitate rapid design for biotechnology applications.

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