487a Genomics Tools for Elucidating the Function of Trait Conferring Genes

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We have developed a variety of functional genomics tools useful for the identification and characterization of trait conferring genes in prokaryotes. These include: a series of broad host range vectors for library screening, a system for transposon-based insertional mutagenesis that produces greater than 105 mutants, a unique promoter probe reporter, that provides for both positive and negative growth selections and allows for the identification of promoters active only under particular conditions, and a DNA micro-array based method for identifying genome-wide, quantitative growth phenotypes corresponding to increased dosage effects. In combination, these tools allow for an integrated approach for engineering microbial phenotypes that builds upon natural evolutionary mechanisms involving increased gene dosage, mutation, or disruption. Towards this end, we have applied these tools to i) identify E. coli genes that improve E. coli growth in minimal media, ii) improve understanding of genetic factors influencing the biofilm phenotype of E. coli (including several genes that increase biofilm growth rates) and iii) identify E. coli genes that affect tolerance to organic-acids that are the focus of bio-refining efforts. Separately, these tools have been applied to improve understanding of the evolution of antibiotic resistance in the clinical pathogen Pseudomonas aeruginosa. Combined, these tools and methods should prove useful in future efforts to further understanding and engineering of useful or interesting phenotypes.