487e A High-Throughput Screen for Poly-3-Hydroxybutyrate for Inverse Metabolic Engineering of Recombinant *Escherichia Coli* and *Synechocystis* Pcc 6803

Keith E. Tyo, Hang Zhou, Hal S. Alper, and Gregory Stephanopoulos

The systems biology paradigm aims at understanding the behavior of complex cellular networks. Inverse metabolic engineering (IME) is an experimental approach to identifying key nodes in such networks that affect a given phenotype through random perturbation and screening. IME serves both the purpose of engineering improved strains *and* uncovering the important metabolic and regulatory events that affect the phenotype. Experimental tools have been developed to generate interesting libraries and identify the genotype of improved strains. An integral component of these tools is high throughput screening methods which can be used to efficiently probe the library diversity. To this end, we have developed a fluorescence-activated cell sorting (FACS)-based staining method for screening large combinatorial libraries and applied it to the identification of high poly-3-hydroxybutyrate (PHB)-accumulating clones in *E. coli* and *Synechocystis* PCC 6803.

Nile red is a well-known stain for PHB granules. Despite this, predictive fluorescence measurements of PHB *in vivo* have only been shown using lethal cell permeabilization methods. Here, we demonstrate a method for quantitative nile red staining of PHB granules in *viable* cells. Exploiting techniques from competent cell protocols, living cells can be prepared that readily take up the stain. We have developed this method for recombinant *E. coli* and *Synechocystis PCC 6803*. This screen has the capability of identifying mutants with enhanced PHB accumulation properties from large combinatorial libraries. As well, this novel staining approach could be generally applicable for transporting membrane-impermeable stains into the cytoplasm. We will present data on the development of the screen and its application to the selection of over-expressing clones.