436h Construction of a Genetic Toggle Switch for Polyhydroxyalkanoate Production in *Escherichia Coli*

Thomas B. Causey, Stephanie Portle, Ka-Yiu San, Nikos V. Mantzaris, and George N. Bennett

The manifestation of cellular phenotype is dependent on the genetic architecture of the cell and the extra-cellular milieu. Environmental conditions and complex interactions among cells of a population control gene expression through intra-connected regulatory networks. Variability among cells of an isogenic population exists due to two factors. First, when cell division occurs the contents are distributed unevenly. Second, regulatory compounds are present at very low concentrations within the cell. Random fluctuations in the concentration of regulatory compounds affect the reaction rates of the regulated processes. Therefore, regulation of gene expression and cell population heterogeneity influence the adaptation dynamics of a cellular population to environmental challenges. The design of gene switching networks is a powerful metabolic tool that allows the transient control of competing metabolic networks. Recently, Gardner et al. (*Nature*. 2000. 403:339-342) described a genetic toggle network (pTAK117) that employs two regulable promoters that are inhibited by a repressor transcribed from the opposite promoter. In order to examine the kinetic behavior of the network, pTAK117 was modified to include an *ssrA* tagged green fluorescent protein (GFP) transcribed from the IPTG inducible promoter P_{trc}. In addition, the *Discosoma* sp. red fluorescent protein (DsRed), obtained from the pDsRed-Express vector (BD Biosciences), was placed under the control of a temperature sensitive promoter (P_{s1con}) controlled by the phage lambda CI protein. The ability of the toggle switch to yield different gene expression ratios was investigated in batch experiments and fluorescence was determined using a FACScan flow cytometer (BD Biosciences). Ultimately the genetic toggle network will be used to control the supply of substrates for polyhydroxyalkanoate (PHA) block copolymer monomer formation in recombinant *Escherichia coli*. This genetic control will provide flexibility for production of PHAs in *E. coli* with a broad range of physical properties.