Quorum-Coupled Bacteriocin Release: Engineering a Champion

Thane A.L. Kubik, Kevin R. McLeod, Christian Jacob, Sonja Georgijevic
University of Calgary

Microorganisms use pheromones to monitor their own population density as well as to detect and interact with other microbial species in a process known as quorum sensing. For instance, bacteria can disrupt the pheromone signals of other competing bacteria, effectively preventing their proliferation. In a similar sense, we, the University of Calgary's iGEM 2008 WetWare team, have exploited the natural communication systems involving Autoinducer-1 (AI-1) from *Vibrio fischeri* and Autoinducer-2 (AI-2) from *Vibrio harveyi*, to create a model biosensor system in *Escherichia coli*. We have engineered the genetic circuits necessary for the production of these pheromones into two populations of *E. coli* (termed Bad guy #1 and Bad guy #2, as per their respective Autoinducer). In addition, our third population of *E. coli* (termed Champion cell) acts as a biosensor by receiving these signal inputs and subsequently initiating transcription of specific *E. coli*-targeted bacteriocins (*i.e.* colicins). The presence of AI-1 induces the Champion to produce a colicin to which Bad guy #1 is susceptible, but to which Bad guy #2 is resistant, and vice-versa for AI-2. An additional aspect of our system is the ability of the Champion cell to report the presence of each specific Bad guy by producing a specific fluorescent protein (*i.e.* either red or green fluorescent protein) in tandem with the specific colicin as determined by the presence of either AI-1 or AI-2. We constructed this system using the molecular cloning methods used in the undergraduate International Genetically Engineered Machine (iGEM) competition. While the *Vibrio fischeri* components of our system were obtained as standardized parts from the iGEM Registry, we cloned and standardized all parts of *Vibrio harveyi* AI-2 quorum sensing system. All of the standardized parts are flanked by specific restriction endonuclease sites (*i.e.* iGEM BioBricks), thereby allowing for a simple and iterative directional cloning strategy for the construction of the necessary genetic circuits.