Biological manufacturing processes face certain barriers, or “borders”, that may be broken down using synthetic biology. For example, cell membranes act as a physical border to efficient product recovery. To break this barrier, BioBricks were constructed for secretion-based recovery of proteins and polyhydroxyalkanoates (PHAs). This library could also be used for secretion of other proteins.

The current inability to use BioBrick parts in organisms other than E. coli represents a second border to efficient cellular production. Organisms like Synechocystis PCC6803, Rhodobacter sphaeroides, and Pseudomonas putida possess unique potential for manufacturing. BioBrick-compatible broad-host-range (BHR) vectors would help break down this barrier by facilitating BioBrick use in multiple organisms.

Downstream processing is typically the most costly aspect in cellular manufacturing. Secretion-based recovery methods focus on compounds, like proteins and PHAs, were investigated as an alternative. PHAs are microbially-accumulated bioplastics (Fig. 3). Proteins, called phasins, bind to PHA granules. Fusion of a signal peptide to phasin promotes protein export. Binding of phasin to PHA may result in secretion of the PHA-phasin-signal peptide complex (Fig. 4). For this project, GFP secretion was studied in parallel to provide insight on secretion mechanisms.

Many parts were needed in this study (Fig. 5), including Silver fusion BioBricks of 5 signal peptides, phasin, and GFP sequences. The signal peptides represent the most common secretion mechanisms. Forty-nine BioBricks were constructed and sequenced. Figure 6 shows a phasin secretion BioBrick device.

A new Silver fusion GFP BioBrick (excitation 395 nm, emission 509 nm) was created using a mutant GFP that is 45 times brighter than wild-type GFP. This part is functional, as seen in Figures 7 and 8.

Current BioBrick vectors are designed for use in E. coli. Other organisms, like photosynthetic cyanobacteria and R. sphaeroides, possess desirable characteristics for biomanufacturing. We would like to extend the use of secretion constructs and other BioBrick parts to these strains by constructing a BioBrick-compatible BHR vector. Vector pCPP33 is functional in P. putida and E. coli. We worked to convert pCPP33 to the BioBrick format. Next, triparental mating and selection were used to assess whether this vector is also functional in R. Sphaeroides and Synechocystis PCC6803.

The goal of this project was to break down BioBrick borders by constructing devices for secretion-based product recovery and for expression of BioBricks in multiple organisms. Some achievements include:

- 49 BioBricks were constructed and sequenced
- Phasin secretion was observed
- Broad-host vector pCPP33 was expressed in R. sphaeroides, P. putida, and Synechocystis PCC6803

Future work includes further testing of secretion constructs and more characterization of BHR vectors.

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