

Efficient Systems for Monitoring Polyhydroxybutyrate Production in Microorganisms

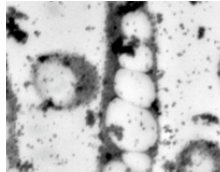


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PHB or not PHB... That is the question

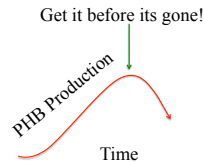
The Utah State University iGEM 2008 project is focused on creating an efficient system for producing and monitoring the production of polyhydroxyalkanoates (PHAs) in microorganisms. The goal of this research is to develop and optimize a method, using fluorescent proteins, for the detection of maximum product yield of polyhydroxybutyrate (PHB, a bioplastic) in recombinant *E. coli* and *R. eutropha*. To accomplish this, we are working to use GFP to identify when the operon containing the PHB biosynthetic genes are being expressed and find the promoter regions responsible.



Intracellular PHB granules.

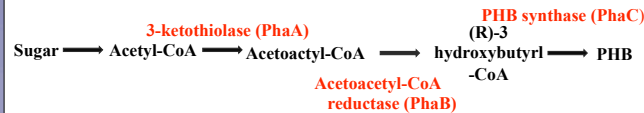
The problem with PHB

Polyhydroxybutyrate (PHB) is a naturally occurring biodegradable thermoplastic, with material properties comparable to petrochemically-derived plastics. The high cost of PHB acts as the primary factor preventing industrial scale PHB production and commercialization. Polymer extraction can be optimized and overall costs reduced with a rapid biosensor system to detect the point at which PHB production is at its maximum.

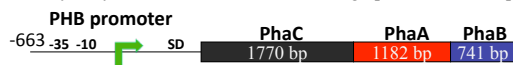


PHB Metabolic Pathways

The metabolic pathway for PHB accumulation in *R. eutropha* involves three biosynthetic enzymes, PhaA, PhaB, and PhaC.



These key enzymes are found on the following operon in *R. eutropha*:



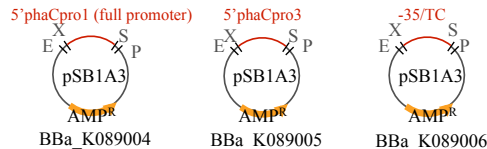
Results

Detection of Intracellular PHB

Gas chromatography was successfully used to detect PHB accumulation in recombinant *E. coli* harboring the PHB biosynthetic genes from *R. eutropha*.

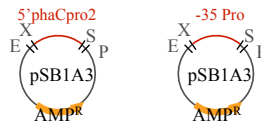
Successfully Created BioBricks

These bioBricks are portions of the PHB promoter region:

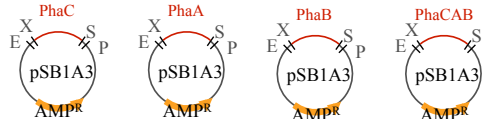


Biobricks In Progress

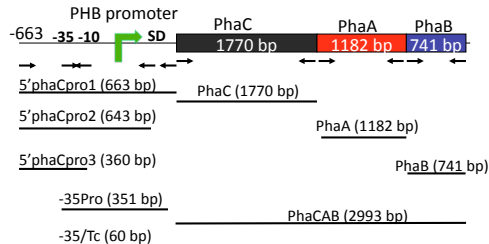
These bioBricks are portions of the PHB promoter region:



These bioBricks are the key biosynthetic enzymes for PHB accumulation:



Sites on the PHB biosynthetic operon where bioBricks are from:



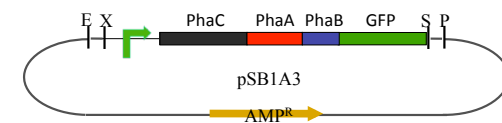
GFP Correlation

PHB Optimization

To catch PHB production at its maximum, we want to have a simple, quantifiable correlation with GFP expression. There are three ideas to do this:

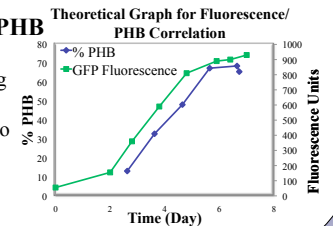
- Plasmid containing PHB biosynthetic and GFP genes in same operon
- Plasmid containing both operons but at different sites in the plasmid
- Two plasmids, each containing one operon

The Goal: Final PHB operon with GFP as reporter



Correlating GFP with PHB

Large PHB promoter region may not be necessary. Using GFP we will look at the different promoter regions to determine which parts are needed for expression.



Conclusions

Polyhydroxybutyrate has great potential as a renewable, biodegradable plastic. Currently, extraction methods of PHB are more costly than production methods for petrochemically-derived plastics. Therefore it is necessary to design a process with higher extraction efficiency. Using a genetic marker, such as GFP, to identify optimal extraction time will reduce these costs by providing maximum PHB yields.

The effective use of GFP as an expression marker will depend on sufficient understanding of both the PHB promoter and PhaCAB cassette. The USU iGEM team has made progress in both of these areas and has created three BioBricks from the PHB promoter region.