Cellfifuge: Self inducing, Auto-aggregating Bacteria

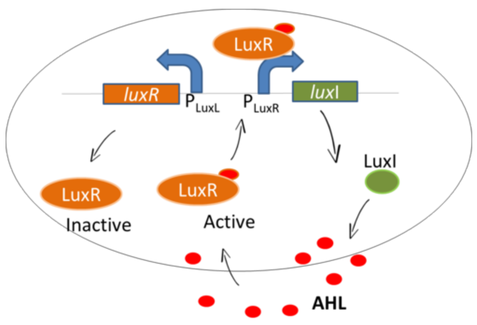
IISc iGEM Team, 2016

Introduction

The biotechnology industry has spread its roots to every niche of our lifestyle, including food, medicine, and bioplastics. The industry underwent rapid progress after the 70's, going from one FDA approved recombinant therapy in 1982 to 151 such therapies in 2009. Yet it has not been able to reach its full potential, it is largely economically inaccessible. The IISc iGEM team thus decided to try and cut the manufacturing costs of these goods by tinkering with the already established processes in the industry. Some of the processes we aimed at were the monitoring of growth of the culture before induction, induction of bacterial culture for protein production and centrifuging large volumes of bacterial culture. These processes make up for about 30% of the final cost in many cases. We intend to genetically modify bacteria such that,

- The bacteria autoinduce protein production such that yield is maximized.
- They self aggregate, after nutrient content in the medium is diminished sufficiently.

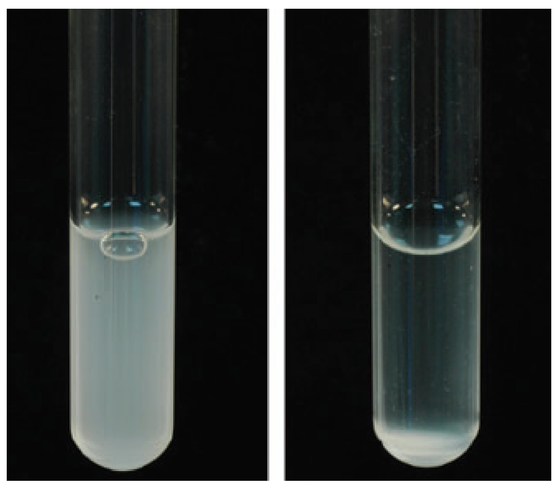
Self induction



Lux system

- **Motivation** – Optimize **protein synthesis rate**
- **Challenge** – Trade off between **division** (leads to higher density) and **synthesis** at each instant
- **Solution** – Density dependent rates of division and synthesis, by **quorum sensing**, experimental optimization
- Two phases
  - **Rapid division, slow synthesis**
  - **Slow division, rapid synthesis**
- Parameters such as division, synthesis rates in both phases, density at transition, etc, can be varied

Auto-aggregation



Aggregation by Ag43 vs Negative Control

- System switched on by **diauxic shift**.
- Arabinose inducible and glucose repressed promoter pBAD/araC regulates the synthesis of autoaggregation surface protein – Ag43.
- Two major phases:
  - **Glucose utilization** – Division of cells and synthesis of protein of interest.
  - **Arabinose utilization on glucose exhaustion** – Over-expression of aggregation protein.

Methodology

The methodology for making parts primarily involves two methods:

1. Gibson Assembly:

- Design and generate all fragments with overlaps on both ends, using PCR.
- Use enzyme cocktail to ligate it and make a plasmid.

2. Restriction Cloning:

- Cut relevant fragments out of parts interested in, using restriction digestion.
- Cleave plasmid backbone with appropriate restriction enzyme at location interested.
- Ligate the cut fragment into the cleaved backbone.

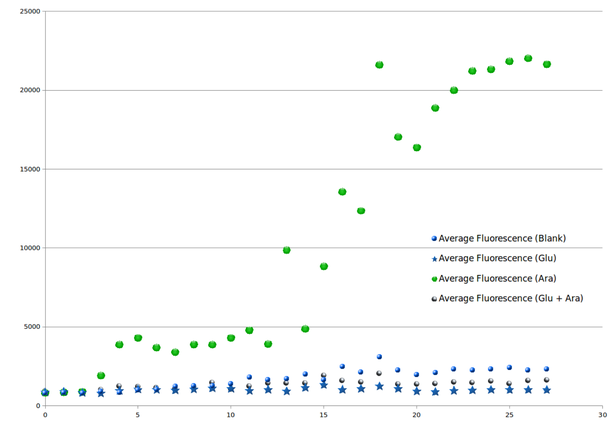
The parts are transformed into *E. coli*, selected using appropriate antibiotic, and verified by restriction digestion. Transformed *E. coli* are then used for experiments.

Interlab Study

- One experiment performed in several labs
- **This year's theme –** Absolute units for fluorescence measurements
- **Method –** Relating relative fluorescence under standard conditions to a chemical standard (FITC) and protein copy number
- **Our contribution –** Data submitted, pointed out errors in protocol.

Parts Improved

pBAD/araC Repressed by Glucose



pBAD/araC Repression

- Demonstrated repression by glucose, of this part, for the first time.
- Verified induction by arabinose.

Quorum Sensing

- Quroum Sensing (QS) – bacterial system with native inducer synthesis
- **Majority of QS parts –** External source of inducer required
- **Plan**
  - Determining time evolution of a recent QS part with native source of inducer
  - Constructing a QS part of our design with the native arrangement of sequences

Results/Conclusions

- Aggregation observed in cells expressing Ag43.
- Repression of pBAD/araC by glucose proved, induction by arabinose verified.
- PCR products required to assemble new Ag43 constructs - 6X His tagged and sfGFP fusion generated.

We still have to do experiments on our Quorum Sensing parts. Then we will transform the bacteria with both the 'Self Induction' and 'Auto-aggregation' part, to generate a finished final product.

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