AgRePaper and E.coli Ink Project
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Introduction
Cellulose is used as raw material for paper, so our team experimented various ways to increase the amount of cellulose produced by agrobacterium and using it to make papers. For this we developed the different parts to insert into the system of Agrobacterium. Among them were the genes used for expression of the Curdlan. Similarly, genetic parts in order to increase the expression of the cellulose, along with the Agrobacterium type Binary vector were also developed. We also worked on recycling of the produced paper by degrading the cellulose to D-Glucose using various enzymes. We worked for the preparation of the biological ink using the sperm whale’s cells by genetically modification to increase amount of myoglobin. Then, we observed the change on the color of the product by altering the formation of myoglobin and the production amount of myoglobin with the insertion of T7 promoter to the cell system.

**AgRePaper Project**

**What’s Agrobacterium tumefaciens?**

Fig.1 A:Agrobacterium tumefaciens has 2 Chromosome and 2 plasmid, Circular and Liner Chromosome , T1 plasmid and At plasmid. B: Scanning electron microscopy reveals several Agrobacterium tumefaciens as they begin to infect a carrot cell. In the process, the bacteria’s genetic material will enter the plant cell. Source: A. G. Mathyssen, K. F. Holmes, R.H.G. Gaullia

**Export Glucan form Agrobacterium.**

Fig.2 A: We dissolved curdlan in water and dried up it. As a result it became the Gel. Therefore, we suggest curdlan would be in place of the role of the glue that connects the cellulose. B: We confirmed Agrobacterium exported glucan. Agrobacterium produced something like white films. We mixed and centrifuged it from Agrobacterium with congo-red liquid. As a result, we confirmed red pellet. But, we didn’t confirm pellet without a mixed it from Agrobacterium with congo-red liquid. As a result, we confirmed red pellet. Therefore, we suggest curdlan would be in place of the role of glucan. Fig.3 A:Synthesis system of Cellulose and Curdlan. Degradation system of Cellulose.

**Synthesis system of Cellulose and Curdlan.**

Cellulose → Curdlan → UDP-Glc-urone

**Degradation system of Cellulose.**

Fig.3 B: Scaning electron microscopy reveals several Agrobacterium tumefaciens as they begin to infect a carrot cell. In the process, the bacteria’s genetic material will enter the plant cell. Source: A. G. Mathyssen, K. F. Holmes, R.H.G. Gaullia

**E.coli Ink Project**

**What’s myoglobin?**

We are planning to use myoglobin as ink. We are also trying to change the color. This Part is translation unit including high efficiency RBS. And that is optimized in E.coli.

**What's Binary Vector?**

We named BbA_K1044006 “pBI107”. This plasmid length is about 9,000 bp. pBI107 is improved form of empty backbone vector obtained from pre-existing Binary Vector of E.coli and Agrobacterium. Selective with Kanamycin, there is possibility of reproduction by both E.coli and Agrobacterium. Colonies were detected and after 24 hours of culturing the colonies with inserted RFP device were confirmed by the colonies which were shining red. We determined the prefix and suffix of the Biobrick cloning site and inserted RFP device (BbA_J04450) into pH107 and cloned E.coli JM109. Then it was cultured in LB-Agar plate with 50 mg/ml kanamycin and IPTG at 37°C. After 4 hours of culturing, colonies were detected and after 24 hours of culturing the colonies with inserted RFP device were confirmed by the colonies which were shining red. From the above result, we found out the existence of prefixes and suffixs as the iGEM standard in pH107 and hence BioBrick was expressed with E.coli JM109.

**Discussion and Conclusions**

We are planning to produce cellulose and curdlan by using binary vector that is our team constructing. We construct the cassette with binary vector and promoter that is more expressing of cellulose and curdlan and then we combine cellulose with curdlan to create AgrePaper. Other hand we produce Cellulase to digestion of cellulose, finally cellulase digests AgroPaper to carbohydrate. We are also planning to change myoglobin’s color in addition to red. Example, pink, green, blue, etc. This chemical reaction was already published on paper. We want to use T7cassette to mass produce myoglobin and create E.coli ink.

**T7 cassette**

Plac  RBS  RNAP  ter  PT7  RBS  part  ter

Fig.7 We suggest this cassette named T7cassette. This cassette includes RNAP and T7promoter. You don’t worry about E.coli strain on using T7promoter.