INDUCTION AND KINETIC MEASUREMENT

After assembly of pZs proD sfGFP Ag43 construct was completed, it was co-transformed with pZE pBAD TEV Ag43 plasmid to conduct induction experiments. Before induction was held, heat release experiment was done with pZs proD sfGFP Ag43 construct and the result was unsatisfying. To choose a backbone, we got help from modelling and started to construct plasmids with different combinations of origins. By the time assemblies were done, we, first wanted to see the result of the induction with pZs proD sfGFP Ag43 + pZE pBAD TEV Ag43 including cells which is BL21 Marionette strain.

In order to facilitate the activity of TEV Protease, there is a protocol [1] which was optimized specifically to TEV Protease, also possessed by the lab we work in. However, since we wanted to show that our system works in intestine, and also want our cells grow and continue to divide as the TEV Protease cleaves the Insulin analog, we decided to simulate the conditions that as closely as to the real life conditions. Therefore, we created those conditions (or tried to create) with Intestine-like Buffer. There are several ways to simulate the conditions but it is very hard to make cells live *in vitro* in the conditions. Adjusting pH and adding the ions the gastrointestinal fluid contains were seemed enough after making some literature research [2].

Also, in order to see the cleavage while cells continue to divide, it was decided to prepare MOPS minimal media that is close in pH to the human gastric fluid's pH.

Essentially, single transformed pZs proD sfGFP Ag43 and Marionette pZs proD sfGFP Ag43 + pZE pBAD TEV Ag43 were tried at MOPS (pH 7.43, same with ileum), Tev Protease Reaction Buffer and Intestine-Like Buffer. Protocols were provided at Experiments page.

According to the first results Marionette pZs proD sfGFP Ag43 + pZE pBAD TEV Ag43 worked better at Intestine-Like Buffer but yield was low.

We moved to the kinetic measurements, which deals with RFU/OD600 values of the newly added backbones, pZA coupled with proD sfGFP Ag43, and pZs and pZA coupled with pBAD TEV Ag43.

Heat release was conducted to the sfGFP containing plasmids which indicated 2-fold difference between pZA and pZs.

BL21 Marionette - pZs proD sfGFP Ag43 + pZE pBAD TEV Ag43 - was again induced with L-arabinose. The data showed a slight better difference (1.5 fold) between induced and uninduced samples but we had to modify the conditions. Therefore, the transformations were completed, and we had four combinations of cells of Marionette strain.

When kinetic measurements of the single transformed pZs proD sfGFP Ag43 and pZA proD sfGFP Ag43 were completed (6 hours, RFU/OD600 every 30 minutes), there was a necessity to see how the system works at constitutive promoter fused protein + inducible promoter (araC) fused TEV protease. Therefore, by also modifying MOPS (pH to 8), we managed to start 16-24 hours kinetic measurements for 4 hour of each. One kinetic measurement of 16 hour was taken at pH 7.43 and one kinetic measurement of 16 hour was taken at pH 8 of MOPS. Lastly, we made a kinetic measurement of 24

hours was taken at pH 8. We used as a control group which we knew is working with double induction but we couldn't see the same pattern with our samples.

To mimic the intestinal conditions [3], we adjusted the settings of a shaker-incubator, and cells grown with LB, were transferred to Intestine-Like Buffer and incubated for 24 h, at 37 C - 40 C (temperature shifting in this interval).

Finally, we transformed an inducible plasmid (with atc) into cells containing our SCIA expressing plasmids (IDT3 and IDT7), we adjusted the cell number, collected the insulins with heat release and performed Western Blotting.

CHARACTERIZATION

For characterization, proD-sfGFP basic part from iGEM Registry (Bba_K1741014) was synthesized. To characterize this part, we cloned this part into pZs, pZa and pZe plasmids which they include pSC101, p15A and pBR322 origin. We characterize the part in all the level of expression, we measured and demonstrated the growth curve, RFU datas. DNA assembly was done via Gibson Assembly method and backbone was digested with compatible enzymes for part to be inserted.

PART IMPROVEMENT

Native Ag43 from registry, Bba_K3246007, was improved by deleting the first 160 amino acids of alpha subunit. Native Ag43 confers to cell to cell aggregation when it was expressed. To use this protein as autotransporter we have deleted the 160 amino acids and it conferred to no cell to cell aggregation and depict truncated form. By this way localized Ag43 proteins carried the protein of interest and was ready to release by TEV protease site presented in a fused way.

GROWTH & FLUORESCENCE MEASUREMENT

Purpose: Understanding the expression level of sfGFP with different ori in different strains

pZs sfGFP Ag43 and pZa sfGFP Ag43 plasmids were separately transformed into B strain (BL21 Marionette) cells and DH5α (MG1655) cells prior to these experiment.

Our protocol for Growth and Measurement was followed. Flourescence and OD_{600} values were measured.

Transformation to E.coli Nissle

Our ultimate goal is to make a probiotic system synthesizing single chain insulin analogs. Therefore, we tried to transform our constructs into E.coli Nissle. Then, we will take kinetic measurements from this strain and optimize it as well.

References

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