We attached the COX-2 (BBa_K2226003) and c-Myc (BBa_K2226002) promoters to the half of the reporter systems that yielded the best results, a method we took from the Peking iGEM team from 2015 (Peking iGEM Team 2015, 2015) (Rossi, Charlton, & Blau, 1994) (Rem, Galarneau, & Michnick, 2001). These reporter systems included the rapamycin-binding domain (FRB) fused to nLuc and the FK-506 binding protein 12 (FKBP) fused to cLuc. The fusion of the promoter to the reporter system made the nonspecific binding sites on the domains to become specific to the COX-2 (BBa_K2226003) and c-Myc (BBa_K2226004) proteins (Tomano & Nobuyuki, 2016). The newly specialized promoter-reporter system would then only come together in the presence of these two proteins. The creation of the FRB-rapamycin-FKBP complex induced the fusion of nLuc and cLuc, in turn generating light in the presence of rapamycin (Peking iGEM Team 2015, 2015).

Promoter Strength Determination

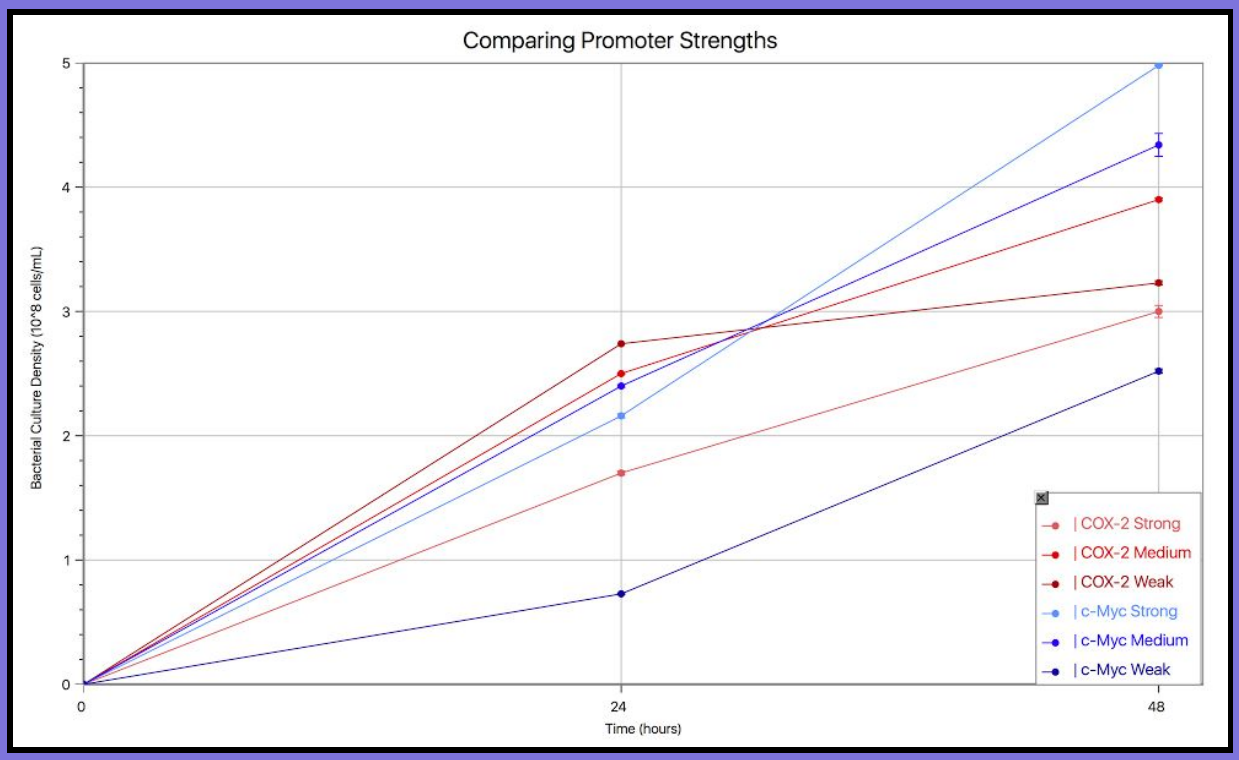

Fig. 1: The optical density measurements were converted to bacterial culture density (10^8 cells/mL) so that we could compare the *E.coli* growth in each solution. By graphing the bacterial culture density of the promoter and gene combinations against time, we were able to determine that the

COX-2 gene with the medium Anderson promoter strength, and the c-Myc gene with the strong Anderson promoter strength were the combinations that optimized *E.coli* growth.

To determine which Anderson promoter strength would optimize COX-2 and c-Myc gene growth, we combined these genes with Anderson promoters of three different strengths (JB23101, JB23106, JB23110). We then transformed them into competent cells to see which promoter strength yielded the most colonies for each respective gene. By counting colonies and performing optical density, we confirmed the best combination of gene and promoter to optimize protein production. As shown in Fig. 1, the JB23106 Anderson promoter combined with the COX-2 gene and the JB23110 Anderson promoter combined with the c-Myc gene yielded the most growth.

Construction of the Promoter-Reporter System

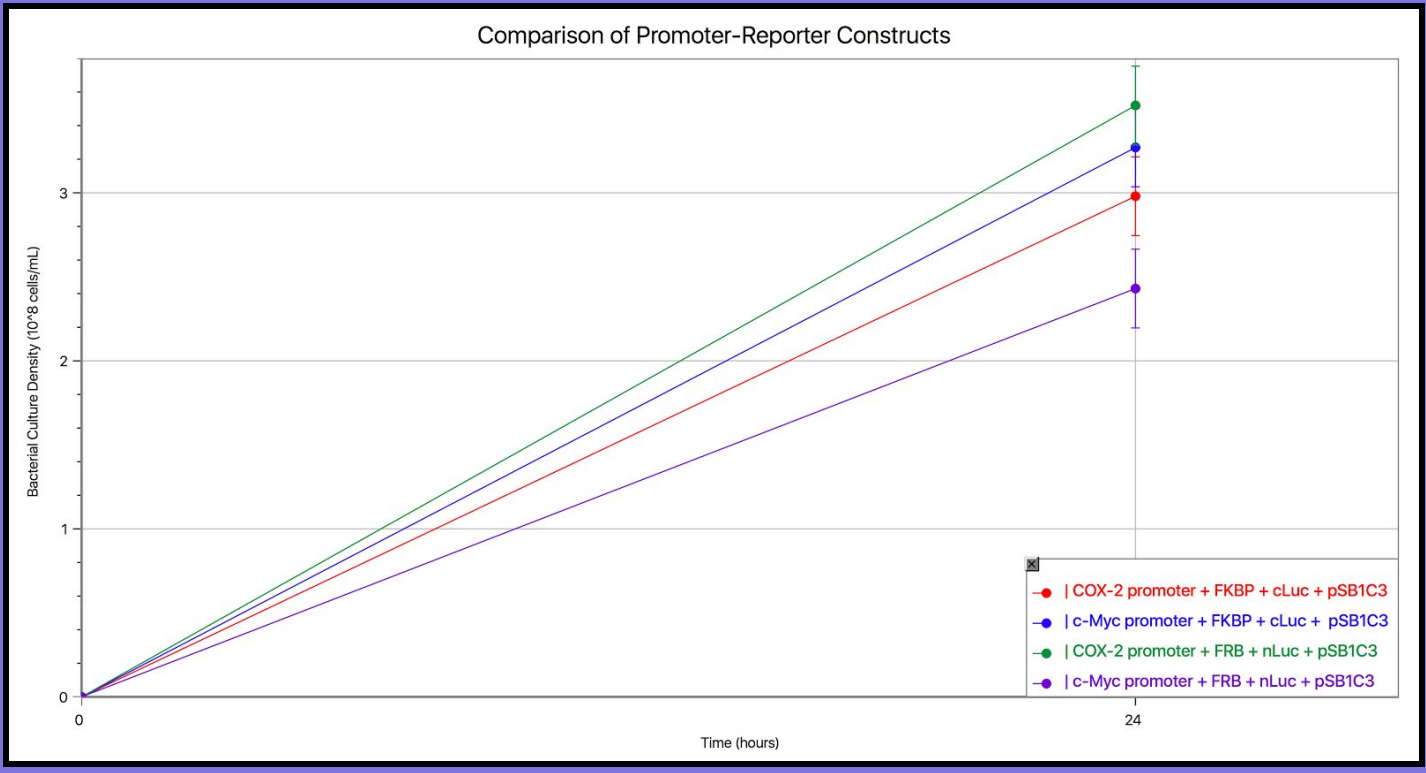

Fig. 2: The optical density measurements were converted to bacterial culture density (10^8 cells/mL) so that we could compare the *E.coli* growth in each solution. After comparing the various constructs, we concluded that the combinations of COX2+FRB+nLuc and c-Myc+FKBP+cLuc were optimal for our construct.

By binding the promoter systems to the non-specific binding proteins, the FRB FKBP domains become specific to the COX-2 and c-Myc proteins (Tomano et al., 2016). To determine the best possible combination of promoter and reporter, we combined the COX-2 and c-Myc promoters to the FRB-nLuc and FKBP-cLuc reporter systems and compared the combinations to see which yielded the most colonies. We did this by transforming the constructs into E. coli and plating them on petri dishes containing lysogeny broth (LB) and performing optical density tests for those cultured in solution. Fig. 2 shows our results from the optical density tests and proves that the combination of the COX-2 promoter with the FRB/nLuc reporter and and c-Myc promoter with the FKBP/cLuc reporter were the best combinations.

Proof of Concept

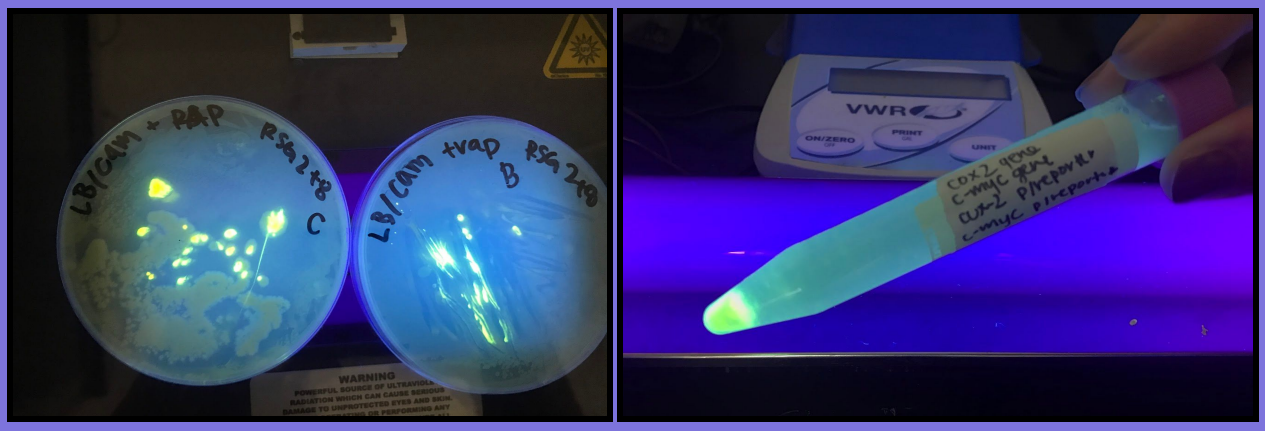

Fig. 3: Qualitative results demonstrate the constructs combining successfully in the presence of rapamycin when transformed in E. coli (a) E. coli on plates and (b) E. coli expanded in solution.

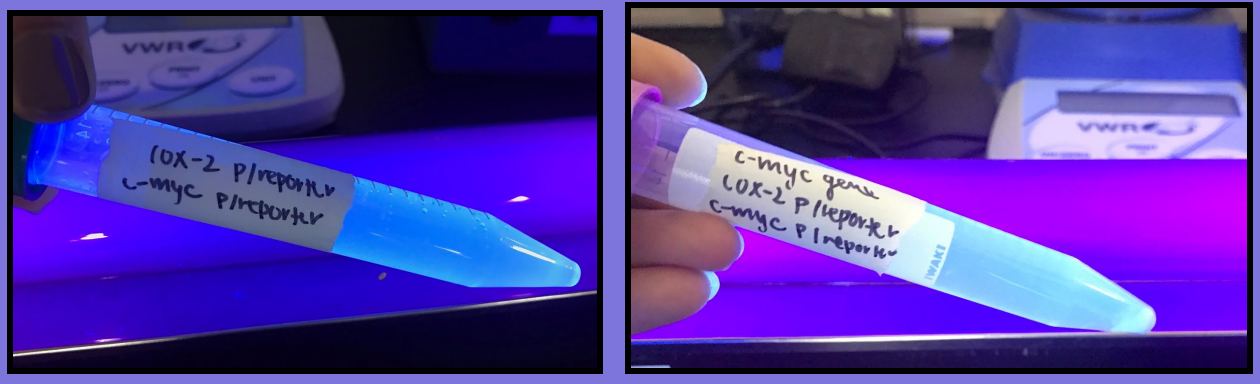

Fig. 4: results prove that neither constructs with only the promoter reporter system (left) nor constructs without rapamycin (right) induces glowing.

In order to test the constructs, we combined the COX-2 promoter-reporter construct with the c-Myc promoter-reporter construct in a solution with the COX-2 and c-Myc gene constructs to

model the conditions in the bodies of patients with CRC, where an excess of COX-2 and c-Myc protein is shed. As shown in Fig. 3 and Fig. 4 above, the combination of the COX-2 promoter-FRB-nLuc construct, c-Myc promoter-FKBP-cLuc construct in the presence of rapamycin and free-floating COX-2 and c-Myc protein successfully induced glowing.

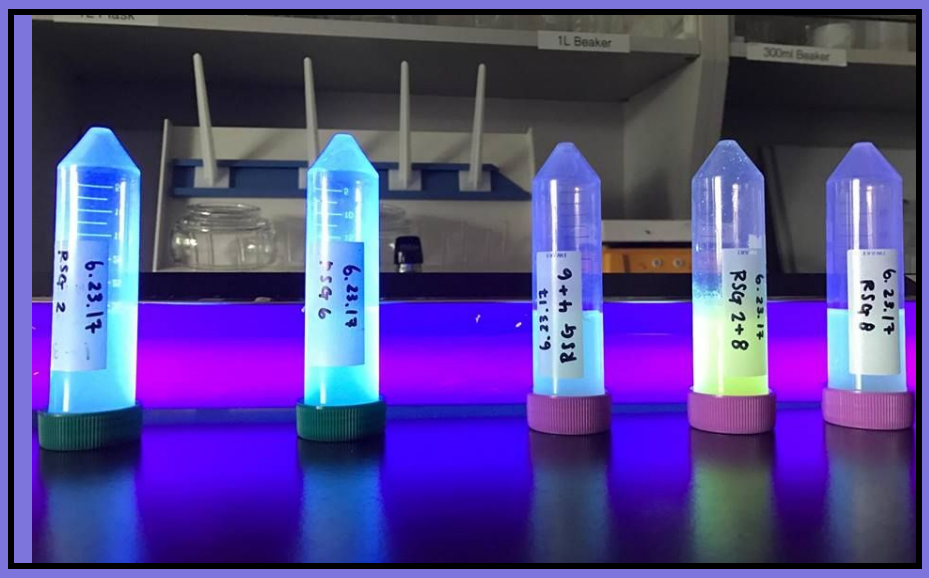

Fig. 5: Qualitative results indicate construct glowing only with all components present

In addition, to make sure the construct worked only with all components present (gene constructs, promoter-reporter system, and rapamycin) we tested out multiple combinations with and without rapamycin, as well as each individual construct/component with and without rapamycin. Fig. 5 shows that the promoters alone will not induce glowing, and glowing was induced only when all the components present.

Confirmation

Fig. 4: Quantitative results show that protein concentration of c-Myc construct is significantly less than the rest of the constructs.

The Bradford assay was used to analyze the protein concentrations of our constructs. We performed the Bradford assay on the 4 constructs that would come together and induce glowing: c-Myc gene, COX-2 gene, c-Myc promoter-reporter, and COX-2 promoter-reporter constructs. Fig. 4 shows that the protein concentrations of the c-Myc construct was significantly lower than the rest of the constructs, indicating that this construct is the limiting factor of our experiment.

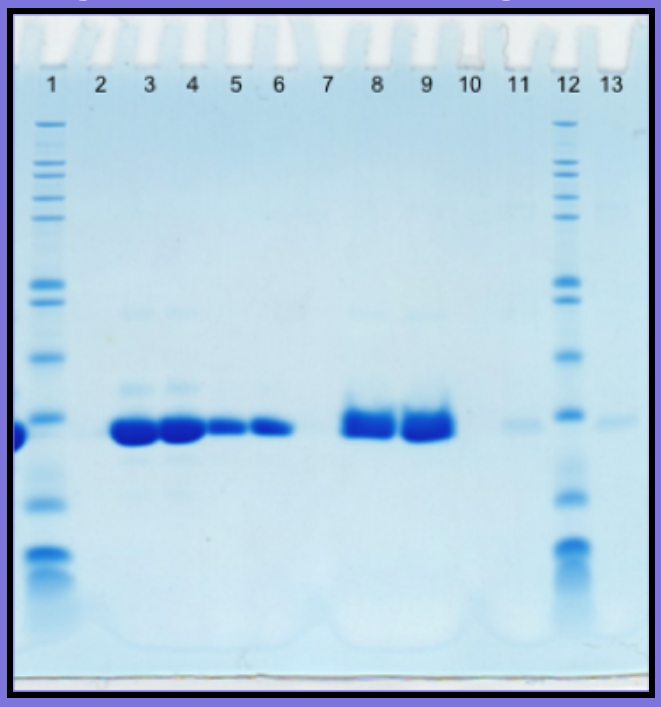

Fig. 7: Lane 1 &12: Protein markers
Lane 3 & 4: COX-2 – FRB– nLuc construct
Lane 5 & 6: COX-2 gene construct
Lane 8 & 9: c-Myc – FKBP –cLuc
Lane 11 & 13: c-Myc gene construct

We used a PAGE electrophoresis to measure the relative protein concentration and reconfirm this result. As intensity of the bands indicates, the c-Myc gene constructs in Lanes 11and 13 had the lowest relative protein concentration. This reinforced our beliefs that the c-Myc was indeed the limiting factor of our experiment.

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