DBTL(Design-Build-Test-Learn) cycle

We adopted the DBTL cycle and tried to make it reproducible by others by showing the basic parts and composite parts used and the lab notebook.

1. Design

We have designed a bacterium that can detect carbon monoxide. We introduce the carbon monoxide sensor protein CooA and the photoprotein luciferase into E. coli. CooA is a carbon monoxide-dependent transcriptional activator. CooA contains heme and activates CooA-dependent promoters when it binds CO. Firefly luciferase is an enzyme that catalyzes a bioluminescent reaction in the presence of Mg2+. We expect that the luciferase produced by E. coli and D-luciferin added to the culture medium will cause the following reaction.

$$\text{luciferase+luciferin+ATP} \leftrightarrow \text{luciferase·luciferyl-AMP+PPi}$$
$$\text{luciferase·luciferyl-AMP+O2} \rightarrow \text{luciferase+oxyluciferin+AMP+CO2+hv}$$

CooA, which is always produced when CO is nearby, binds to CO. pCooM has the gene for luciferase downstream, so we can add luciferin to the medium to output light. Since our E. coli is used as a CO detector, we need to adjust the CO sensitivity and make it emit light at the optimal CO concentration. We believe this can be achieved by using promoters with different strengths and RBS. For example, by combining two CooA-dependent promoters of different strengths and two RBSs of different strengths, we can construct four different patterns. In fact, we designed a part that combines pCooM, luciferase, and RBS of two different strengths, and registered it in the Parts Registry. (BBa_K3720002, BBa_K3720003) In addition, we used them to design the following plasmids. Details can be found in the methodology.

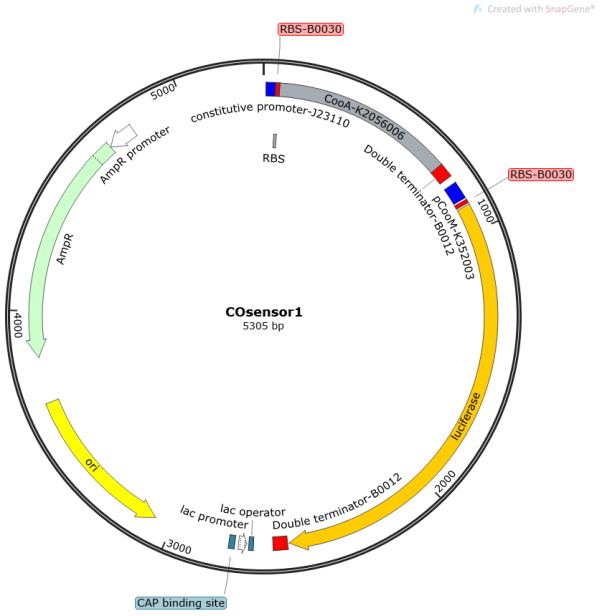


Fig. 1 Plasmid design (using strong RBS)

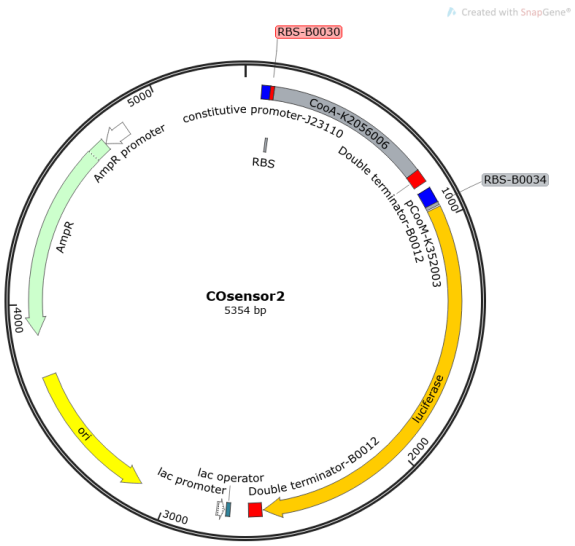


Fig. 2 Plasmid design (using weak RBS)

2. Build

Modeling was used to predict the relationship between carbon monoxide concentration and luciferase expression.

$$CO + CooA \underset{k_2}{\overset{k_1}{\rightleftharpoons}} CooAco$$

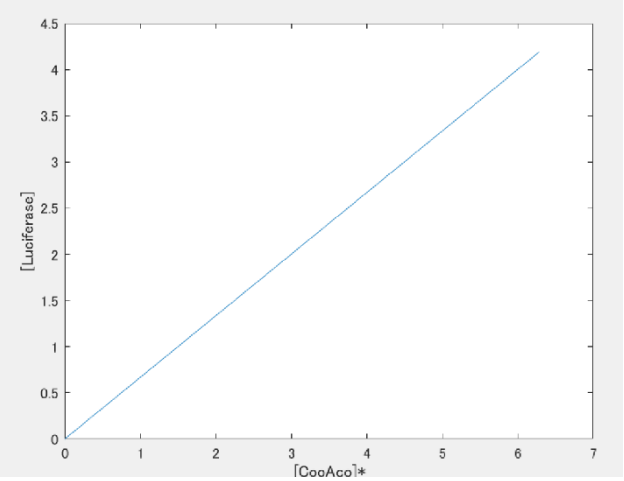
From the above reaction equation, the rate reaction equation for the formation of CooAco is as follows.

$$\frac{t[CooAco]}{dt} = k_1[CooA][CO] - k_2[CO]$$

Here, the concentration of luciferase is expressed by the following equation.

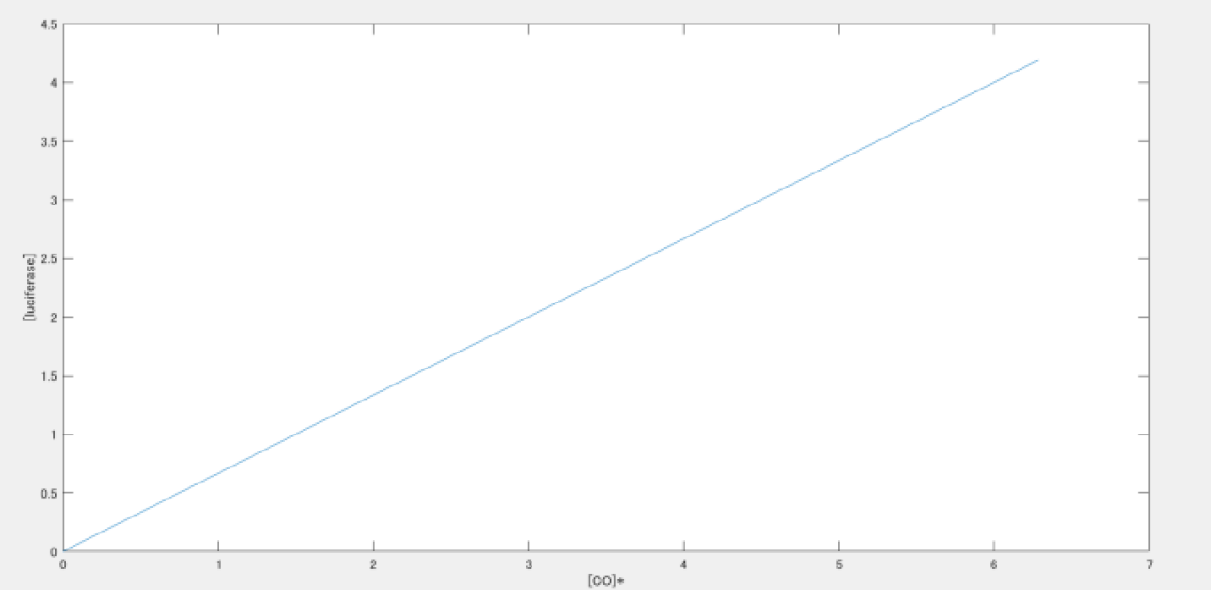
$$[luciferase] = \frac{\gamma[CooAco]^*}{\alpha} + \left(y_0 - \frac{\gamma[CooAco]^*}{\alpha}\right)e^{-\alpha t}$$

Assuming that t is very large, this equation leads to the following graph. This graph predicts the relationship between CooAco and luciferase expression levels at steady state.



CooA continues to be transcribed, but the amount of protein will eventually reach a steady state. After a sufficient amount of time, the amount of CooAco can be considered to depend on the CO concentration, and the following equation can be obtained.Then the following graph is derived.

$$[luciferase] = \frac{\varepsilon[CO]*}{\alpha} + \left(y_0 - \frac{\varepsilon[CO]*}{\alpha}\right)e^{-\alpha t}$$



A plasmid without insert was used, but the ligation was not going well due to the low concentration of insert. We also wondered if The enzyme in the Gibson Assembly Master Mix could have had a negative effect. Therefore, we purified the insert and further purified the Gibson Assembly. We faced the problem of difficult ligation because the length of the insert was too long for the vector, and we assembled a new DNA using only the Gibson Assembly. We speculate that the large number of parts to assemble reduces the success rate of the Gibson Assembly, and the Gibson assembly was done in two parts. Amplification of insert by PCR, checking whether the Gibson Assembly is successful by performing a blue-white screen and the use of longer vectors will be added to future experiments. More details can be found in the notebook and results.

3. Test

Next, we predicted that there would be a negative effect on growth based on the effect of the CooA gene being expressed normally and the effect of anaerobic conditions as the CO concentration increased. We thought that we could predict just the right balance between logistically suppressed growth and light intensity.

$$\frac{d}{dt}N = \left(1 - \frac{N}{K}\right) * N * r * f([CO]) * g([CooA], [luciferase])$$

$$f(X_{CO}) = \frac{1}{(1 + a_1 * X_{CO})}$$

$$g(X_{CooA}, Y_{luciferase}) = \frac{1}{\left(1 + \left(a_2 * X_{CooA} + a_3 * Y_{luciferase}\right)\right)}$$

We considered the negative effect of CO on growth at f, and the negative effect of the production of proteins that are not originally produced by E. coli at g. Here is the equation.

$$N = \frac{KN_0}{N_0 + (K - N_0)e^{\frac{-rt}{(1+a_1)(1+Z)}}}$$

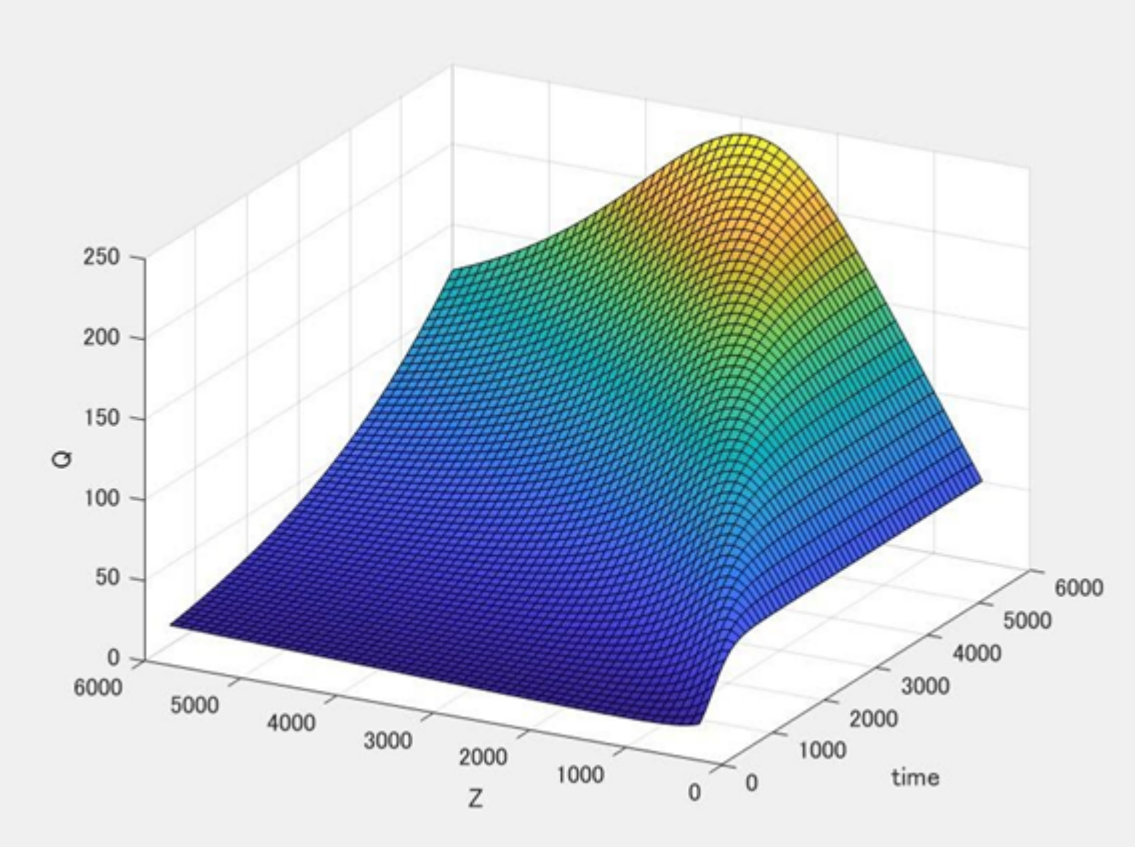
Z was defined as follows.

$$a_2 * X_{CooA} + a_3 * Y_{luciferase} = Z$$

Z represents the amount of light and how responsive it is as a sensor.

Q=NZ

Q represents the ability of the E. coli aggregate to act as a sensor and the degree of luminescence. We took the CO, Z, and Q axes and graphed them as shown below.



More details can be found on the modeling page.

We didn't have a suitable device to measure bioluminescence in our lab, so we tried to measure it by setting the LED intensity of the absorptiometer (PiCOEXPLORER) to 0. We think that it is possible to measure the luminescence of E. coli at the CO concentration by putting a sample, PiCOEXPLORER, and a CO concentration meter in a closed container and injecting CO into it. This method is possible because PiCOEXPLORER is compact and can be remotely controlled with a smartphone. As a result, the CO-injected sample appeared darker green, but PiCOEXPLORER was unable to measure luminescence.

4. Learn

We discussed with Hiroto Tanaka of the National Institute of Information and Communications Technology (NICT), Nagaura farm, Micro bio factory Inc, Figaro Engineering. The discussions with people from various backgrounds gave us a multifaceted perspective. For example, the discussion with Figaro Engineering gave us a hint on how to make the CO detection system work practically. We also went to a cattle barn to observe the actual site where the results would be used. Details can be found in the human practices.