Results

We were not able to confirm the success of the transformation. Electrophoresis results showed that the E. coli that we attempted to engineer appears to have the same plasmid as the vector. However, we observed a change in the color of the medium when we injected the E. coli with carbon monoxide.

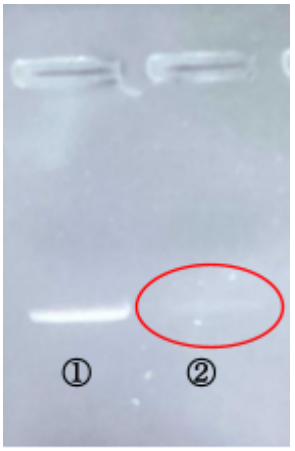
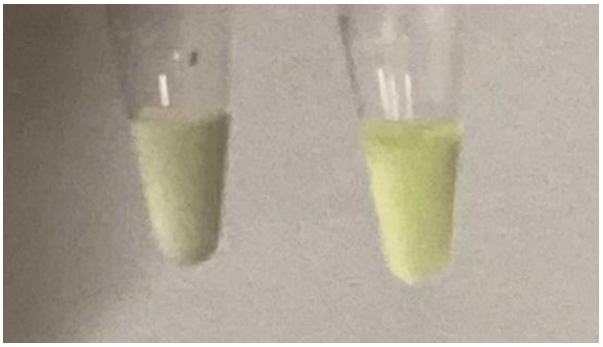


Fig. 1 Electrophoresis results▸
①Vector
②Plasmid DNA from engineered E. coli



◂Fig. 2 Color changes in bacterial culture
Left: LB medium+luciferin
Right: LB medium+luciferin+CO gas

Since there is no proper device in our lab for observing bioluminescence, we tried to measure the luminescence intensity using an absorption spectrophotometer (PiCOEXPLORER). However, we could not measure the luminescence of the CO-injected sample with the PiCOEXPLORER. Furthermore, we could not see the luminescence of the color-changed samples with the naked eye, even in the dark.

During our lab work, we have discussed the reasons why we could not confirm the success of the transformation and have taken solutions each time. The process is as follows.

Step 1
We tried to assemble the DNA by Gibson Assembly and ligation.
→The plasmid without insert was introduced.
Discussion)
・The ligation may not have gone well because the concentration of the insert was too low.
・The enzyme in the Gibson Assembly Master Mix may have had some adverse effects on ligation.

Step 2
We performed purification to increase the concentration of the insert. In addition, we performed another purification after Gibson Assembly.
→The plasmid without insert was introduced as before.
Discussion)
・The length of the insert is too long for the vector, hence ligation may not be possible.

Step 3

We ordered new DNA and tried to assemble the DNA using only Gibson Assembly without ligation.
→There were no colonies in the LB Ager Plates.
Discussion)
・Because of the large number of parts to be assembled, the success rate of Gibson Assembly may be low.

Step 4
We did the Gibson Assembly in two parts.
→The plasmid without insert was introduced as before.
Discussion)
・We still think that the concentration of the insert was too low.
・In fact, the transformation has been successful.

Next Step
Based on the failures so far, we will add the following tasks to the experiment.

・Amplify the insert by PCR.
・Check whether the Gibson Assembly is successful by performing a blue-white screen.
・Use longer vectors.