AUG 9 th

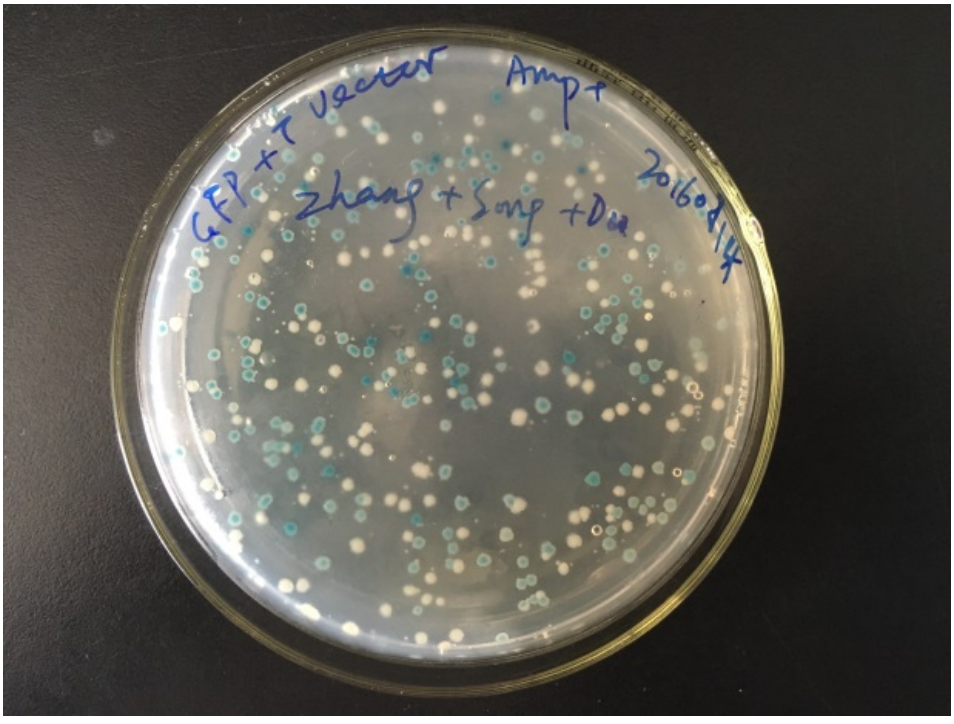
Since our GFP was located on the model vector, we had to clone and, also, to amplify it for our further process. Therefore, we amplified the GFP gene by Polymerase Chain Reaction and tested it by gel electrophoresis. Next, we strictly followed the commercialized gel-recycle kit to attain GFPs with correct sequence.

AUG 10 th

We added a basic group, adenine, on the very end of the target gene, purified GFP, since the DNA polymerase that the target gene needed was Q5 which required a cohesive end instead of a blunt one. Afterwards, we connected the modified GFP gene and T-vector at 25 ℃ in 5 minutes. Furthermore, we transformed *E. colis* (DH5α) by thermal stimulation method and cultured the bacteria over night at 4 ℃. Since the LB media contained x-gal+2PTG and ampicillin, we were able to exactly filtrate bacteria which contained with target genes.

AUG 11 th

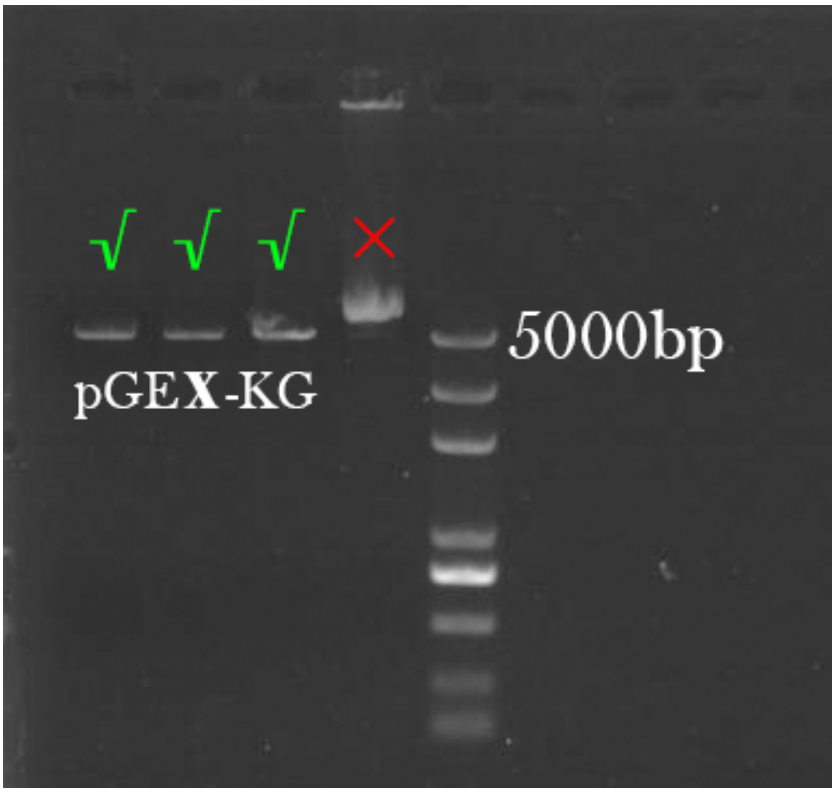
While the bacteria had grown blue and white in the media because of IPTG, we could easily select the correct bacteria with white color and culture them in liquid LB media for numerable amount. At the end of the day, we extracted enough plasmid and obtained T-vectors with GFP inside.



AUG 12th

We cut the expression vectors with two restriction enzymes: Bam H1 and Nco 1 in 37℃ for 3-5 hours in order to link GFP into the vector on the next step. After that, we tested the pGEX-KG

sections by gel electrophoresis to make sure our pGEX-KG is completely disconnected.

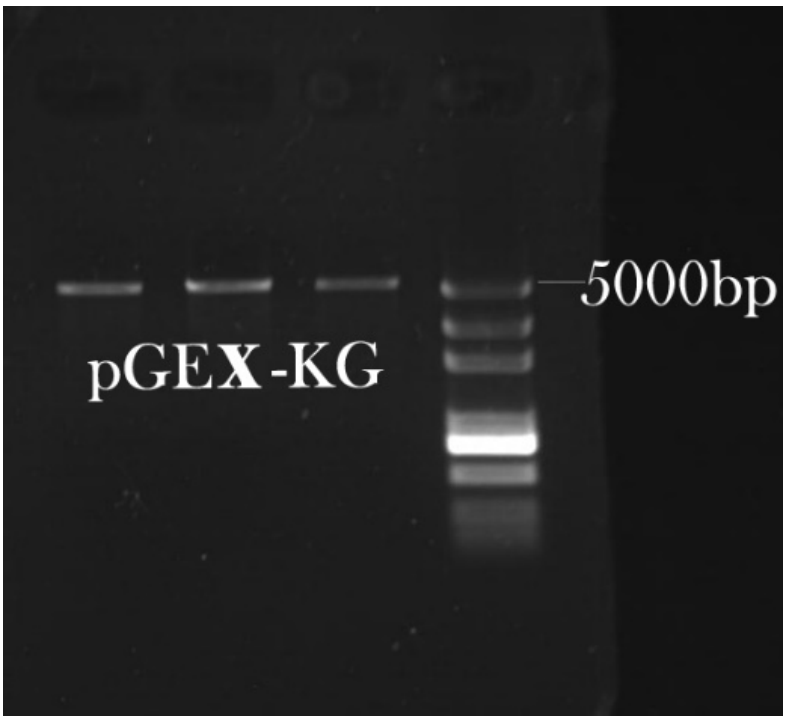


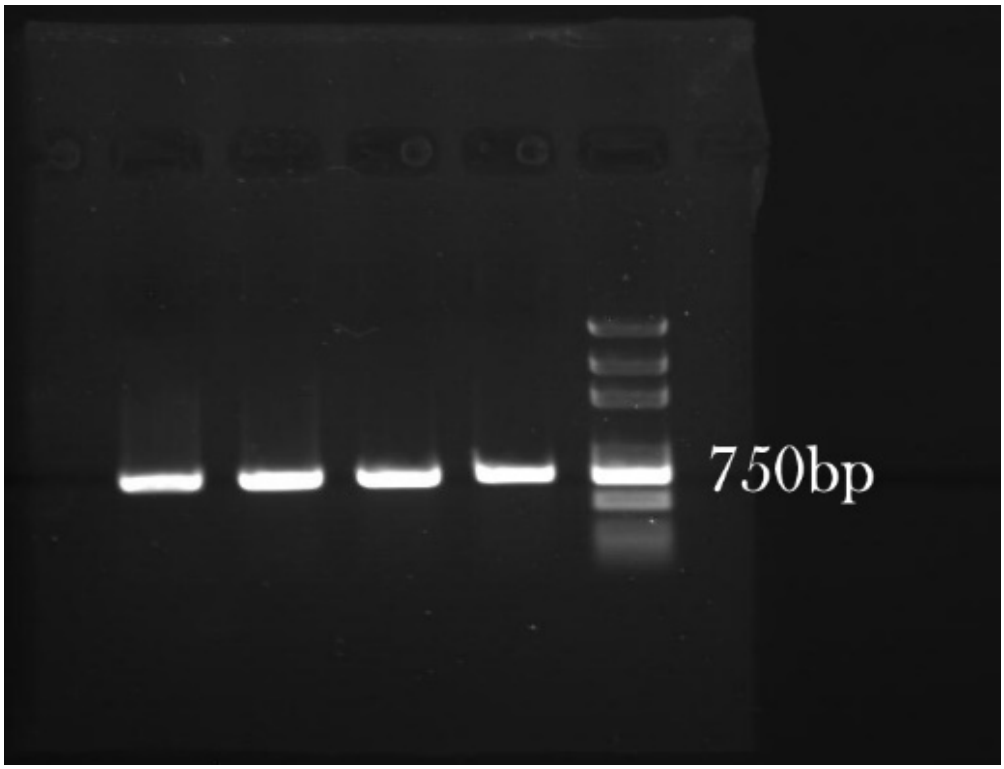
As it is shown above, the column with "×" is not ideally separated.

AUG 13th

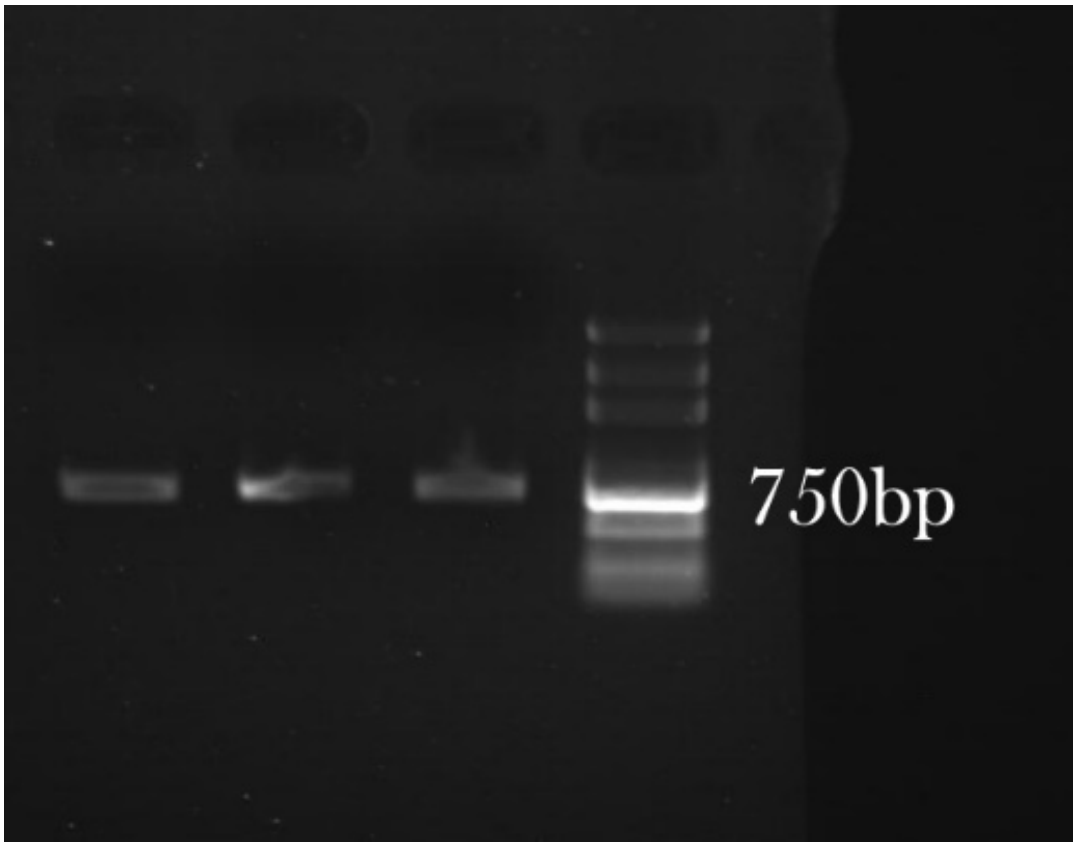
We purified the pGEX-KG sections and tested its base pairs (ca. 5000 bp). Meanwhile, we amplified GFPs containing both Bam H1 and Nco 1 restriction enzymes by polymerase-chain reaction.

The picture below shows the purified pGEX-KG vectors.



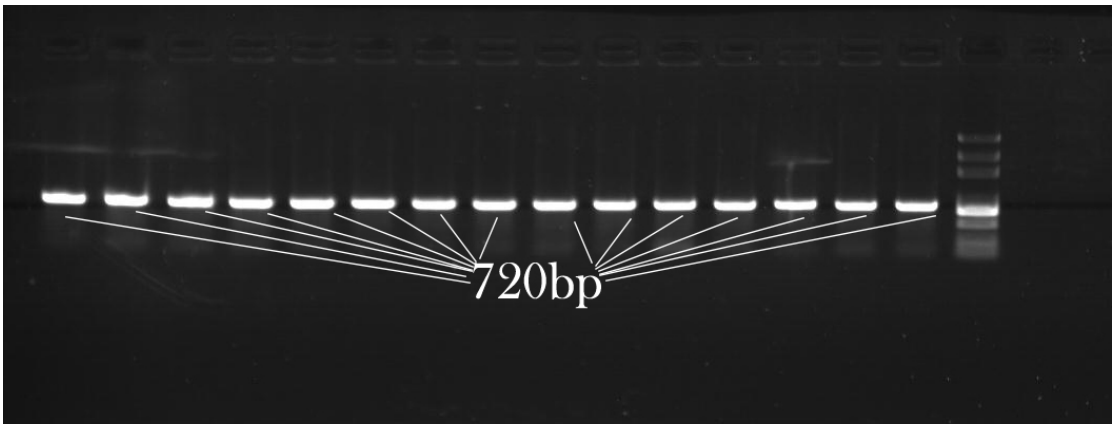


The picture above shows the testing result of GFPs with restriction enzymes.



The picture above shows the result of our purification of GFPs containing restriction enzymes.

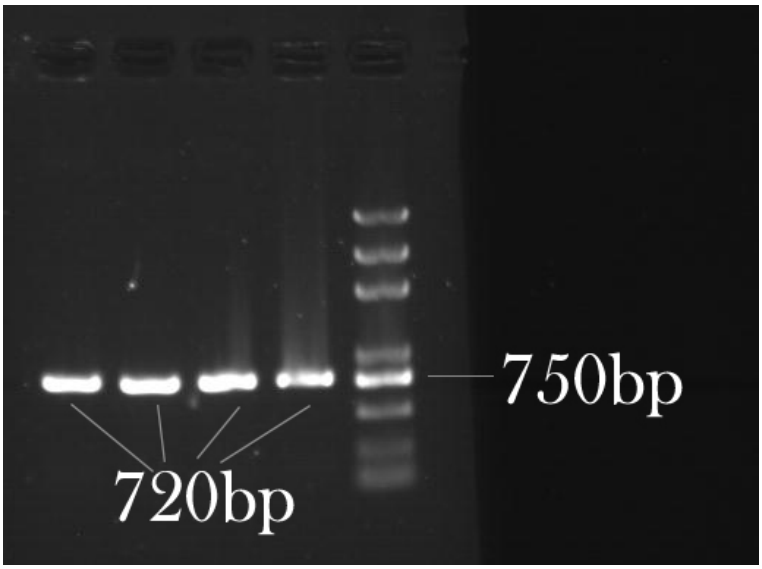
AUG 15th



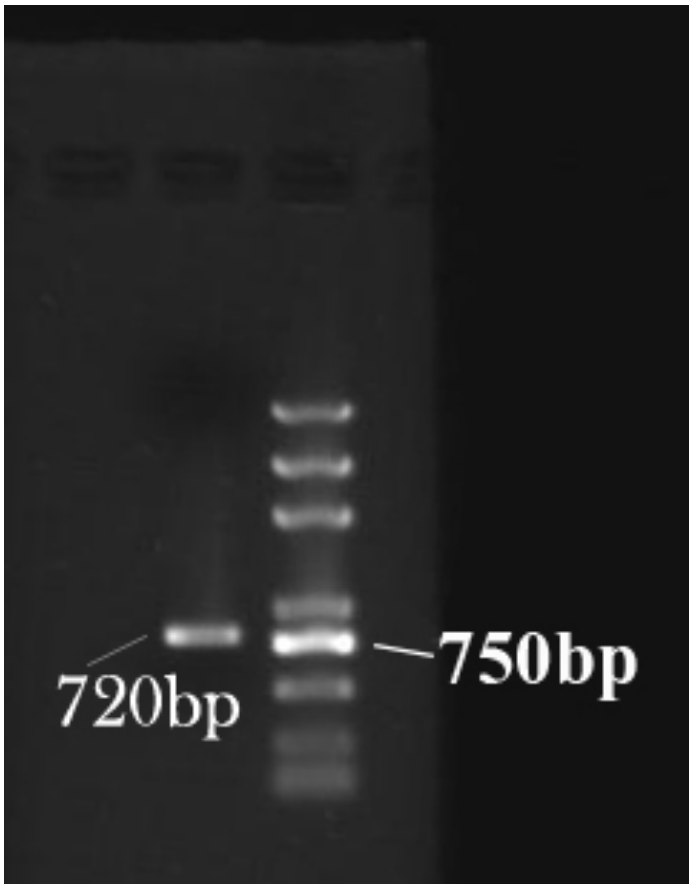
In order to make sure that the GFP gene that we extracted was completely correct, we used T-vector to connect GFP and to measure the sequence. After connection, we tested the T-vector connecting with GFP gene which contains Bam H1 and Nco 1 restriction enzymes, and as you can see, our results were all located on around 720 base pairs, whichwere exactly what we needed.

AUG 16th

We added TEV restriction site into the GFP gene by PCR because we wanted to purify the target gene easier by cutting TEV and the thrombin site that originally contained in pGEX-KG. After that, we made purification by recycle gel electrophoresis to preserve only GFPs with TEV.



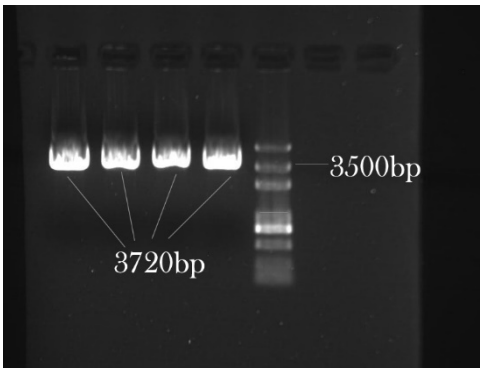
The picture above shows the GFP gene with TEV restriction site.



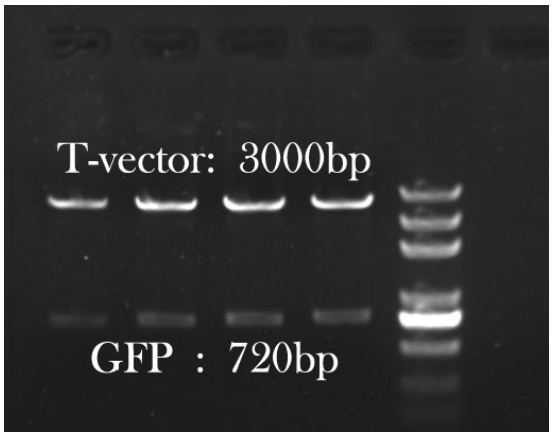
Picture above shows the GFP gene with TEV after purifying.

AUG 17th

We transmitted the GFP gene with TEV onto T-vector and planned to measure the sequence again. After connection, we tested the GFP roughly with gel electrophoresis and purified afterwards. By knowing the sequence is right, we cut the T-vector with Bam H1 and Nco 1 restriction enzymes to get perfectly correct gene. At the same time, we cut the pGEM-KG vector with Sal I and Hind III restriction enzymes and connected these two parts over night at 4℃.



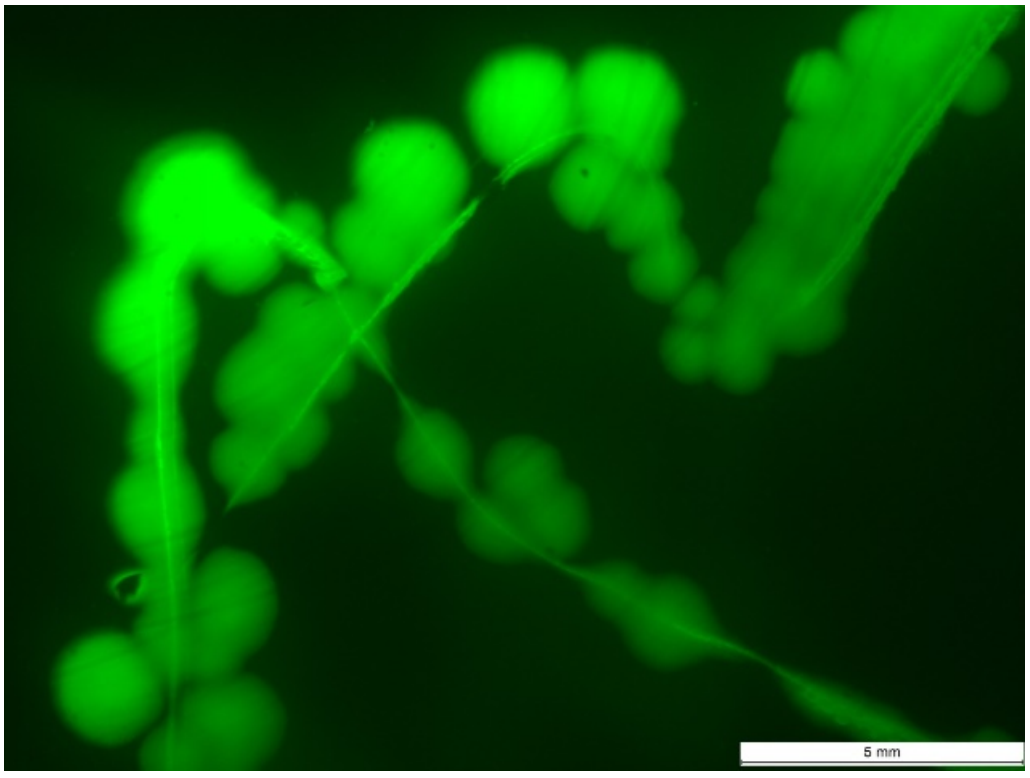
Picture above shows the GFP connecting T-vectors around 3720 bp.



Picture above shows the GFP and T-vectors' section.
http://2016.igem.org/File:T--CIEI-BJ--notebookBJ10.jpg

AUG 18th

The same as we did last week, we used thermal stimulation method to transform target vector into *E. colis* (DH5α), amplifying GFPs and culturing them in liquid media to extract enough plasmid. After selecting the correct bacteria by blue-white selection, we amplified for more and extracted plasmid to get pGEX-KG vectors with GFP containing TEV restriction site. Finally, we transformed the modified vector into *E. coli* BL21 , which allowed us to observe green fluorescence under the fluorescent microscope after bacteria were well-grown.



The picture shows that our gene path of **pGEX-KG containing GFP with TEV** has been successfully built.

AUG 19th

Beginning from today, our aim changed to be to insert *SmCPS1*, the key enzyme in forming Tanshinone, to the target vector that we built several days before. The first step was to clone the target gene from formal vector and amplified it with primers by PCR. Afterwards, to obtain the precise part of gene, we used gel electrophoresis to purify the section and recycle it. Same pattern as last week, we added adenine with dATP for 30 minutes at 72℃ after the sequence to create the adhesive end replacing the blunt one. Again utilizing PCR, we connected T-vector with the *SmCPS1* gene containing adenine.

AUG 20th

In the morning, we used thermal stimulation method to transform *E. coli*(DH5α) and culturedthem on solid media which included LB nutrient solution, ampicillin and x-gal+2PTG that is used in blue-white screen process. After cultivation for 8 hours at 25℃, we selected the white bacterial colony for its recombinant plasmid and measured the sequence. Then, we cultured the correct bacteria and amplified, extracted enough target plasmid as T-vectors containing *SmCPS1*.

AUG 21st

Since three of the restriction sites on *SmCPS1* gene are the same as the sites on official BioBricks, we had to use site-specific mutagenesis to change the basic groups without affecting its expression protein. Afterwards, we amplified the specific *SmCPS1* gene with mutagenesis and Sma I and Xho I, two restriction enzymes we needed by PCR, and tested it by gel electrophoresis.
At the end of the day, we recycled and purified correct gene on the gel.

AUG 22cd

Reusing the structure last week, we added adenine at the end of the target gene to create the adhesive ends since the DNA polymerase we used for amplifying gene was Q5. Then, we connected *SmCPS1* product containing adenine with T-vector at 25℃ in 5 minutes. Afterwards, we transformed vectors into *E. coli* (DH5α) by thermal stimulation method and cultured it with the same media we used in the past. With the extraction of plasmid from the correct white bacterium colony, we obtained **T-vectors with *SmCPS1* as well as Sma I and Xho I**, two key restriction enzymes, contained on the gene.

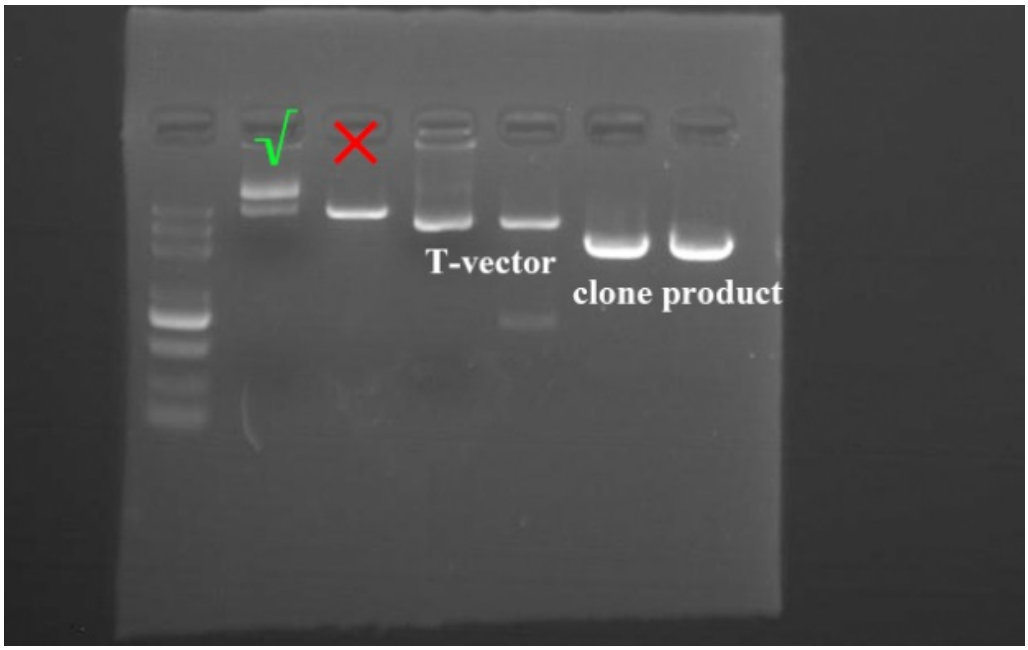
AUG 23rd

After successfully building two separated sections of gene in vectors, we were finally able to connect *SmCPS1* and pGEX-KG containing TEV restriction site and GFP. Firstly, we cut T-vectors which contain our target gene by Sma I and Xho I restriction enzymes. Then, pGEX-KG vector was cut by using Sma I and Sal I since Sal I and Xho I are isocaudarners which enabled us to connect these sections together. After both of the sections are tested by gel electrophoresis and recycled, we

connected them by using PCR over night at 4 ℃.

AUG 24th

Using the same method to culture and select, we got pGEX-KG vectors containing *SmCPS1* gene. Then we transformed these vectors into BL21 *E. coli* and cultured them, which allowed us to observe the green fluorescence under fluorescent microscope. Therefore, it showed that our gene path, **pGEX-KG vector containing *SmCPS1***, was successfully built.



The first two columns show the pGEX-KG vectors cutting by Sma I and Sal I, which clarifies a successful one and unsuccessful one.
The next two columns show T-vectors cut by Sma I and Xho I.
The last two columns show the final product of pGEX-KG vector containing SmCPS1, which we called it clone product.