Genetically Engineering Plants using Agrobacterium

Engineering Goal

To engineer plasmids and duplicate them using E.coli.

Background/ Introduction

Due to global warming, food shortage have become more and more serious through consistently increased temperature as well as less and less precipitation or even drought which greatly restricts the survival of crops, resulting in serious food shortages. However, cacti can survive living at high temperatures without much water. Beside the way their stem and leaves grow, they have two other characteristics to help them survive in such conditions for which other crops could also adopt. One of them is the water storage root system which often contains tubers or bulbs, the other one is that they tend to collect and store CO_2 during night so that they don't have to do it in the morning which would mean more moisture loss.

However, if we could engineer the DNA of crops by inserting drought resistant genes or making the plants develop different types of root systems that store water this would help the crops survive harsh conditions over long periods of time. This would allow us to provide a more consistent food supply to people in drought-prone areas.

While there are studies on using agrobacterium to infect plants, there aren't many on using E.coli to replicate plasmids with agrobacterium vectors for future infection. And that's what makes our research unique.

Methods

1. Pour 2 plates of kanamycin LB agar and 2 plates of ampicillin LB agar to grow the plasmids on.
2. Get your plasmids and spread the pDGB3_alpha1 on the kanamycin and the pCfB2793 on the ampicillin plates using an inoculation loop
3. Put all the agar plates into the incubator. Make sure to turn the plates upside down with the bacteria are facing down.
4. Gather one colony for each plasmid and put it into an overnight culture.
5. Use the Mini Prep Kit to extract the DNA from the culture.
6. Use the plasmids, the High-Fidelity 2X Master Mix, and the primers to run a PCR for both the plasmids.
7. Follow the instructions on the HiFi Master Mix for each plasmids.
8. Add a stain to the DNA so you can see it once you run the gel.
9. Run an electrophoresis gel for both types of DNA.
10. Use sterilized equipment to cut DNA out of the gel
11. Use the DNA Gel Extraction Kit to extract the DNA from the gel.
12. Use the HiFi Assembly Kit to combine the purified plasmids DNA from the Gel Extraction Kit.
13. Spread the product from the HiFi Assembly Kit on an kanamycin agar plate, incubate overnight.
14. Follow the Miniprep Kit instructions and receive your purified plasmid.

Result

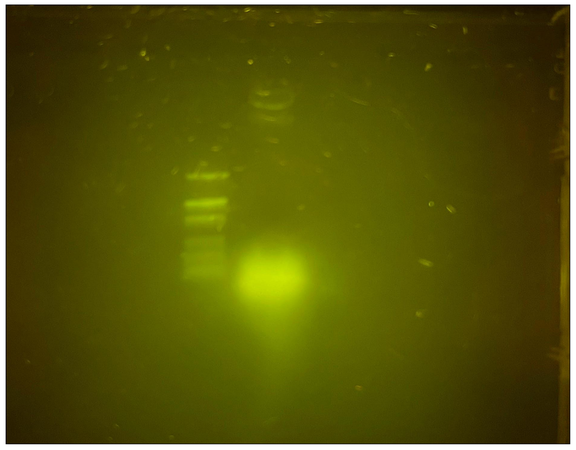

Figure 1, picture of pDGB3_alpha1 plasmid

We successfully did the master mix and ran the electrophoresis gel to extract the desired genes for pDGB3_alpha1 plasmid. Figure 1 is the electrophoresis gel. The column on the left is the DNA ladder that shows the length of DNA. The one on the right is DNA from pDGB3_alpha1plasmid.

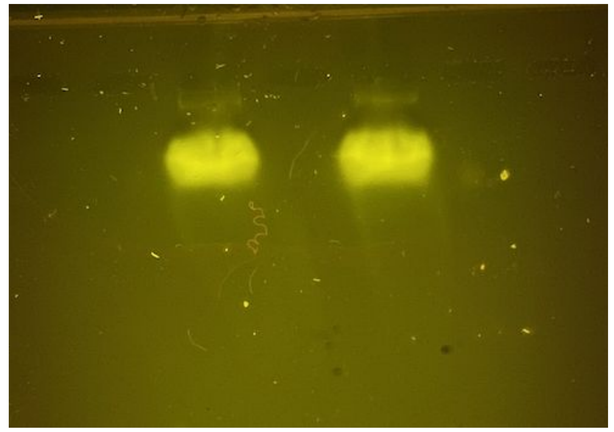

Figure 2, picture of our final plasmid

Figure 2 is one of our final plasmid, which includes the two genes from the two plasmids. A testing primers were used on the plasmid which would check whether the two plasmids combined. As the image shows, the plasmid is glowing, which means that there is in fact DNA. This show that the primers successfully connected to our plasmid, meaning that the Gibson assembling was successful.

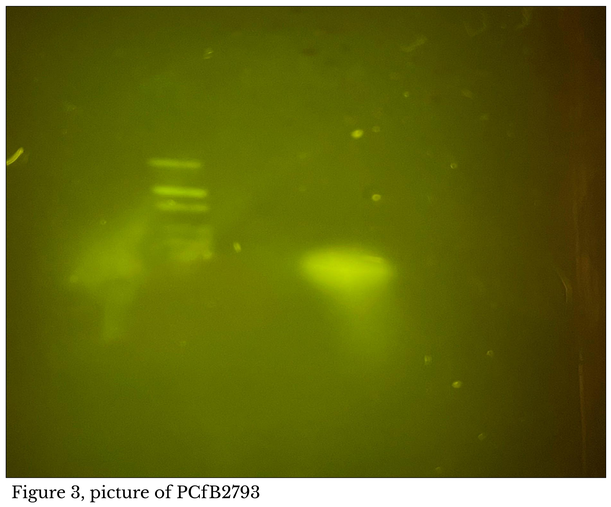

Figure 3, picture of PCfB2793

We also successfully did the master mix and ran the electrophoresis gel to extract the desired genes for pCfB2793 plasmid. Figure 3 is the electrophoresis gel. The column on the left is the DNA ladder that shows the length of DNA. The one on the right is DNA from pCfB2793 plasmid.

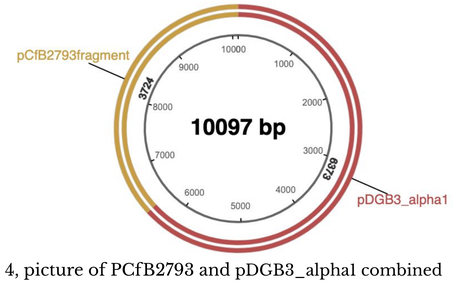

Figure 4, picture of PCfB2793 and pDGB3_alpha1 combined

Discussion

How to improve in the future:
1. When doing the Gibson assembling, we were not sure how to calculate pmol and get an good understanding of the ratio of vector and desired gene that was needed. In the future, we should dig deeper into the calculation to get a more accurate ratio.
2. In the future we should label the centrifuge tube better, with what step is each result come from.

The next steps towards the larger goal:
1. Work on the transformation to E.coli process again and try to get that working.
2. Use the copied plasmid DNA to infect Agrobacterium.
3. Use the engineered Agrobacterium to infect plant.

Conclusion

Impact of our project
The key impact of our project is that we can use the skill of engineering DNA to change the gene of plant and help it get certain characteristics.

Completion of our engineering goal
The goal for our first step of the project is mostly achieved, we have successfully engineered and put two plasmid together

Abstract

This project studied the possibility of using agrobacterium as a vector for transporting drought-resistant genes into plants. This is important because drought is a big cause of food shortages world-wide and drought-resistant plants could help prevent this. We worked to engineer a plasmid that could successfully be placed into agrobacterium to give it a desired gene. We ended the project with a plasmid that could be placed into agrobacterium and carried the GFP gene. We had some trouble growing it in E. coli, but this was most likely due to the e.coli being stored in a faulty freezer. If we were to continue this project we would continue by growing the plasmid in E. coli and eventually actually putting it into agrobacterium. Unfortunately, due to time constraints, we weren't able do this yet.

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All photos taken by exhibitor