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Using CRISPR vectors to study the molecular function of a mutated *Arabidopsis* AS2 gene

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Abstract

The purpose of this study is to understand the molecular functioning of the *Arabidopsis* AS2 gene. In our experiments, we used CRISPR technology to create mutations in the AS2 gene. These mutations are predicted to change AS2 protein structure and function, which may result in a mutated leaf. Analyzing the effects of new mutations allows us to better understand protein function.

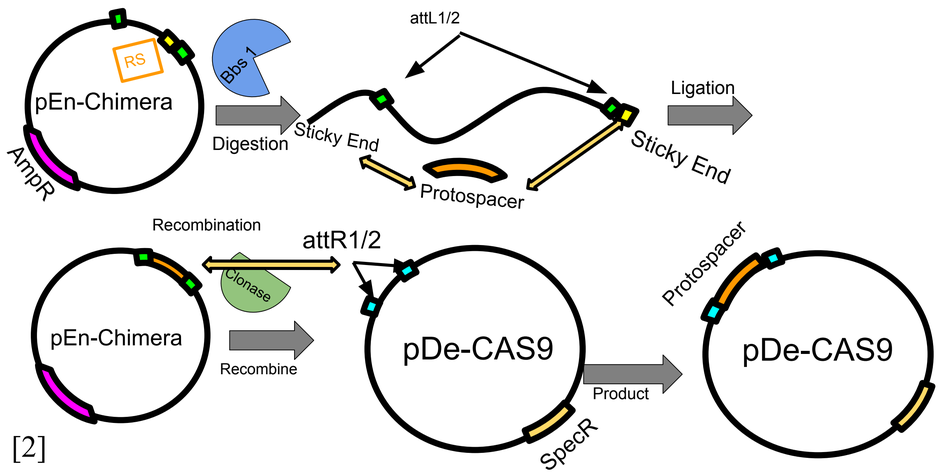
Introduction

AS2 (ASYMMETRIC LEAVES 2) is the gene that encodes a protein required for the proper development of leaves in plants. The normal function of AS2 is to regulate KNOX gene expression. The KNOX genes are a group of genes that when expressed keep cells in an undifferentiated state. Once leaves begin to form, the KNOX genes must be turned and kept off for normal leaves to develop. The role of AS2 is to keep knox genes off while leaves develop. Little is known about the mechanism AS2 employs to keep the KNOX genes off.

Our goal is to use CRISPR to make mutations in two different regions of the AS2 gene of *Arabidopsis*. Two small pieces of the gene were identified, named 0-2370 and 2-2184, can be used to guide the CRISPR-Cas9 proteins to the AS2 gene in *Arabidopsis* and create mutations. Once the CRISPR-Cas9 system with the guide sequences are transformed into plants, the CRISPR system makes mutations in the AS2 gene. Ultimately, we will analyze mutant plants to learn more about AS2 function.

Methodology

Figure 1

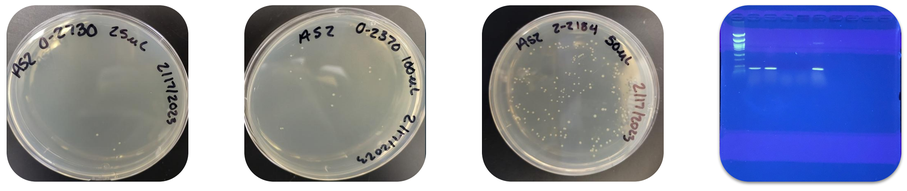


[2]

Figure 1: Overview of creating CRISPR tools. The small pieces of AS2 (aka protospacer), 0-2370 and 2-2184, are inserted into a BbsI digested pEn-Chimera vector by a ligation reaction. The resulting vector was transformed into bacteria (E. coli) and selected on petri plates. E. coli bacteria from the plate were then grown in liquid broth media culture and the DNA from these cultures were isolated. PCR (polymerase chain reaction) and gel electrophoresis were used to confirm that the protospacers were properly cloned, and the resulting vector was copied through PCR to replicate the DNA segment.

Results and Discussion

Figure 2



(A) (B) (C) (D)

(A) 25 µl of E. coli transformed with the 0-2370 pEn-Chimera vector.
(B) 100 µl of E. coli transformed with the 0-2370 pEn-Chimera vector.
(C) 50 µl of E. coli transformed with the 2-2184 pEn-Chimera vector.
(D) PCR confirmation of the presence of the 2-2184 vector in E. coli. The 0-2370 vector was not present in the PCR which was shown through gel electrophoresis.

Conclusion

So far, we have confirmed we inserted 2-2184 into the pEn-Chimera vector, but we have not confirmed the 0-2370 vector. In the future, we will swap 0-2370 and 2-2184 into pDe-Cas9 vector, transform our vectors to *Arabidopsis* plants, and analyze the plants to determine if we created mutations in the AS2 gene.

References

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