Analysis of insulator activity in CLOI-3 subclones for transformation into *Arabidopsis thaliana*

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Summary

The identification and characterization of functional plant insulators are key steps in the understanding of plant gene function within the context of chromatin structure*. Professor Johnson's lab has identified an insulator in the model plant, *Arabidopsis thaliana*, and the objective of this project has been to help define the functional sequences in this specific insulator designated as CLOI-3.

Introduction

Insulators are DNA sequence elements that when inserted between a promoter of a gene and the enhancer, a sequence that boosts expression, prevent the enhancer's influence on the gene. While a gene has its own enhancer, enhancers introduced during transformation, enhancers of neighbouring genes, or the influences of DNA rearrangement can cause a detrimental misexpression of a gene. As such, insulators can also be used for blocking these aberrant enhancer-promoter interactions*.

Currently, it is a matter of finding insulators that can not only properly modify gene expression, but also be properly transformed into specific sites on *A. thaliana*'s genome, and the subclone candidates for analysis in this experiment are CLOI-3-4, CLOI-3-2-2, and CLOI-3-2-26. These subclones are derived from the original CLOI-3 insulator that were cut with combinations of restriction enzymes. This creates smaller base pair DNA strands that help to pinpoint the location of the functional insulator sequence in the original CLOI-3.

Since *A. thaliana* is applicable to research in human health, industry and medicine, finding the means for gene manipulation is important. For example, *A. thaliana* possesses genes that prompt disease in humans and can be more easily manipulated in the plant than in a human or animal model. Also, molecular geneticists can use this model plant to study the conversion of crops into renewable energy sources or the plant's DNA repair mechanisms for medicinal purposes.**

Methodology

Step	Method Description
1	Original Insulator Clone: CLOI-3 Library of subclones created using restriction enzymes. Figure 1 shows the various subclones of CLOI-3 created from restriction enzyme cuts.
2	Selected subclone is cloned into the text vector, pB31. Figure 2 shows the components of the vector.
3	Heat shock transforms the pB31 vectors with the subclone insert into *Escherichia coli*.
4	Streaking and patching of *E. coli* onto agar plates with kanamycin selects for properly transformed bacterial colonies.
5	Colony PCR performed on transformed colonies to increase the DNA content for gel electrophoresis screening. Possible addition of restriction enzyme to certain colonies before PCR and the screening.
6	Plasmids of transformed colonies prepared for sequence analysis to confirm that the insert has the correct subclone sequence.
7	Insulators placed into Tumor-Inducing plasmids of *Agrobacterium tumefaciens* with a GUS marker for transformation into *A. thaliana*. (In Progress)

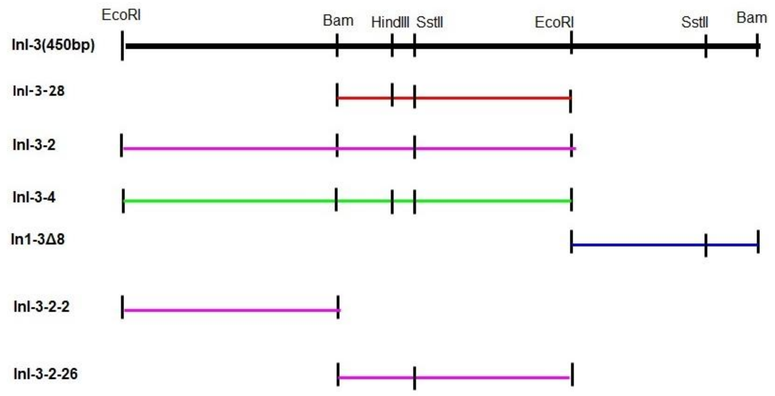


FIGURE 1: **Schematic map of InI-3 and subclones with enzyme restriction sites**

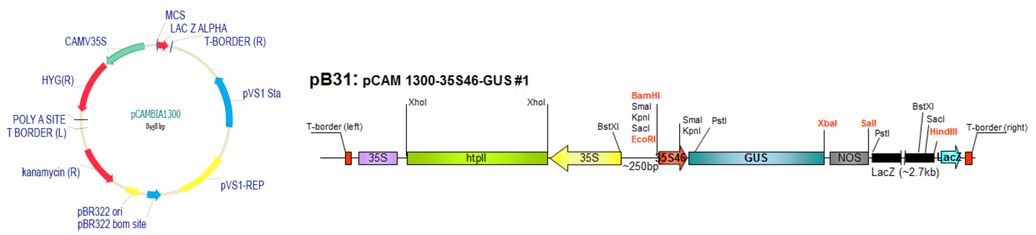
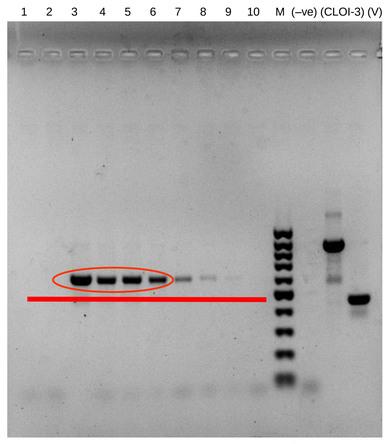


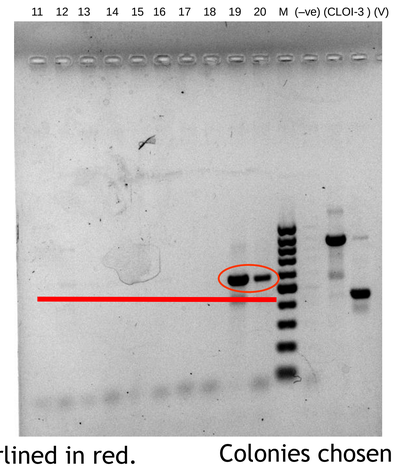
FIGURE 2: **Components of pB31 base vector**

Results

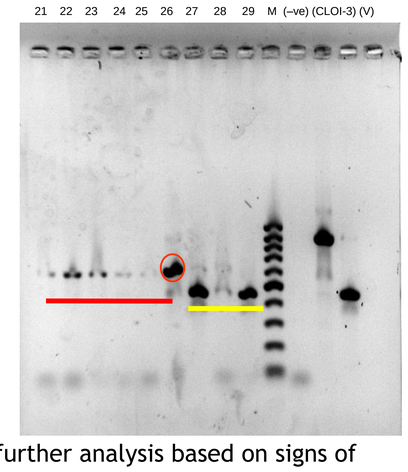
Gel 1A:



Gel 1B:



Gel 1C:

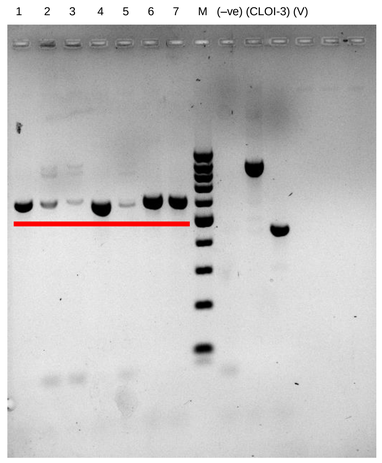


Two CLOI-3-2 derivative colonies are underlined in red. CLOI-3-14 colonies are underlined in yellow.

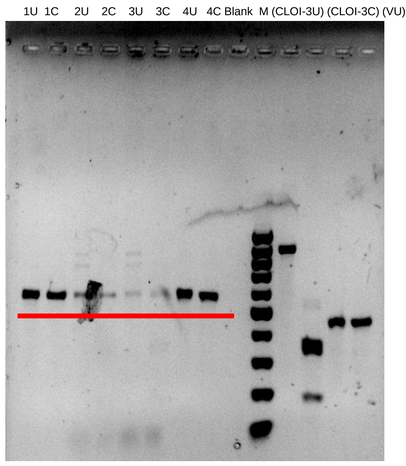
Colonies chosen for further analysis based on signs of proper insulator inserts are circled in red.

Bands found in between (CLOI-3) and (V), pB31, controls are positive signs for potential inserts.

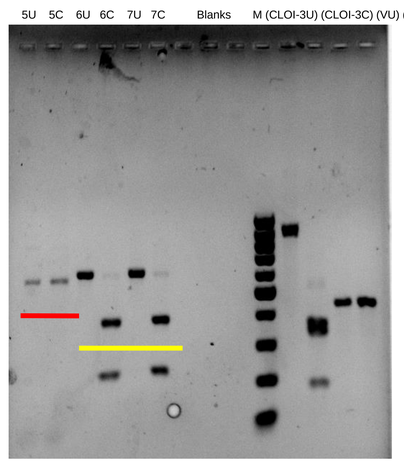
Gel 2:



Gel 3A:



Gel 3B:



Chosen colonies confirmed for a second time to have inserts by Gel 2. Gel 3A and 3B show the differentiation of the two possible CLOI-3-2 derivatives by cutting with restriction enzyme, Sst II, to identify CLOI-3-2-2 (red line) and CLOI-3-2-26 (yellow line) subclones.

C=Cut, U=Uncut

Conclusion

CLOI-3-4 and CLOI-3-2 were derived from EcoRI restriction enzyme cuts. On analysis of Gel 1C, Lanes 27, 28 and 29 did not show the presence of the CLOI-3-4 subclone insert which suggests it was not properly cloned into pB31. Thus, CLOI-3-4 colonies did not continue on in this experiment.

Since CLOI-3-2 was confirmed by the Graduate Student to be a viable insulator, it was cut again using BamHI and EcoRI resulting in CLOI-3-2-2 and CLOI-3-2-26 which showed the presence of inserts as seen in the Gel 1 set. The seven chosen CLOI-3-2 candidates were reconfirmed to have inserts in Gel 2 though at that point they could be either CLOI-3-2-2 or CLOI-3-2-26.

However, the Gel 3 set differentiates the two derived subclones of CLOI-3-2 by using the Sst II restriction enzyme which cut the site available only on CLOI-3-2-26. Therefore, in the Gel 3 set, Lanes 1 to 4 can be identified as CLOI-3-2-2, and Lanes 6 and 7 can be identified as CLOI-3-2-26 due to the presence of multiple cut bands.

Lanes 1, 4, 6, and 7 of the Gel 3 set were chosen for sequence analysis and were confirmed to have the correct sequences. Recently, these subclones were transformed into *A. tumefaciens* and are awaiting final transformation into *A. thaliana*. When the transformed plants mature, the presence or lack of GUS staining in the plants will indicate if CLOI-3-2-2 and/or CLOI-3-2-26 contain the functional insulator sequence or not.

References

"Genetics: the early days." *WageningenUR*. *n.p.* 15 July 2003. Web. 28 December 2012.**

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