



Determining boundaries with enhancer blocking insulators in *Arabidopsis thaliana*

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Introduction

Regulation of a gene is achieved in part by sequences called insulators. An insulator (Ins) is a genetic element that blocks the interaction between enhancers and promoters. Insulators therefore determine the set of genes an enhancer can influence. That prevents unwanted interactions with other genes. In this project, "Ins" sequence taken from another species will be tested in order to determine whether it is functional in a model plant: *Arabidopsis thaliana*. Being able to prove that the exact same sequence is conserved along species infers a trans-species molecular conservation along the evolutionary manner and widely affects the biotechnological advancements and genetic engineering for the desired crops.

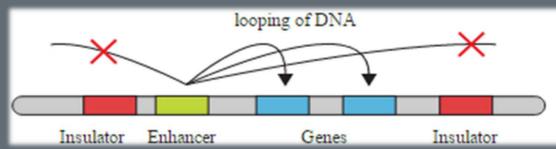


Figure 1. Idealized representation of the mode of action of the insulators. The mechanism shows the independence of the location of the insulator on the repression of an enhancer.

Methodology

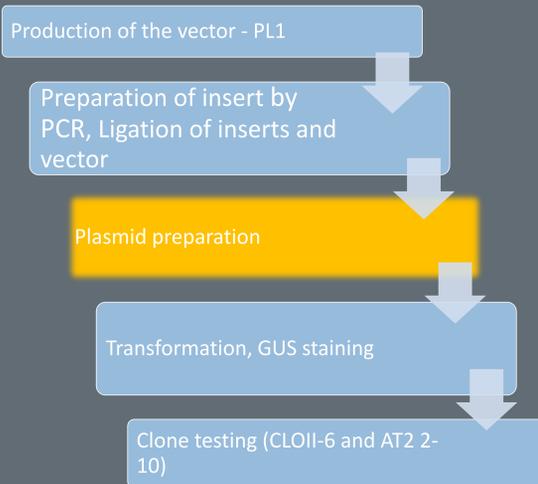


Figure 2. Plasmid Vector pL1: pCAM 1391 Napin. The GUS staining is used to determine the presence of a potential insulator. The hygromycin resistant gene is driven by 35S enhancer/promoter which influences neighboring genes. The second gene, GUS, responsible for the blue plant staining is driven by the Napin seed specific promoter and the 35S enhancer.

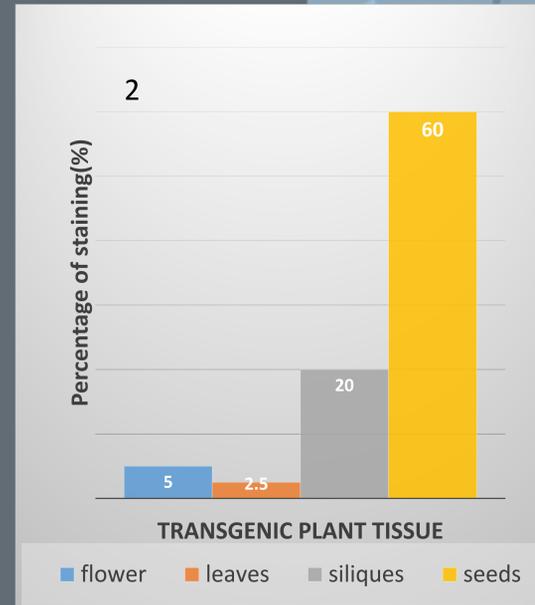
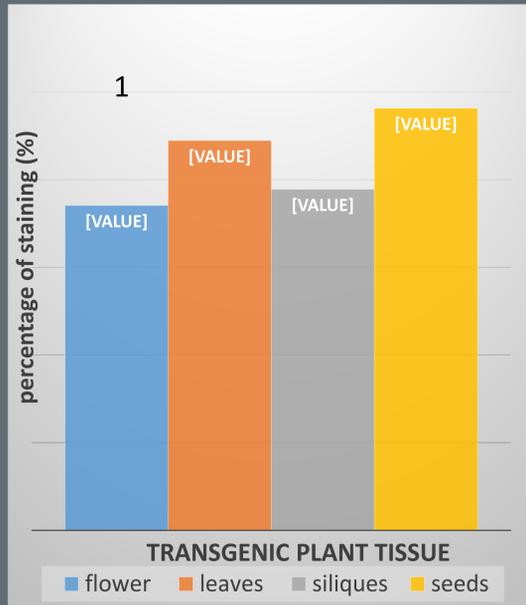


Figure 3. Staining results for the pL1 vector (control) and 2 putative clones. 1) The control shows high staining in all four tissues indicating no insulator function. 2) AT2-10 showing moderate levels of GUS staining in siliques and seeds while 3) CLOII-6; displays no GUS staining from all samples

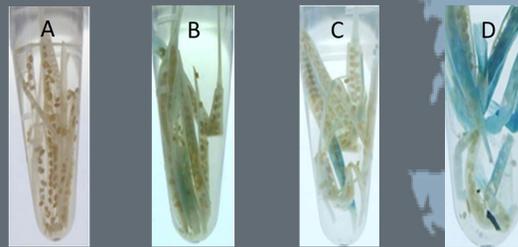


Figure 2. Candidate clones indicating different levels of insulator activity. GUS (GUS: β -glucuronidase) staining: a histochemical technique used for the analysis of the activity of a promoter. The first Assay (A) expresses no staining: high insulator activity while the last (D) shows high staining meaning the absence of insulator activity.

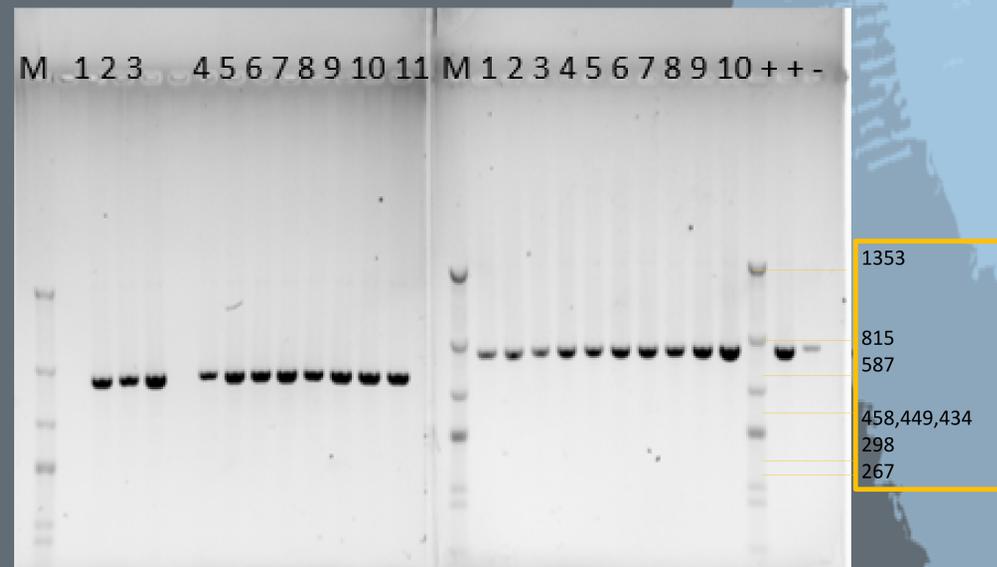


Figure 4. Gel electrophoresis using the AT2 2-10pL1 Salk primers. Gel electrophoresis is performed using DNA Marker PhiX HaeIII in order to confirm the expected transgene used to generate transgenic plants.

Conclusion:

- In the absence of an insulator, the 35S enhancer acts on the Napin promoter so that there is non-specific staining which explains why the staining is in all tissues. However, in the presence of an insulator, the 35S enhancer can not influence the Napin promoter and therefore we only see staining in seeds and no other tissues.
- CLOII-6 is a good candidate for a potential insulator sequence. Sampling errors can be avoided by including several individuals in the color interpretation of different tissues
- The future potential use of functional insulators with different enhancer/reporter system can influence gene selection of desirable traits in plants.

References:

- Figure 1 was uploaded by Squidonius at [https://en.wikipedia.org/wiki/Insulator_\(genetics\)](https://en.wikipedia.org/wiki/Insulator_(genetics))
- Singer et al. (2011). Enhancer-promoter interference and its prevention in transgenic plants. *Plant Cell*, 30, 723-731.
- Valenzuela, L., and Kamakaka, R.T. (2006). Chromatin Insulators. *Annual Review of Genetics*, 40, 107-138.

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