Characterization of the roles of insulators in the regulation of genes using the model plant A. Thaliana

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Introduction

Regulatory elements associated with specific DNA sequences in a eukaryotic nucleus, such as insulators, have the potential to maintain normal expression patterns by blocking unwanted communication between enhancers and promotors. Insulators not only keep in check the interaction between the enhancer and promoter, they also play a role in correcting transgenes misexpression. Although a recent proposed model based upon experiments of *Cai* and *Georgiev*, who studied the insulators from the *Drosophila melanogaster* *"gypsy"* element raises great interest, scientists have not isolated and examined plant insulators in any detail. In our lab, a transgenic *A. thaliana* system was developed in which CaMV 35S promoter influences the correct seed-specific expression of the napin transgene. Two non-plant insulators, UASrpg and BEAD-1C, block non-specific interaction to a level that was previously reported for the gypsy element in transgenic *D. melanogaster*. This result is the first published demonstration of insulator activity in a plant. Given the potential importance of insulators in plant gene expression and possible key roles in plant biotechnology for the control of tissue-specific transgene expression, we have embarked on a research program to isolate and study plant insulators in order to exploit our previous finding.

Objectives: Carry on the project by looking for possible insulator candidates with the expected length of around 150 base pairs in the cloned library. Draw hypothesis for the ones that doesn't turn out to be 150 base pairs as expected and test it.

Hypothesis: We will see sequences with 150 base pairs. There are two possibilities for the ones that turned out to be 450 base pairs . First is ligation, where a Bam site is ligated to another Bam site and same for Eco sites. As a result, we get three 150 base pair BamH1-Insulator-EcoRI fragments ligated together. The second possibility is hybridization, where the ends of a chain is so similar to the ends of another chain that they are hybridized together and amplified by PCR.

Methods

1. We cut the sequences using Bam and Eco restriction enzymes and then perform PCR screening on these samples to first verify the length of the sequences. We expect around 150 base pairs chains using random synthesis.
2. To test our hypothesis, we will use bioinformatics to find the specific location and the number of different restriction enzymes on a chain. We will observe Bam and Eco sites in the middle of the chain if the hypothesis is true.
3. Colony PCR is a technique used to screen for the presence and the size of plasmid inserts. It's another technique we will to screen for sample size and look for candidates .

Acknowledgements

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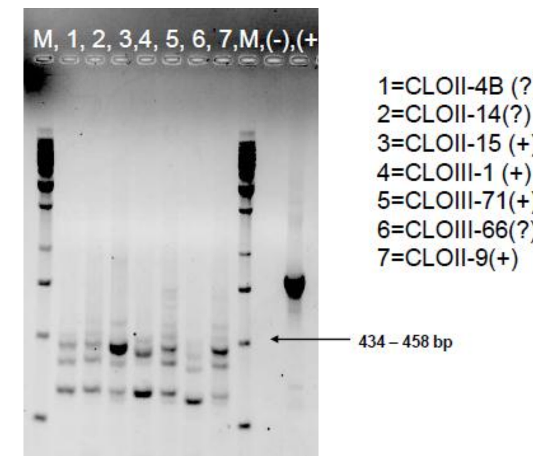

Figure 1. PCR screening of stored pGEM-t clones with M13 primers

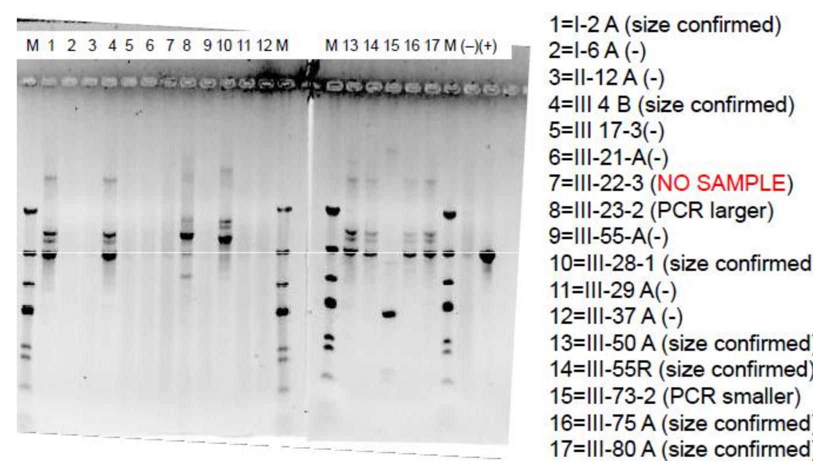

Figure 2. PCR screening of stored PCR products with CLO (insulator) primers

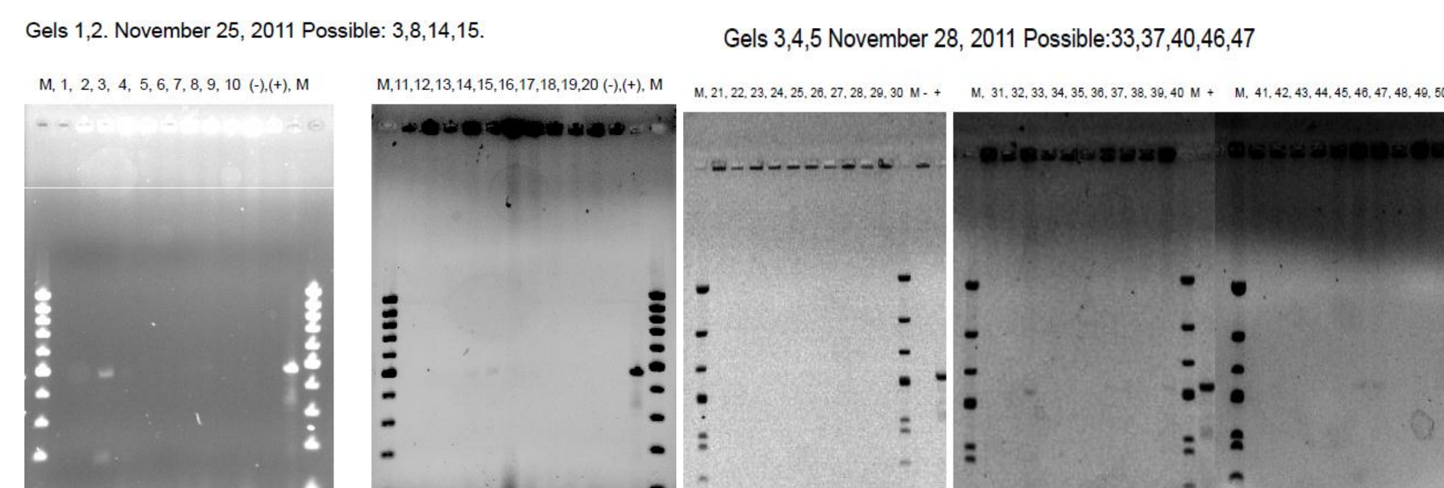

Figure 3. colony PCR screening of pLI CLOI-3 transformants

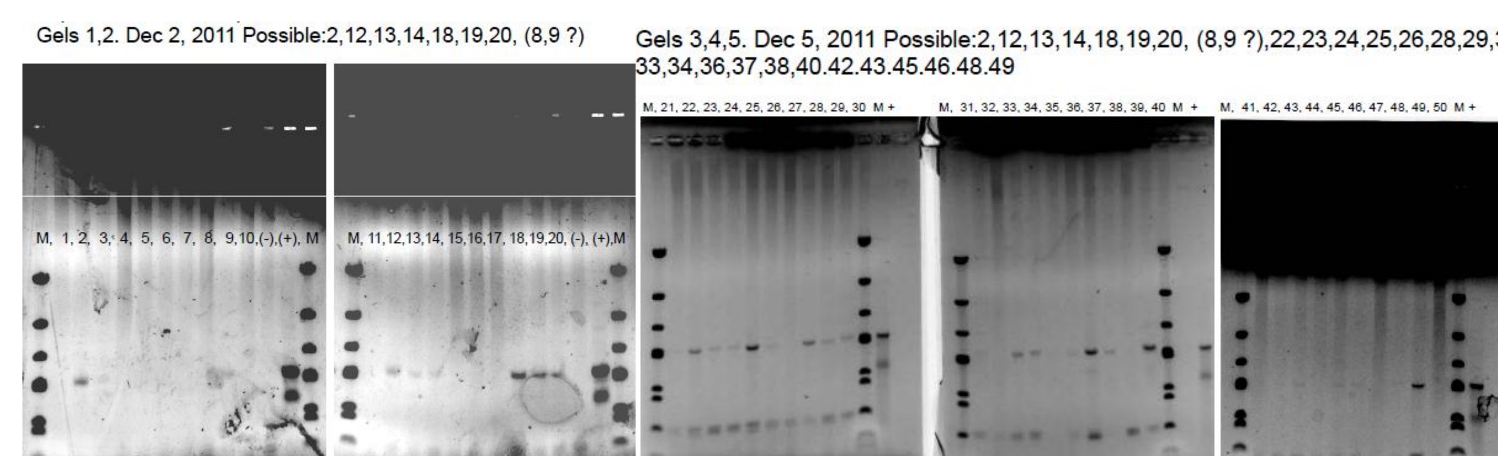

Figure 4. colony PCR screening of CLOIII-78 transformants

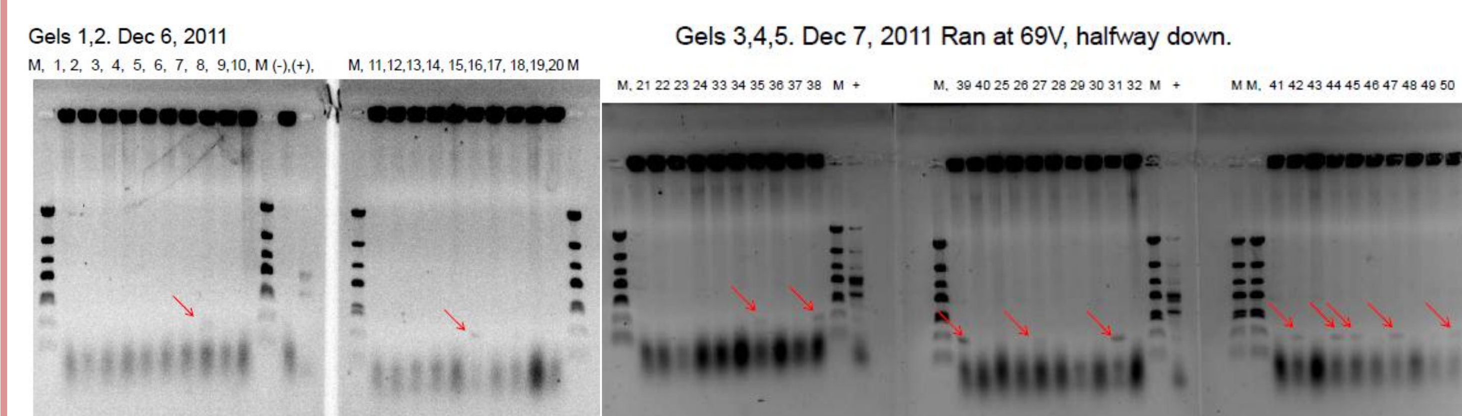

Figure 5. colony PCR screening of CLOII-10 transformants

Results

Table 1: observations summarized in spreadsheet format showing information such as the different transgenic, base pairs (bp), DNA name, restriction sites, and confirmation of the size.

Transgenic	on FC	bp	DNA Name	Restr. Sites (internal from master list with positions). (Searched for BamH1, Sma1, Kpn1, Sac1, EcoR1, HindIII, Sph1, Pst1 Bgl II, Mun1/Mfe1)	Restr. Sites R,B at ends	Gr	PCR 1:50 dilution (M13 primers)	PCR undiluted (M13 primers)	PCR undiluted (Clo primers)	Location	Gel purified
CLOI-4B		440	PCR frag	no sequence	E,B	III			?	Clo cloning Oct 2011	
CLOII-9	3	440	PCR frag	HindIII(203), KpnI(420), Eco, BamH, SacI	E,B				size confirmed	Clo cloning Oct 2011	
CLOII-14	2	450	PCR frag	BamHI 149 ; EcoRI 1,297; SacI 341	E				?	Clo cloning Oct 2011	
CLOII-15	2	446	PCR frag	BamHI 148 ,441; EcoRI 1,298	E,B	IV			size confirmed	Clo cloning Oct 2011	
CLOIII-1	2	440	PCR frag	BamHI 145,435;EcoRI 1,293; SacI 95	E,B				size confirmed	Clo cloning Oct 2011	
CLOIII-66	1	435	PCR frag	Eco, BamH1 *	E,B				?	Clo cloning Oct 2011	
CLOIII-71	1	441	PCR frag	BamHI 149 ,219, 436; EcoRI 1, 295; SacI 338	E,B				size confirmed	Clo cloning Oct 2011	
CLOI-2	5	446	pGEM-CLOI-2A	BamHI 148, 441; EcoRI 1, 298.	E,B	IV	size confirmed			Clo cloning Oct 2011	Yes
CLOI-6	3	438	pGEM-CLOI-6A	BamHI 149,433; EcoRI 1, 285	E,B		negative	size confirmed		Clo cloning Oct 2011	Yes
CLOII-12	4	427	pGEM-CLOII-12A	BamHI 139,422 ; EcoRI 1,45,287; KpnI 18,278	E,B		negative	PCR larger		Clo cloning Oct 2011	
CLOIII-4	5	440	pGEM-CLOIII-4B	BamHI 149,435; EcoRI 1, 291	E,B	III	size confirmed			Clo cloning Oct 2011	Yes
CLOIII-17	5	443	pGEM-CLOIII-17-1	BamHI 146, 438; EcoRI 1,291	E,B		negative	negative		Clo cloning Oct 2011	
CLOIII-21	3	443	pGEM-CLOIII-21B	BamHI 147,438; EcoRI 1, 295; SmaI 54	E,B		negative	negative		Clo cloning Oct 2011	
CLOIII-22	4	425	pGEM-CLOIII-22-2	BamHI 136, 420; EcoRI 1, 272	E,B		negative	size confirmed		Clo cloning Oct 2011	Yes
CLOIII-23	2	430	pGEM-CLOIII-23-1	SacI, Eco, BamH1 *	E,B		PCR larger			Clo cloning Oct 2011	
CLOIII-28	3	733	pGEM-CLOIII-28-2	BamHI 139, 436, 728; EcoRI 1, 288, 584	E,B		size confirmed			Clo cloning Oct 2011	Yes
CLOIII-29	3	427	pGEM-CLOIII-29A	BamHI 138,422;EcoRI 1,283; KpnI 178	E,B		negative	PCR larger		Clo cloning Oct 2011	
CLOIII-37	3	439	pGEM-CLOIII-37A	BamHI 149,367,434; EcoRI 1,296	E,B		negative	size confirmed		Clo cloning Oct 2011	Yes
CLOIII-44	2	436	PCR frag	BamHI 141,431;EcoRI 1, 282; SphI 369	E,B					Clo cloning Oct 2011	
CLOIII-47	2	416	PCR frag	BamHI 132,411; EcoRI 1, 266	E,B					Clo cloning Oct 2011	
CLOIII-50	3	443	pGEM-CLOIII-50A	BamHI 143, 438 ; EcoRI 1,290; SphI 84	E,B		size confirmed			Clo cloning Oct 2011	Yes
CLOIII-55A	3	432	pGEM-CLOIII-55A	BamHI 63,143,427; EcoRI 1,282 ; SacI 366	E,B		negative	size confirmed		Clo cloning Oct 2011	Yes
CLOIII-55R	4	432	pGEM-CLOIII-55A	BamHI 63,143,427; EcoRI 1,282 ; SacI 366	E,B		size confirmed			Clo cloning Oct 2011	Yes
CLOIII-73	4	712	pGEM-CLOIII-73-1	BamHI 149, 411, 707; EcoRI 263, 559	E,B		PCR smaller			Clo cloning Oct 2011	
CLOIII-75	3	437	pGEM-CLOIII-75A	BamHI 102, 138, 432: EcoRI 1, 284; HindIII 163; KpnI 18, 28	E,B		size confirmed			Clo cloning Oct 2011	Yes
CLOIII-80	3	451	pGEM-CLOIII-80A	BamHI 149, 446; EcoRI 1, 297; HindIII 127	E,B		size confirmed			Clo cloning Oct 2011	Yes

Discussion

About half of the clones turned out to be 150 base pairs as expected. As we can see in table 1, a few transgenic that turned out to be more than 150 base pairs are studied in further detail. We have their size confirmed and we have found internal Eco and Bam sites with specific location, which prove our hypothesis of ligation. We used PCR screening to screen for the size of these transgenic, and then rescreened all the ones that did not have the expected size to verify that they are truly larger than 150 base pairs. There are a few promising candidates so far but they require further tests before using them for the next step.

Supplementary Work

The next step of the project will be to test and confirm the restriction sites in the middle of the chains. We can also break down the 450 base pair chains into smaller chains to test for the restriction sites. The graduate student, *Batool*, will clone the possible candidate sequences from the first vector into the second vector then from the second vector into the third vector to see it the gene is expressed only when expected. Another graduate student, *Lara*, will clone the candidate sequences from the first vector straight into the third vector. If we get a good result, we will use yeast as our next model.

References

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