What Are the Roles of Titan1 and APRR2 Genes in Seed Development?

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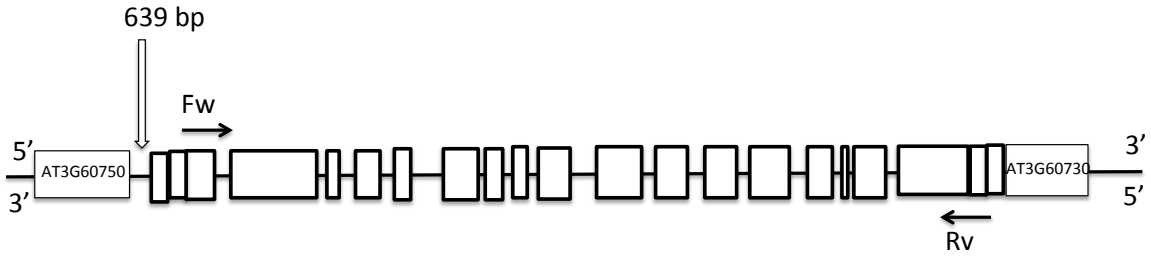
HC70AL Spring 2011

What is AT3G60740?

- Known as Titan1
- Chromosome 3
- Reverse Orientation
- 6,120 bp
- 17 exons, 16 introns
- Encodes tubulin-folding cofactor D
- 1,254 AA

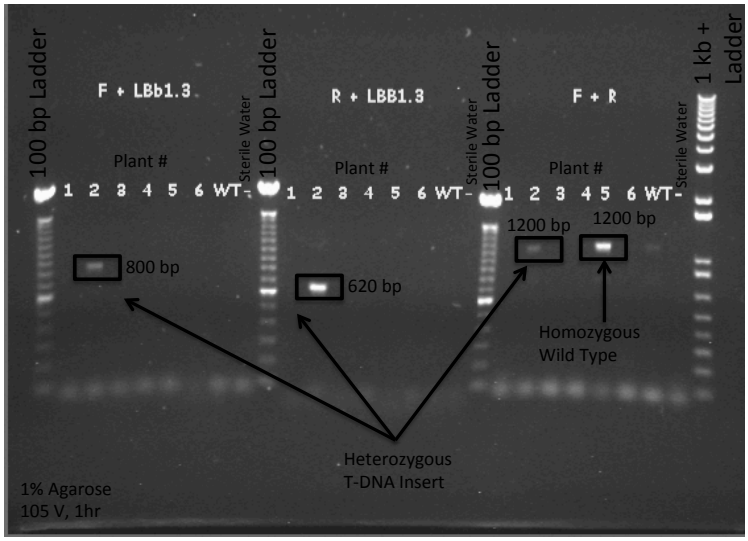
What is the Structure of Titan1?



= Start = Exon

= Stop = UTR

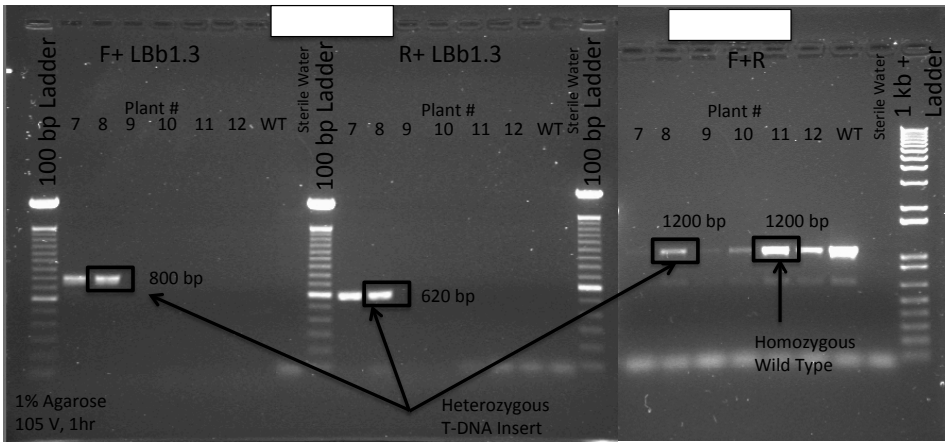
What Are the Genotypes of My Plants #1-6?



Expected Wild Type Size: 1,138 bp
Expected T-DNA Insert (FW+LBb1.3) Size: 807 bp
Expected T-DNA Insert (RV+LBb1.3) Size: 637 bp

Heterozygous T-DNA: Plant #2
Homozygous WT: Plant #5

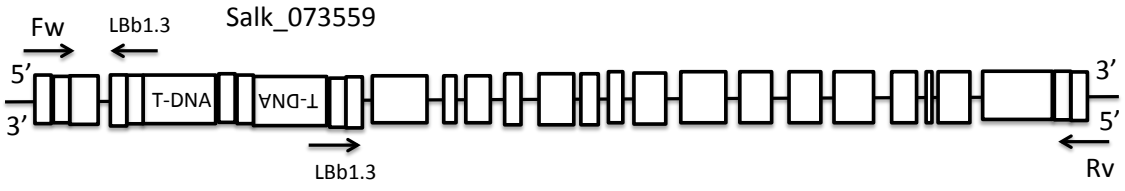
What Are the Genotypes of My Plants #7-12?



Expected Wild Type Size: 1,138 bp
Expected T-DNA Insert (FW+LBb1.3) Size: 807 bp
Expected T-DNA Insert (RV+LBb1.3) Size: 637 bp

Heterozygous T-DNA: Plants #7, 8
Homozygous WT: Plants # 9, 10, 11, 12

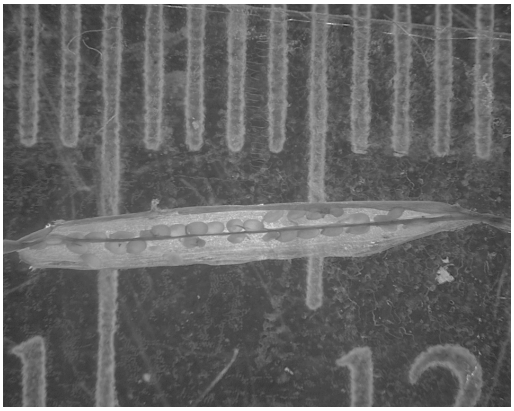
Where is the T-DNA Insert?



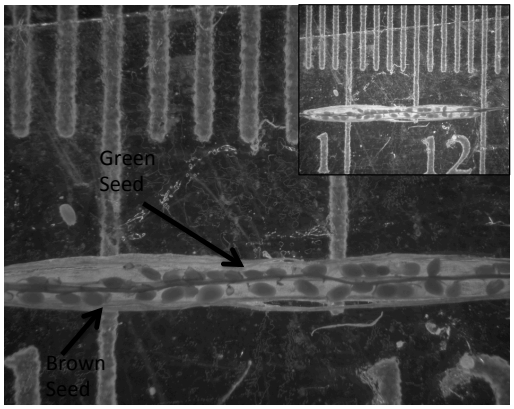
= Start = Exon

= Stop = T-DNA Borders

Is the T-DNA Insert Seed Lethal?



Wild Type Silique (Plant #12)
Green to White/Brown Seeds- 34:0



Heterozygous Mutant Silique (Plant #7)
Green to White/Brown Seeds- 40:13

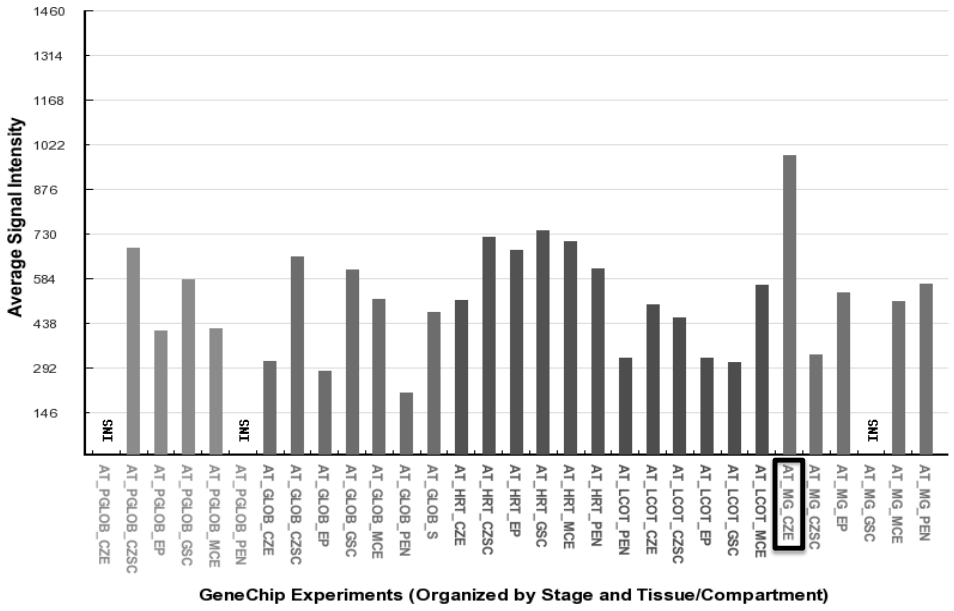
What Do My Results Mean?

Plant#	Genotype	Silique #	Length of Silique (cm)	Total Seeds	Total Mutant Seeds (White/ Brown)	Total WT Seeds (Green)
12	Homozygous WT	1	1.3	34	0	34
12	Homozygous WT	2	1.1	37	0	37
7	Heterozygous Mutant	3	1.3	53	13	40
2	Heterozygous Mutant	4	1.1	47	13	34
2	Heterozygous Mutant	5	0.7	18	3	15
8	Heterozygous Mutant	6	0.9	36	6	30
7	Heterozygous Mutant	7	0.7	30	8	22
8	Heterozygous Mutant	8	0.8	35	7	28
7	Heterozygous Mutant	9	0.7	35	11	24

Null Hypothesis: There is no significant difference between the expected 3:1 (green: white seeds) ratio and my observed results.

$\chi 2$ Value= 3.7597
p-Value= $0.05 \leq p \leq 0.1$

Where and When is Titan1 Expressed During Seed Development?



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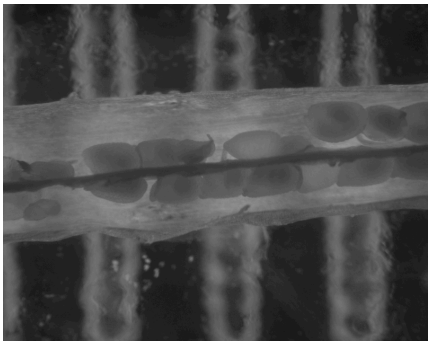
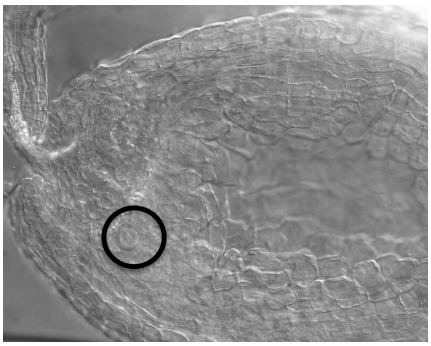
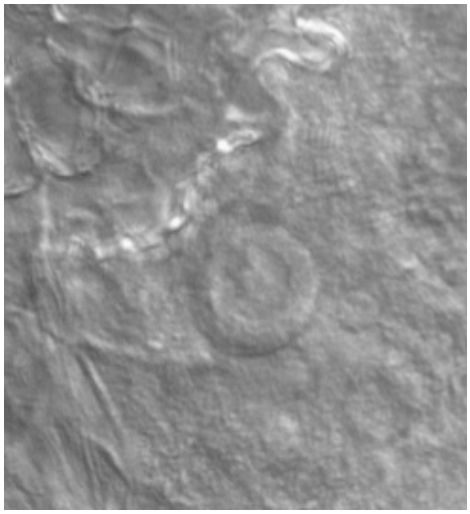
What is the Phenotype of the T-DNA?



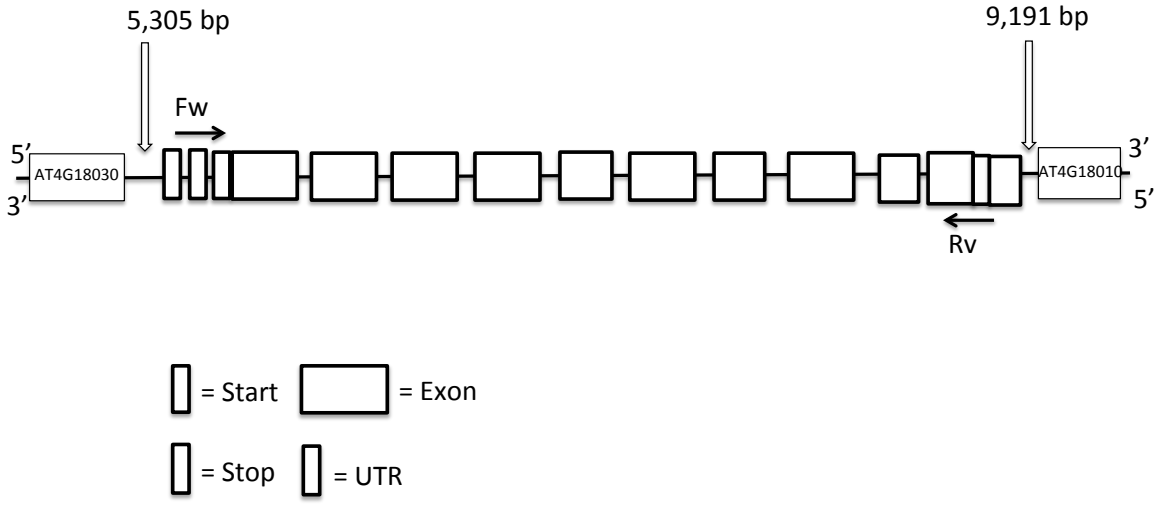




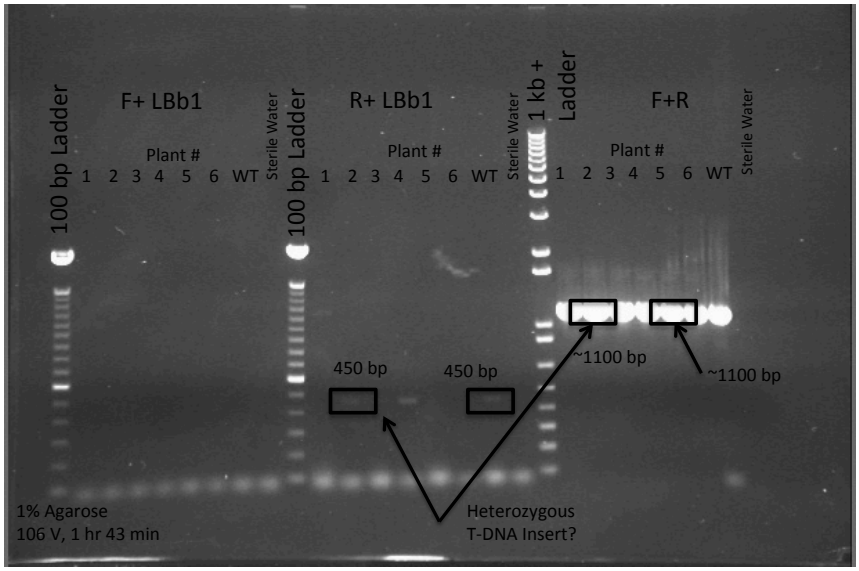
What is AT4G18020?

- Known as APRR2
- Chromosome 4
- Reverse Orientation
- 4,153 bp
- 10 exons, 10 introns
- Encodes pseudo-response regulator 2
- 535 AA

What is the Structure of APRR2?

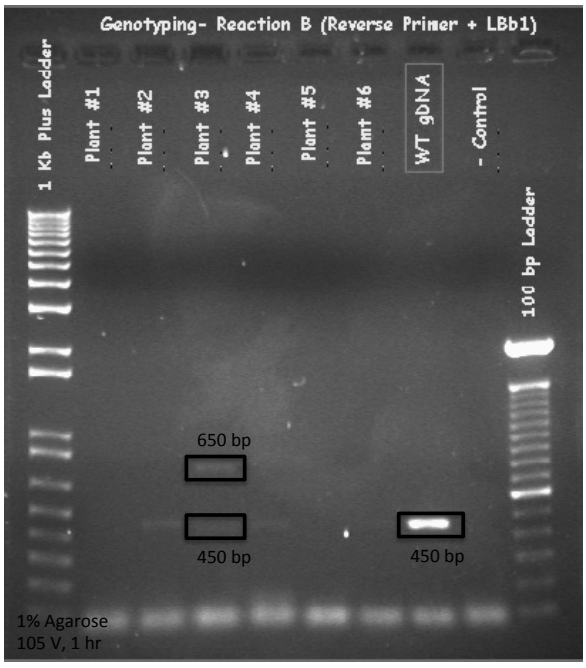


What Are the Genotypes of My Plants?



Expected Wild Type Size: 1,142 bp Expected T-DNA Insert (FW+LBb1) Size: 656 bp

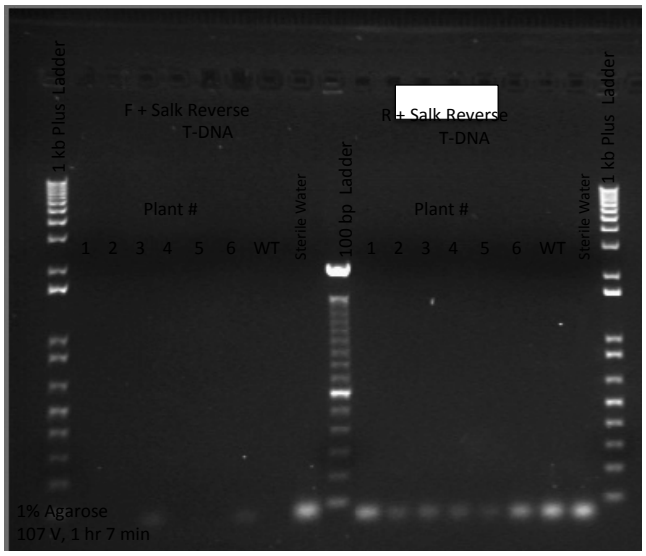
What Are the Genotypes of My Plants?



Possible problems?

- Contamination
- PCR Conditions were off
- Primers did not anneal properly
  - Non specific binding

What Are the Genotypes of My Plants?



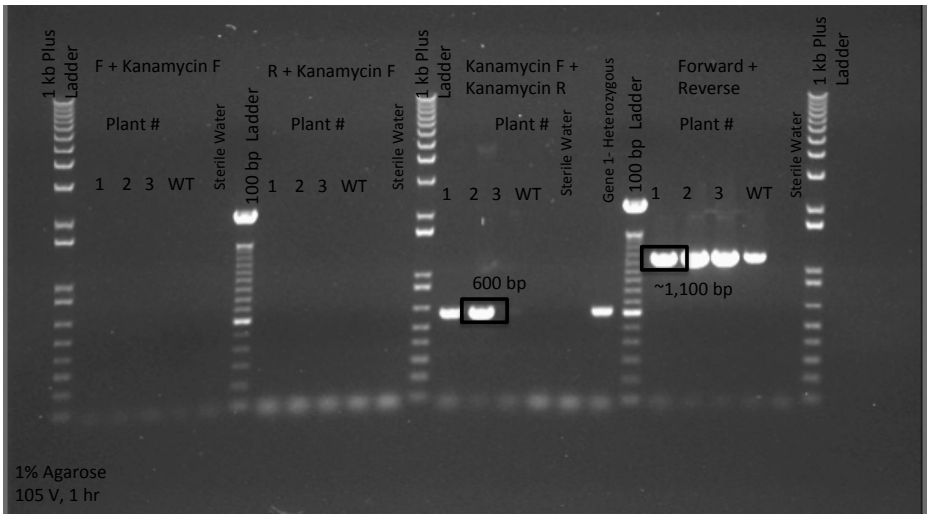
Possible problems?

- Huge rearrangement of T-DNA
  - Flipped
  - Deletion
- No T-DNA

Next Step?

- Kanamycin primers

What Are the Genotypes of My Plants?

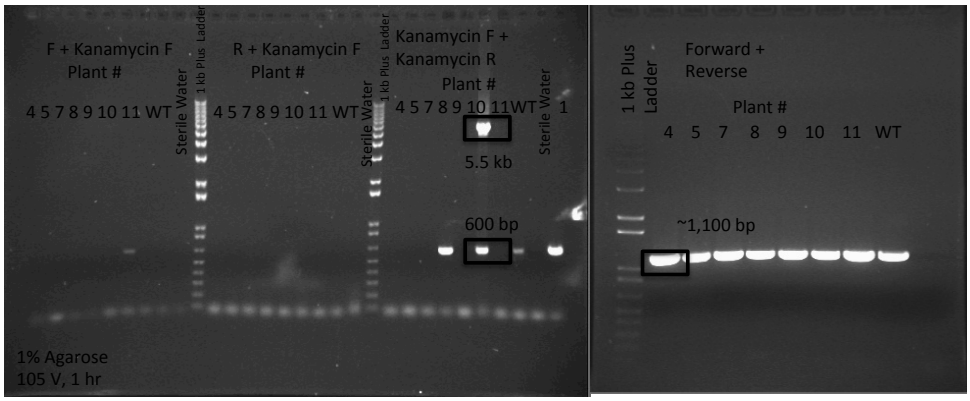


Expected Wild Type Size: 1,142 bp

Expected T-DNA Insert (F+ Kanamycin F OR R+ Kanamycin F) Size: 1,500 bp

Expected Kanamycin F+ Kanamycin R Size: 600 bp

What Are the Genotypes of My Plants?

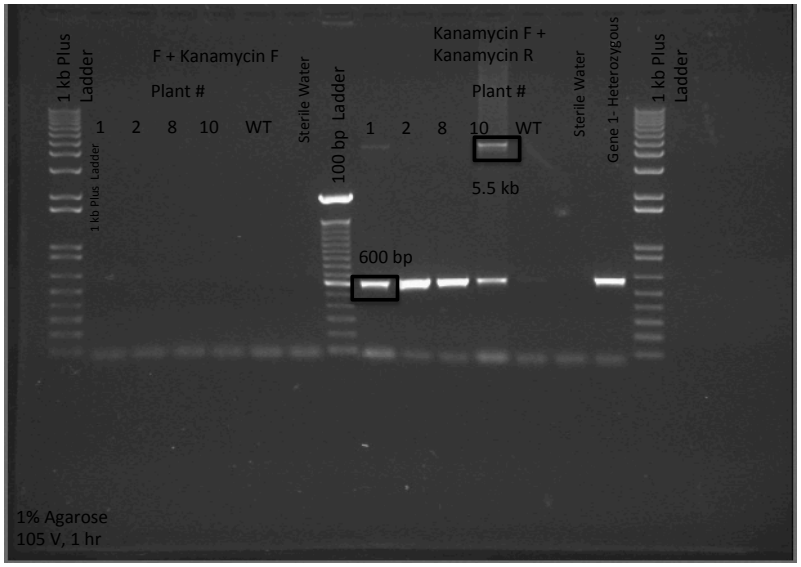


Expected Wild Type Size: 1,142 bp

Expected Kanamycin F+ Kanamycin R Size: 600 bp

Expected T-DNA Insert (F+ Kanamycin F OR R+ Kanamycin F) Size: 1,500 bp

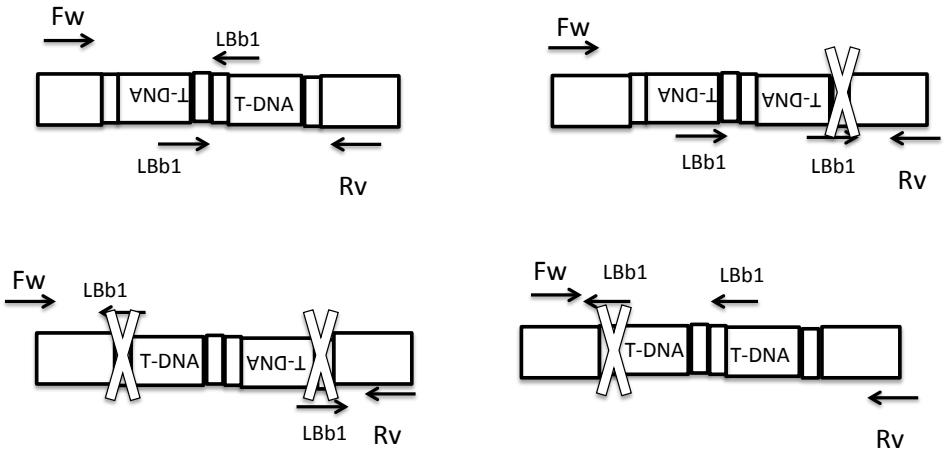
What Are the Genotypes of My Plants?



Expected Kanamycin F+ Kanamycin R Size: 600 bp

Expected T-DNA Insert (F+ Kanamycin F OR R+ Kanamycin F) Size: 1,500 bp

What Are the Possible T-DNA Orientations That Are Leading to a Lack of PCR Products?



Assuming T-DNA is in My Gene, Is the Mutation Seed Lethal?

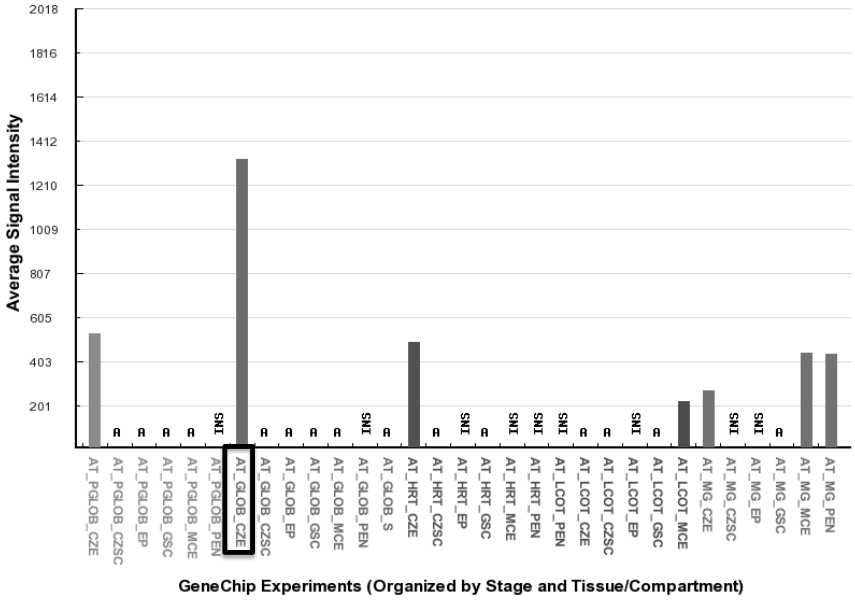
Silique #	Plant #	Genotype	Length of Silique (cm)	Total Green Seeds (Wild Type)	Total White/ Brown Seeds (Mutant)
-	6	WT	0.8	22	0
-	6	WT	0.9	21	0
1	10	Ht	0.9	31	0
2	10	Ht	0.9	34	0
3	10	Ht	0.6	7	0
4	10	Ht	0.8	16	0
5	10	Ht	1.1	38	3
6	10	Ht	0.6	13	0
7	8	Ht	1.2	49	0
8	8	Ht	1.0	33	0
9	8	Ht	0.5	-	-
10	8	Ht	0.9	45	1
11	8	Ht	1.2	40	0
12	8	Ht	1.1	42	0
13	8	Ht	1.1	37	0
14	8	Ht	1.1	32	0
15	10	Ht	1.1	33	1
16	10	Ht	0.7	37	2

Null Hypothesis: There is no significant difference between the expected 3:1 (green: white seeds) ratio and my observed results.

$\chi 2$ Value= 58.04
p-Value= 0.001

0.1% Probability deviation from expected ratio is due to chance.

Where and When is APRR2 Expressed During Seed Development?

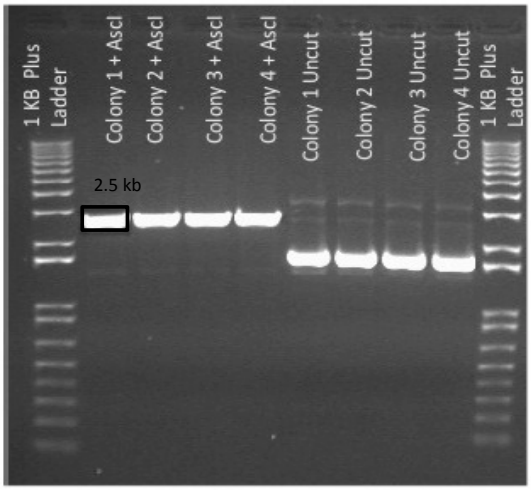


http://seedgenenetwork.net/

When and Where is APRR2 Transcribed?

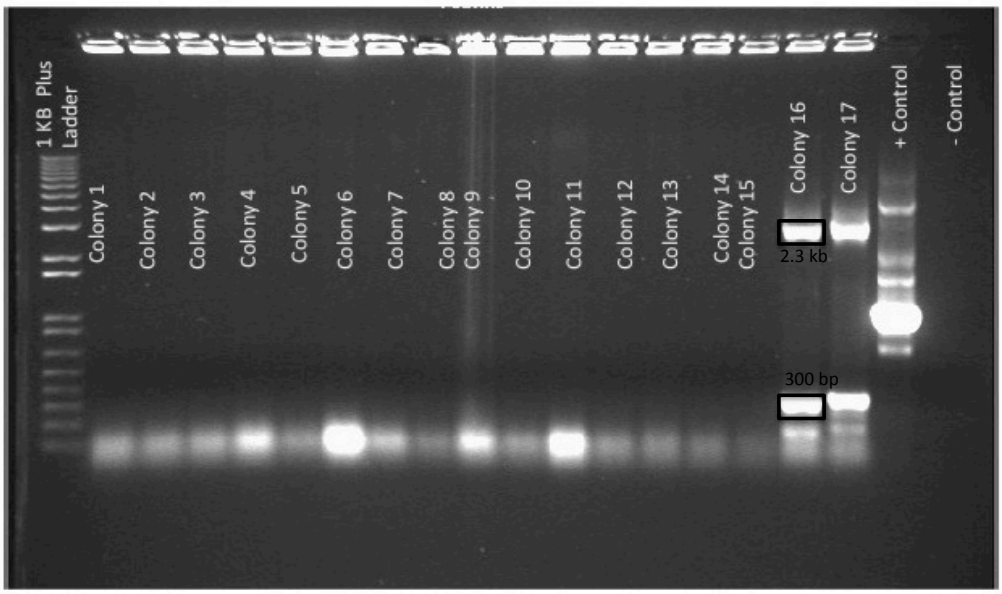
- Another way to determine level of gene expression
- Knowing when and where gene is expressed will shed information about its function
- Transcription factors bind to sites in upstream region allowing RNA Polymerase to begin transcription
- Use reporter genes fused to promoter region to determine promoter activity

Can I Clone the APRR2 Upstream Region?



Expected cut plasmid without insert
Size: 2.5 kb

Can I Clone the APRR2 Upstream Region?



Expected PCR Product for Whole promoter + Vector Size: 2.3 kb

Expected PCR Product for Vector Size: 300 bp

What is the Significance of My Results?

- Titan1
  - Knockout is most likely seed lethal
  - Mutant phenotype: enlarged nucleoli
- APRR2
  - Knockout is most likely NOT seed lethal
  - No mutant phenotype observed

What Further Research Can Be Done?

- Screen more plants for APRR2 or retry genotyping → sequencing
  - Reorder seeds from salk-line and re-sow seeds?
- Repeat experiment: Cloning APRR2 upstream region
  - Once reliable results are produced, proceed with experiment.
  - Transform Arabidopsis plants using Agrobacterium containing Ti helper plasmid and T-DNA plasmid