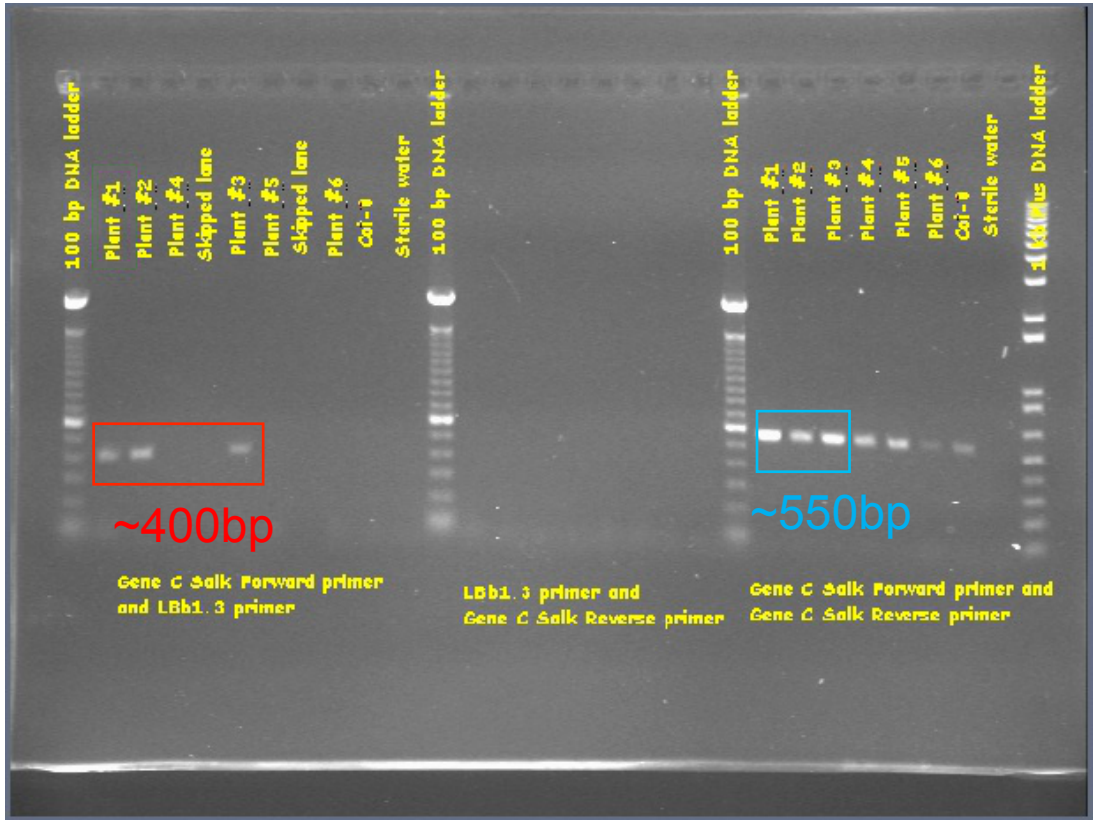
What are the functions of AT4G12750 (Hox7d) and AT5G27910 (NF-YC8)?

Ham Kok Wing

How did we determine the knockout?



- gDNA isolated from 6 plants, 5 potential mutants.
- PCR product formed from reactions with primers chosen between gene-specific Salk forward, gene-specific Salk reverse and LBb1.3 primers.
- Plants #1-3 are heterozygous for the T-DNA insert in the forward orientation.

What is AT4G12750?

- Also known as Hox7d.
- Codes for a homeodomain-like transcriptional regulator.
  - 1131 aa long.
  - Protein structural domain that binds DNA.
  - Involved in regulation of transcription.
- Orientated reverse to the direction of chromosome 4.

What is the structure of Hox7d?

5’ 3’

Start Stop

UTR

Start/Stop codons

Exon

Intron

3UTRs, 17 Exons, 17 Introns, 1 Start codon, 1 Stop codon
222bp, 485bp, 89bp, 3bp, 49bp, 199bp, 357bp, 267bp, 62bp, 81bp, 231bp, 99bp, 60bp, 74bp, 218bp, 56bp, 139bp, 99bp, 87bp, 155bp, 132bp, 199bp, 141bp, 303bp, 123bp, 154bp, 456bp, 301bp, 387bp, 113bp, 600bp, 112bp, 105bp, 96bp, 223bp, 208bp, 20bp, 3bp, 195bp.

Where is the T-DNA insert?

UTR

Start/Stop codons

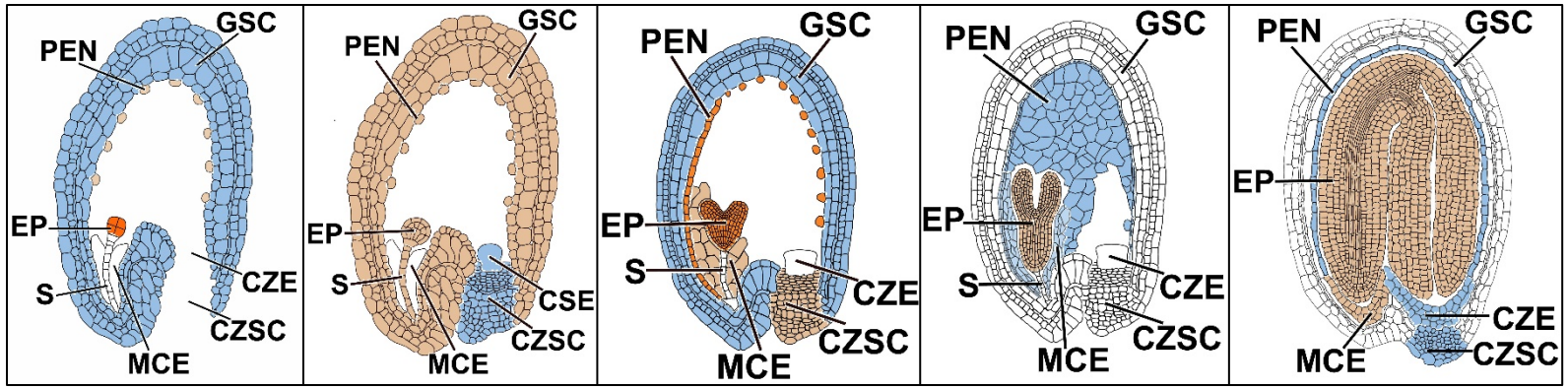
Exon

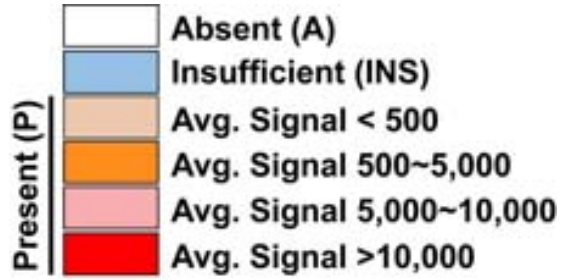
Intron

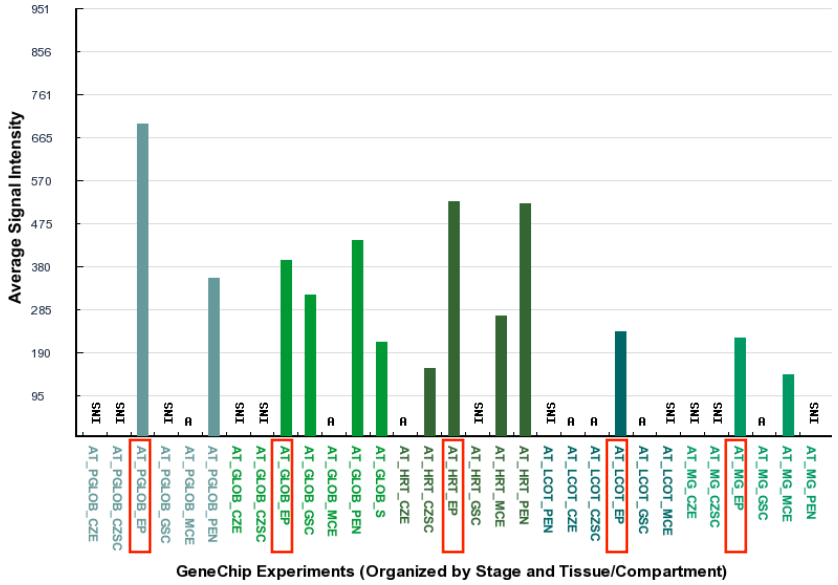
T-DNA insert

- Insertion site in intron 15, at nucleotide 7503226.
- Forward primer at exon 2.
- Reverse primer at exon 4.

Where is the gene expressed?



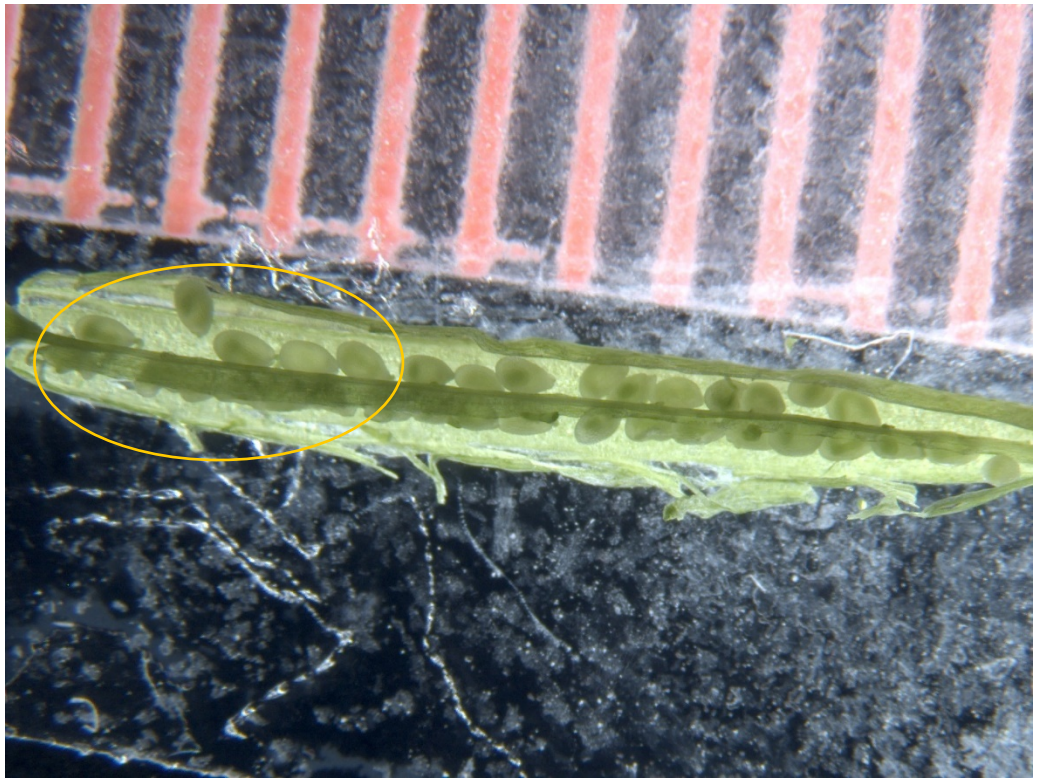




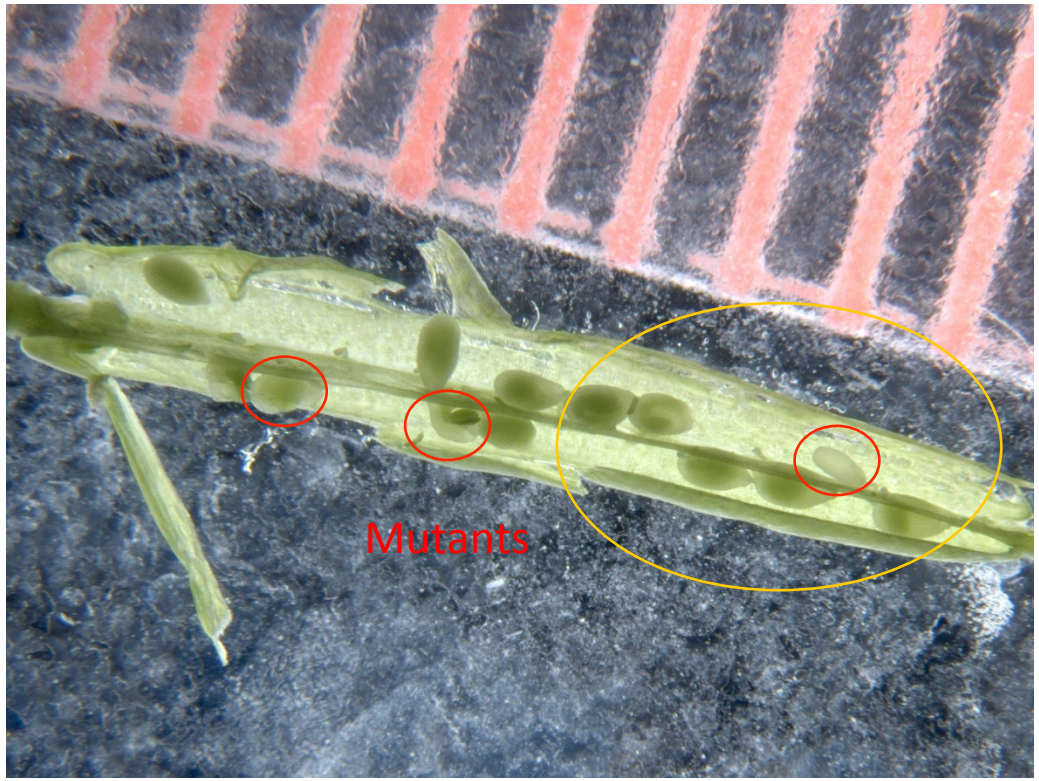
- The gene is active in the embryo proper at all stages.

What phenotypes were observed?

Observing a wild type silique with a light microscope

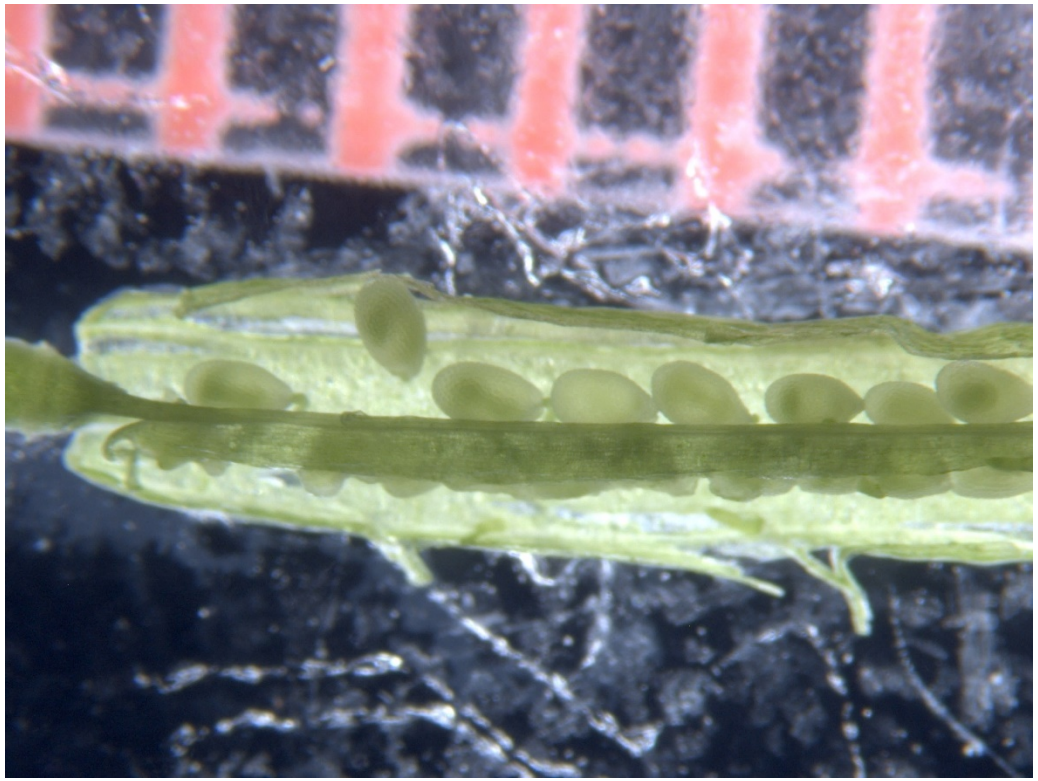


Observing a mutant silique with a light microscope

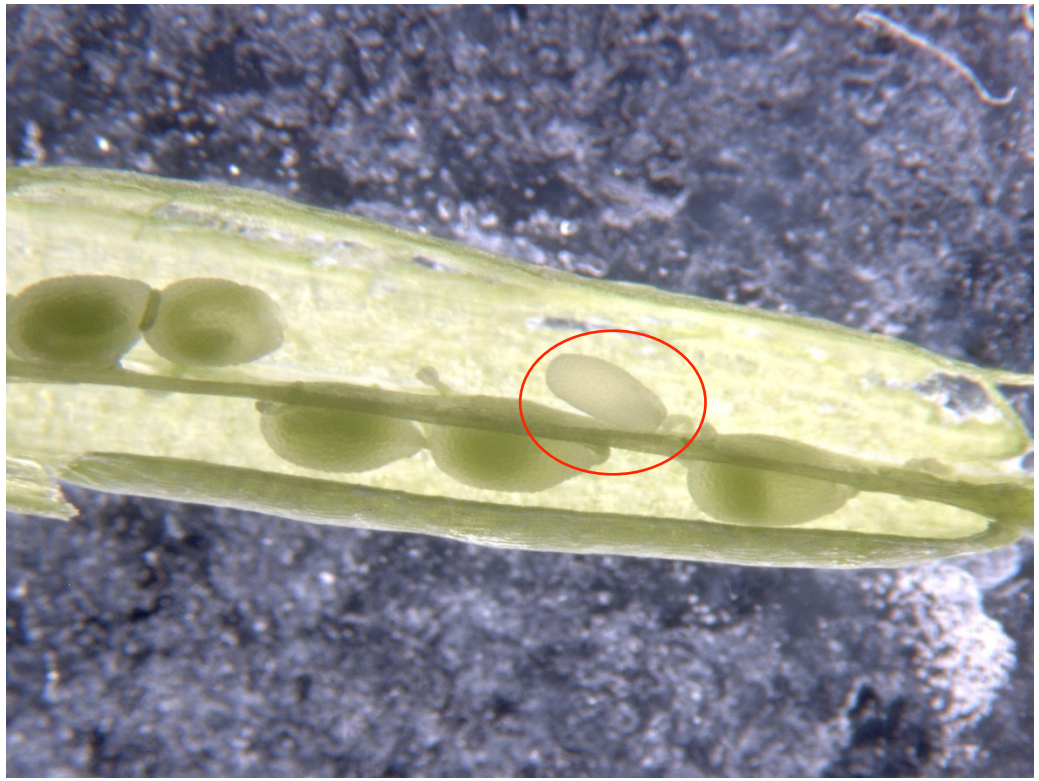


What phenotypes were observed?

Taking a closer look at a wild type silique

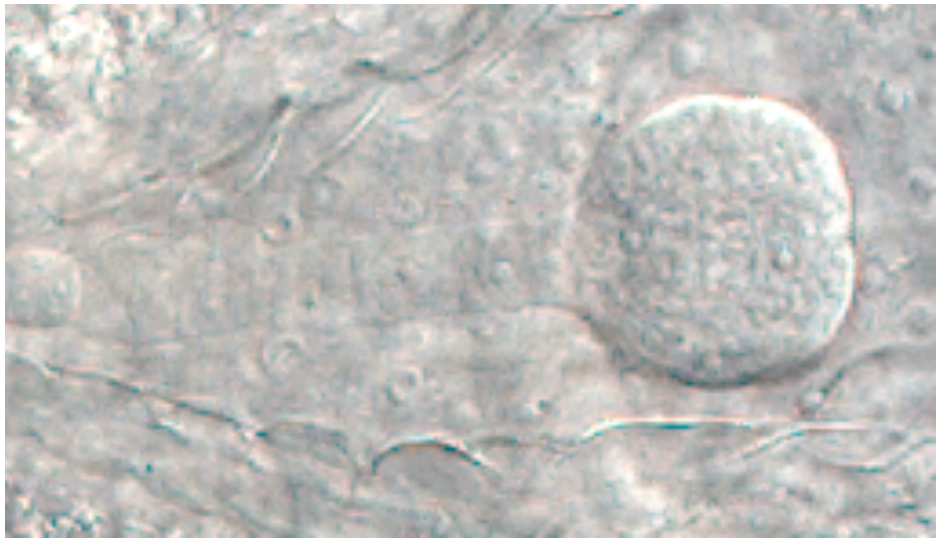


Taking a closer look at a mutant silique



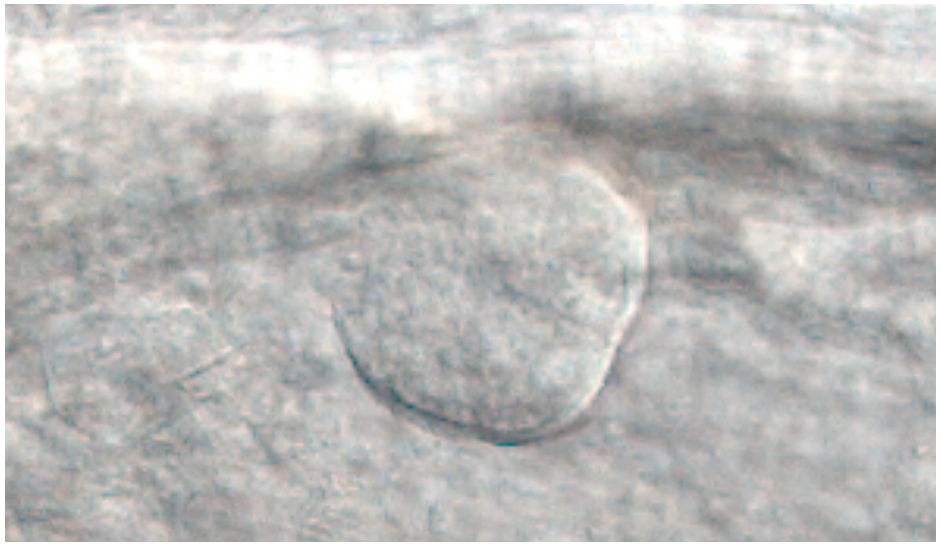
What phenotypes were observed?

Observing a wild type seed with a Nomarski microscope



A globular stage embryo with normal phenotype.

Observing a mutant seed with a Nomarski microscope



A globular stage embryo with abnormal phenotype.

What phenotypes were observed?

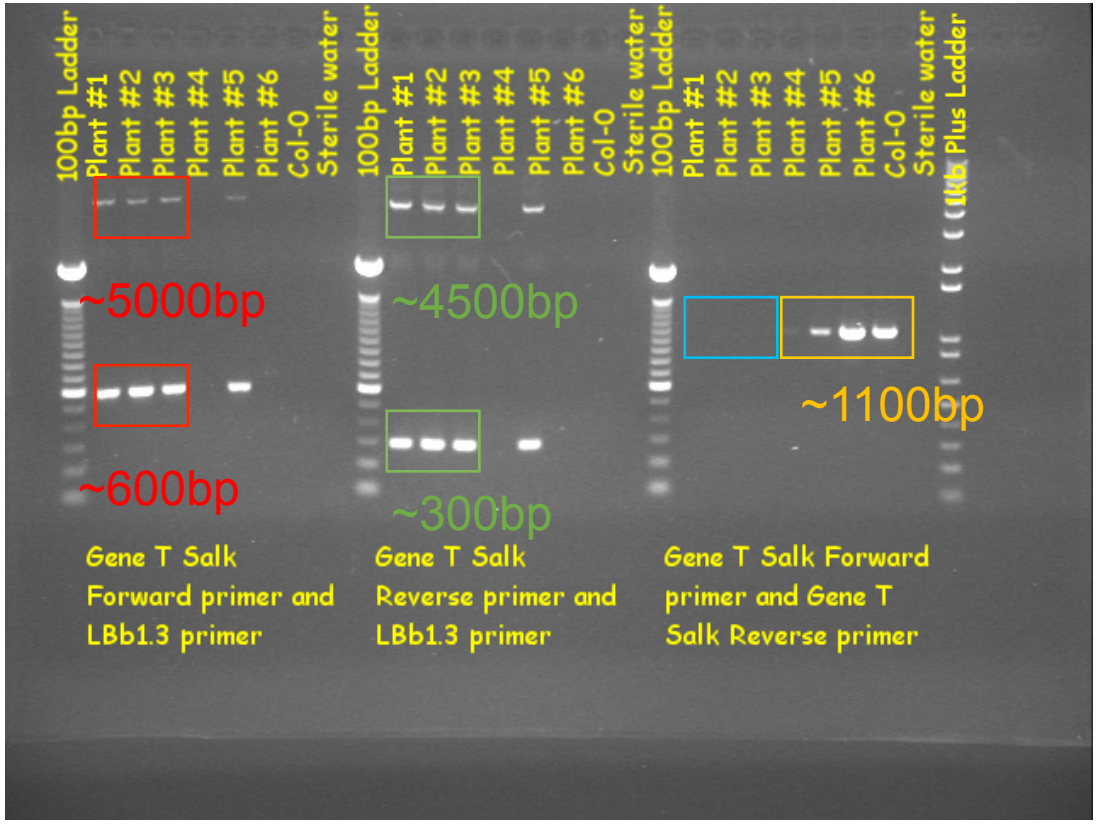
Observing a mature mutant seed with a Nomarski microscope

A mature seed for which no embryo could be found.

What do the observations mean?

- A close to 3:1 ratio was observed.
- Chi-squared tests agreed with the null hypothesis of a 3:1 ratio.
- Deformities at the globular to heart stages indicate that the gene becomes important for embryo development at this point.

How did we determine the knockout?



- gDNA isolated from 6 plants, 5 potential mutants.
- PCR product formed from reactions with primers chosen between gene-specific Salk forward, gene-specific Salk reverse and LBb1.3 primers.
- Plants #1-3 are homozygous for the T-DNA insert in both orientations.

I would like to acknowledge Kelli Henry for providing the PCR results.

What is AT5G27910?

- Also known as NF-YC8.
- Codes for a transcription factor.
  - 187 aa long.
  - Functions in DNA binding.
  - Involved in regulation of transcription.
- Orientated reverse to the direction of chromosome 5.

What is the structure of NF-YC8?

UTR

Start/Stop codons

Exon

Intron

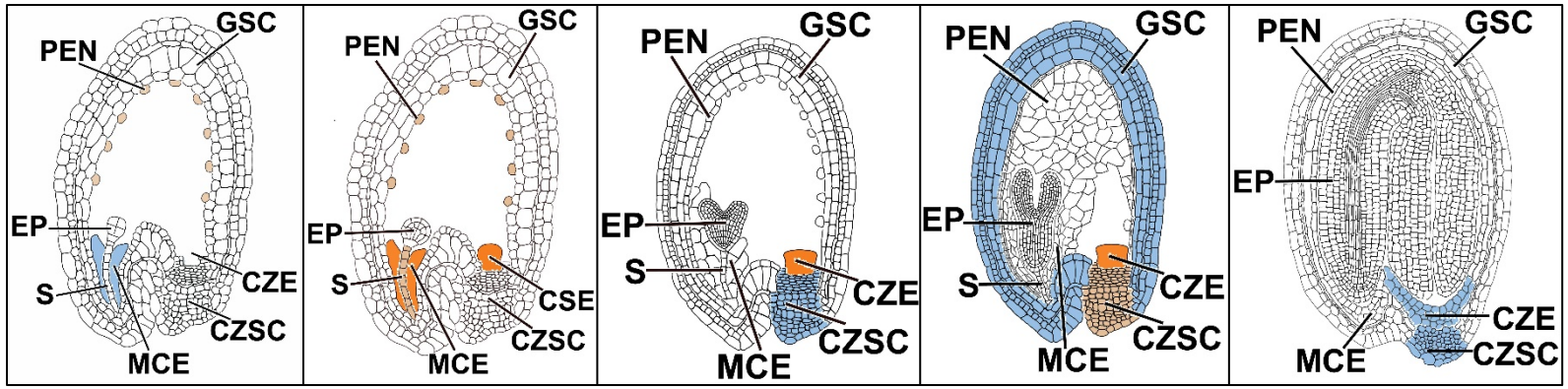
1 Exon,1 Start codon, 1 Stop codon
3bp, 558bp, 3bp.

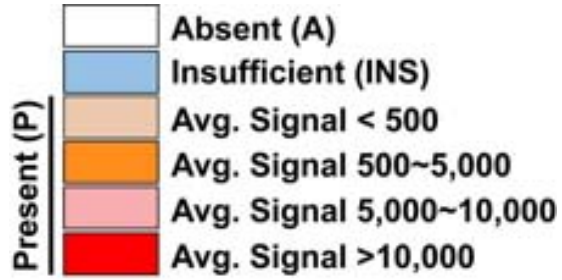
Where are the T-DNA inserts?

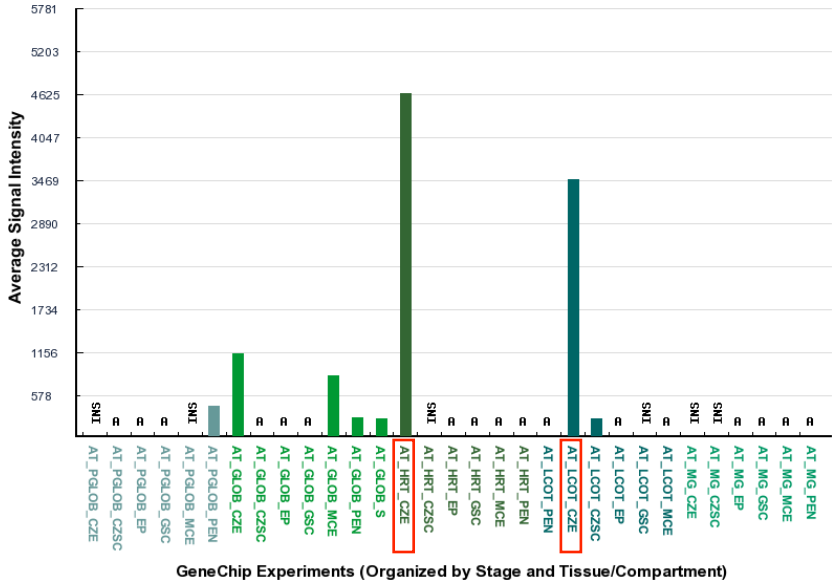
- UTR
- Start/Stop codons
- Exon
- Intron
- T-DNA insert
- Upstream/Downstream regions

- Upstream region: 93bp, 204bp
- Downstream region: 213bp
- Forward orientation at nucleotide 9941148.
- The insertion site for the forward orientation is expected as per the TAIR website; however, the reverse orientation insertion is not expected.
- To form a ~300bp product, the reverse T-DNA insert cannot be attached to the forward T-DNA insert, as this would produce a ~1000bp product, which does not show on the gel.
- Primers should be specific enough, as a check was done allowing for 5 base pair changes anywhere along the primers.
- The data gathered is insufficient to determine the other two insertion sites.

Where is the gene expressed?



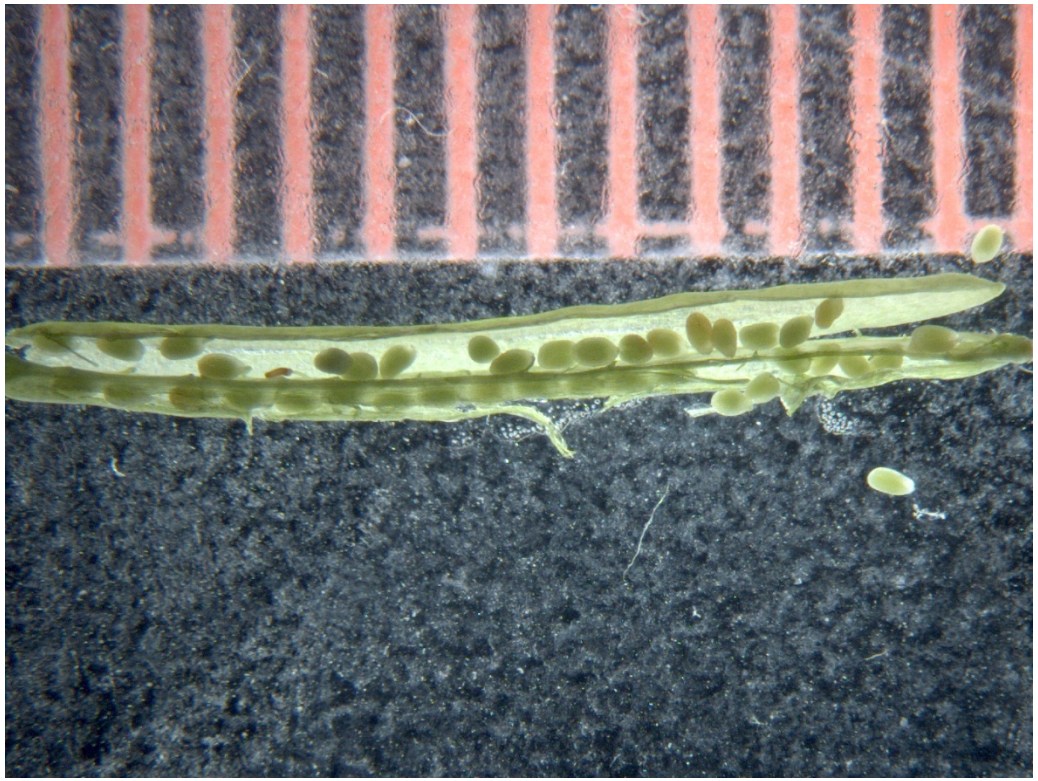




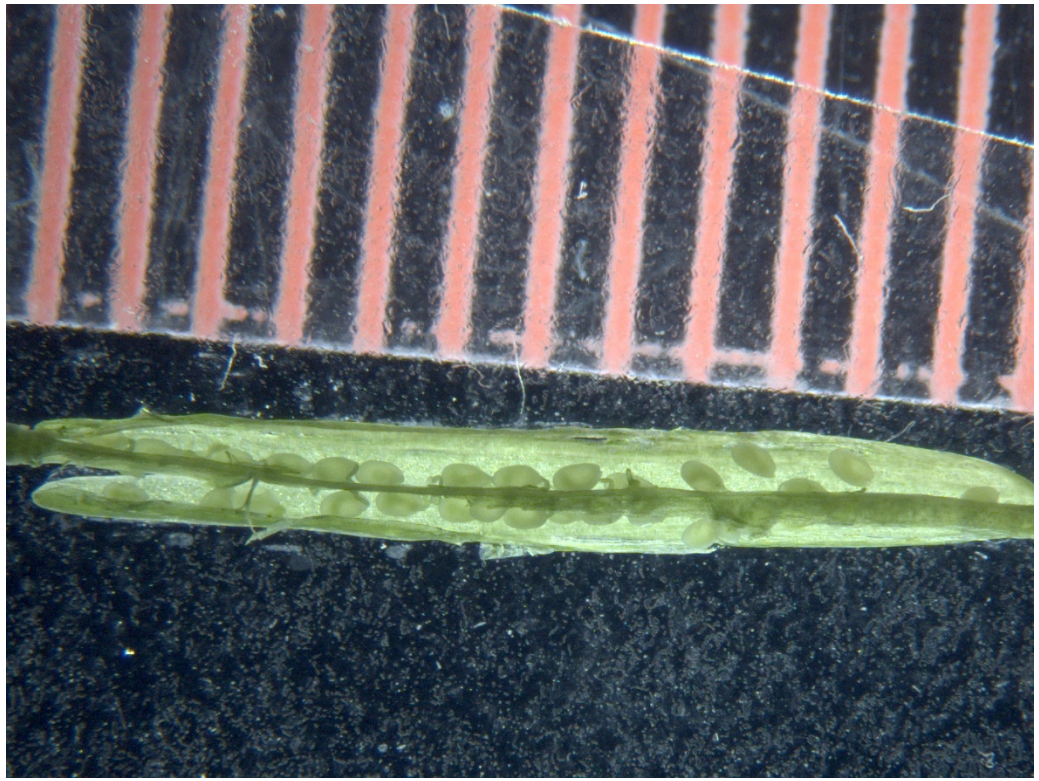
- The gene is very highly active in the chalazal endosperm during the heart and linear cotyledon stages.
  - The chalazal endosperm is the subregion with the most seed-specific mRNAs.

What phenotypes were observed?

Observing a wild type silique with a light microscope



Observing a mutant silique with a light microscope

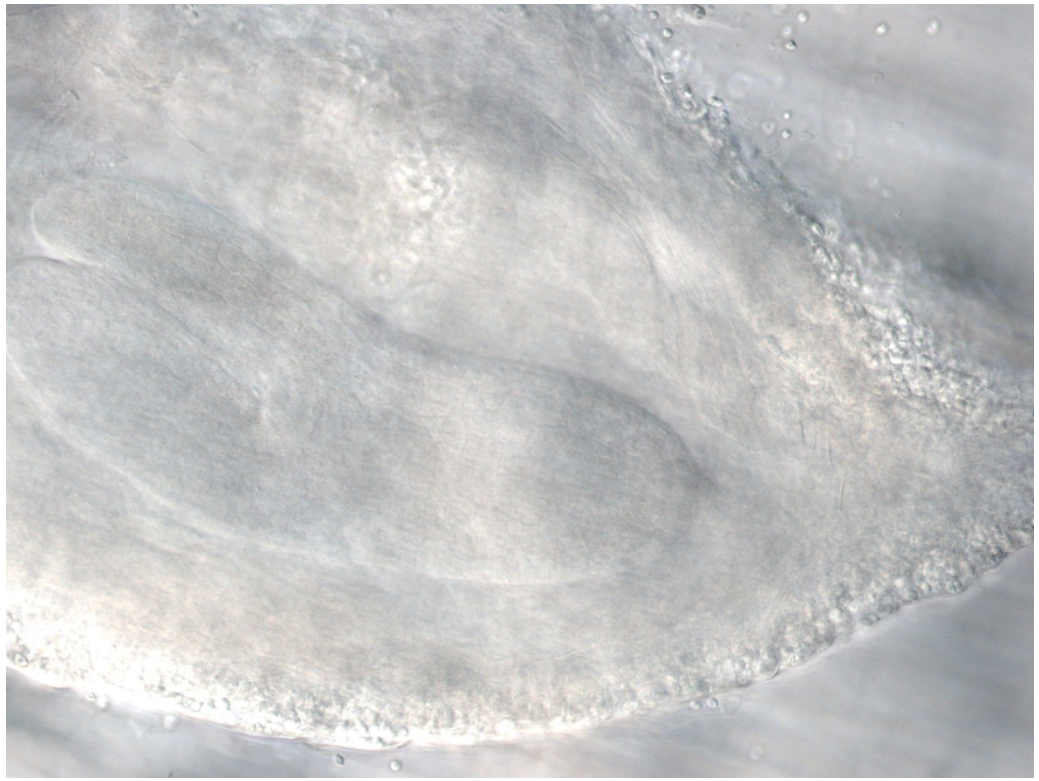


Both wild type and mutant seeds have similar phenotypes.

What phenotypes were observed?

Observing a wild type seed with a Nomarski microscope

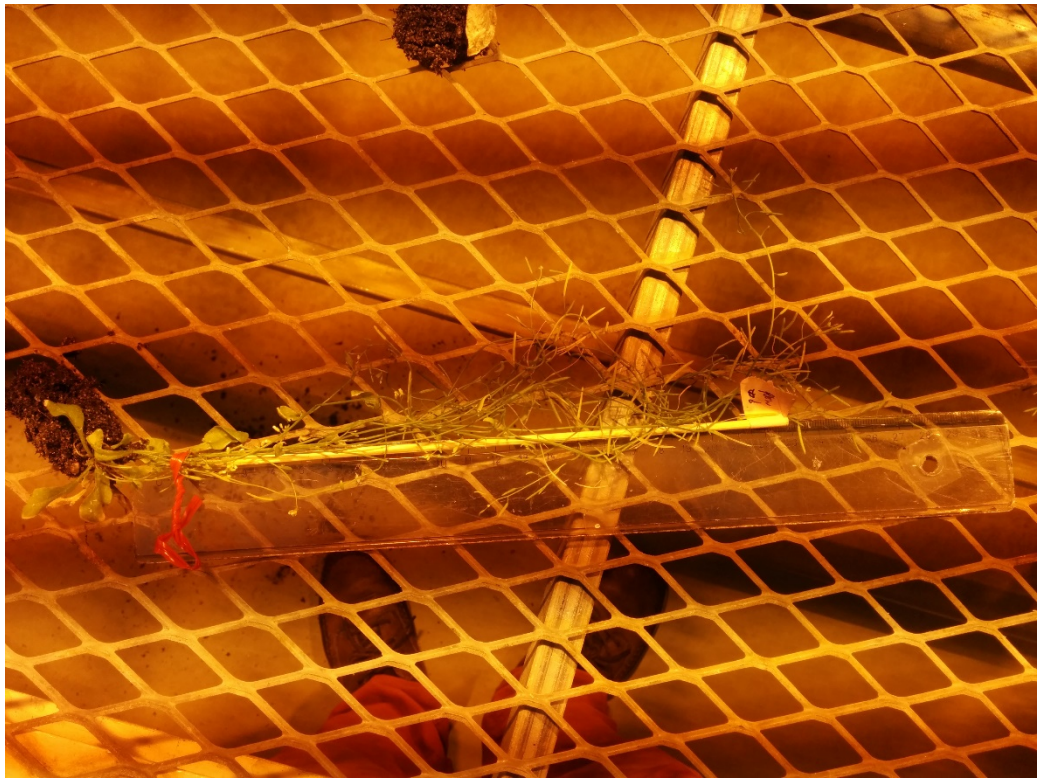
Observing a mutant seed with a Nomarski microscope



Both wild type and mutant seeds have similar phenotypes.

What phenotypes were observed?

Measuring main stem height



Measuring root length

What phenotypes were observed?

Measuring main stem height

Plant #	Genotype	Height (cm)
1	Homozygous	48
2	Homozygous	44
3	Homozygous	48
4	Wild type	39
6	Wild type	30

Measuring root length

Plant #	Genotype	Length (cm)
1	Homozygous	3.9
2	Homozygous	6.5
3	Homozygous	9.3
4	Wild type	3.6
6	Wild type	6.1

- Average mutant stem height: 46.7cm; average wild type stem height: 34.5cm
  - Average mutant stems are taller.
- Average mutant root length: 6.57cm; average wild type root length: 4.85cm
  - Average mutant stems are longer.
- Sample size is insufficient to draw any conclusions.

What do we do next?

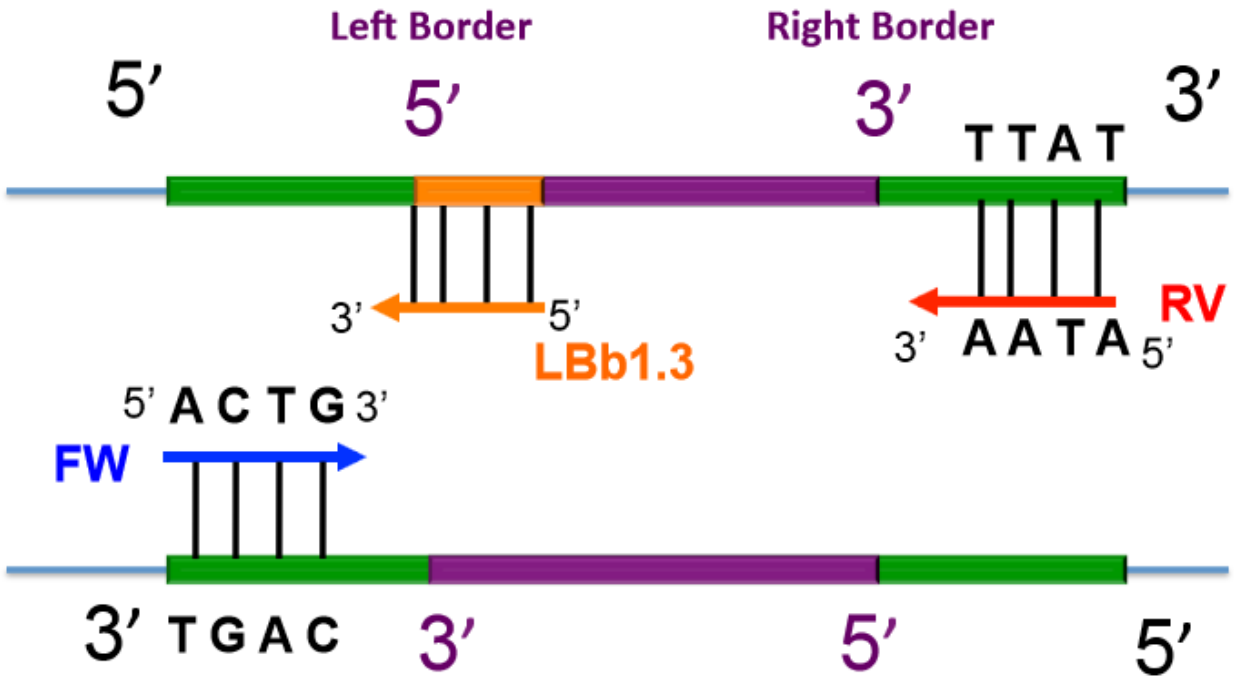
- Further experimentation required.
  - Determination of other T-DNA insertion sites.
  - Isolation of seed subregions and comparison mRNA levels between wild type and mutant seeds at different stages of growth.
  - Knockouts of gene subfamily.
  - Observation of more mature plants.

I would like to express my gratitude to Kelli Henry, Mike Lyons and Professor Bob Goldberg for facilitating this unique and wonderful experience. Thank you!

How do we genotype plants?

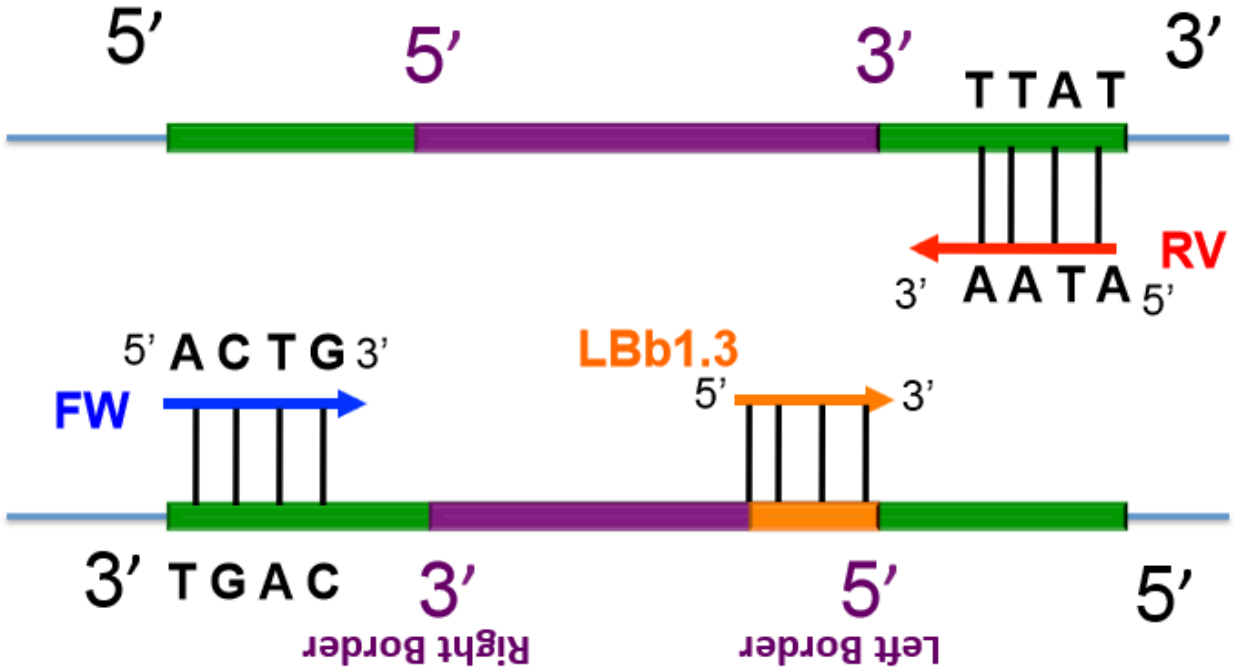
- **Form solutions with a combination of two primers chosen between the Gene-specific Forward, Gene-specific Reverse and left border primers, and perform polymerase chain reaction (PCR)**
- **Run the PCR products through gel electrophoresis.**
- **Interpret the results.**

What happens if T-DNA is present in the forward orientation?



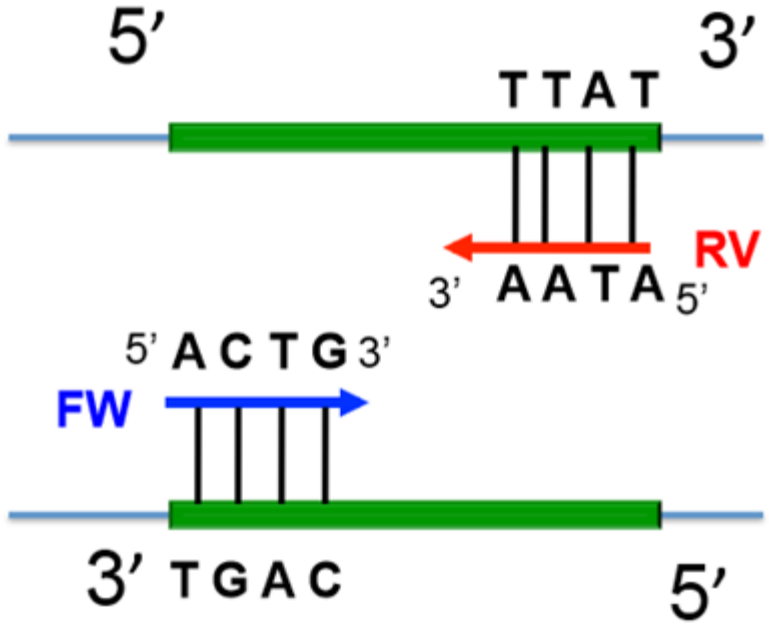
A PCR product is formed between the gene-specific forward and left border primers.

What happens if T-DNA is present in the reverse orientation?



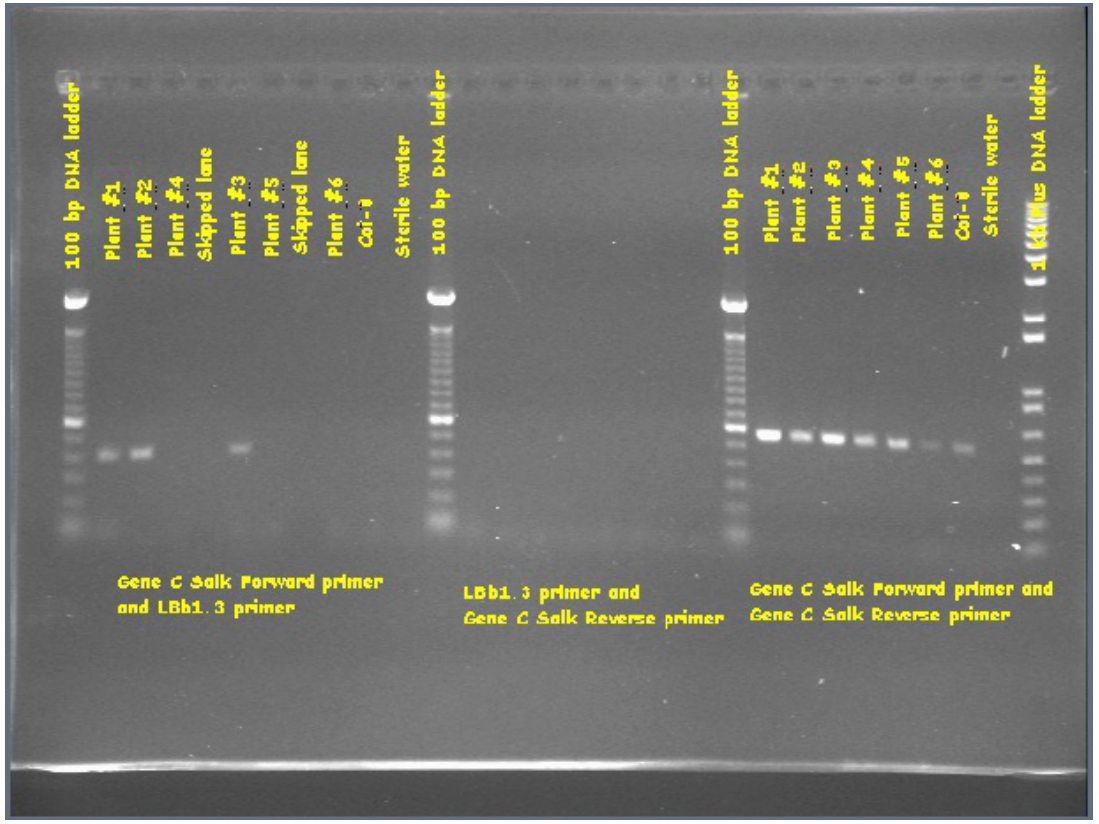
A PCR product is formed between the gene-specific reverse and left border primers.

What happens if no T-DNA is present?



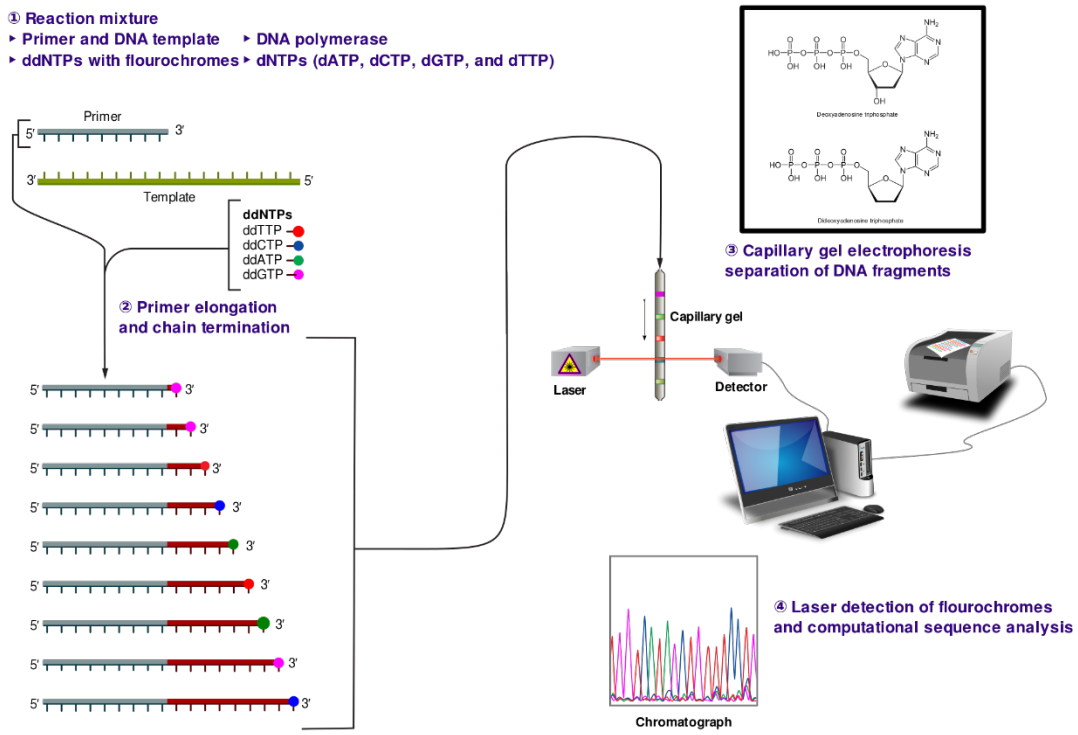
A PCR product is formed between the gene-specific forward and gene-specific reverse primers.

How do we interpret gel electrophoresis results?



- If a band shows up for a gene-specific forward or reverse primer and Lbb1.3 primer, T-DNA is present in that orientation in the gene of interest of the DNA sample.
- If a band shows up for gene-specific forward and reverse primers, a wild-type allele is present in the gene of interest in the DNA sample.

What is Sanger sequencing?



- Dideoxynucleotidetriphosphates (ddNTPs) terminate DNA chain elongation because they cannot form a phosphodiester bond with the next deoxynucleotide.
- Four reactions are done, with one of each ddNTP and all four dNTPs.
- The resultant products are run through a gel and the resultant bands will produce a visualization of the DNA sequence.