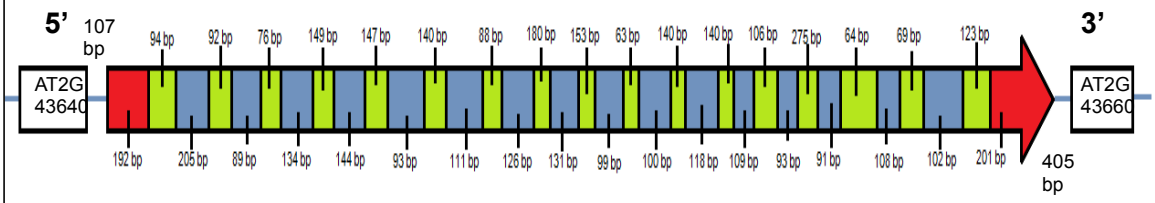
Of EMB2777 and F14J22.20 - exons & introns

by Hanbee O

What are the Properties of the Protein EMB2777?

- Embryo defective / seed lethal transcriptional factor
  - Sas10 / U3 ribonucleoprotein (Utp) family
  - Gene-silencing
  - 654 aa
- Coded by the gene AT2G43650
  - Located in Chromosome 2
  - 4108 bp
  - Forward (5'→3')

What is the Structure of AT2G43650?



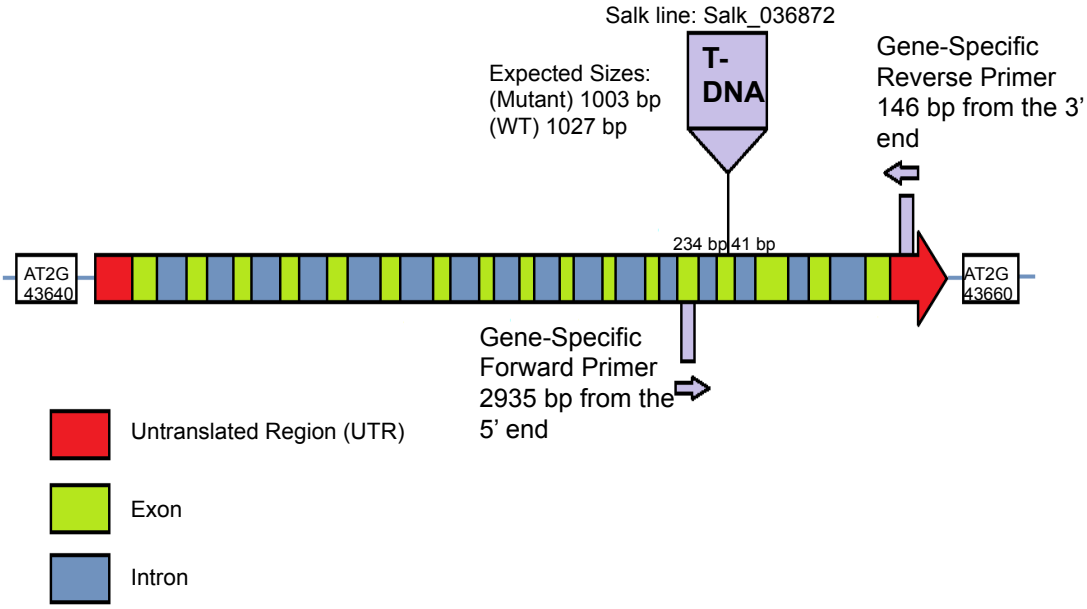
(bp = base pairs)

Untranslated Region (UTR)

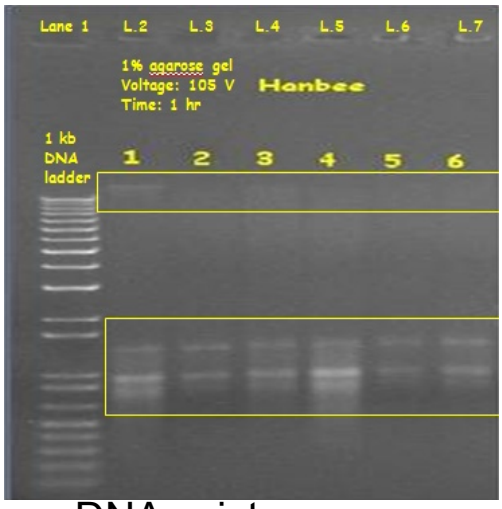
Exon

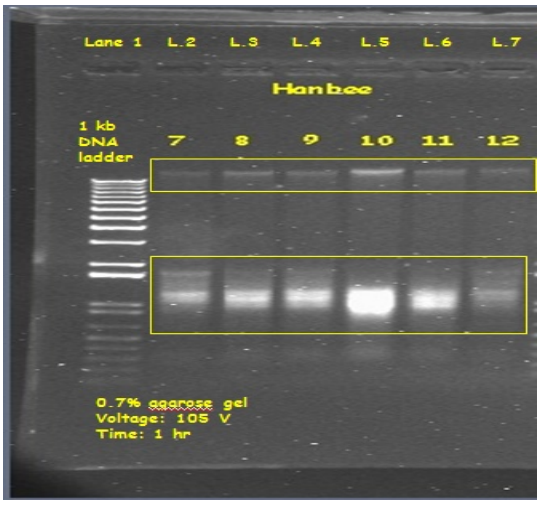
Intron

Where is the T-DNA Insertion Site for AT1G49560?



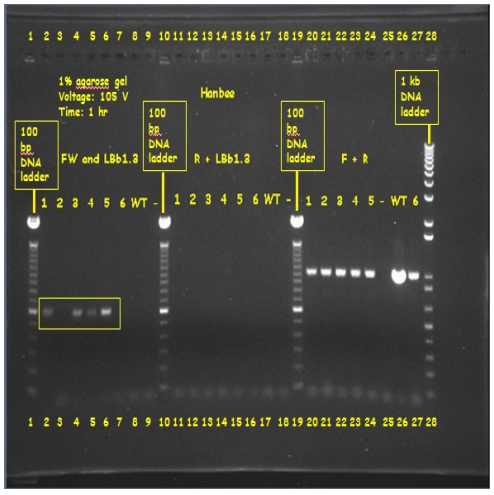
What are the Genotypes of Plants?

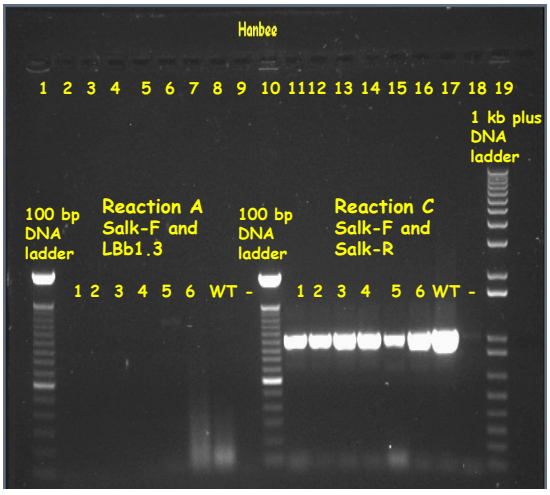




- gDNA exists

What are the Genotypes of Plants?





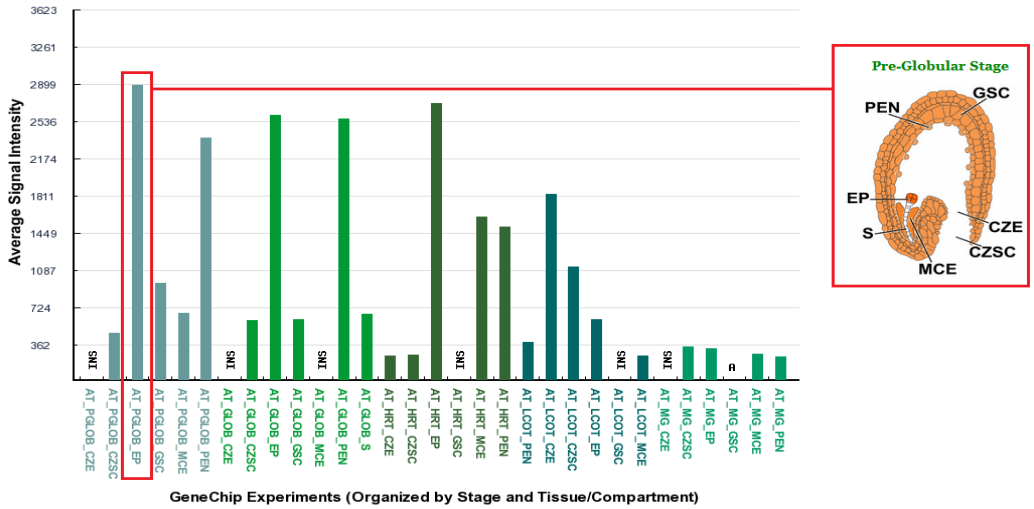
What are the Genotypes of Plants?

Plant #	Mutant?	WT?	Genotype?
1	**X**	**X**	**Heterozygous**
2	---------	X	Homo. WT
3	**X**	**X**	**Heterozygous**
4	**X**	**X**	**Heterozygous**
5	**X**	**X**	**Heterozygous**
6 (WT)	---------	X	Homo. WT
7	---------	X	Homo. WT
8	---------	X	Homo. WT
9	---------	X	Homo. WT
10	---------	X	Homo. WT
11	---------	X	Homo. WT
12 (WT)	---------	X	Homo. WT

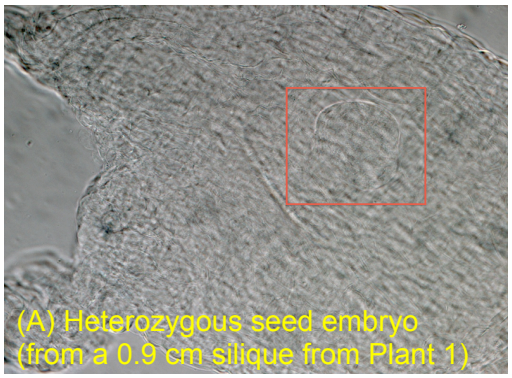
Genotype	Plant #
Heterozy-gous	1, 3, 4, 5
Homo. WT	2, 6, 7, 8, 9, 10, 11, 12

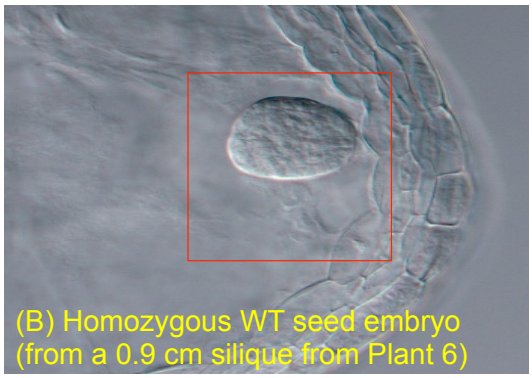
No homozygous mutant!
Likely to be seed lethal

When and Where is the Gene Active in Seed Development?



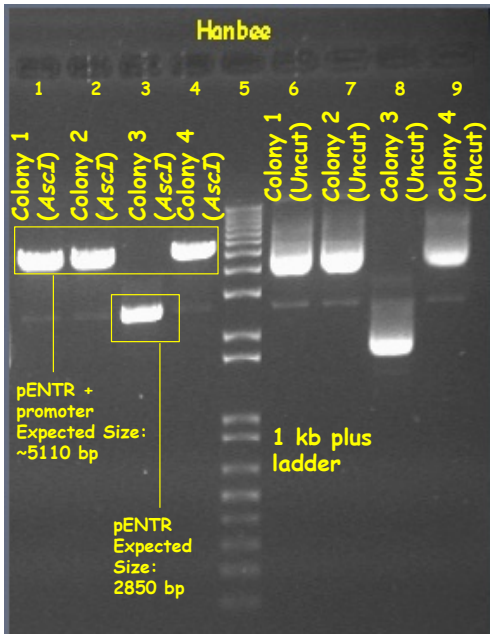
What are the Differences in Seed Phenotypes?





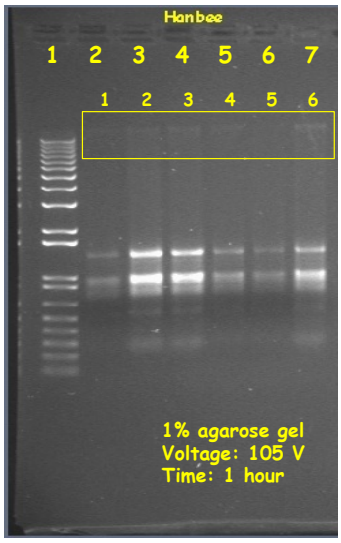
- Embryo's morphology affected by the mutant gene's presence
- Changes in seed coat hard to observe, but WT embryo's seed coat seems to be thicker and more developed

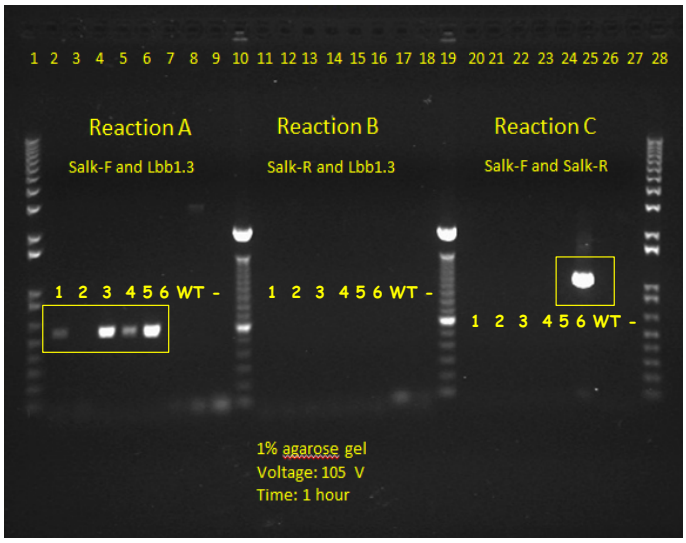
How Can I Observe the Activity of the Promoter of a Gene of Interest in an Arabidopsis Plant?



- Using promoter region and reporter genes (GUS and GFP), we can visualize the activity of the gene of interest at cellular and tissue levels
- Transformation, Selection, Genotyping, Sequencing
- In the end, transform Arabidopsis plants and observe the gene expression

What are the Genotypes of Plants?





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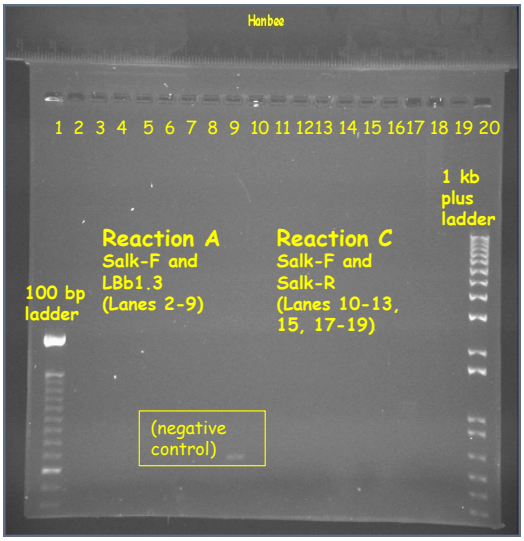

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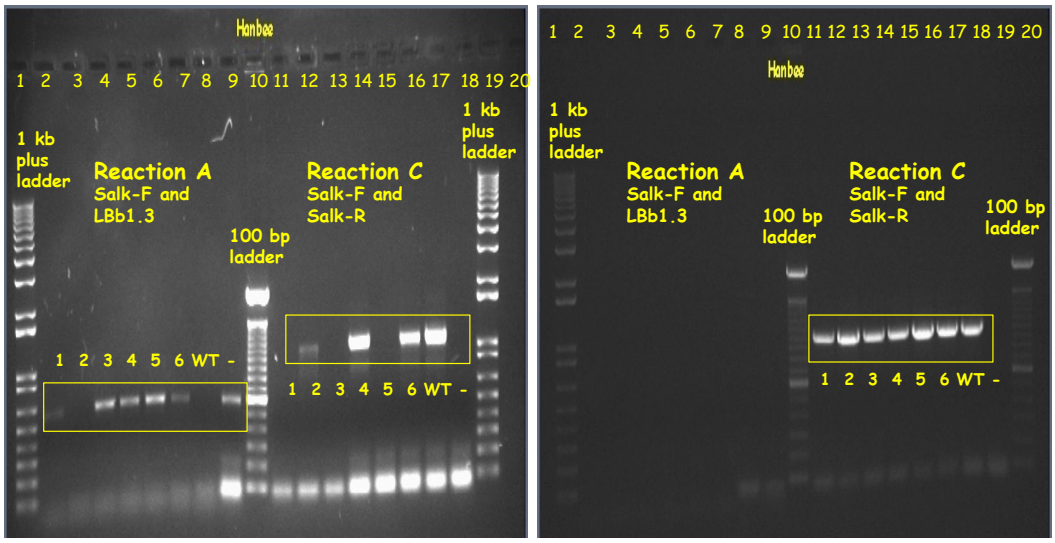


What are the Genotypes of Plants?

	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6 (WT)	+ Control	- Control
Trial 1	T	---------	T	T	T	WT	---------	---------
Trial 2	T	WT	T	T & WT	T	WT	T & WT	T
Trial 3	---------	---------	---------	---------	---------	---------	---------	T
Trial 4	T	WT	T	T & WT	T	T & WT	WT	T
Pheno-type?	Homo. mutant	Homo. WT	Homo. mutant	?????	Homo. mutant	?????	?????	?????

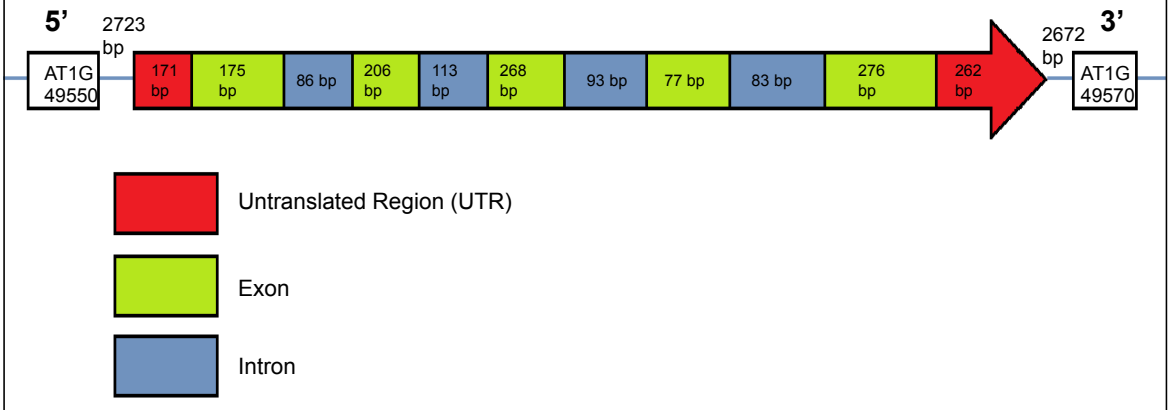
Genotype	Plant #
Homozygous mutant	1, 3, 5
Homozygous WT	2

Homozygous mutants are present!
Mutation is not seed lethal

What are the Properties of the Protein F14J22.20?

- Myb-like DNA-binding domain protein
  - Homeodomain-like superfamily
  - DNA-dependent
  - 333 aa
- Coded by the gene AT1G49560
  - Located in Chromosome 1
  - 1810 bp
  - Forward (5'→3')

What is the Structure of AT1G49560?



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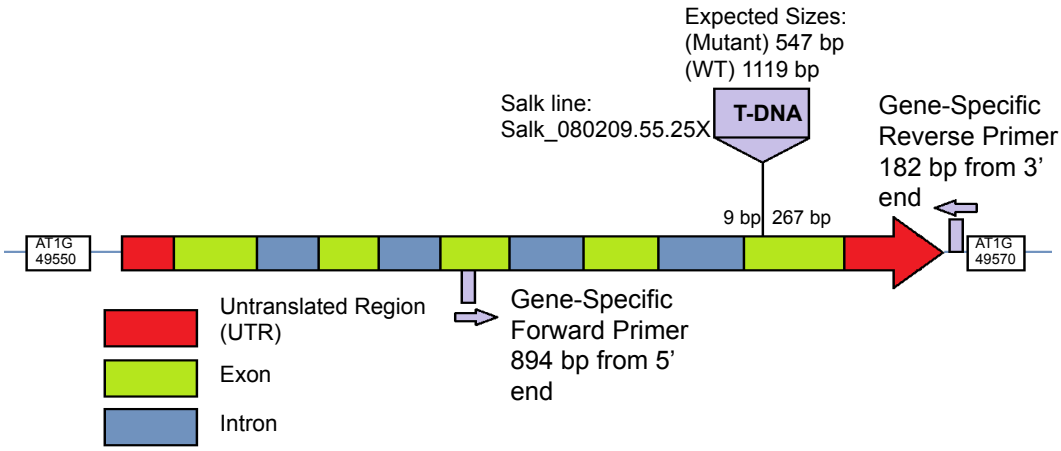

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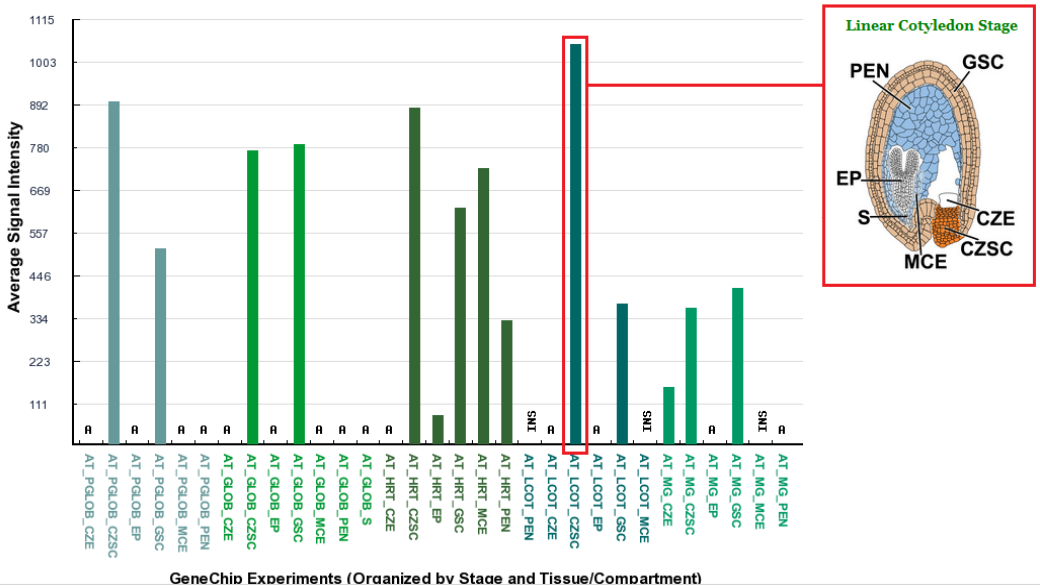

What are the Differences in Seed Phenotypes?

- Pictures could not be saved due to technical difficulties
- No distinguishable difference between homozygous mutant and homozygous WT seeds in neither the chalazal seed coat nor the seed coat
- What next?

What is the Significance of the Results?

AT2G43650	AT1G49560
Knockout of the gene is likely to be seed lethal	Knockout of the gene is not lethal
Mutation has a significant impact on embryo / seed development	Mutation does not have a significant impact on embryo / seed development
Mutant gene affects the morphology of the embryo (and possibly the seed coat)	No mutant phenotype observed

What Future Researches Can be Done?

- Observation of seed phenotypes with better visualizing equipments in order to observe subtle phenotype changes caused by gene mutation (especially AT1G49560)
- Is gene AT1G49560 dominant or recessive?
- Transforming Arabidopsis plants with promoter regions and reporter genes to see where genes are expressed at

Acknowledgements

- Thank **YOU**!
- Prof. Goldberg, Kelli, Brandon, Eden, Elaine, and others in the Goldberg Lab
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