

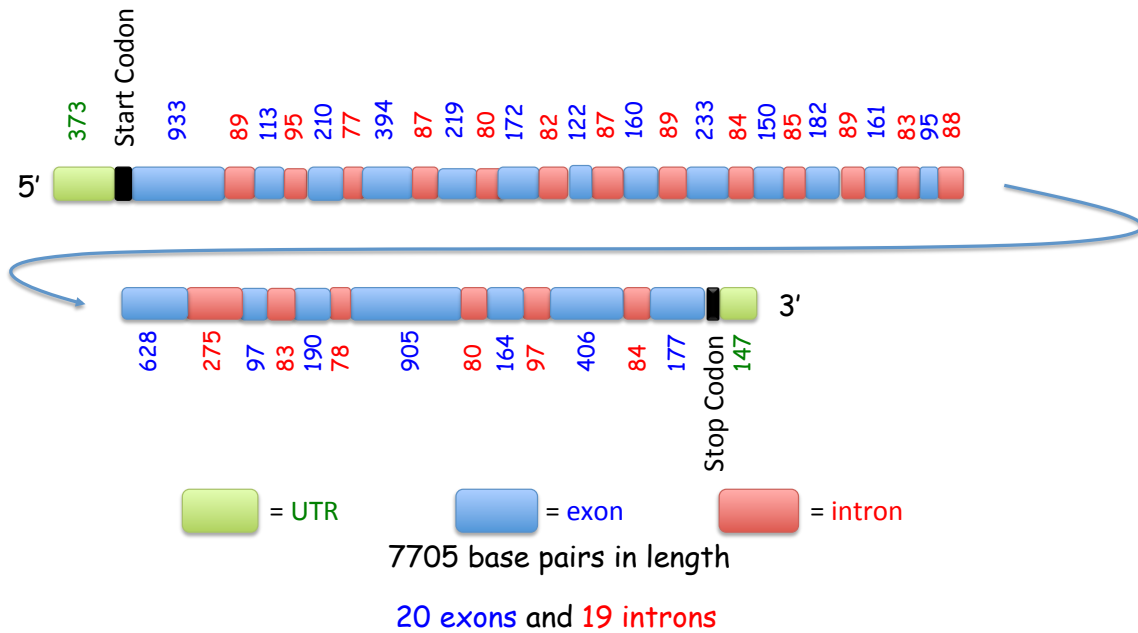
What are the functions of AT1G01040 and AT4G00950?

Mike Lyons

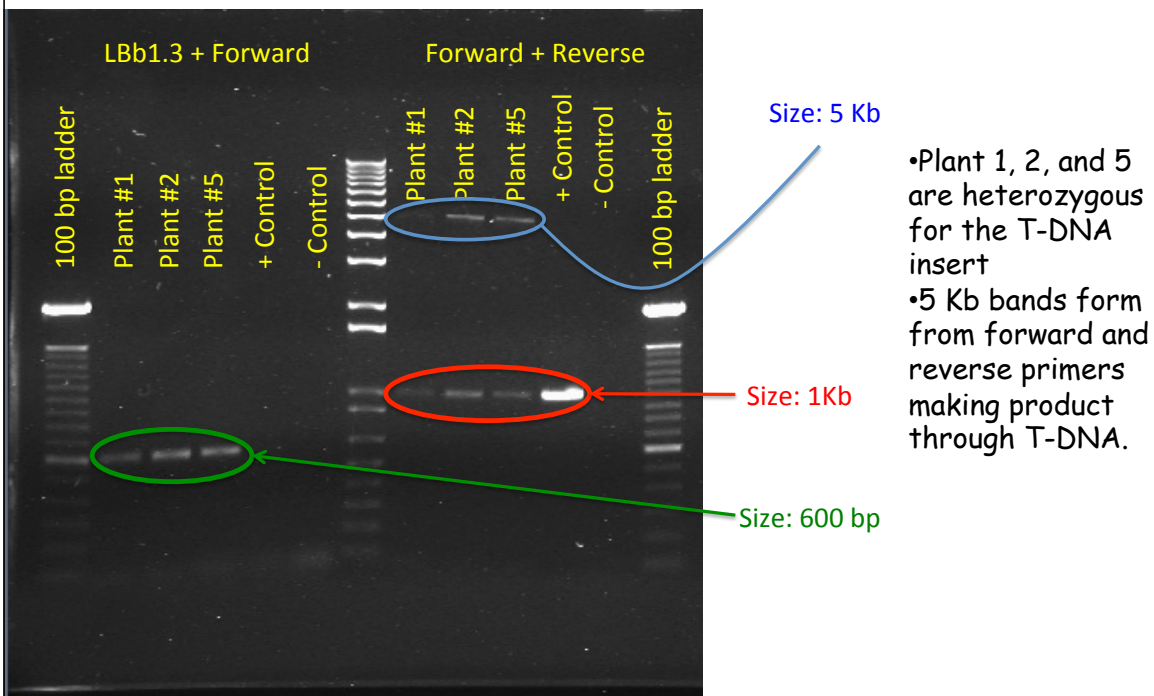
What is AT1G01040?

- Known as Dicer-Like 1
- Codes for Dicer Homologous protein
 - 1910 Amino Acids long
 - Involved in miRNA processing. Found in nucleus.
- Orientation in same direction as chromosome 1.

What is the structure of AT1G01040?

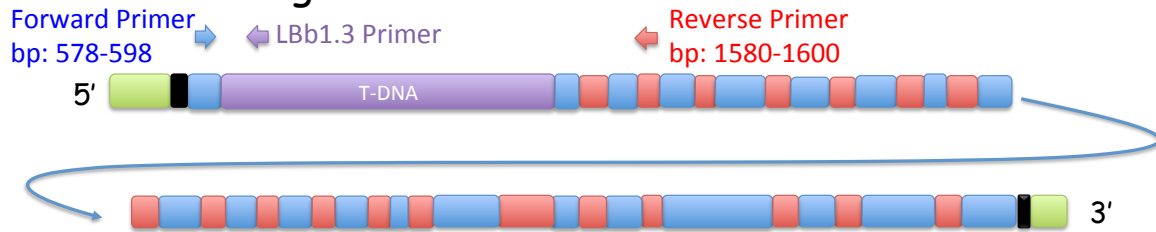


What are the genotypes of my plants?



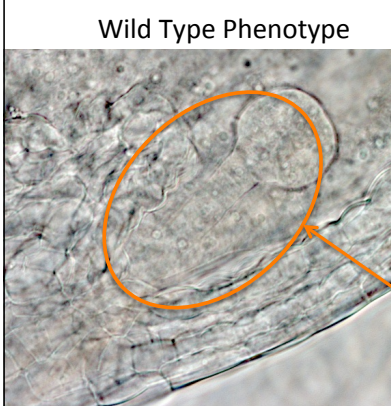
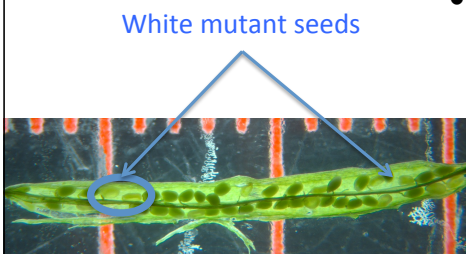
Where is the T-DNA inserted?

- T-DNA inserted at nucleotide 973
 - Expected size of PCR product matches gel results.



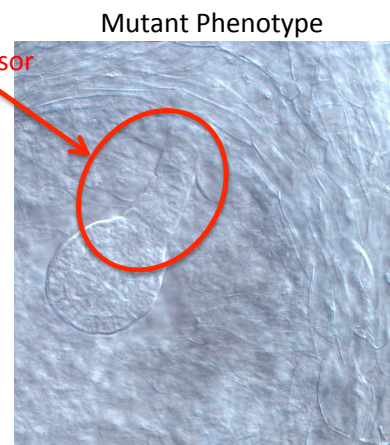
What are the effects of T-DNA insert?

- Light microscopy revealed a 1:3 ratio of white to green seeds in heterozygous siliques.

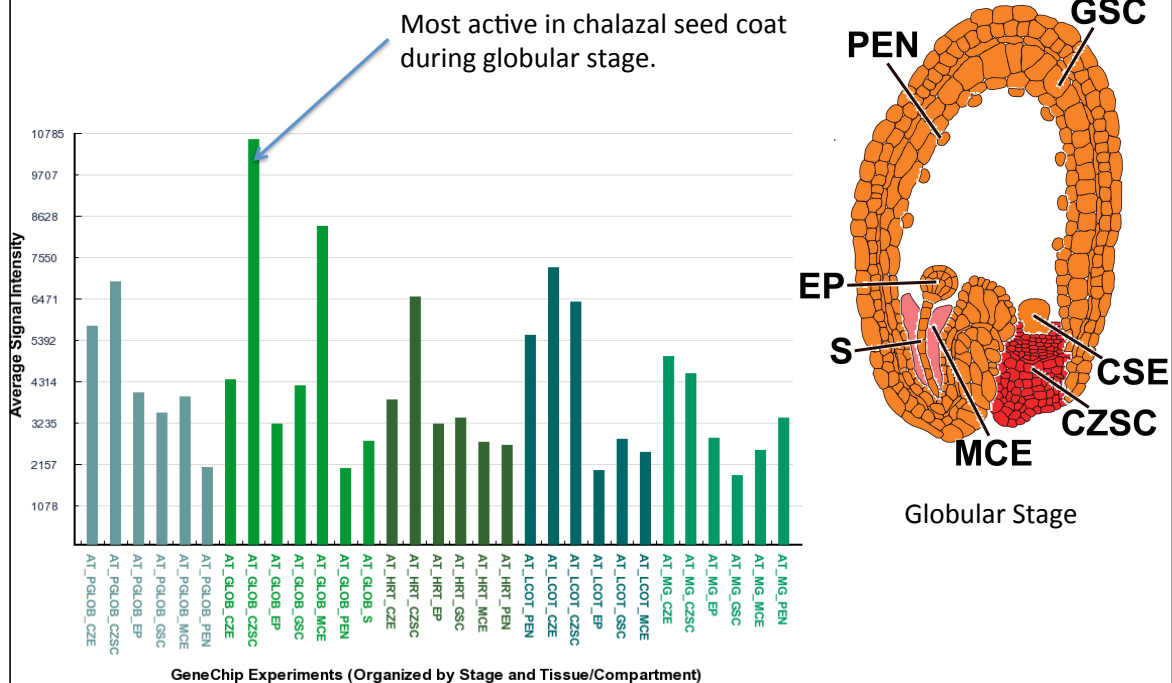


• Nomarski microscopy reveals abnormal suspensor in mutant seeds.

2 cell line suspensor



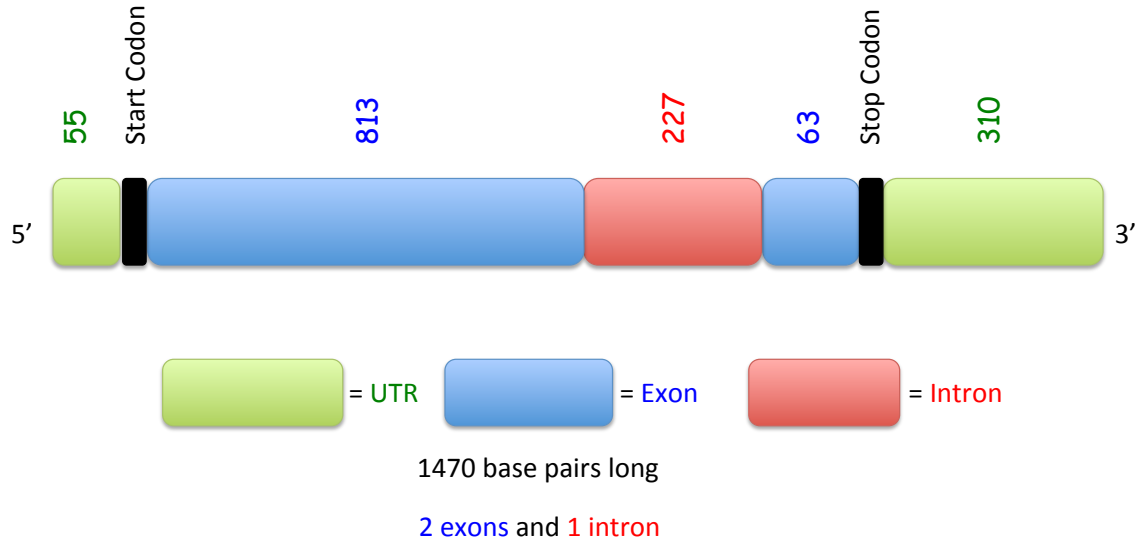
Where is the gene active?



What is AT4G00950?

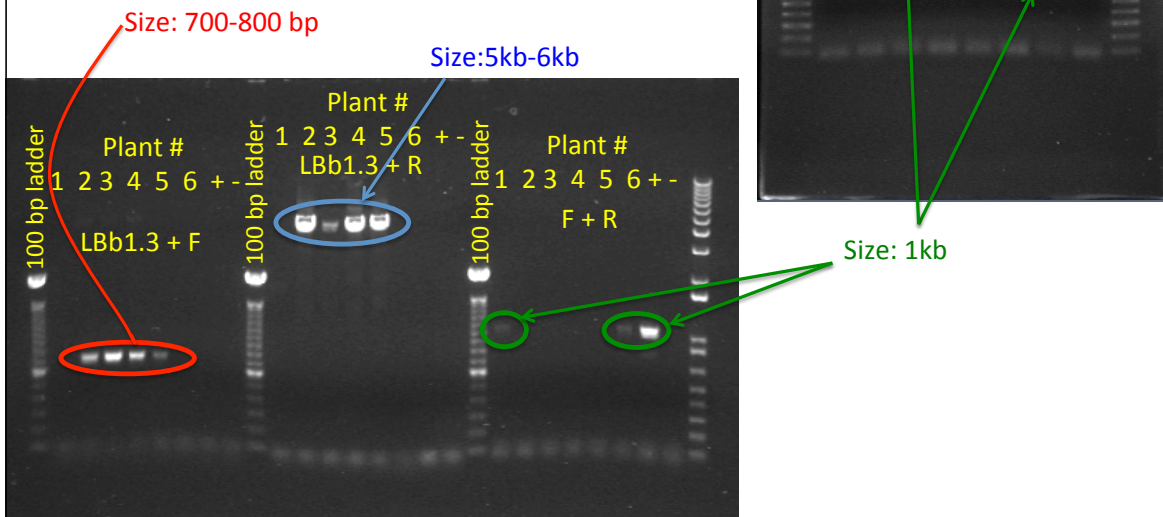
- Maternal Embryo Effect Arrest (MEE 47)
- Orientation: Reverse to chromosome 4.
- Codes for Transcription Factor of unknown function.
 - 291 amino acids in length

What is the structure of AT4G00950?



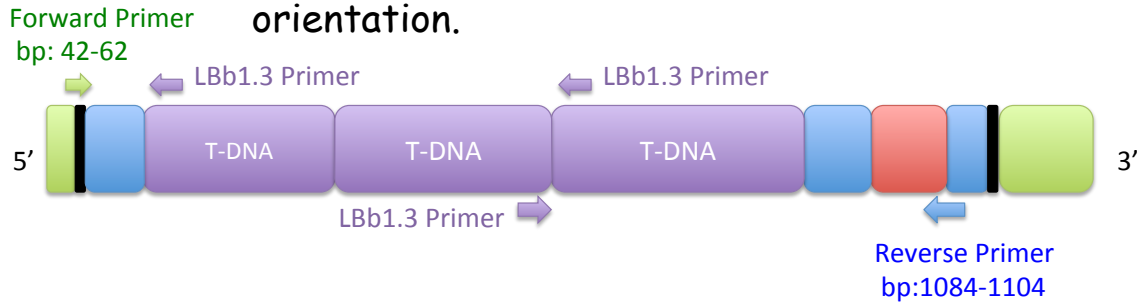
What are the genotypes of my plants?

- Gene is not seed lethal because of the presence of homozygotes for T-DNA insertion.



Where is the T-DNA inserted?

- Inserted somewhere between base pairs 567 and 637.
- How did I get the different sized bands?
 - T-DNA in center has opposite orientation.

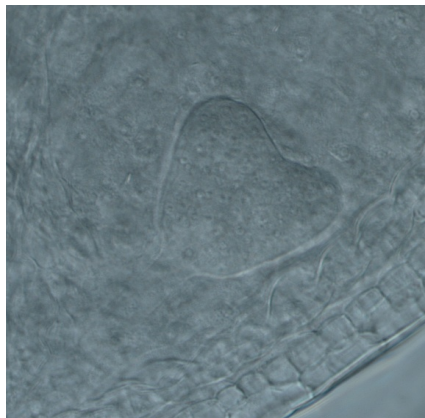


What are the effects of T-DNA insert?

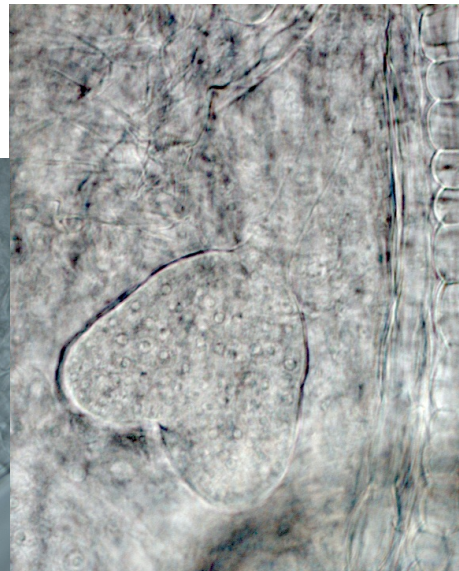
- No observable phenotype differences between mutant and wild type seeds.

- Possible related protein that has similar function?
- AT4G27810: e value=5e-4
- AT5G53030: e value=6e-4

Mutant Phenotype

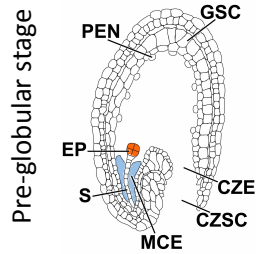
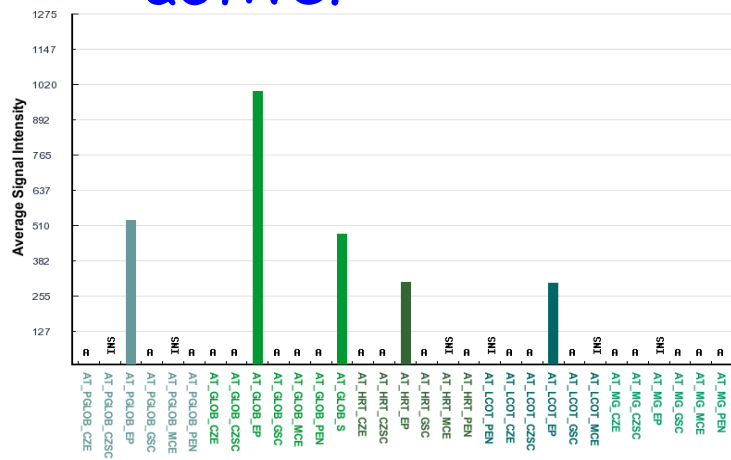


Wild type phenotype

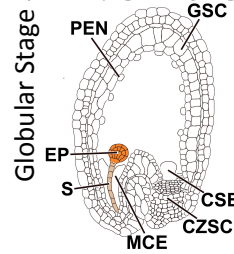


When and Where is AT4G00950 active?

- Gene is most active in the embryo proper during early stages of development.



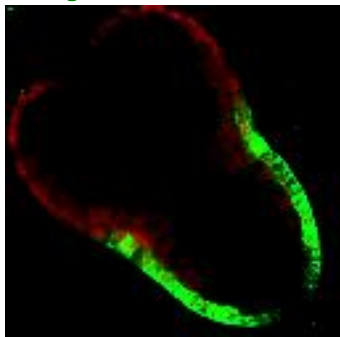
GeneChip Experiments (Organized by Stage and Tissue/Compartment)



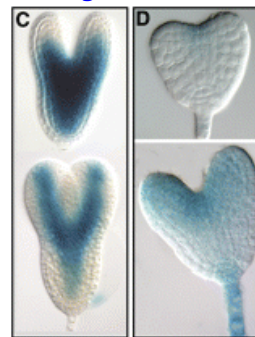
What is another experiment to show where the gene is active?

- Genetically engineering Arabidopsis with reporter genes such as GFP and GUS and promoter of specific gene.

Glowing Fluorescent Protein

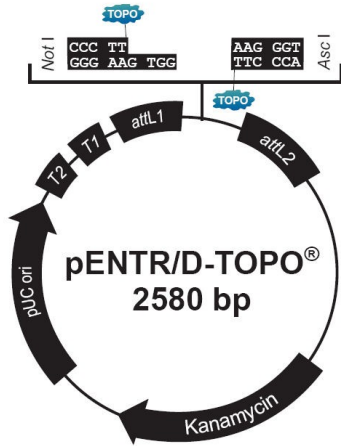


Beta-glucuronidase



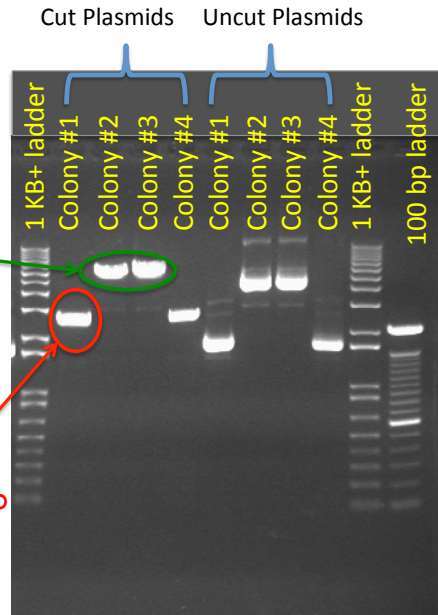
Can we clone the promoter sequence for AT4G00950?

- Promoter sequence ligated to pENTR vector for colony 2 and 3
- Expected Promoter Size: 3.5 Kb



Size: around 6kb

Size: 2.5 kb



Did I get a useful promoter sequence?

- Successful inserted promoter into pENTR vector
- Mutations due to ExTaq polymerase in the TA PCR reaction
 - Should not continue in experiment with these colonies

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tattatcacagcttgacgttt
|||||
tattatcacaacttgacgttt
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What is the next step?

- Very little is known about AT4G00950 gene.
- Future experiments could include double knockout for AT4G00950 and related gene.

Thank you

It has been a fantastic
experience.