

What are the roles of AT1G21970 (LEC1) and AT5G58850 (MYB119) in seed development?

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Overview

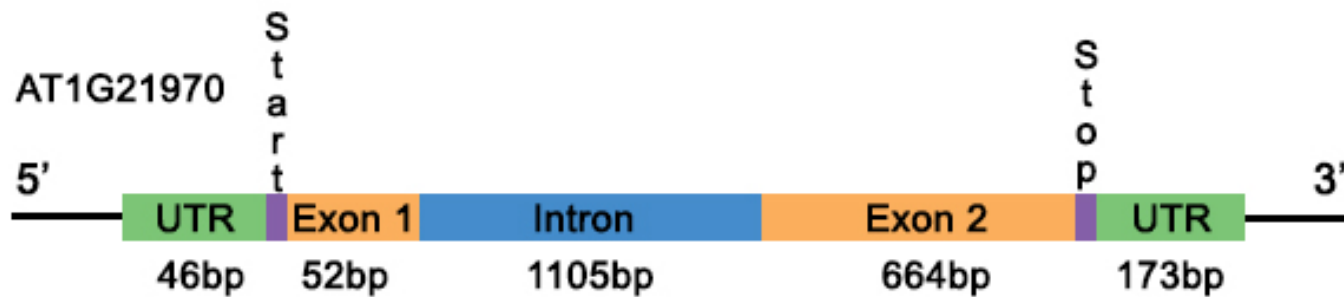
- What is the gene?
- What is the structure of the gene?
- What are the genotypes of the plants?
- Where is the T-DNA insert?
- What are the effects of T-DNA insert?
- Where is the gene active?

AT1G21970 (LEC1)

What is AT1G21970?

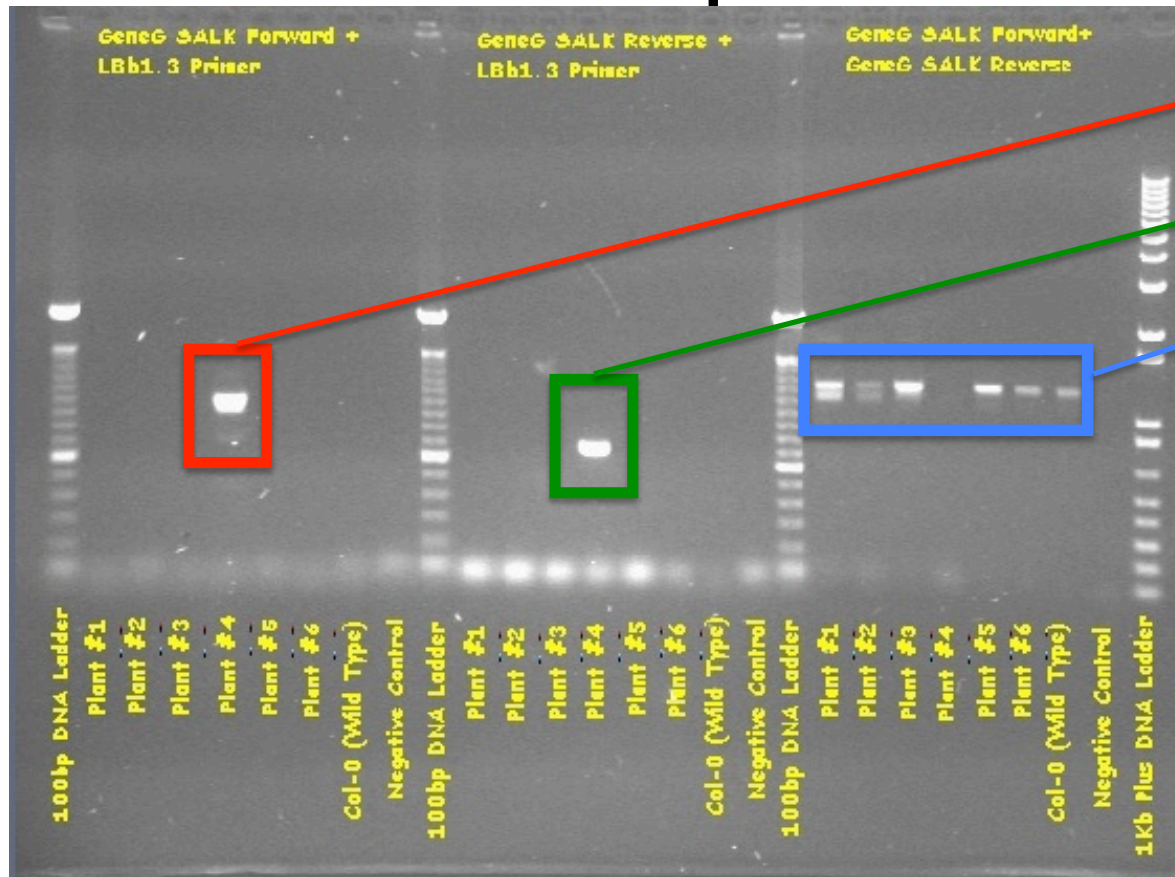
- Leafy Cotyledon 1 (LEC1)
- Transcriptional activator
 - 238 amino acids
- Oriented in the opposite direction of chromosome 1

What is the structure of the gene?



2040 bp in length
2 exons and 1 intron

What are the genotypes of the plants?



Size: ~900 bp

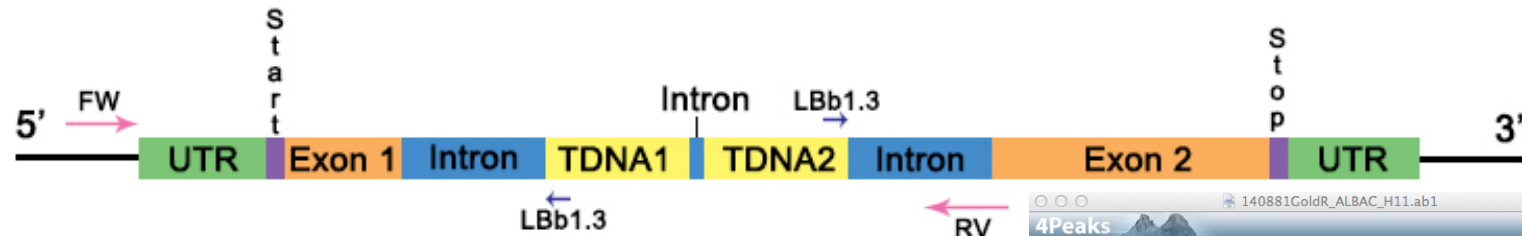
Expected size: 736 bp

Expected size: 1246 bp

- Plant 4 is homozygous for both T-DNA inserts
 - No product formed with FW +RV reaction
- Plants 1-3, 5,6 are wild type
 - Only product in FW+RV reaction

Where is the T-DNA inserted?

AT1G21970



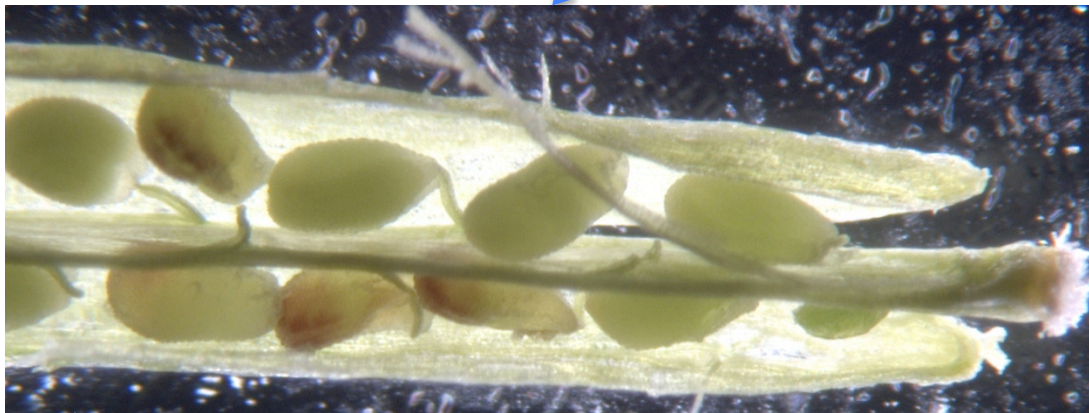
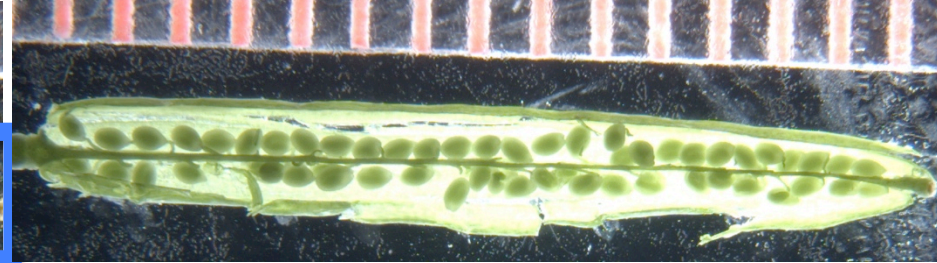
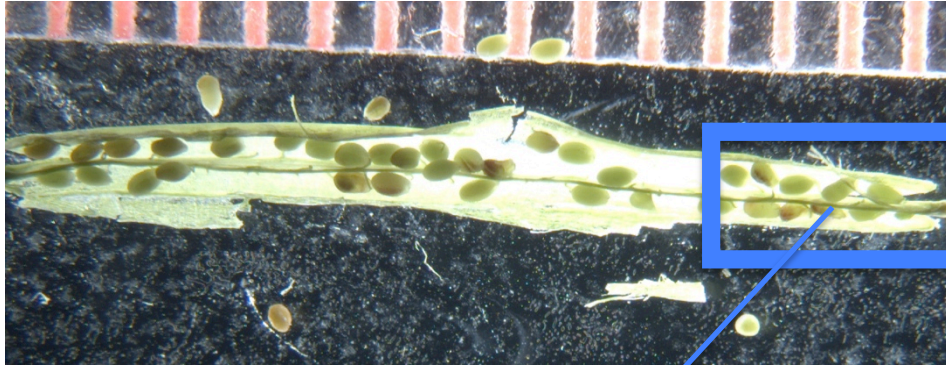
- T-DNA1
 - Found using FW+LBb1.3
 - Left border faces 5' end of gene
 - Inserted in intron
- T-DNA2
 - Found using RV+LBb1.3
 - Left border facing 3' end of gene
 - Inserted in intron
- Expected SALK T-DNA insertion site is 5 bp off from T-DNA2 towards 3' end
- T-DNA1 & 2 insertion sites are 42 bp apart
 - Possibly the result of bad sequencing



What are the effects of T-DNA insert?

(A) Mature mutant silique

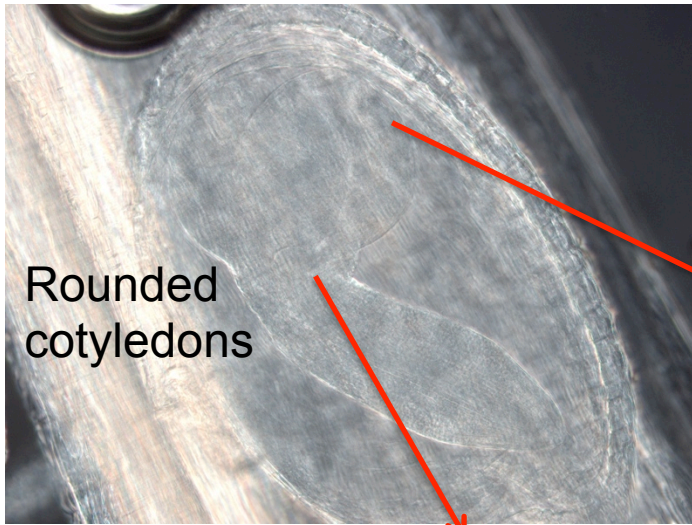
(B) Mature wild type silique



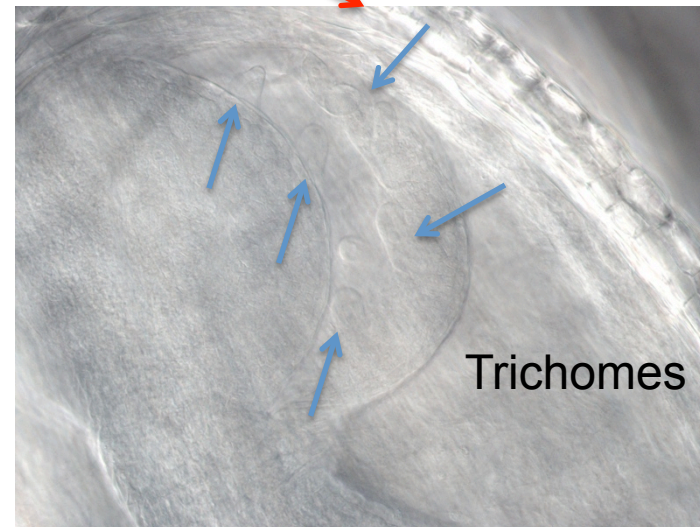
- Light microscopy showed anthocyanin deposits in 23 out of 44 seeds
- Chi-squared test: $p < 0.01$
 - Significant difference!

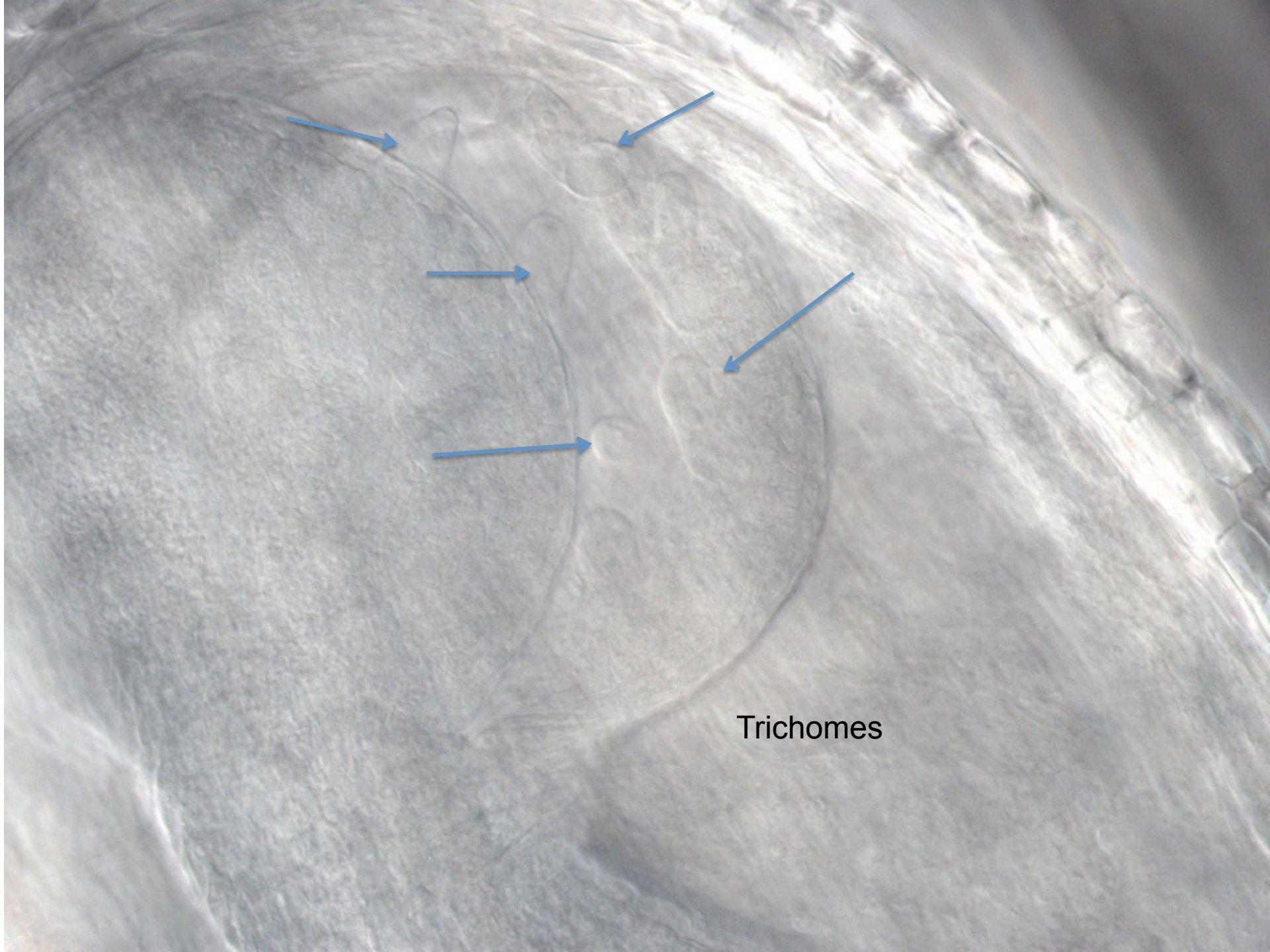
What are the effects of T-DNA insert?

(A) Mutant mature seed



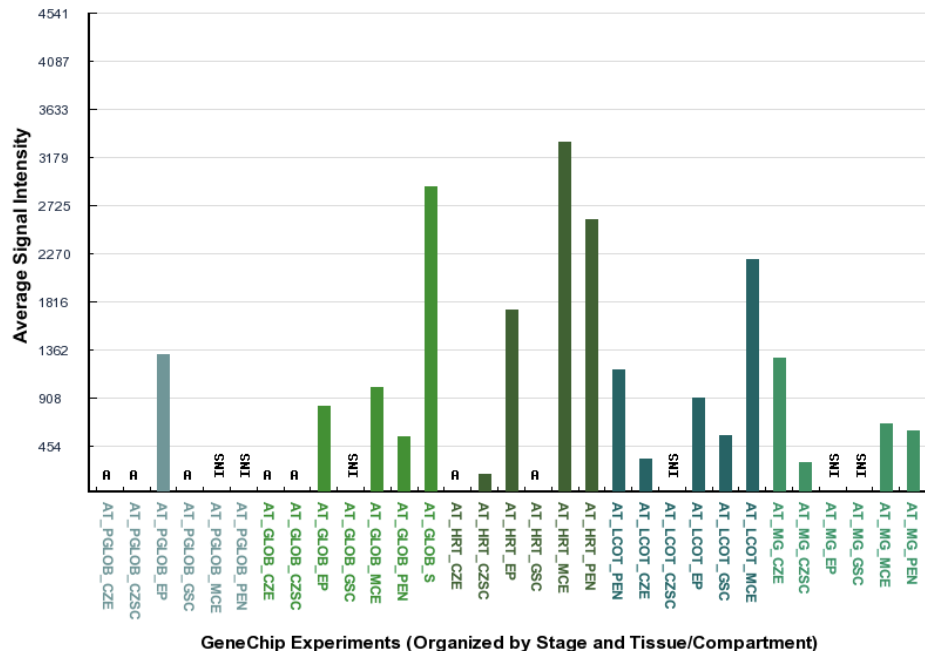
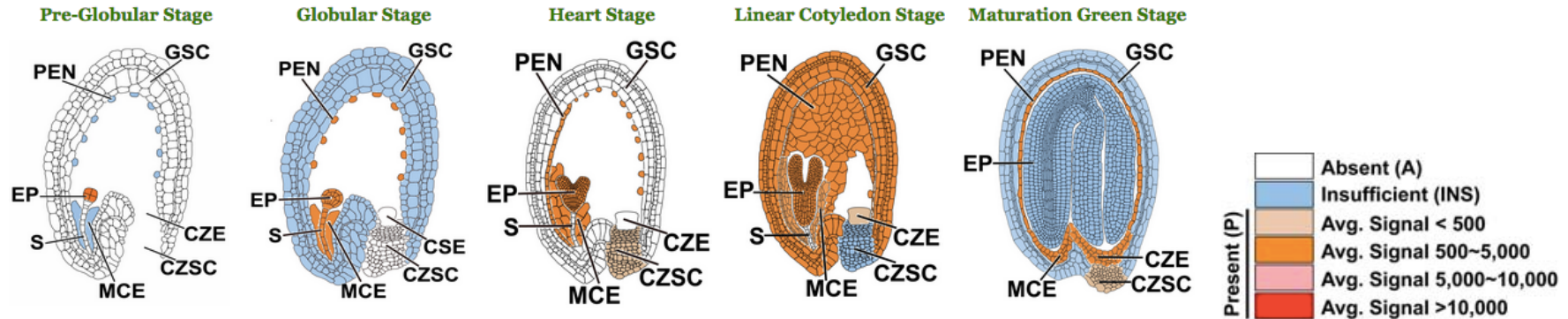
(B) Wild type mature seed





Trichomes

Where is the gene active?



- Gene expressed in many places at many different stages, but especially in cotyledon stage

What is the function of LEC1?

- LEC1 mutants prematurely develop features normally observed in leaves
 - Trichomes, activated shoot apical meristem
 - Specifies the identity of cotyledon during late embryogenesis
- Anthocyanin deposit & GeneChip indicates that LEC1 has more interactions in embryogenesis and seed development

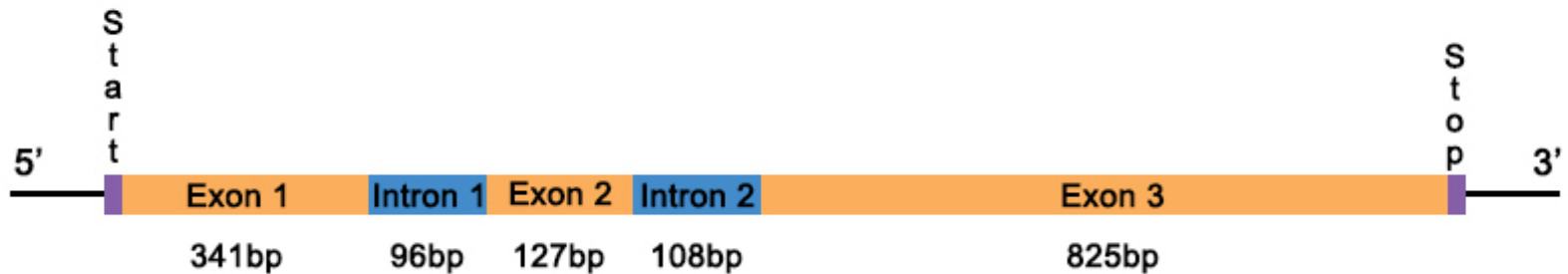
AT5G58850 (MYB119)

What is AT5G58850?

- MYB119
- Putative transcription factor
 - Binds to DNA
 - R2R3-type family
 - 430 amino acids
- Oriented in the same direction as chromosome 5

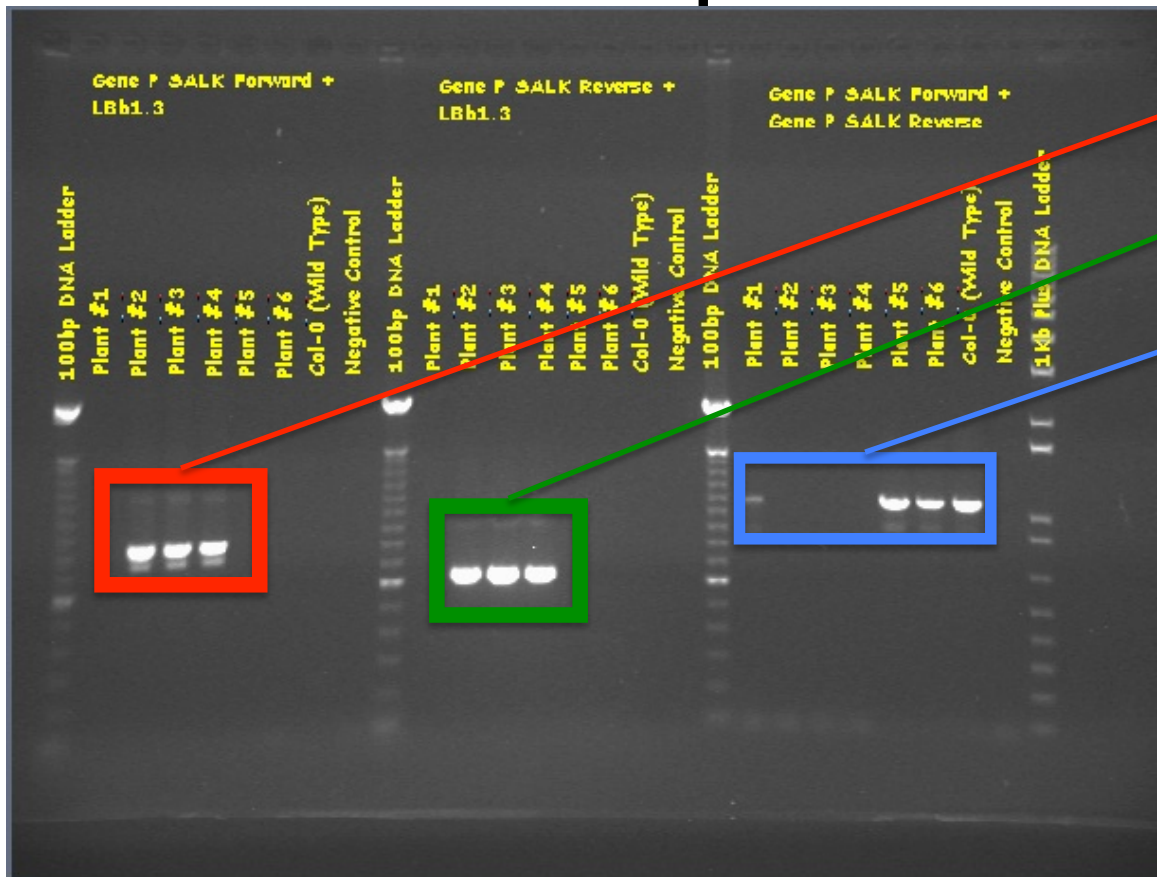
What is the structure of the gene?

AT5G58850



1497 bp in length
3 exons and 2 introns

What are the genotypes of the plants?



Size: ~800 bp

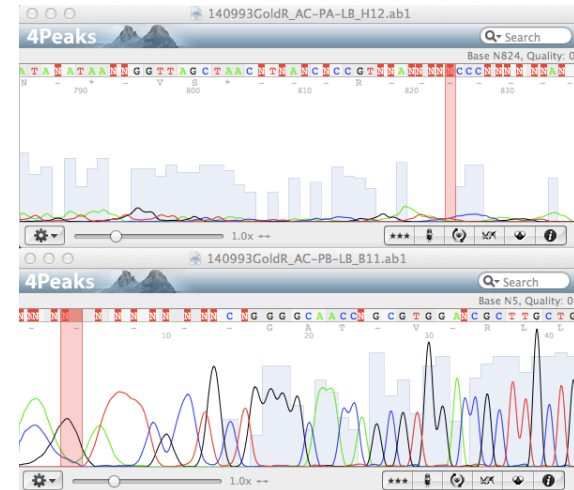
Expected size: 634 bp

Expected size: 1101 bp

- Plants 2-4 are homozygous for both T-DNA inserts
 - No product formed with FW +RV reaction
- Plants 1, 5, 6 are wild type
 - Only product in FW+RV reaction

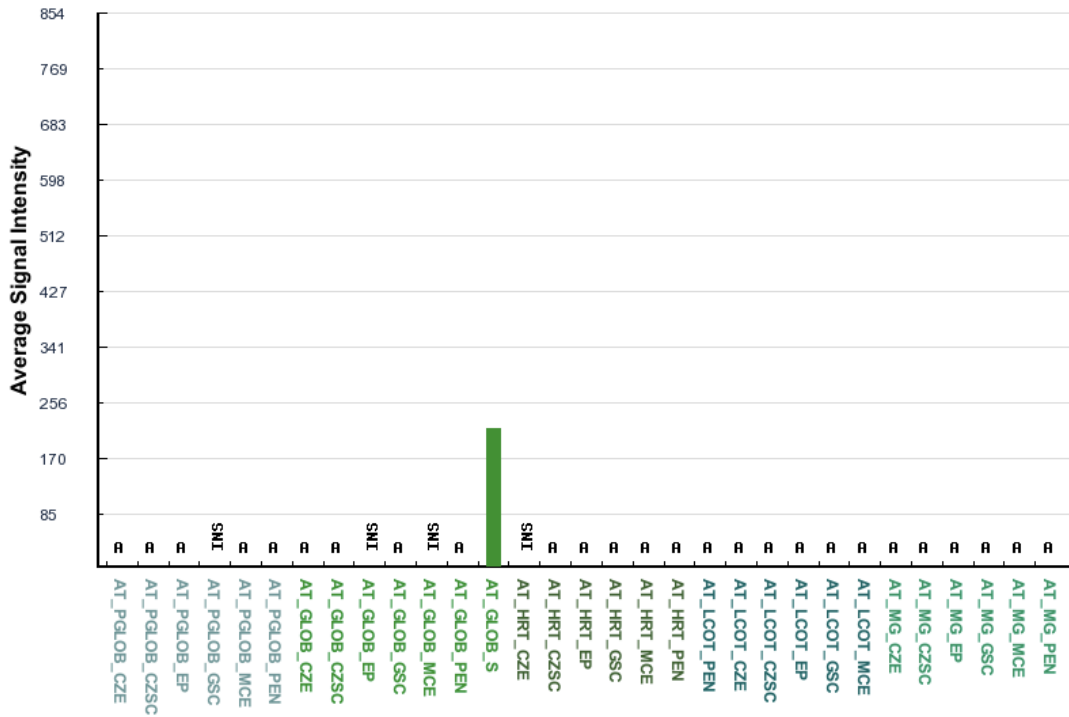
Where is the T-DNA inserted?

AT5G58850



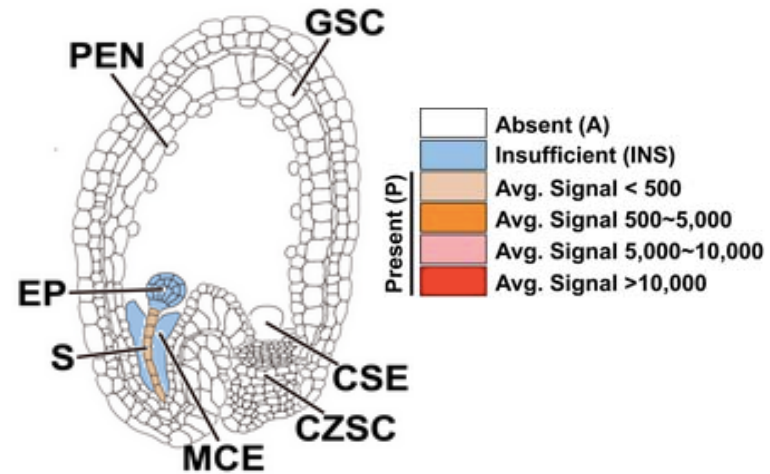
- T-DNA1
 - Found using FW+LBb1.3
 - Left border faces 5' end of gene
 - Inserted in front of start codon
- T-DNA2
 - Found using RV+LBb1.3
 - Left border facing 3' end of gene
 - Inserted 18 bp downstream from start codon
- Expected SALK T-DNA insertion site is 69 bp off from T-DNA2 downstream
- TDNA1 & 2 insertion sites are 18 bp apart
 - Possibly the result of bad sequencing

Where is the gene active?



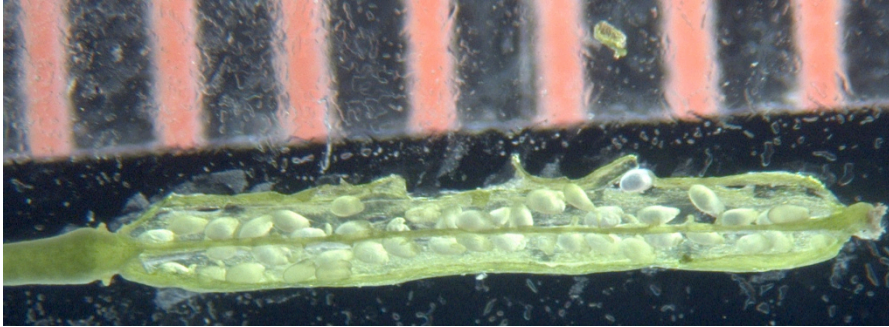
GeneChip Experiments (Organized by Stage and Tissue/Compartment)

Globular Stage

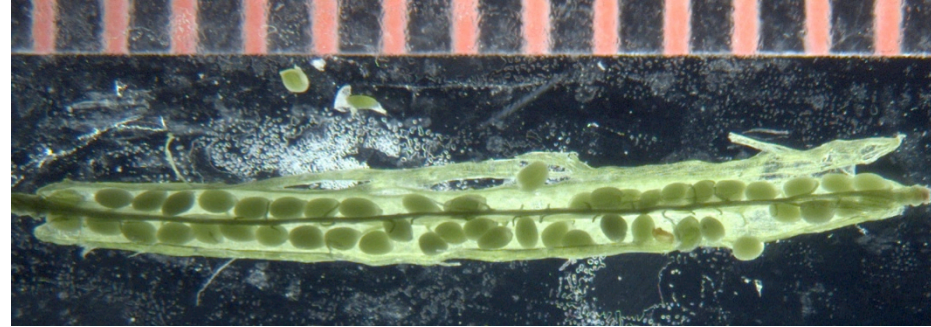


- Gene expressed at low but detectable levels at globular stage in the suspensor

What are the effects of T-DNA insert?

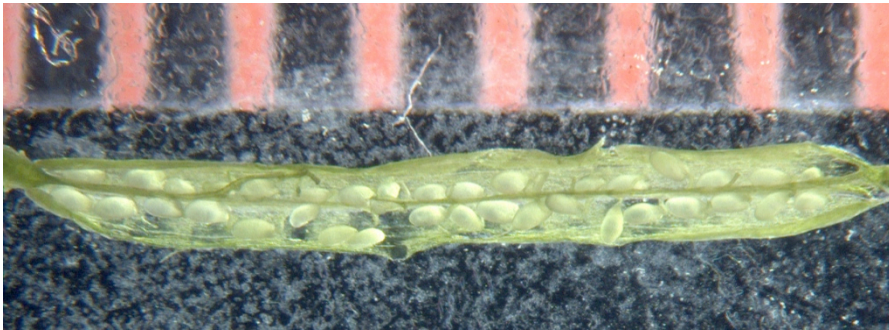


(A) Early WT silique

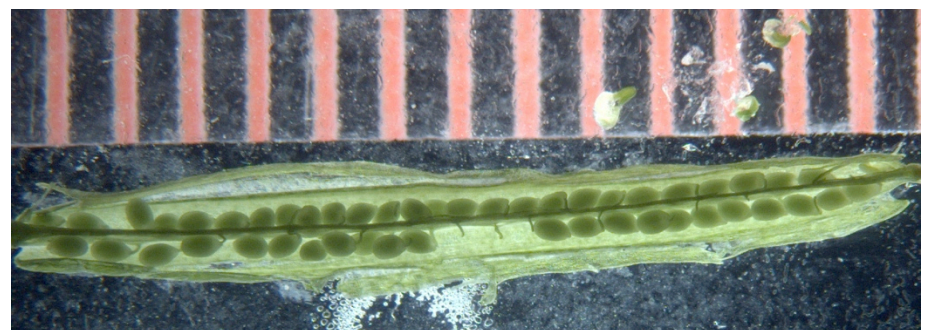


(C) Mature WT silique

(B) Early mutant silique



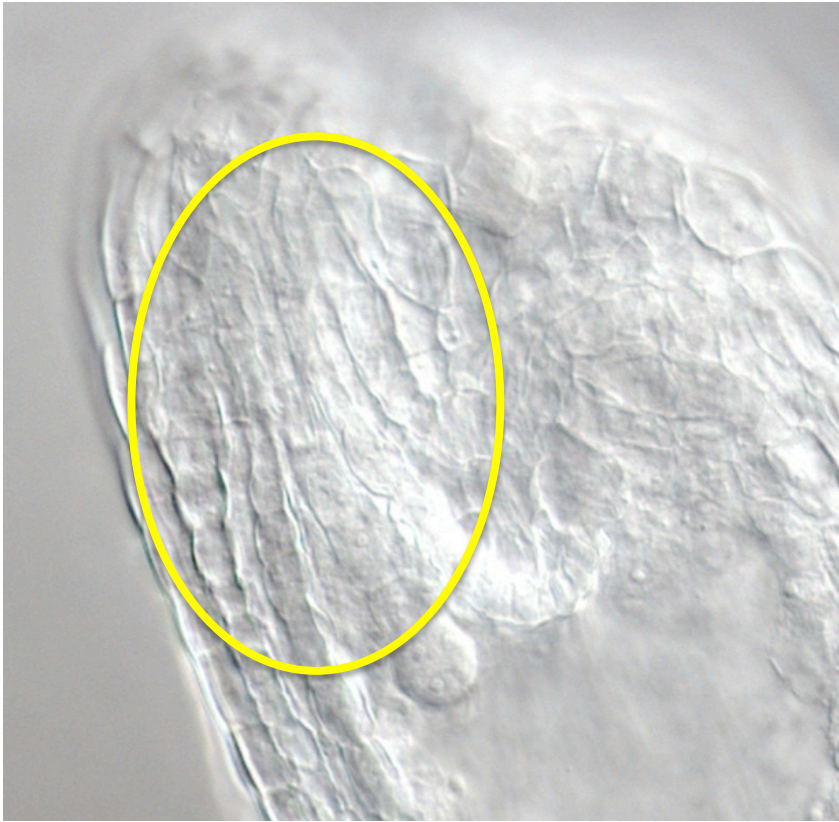
(D) Mature mutant silique



No observable difference!

What are the effects of T-DNA insert?

(A) Early WT seed



(B) Early mutant seed

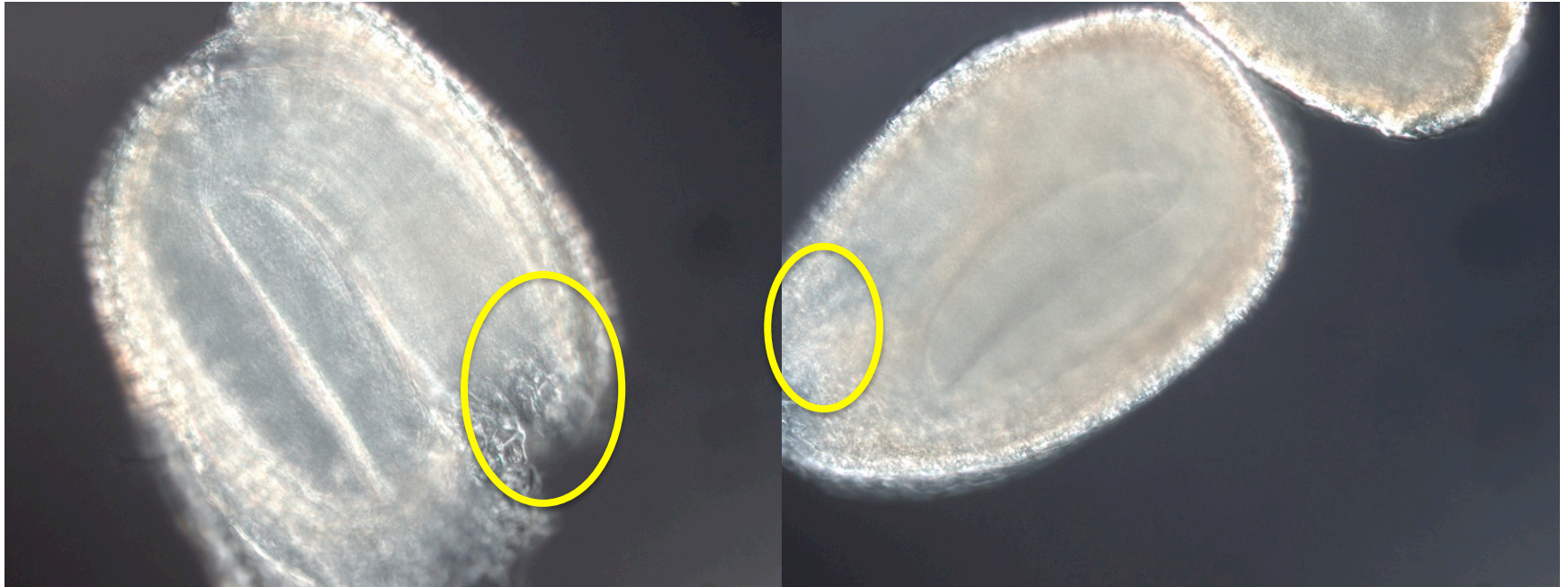


No observable difference!

What are the effects of T-DNA insert?

(C) Mature WT seed

(D) Mature mutant seed



No observable difference!

What are the effects of T-DNA insert?

Can we attribute the difference to T-DNA?

- **Avg. WT height: 25cm**
- **Avg. mutant height: 37cm**
- Population size is too small to accurately determine whether it's significant



(A) WT Plant

- 21 leaves, 0.6-2.4cm
- 21cm stem, 15 branches
- 67 siliques, average 43 seeds
- No difference in flower

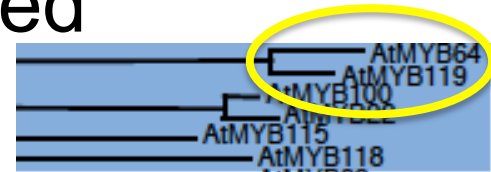


(B) Mutant Plant

- 48 leaves, 0.6-3.2cm
- 45cm stem, 27 branches
- 426 siliques, average 48 seeds
- No difference in flower

What is the function of MYB119?

- No changes in phenotypes observed
- 2013 study by Rabiger and Drews
 - MYB64 and MYB119 (66% AA similarity) act redundantly to promote transition from nuclear division to cellularization-differentiation during female gametogenesis
 - *myb64* and *myb119* double mutant showed phenotypic change where gametophyte is uncellularized and supernumerary nuclei
 - No phenotype observed in *myb119* single mutant



Recap: What are the roles of LEC1 and MYB119 in Seed Development?

- LEC1
 - Specifies and regulates development of cotyledons
- MYB119
 - Does not affect seed development in observable way

ACKNOWLEDGEMENTS