WRKY 59 and Agamous-Like 69 Genes

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HC70AL Spring 2009

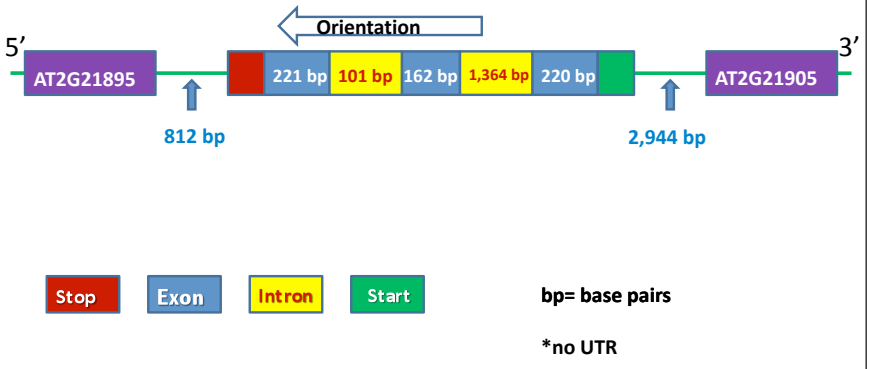
What is AT2G21900?

- 2,074 Base Pairs
- 609 Exon Base Pairs
- On Chromosome 2
- 3′ ⟶ 5′ Orientation
- Encodes for ATWRKY59
  - Member of WRKY Transcription Factor
- 202 Amino Acids

What is a WRKY Gene?

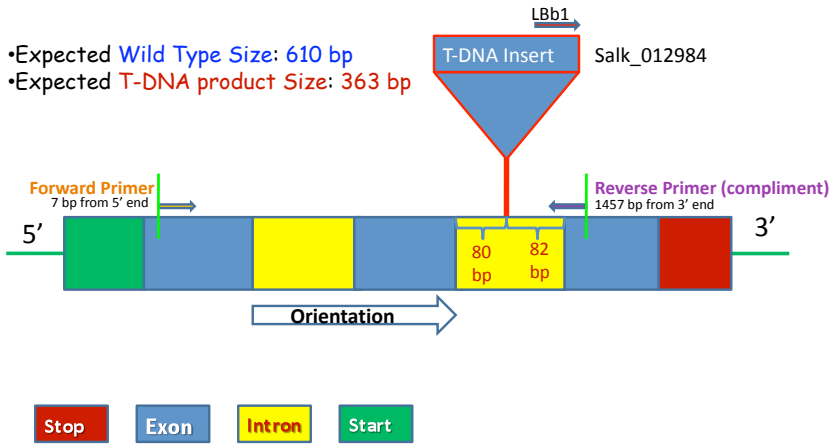
- ATWRKY59
  - member of WRKY Transcription Factor
- Helps plants react to stresses
  - wounding, drought, temperature changes, and pathogens
- Senescence
- Trichome development (epidermis outgrowths)

What is the Structure of AT2G21900?

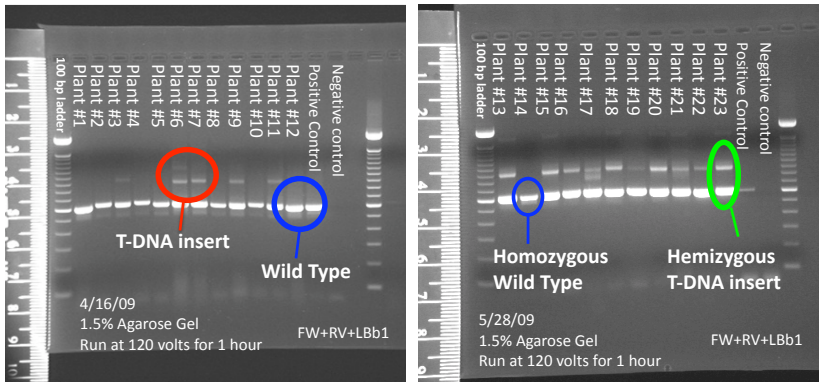


Where is T-DNA Insertion Site?
(COMPLEMENT STRAND)

•Expected Wild Type Size: 610 bp
•Expected T-DNA product Size: 363 bp



Genotyping Results



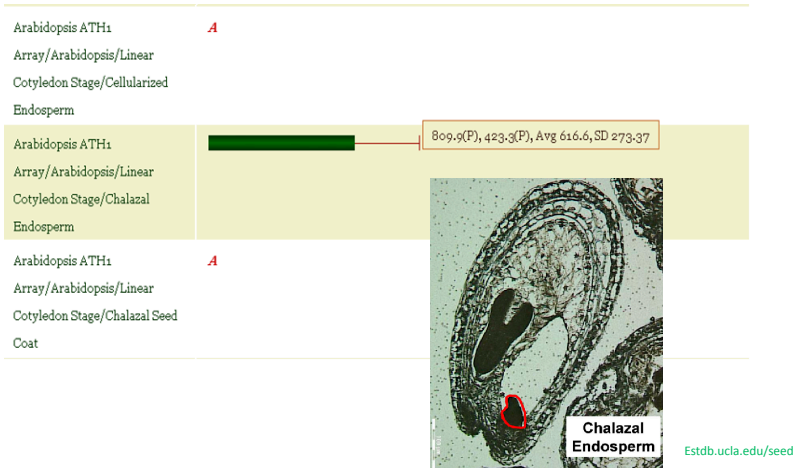
•Expected Wild Type Size: 610 bp
•Expected T-DNA product Size: 363 bp

What Do My Results Mean?

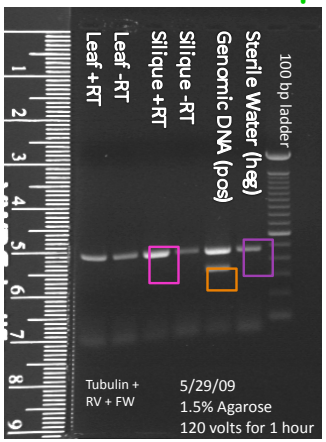
Phenotype	Number of Individuals	Percentage of Phenotype Occurrence
Homozygous Wild-Type	8	34.7%
Hemizygous T-DNA Insert	15	65.2%
Homozygous T-DNA insert	0	0%
Total Number of Individuals	23	

- T-DNA insert is most likely lethal

Where is the Gene Active?
Gene Chip Data Summary



RT-PCR Analysis of Leaf and Silique Tissue



- **No visible results for Leaf +RT or Silique +RT**
- **Low Expression of Gene**
- **Expressed in few cells**
- **Expressed during a different time in development than analyzed**
- **No Transcription in those tissues**

Tubulin RNA

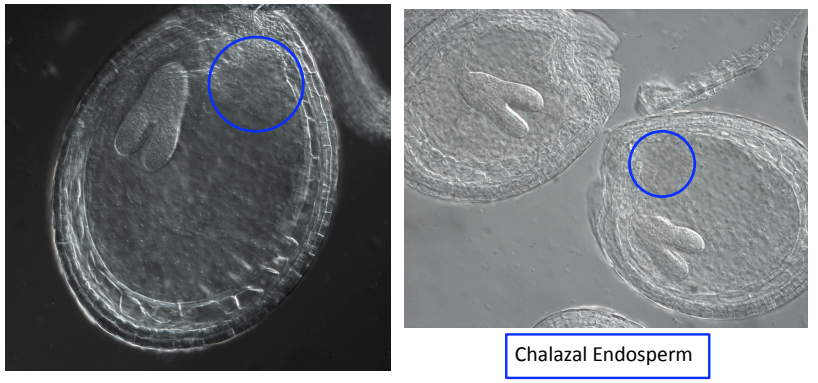
AT2G21900 RNA

DNA Contamination

Phenotypic Differences

Light Microscopy and Nomarksi Microscopy

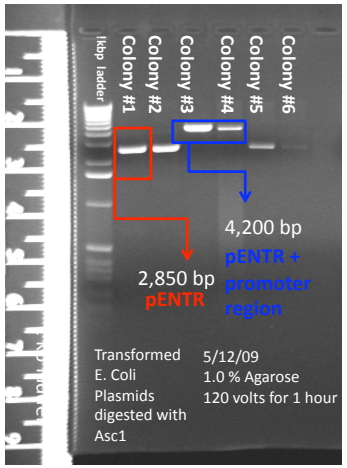
Wild-Type Homozygous **Hemizygous T-DNA Insert**



Chalazal Endosperm

- **There were no notable mutant phenotypes seen through light microscopy or Nomarksi microscopy**
- **Chalazal Endosperm is hard to visualize**

What is Another Way to Study Gene Expression?



- Promoters bind to transcription factors which initiates transcription
- Amplification, Ligation, Transformation, Selection, Digestion, Genotyping, Sequencing
- Can be engineered to GFP or GUS to visualize gene transcription

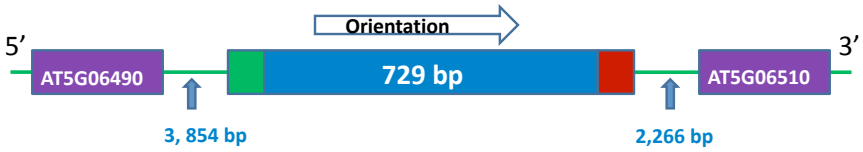
What is AT5G06500?

- On Chromosome 5
- 729 Exon Base Pairs (No Introns or UTR)
- 5'⟹3' Orientation
- Encodes for Agamous-Like 96
  - Responsible for development of flower organs
- 243 Amino Acids

What is Agamous-like 96?

- Part of Agamous Family of Transcription Factors
  - Essential for development of reproductive organs in flowers
  - Also important for the development of tissues derived from double fertilization in flowering plants.

What is the Structure of AT5G06500?

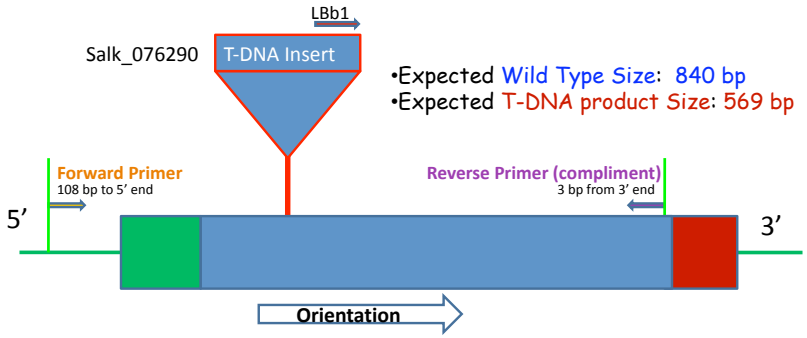


Stop Exon Start

bp= base pairs

*no UTRs or Introns

Where is the T-DNA Insertion Site in AT5G06500?

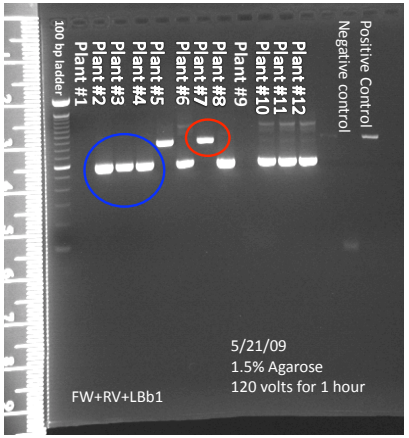


Stop Exon Start

Genotyping Results

•**WILD-TYPE**
•Expected :**840 bp**
•Observed: **~850 bp**

•**T-DNA Insert**
•Expected: **569 bp**
•Observed: **~575 BP**



Homozygous T-DNA

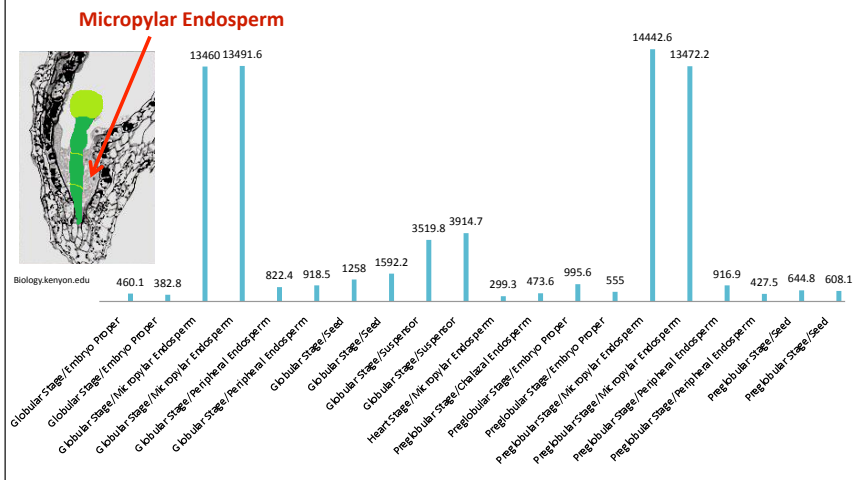
Wild Type

What Do My Results Mean?

Phenotype	Number of Individuals	Percentage of Phenotype Occurrence
Homozygous Wild-Type	2	20.0%
Hemizygous T-DNA Insert	0	0.0%
Homozygous T-DNA insert	8	80.0%
Total Number of Individuals	10	

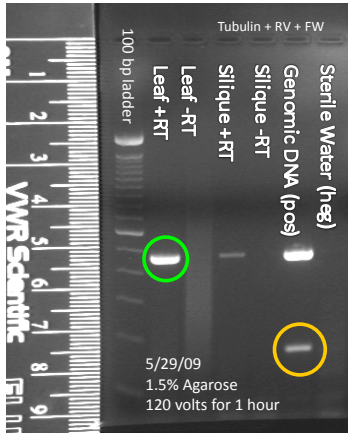
- The T-DNA insert does not cause seed mortality

Where is the Gene Active: Gene Chip Data Summary



Where is the AT5G06500 Active?

- Again no visible results for either silique or leaf tissue
- **Micropylar Endosperm and Suspensor form a small portion of the seed**
- **Thus, the gene may be expressed, but not at detectable levels**

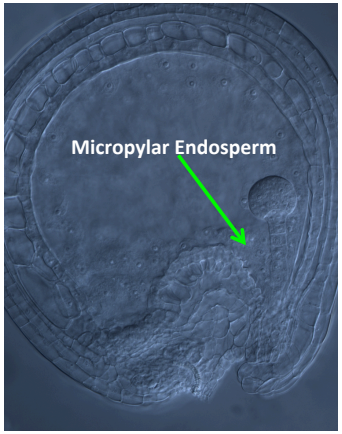


RT-PCR Product
Expected: 249 bp
Observed: ~200bp

Tubulin
Observed: ~470bp

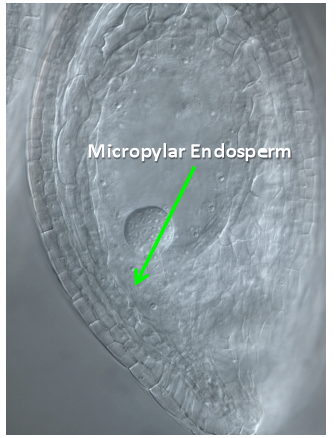
Phenotypic Differences

Homozygous T-DNA Insert



Plant 10

Homozygous Wild-Type



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Significance of Results

AT2G21900

- The knock out of gene AT2G21900 most likely causes seed lethality
- No mutant phenotypes observed for the knock-out lines
  - Tissues where expressed are hard to visualize with light and Nomarski microscopy

AT5G06500

- The knock out of gene AT2G21900 does NOT cause seed lethality
- No mutant phenotypes observed for the knock-out lines
  - Tissues where expressed are hard to visualize with light and Nomarski microscopy

What Further Research Can Be Done?

- Use more complex phenotype analysis tools to analyze the Micropylar Endosperm, the Suspensor, and Chalazal Endosperm.
- Screen more plants for gene one to see if there are any homozygous T-DNA insert plants
- Do more RT-PCR experiments for both genes to try and detect where the genes are transcribed

Acknowledgements

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