



What Role do HOX7D and MYB89 Play in Seed Development?

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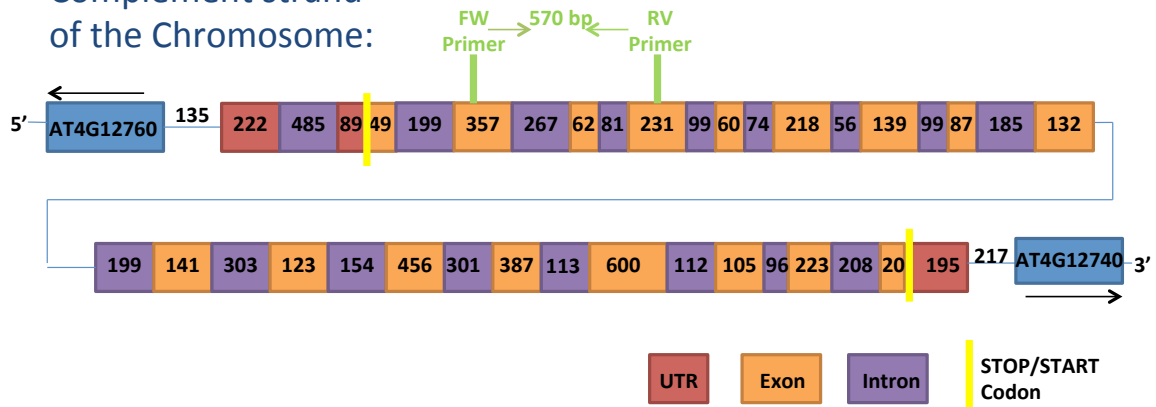
What is HOX7D?

- Homeodomain-like transcription factor
 - Binds to DNA in two locations
 - Helix-turn-helix structure
- Encoded by AT4G12750
 - Chromosome 4
 - 6,903 base pairs
 - Opposite orientation as the chromosome (3'→ 5')
- 191 amino acids

What is the Structure of HOX7D?

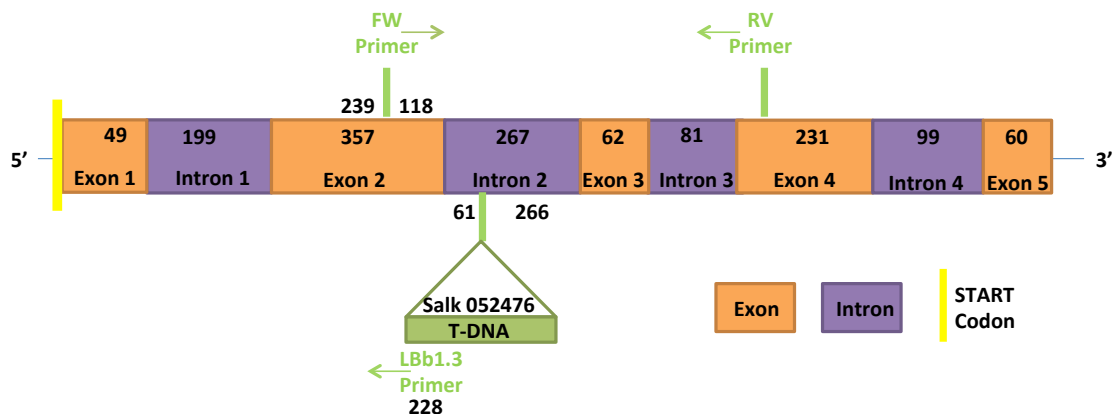
- 16 exons and 17 introns
- Product Size = 570bp

Complement strand
of the Chromosome:



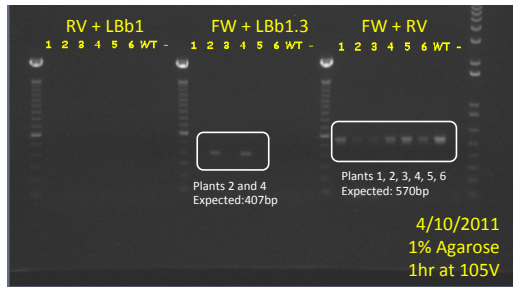
Where is the T-DNA Insertion Site?

- Second Intron
- Opposite orientation as the gene
- Product Size = 407bp

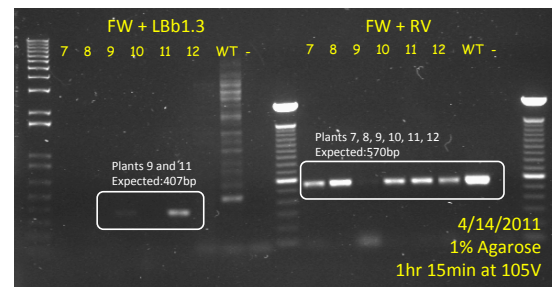


Which Plants have the T-DNA Insert?

PCR Product from Genomic DNA Plants #1-6



PCR Product from Genomic DNA Plants #7-12



- Plants 2, 4, 9, and 11 are heterozygous for the T-DNA insert
- Expected Sizes
 - WT = 570bp
 - T-DNA = 407bp
- Product with Forward and LBb1.3 means T-DNA insert has the opposite orientation as the gene

What is the Function of HOX7D?

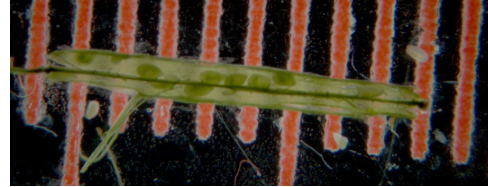
- Heterozygous plants = larger leaf size
 - Mutant: 1.25-2.50cm
 - WT: 0.75-2.00cm
- Heterozygous plants = taller stem height
 - Mutant: 29cm
 - WT: 31.5-35.5cm



What Role does HOX7D play in Seed Development?

Light microscopy reveals 3:1 Ratio

siliqua #	plant #	siliqua length	Genotype	seed ratio white: green	
1	6	1.2cm	WT	0	45
2	12	1.3cm	WT	0	36
3	4	1.2cm	HZ	6	24
4	11	1.0cm	HZ	4	21
5	11	0.5cm	HZ	1	6
6	2	0.65cm	HZ	4	11
7	11	0.5cm	HZ	1	5
8	2	0.6cm	HZ	11	0
9	9	0.7cm	HZ	4	16
10	11	0.9cm	HZ	4	16
11	11	0.6cm	HZ	4	10
12	9	0.7cm	HZ	0	10
13	11	0.9cm	HZ	4	13

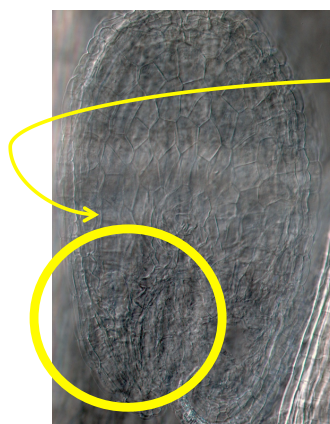


siliqua #	chi-squared	p-value
3	2.8	0.05<p<0.1
4	2.8933	0.05<p<0.1
5	0.90476	0.1<p<0.5
6	1.2667	0.1<p<0.5
7	0.6667	0.1<p<0.5
8	33	p<0.001
9	1.8667	0.1<p<0.5
10	1.8667	0.1<p<0.5
11	1.2381	0.1<p<0.5
12	3.3333	0.05<p<0.1
13	1.4167	0.1<p<0.5

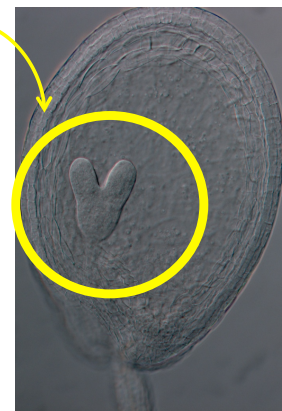
A Chi-Squared test shows that although there is some deviation from the exact 3:1 ratio, the null hypothesis cannot be rejected except for silique 8

What Role does HOX7D play in Seed Development?

- **No Homozygous Plants Suggests Seed Lethality**
- Development arrested in the Globular Stage
 - Visualized in Silique 5 and 13
 - Age of siliques made this difficult in other siliques

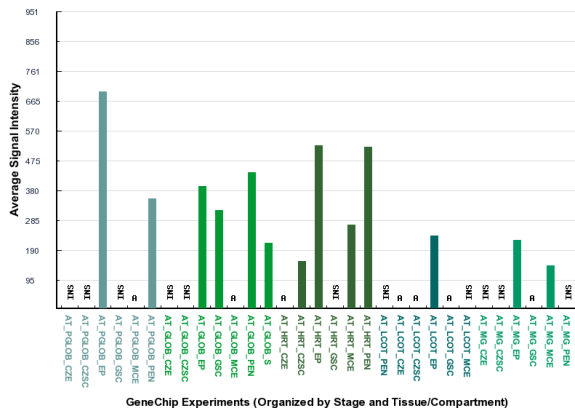


Heterozygous
Silique 13
Globular Stage



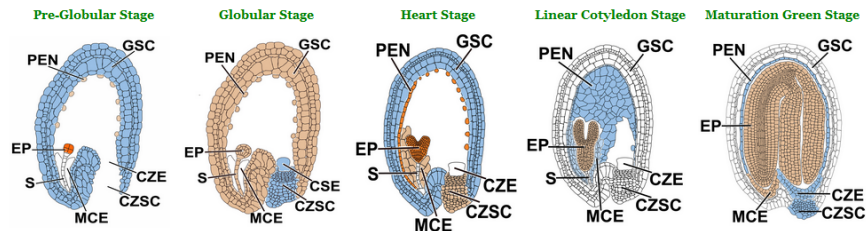
Wildtype
Silique 2
Late Heart Stage

When and Where is HOX7D Active?



AT4G12750 is widely expressed during seed development and merits further research!

GeneChip Experiments (Organized by Stage and Tissue/Compartment)



http://seedgenenetwork.net/nlotorobe?name=254778_at

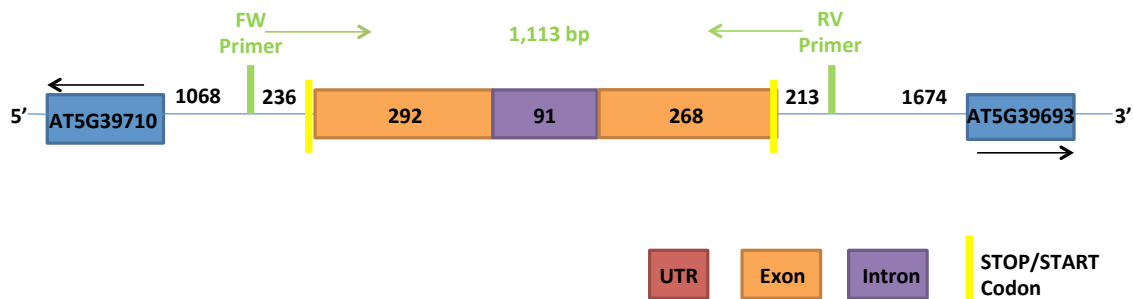
What Gene am I Working With?

- Putative Transcription Factor MYB(myeloblastosis)89
 - Binds to DNA
 - Similar to the helix-turn-helix structure
 - MYB Family first discovered in oncogenic transformation
- Encoded by AT5G39700
 - Chromosome 5
 - 664 base pairs
 - Opposite orientation as the chromosome (3'→ 5')
- 190 amino acids

What is the Structure of MYB89?

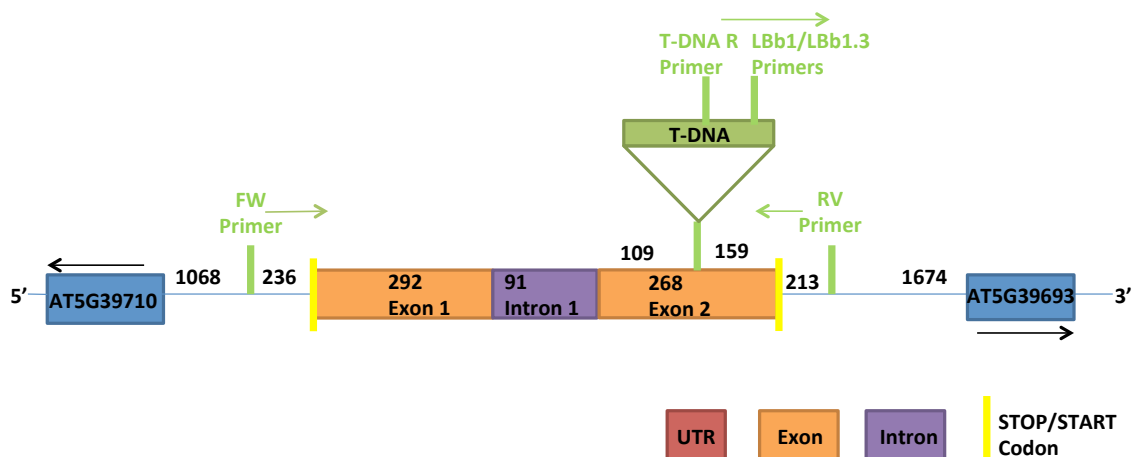
- 2 exons and 1 intron
- Product size = 1,113bp

Complement strand
of the Chromosome:



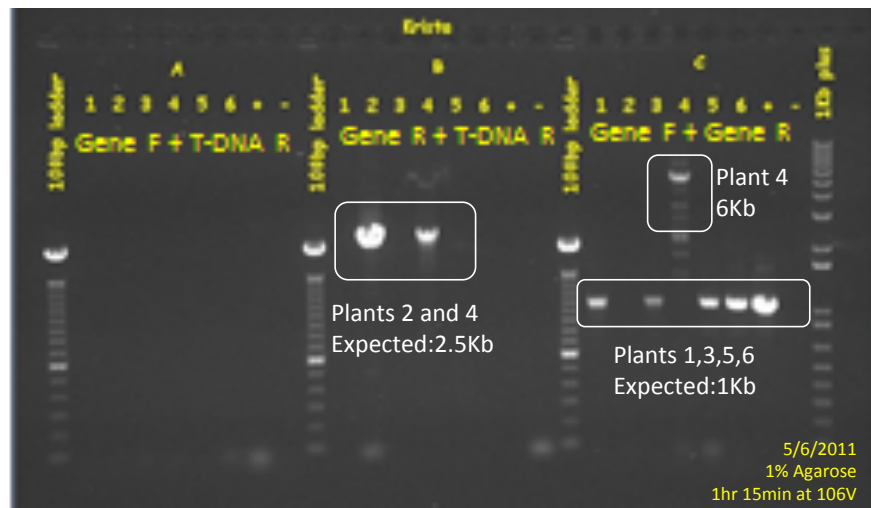
Where is the T-DNA Insertion Site?

- Second Exon
- Same orientation as the gene
- Product size with T-DNA R = around 2.5Kb



Which Plants have the T-DNA Insert?

Plants 2 and 4 are **Homozygous** for the T-DNA insert!

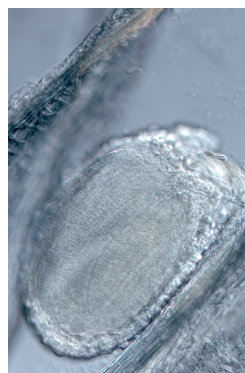


Plant 4 = expected product in T-DNA, no WT reaction

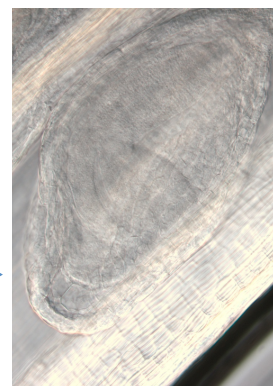
Plant 2 = product in both T-DNA and WT reaction
 WT = 6Kb = 1Kb + 5kb (T-DNA)

What is the Function of MYB89?

- No observable phenotype – does it play a role in seed development?
- Most siliques were too old
 - Shriveled seeds are difficult to observe
 - Can't see development stages where AT5G39700 is expressed
- Future research should study Nomarski images of younger siliques

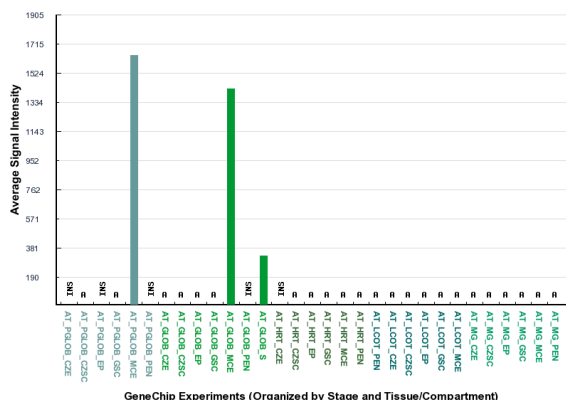


Wildtype
 Silique 2
 Mature Green Stage

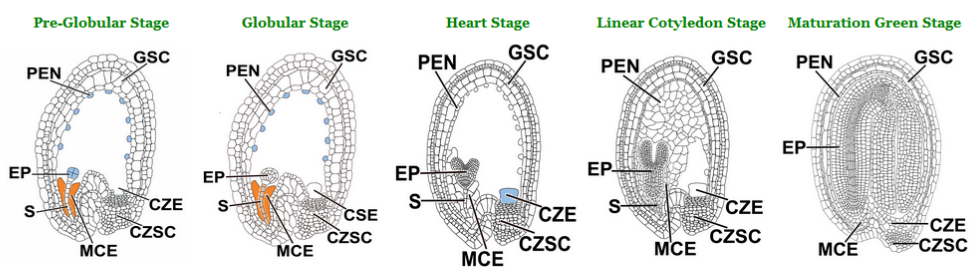


Homozygous
 Silique 7
 Mature Green Stage

When and Where is MYB89 Active?

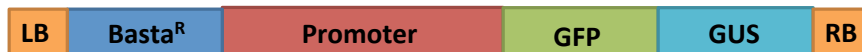


- Micropylar Endosperm during pre-globular and globular stages
- Suspensor during globular stage

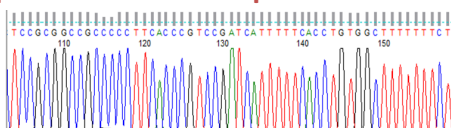
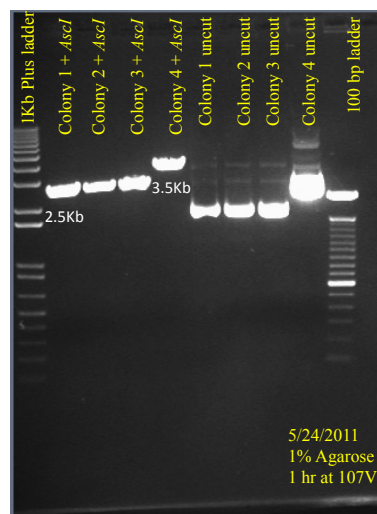


http://seedgenesnetwork.net/plotprobe?name=249414_at

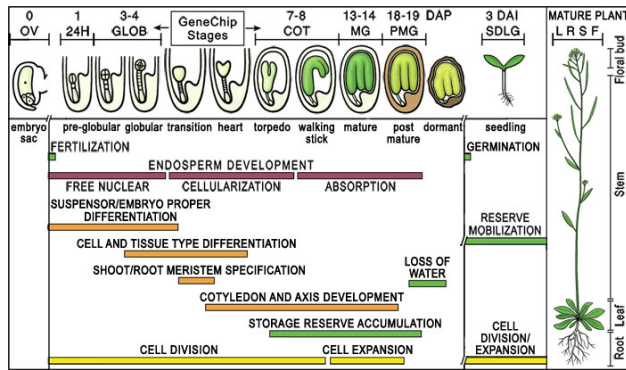
When and Where is MYB89 Expressed?



- Accumulation of mRNA measured by the Seed Gene Network, but how do you measure transcription?
- Do I have a recombinant plasmid?
- Do I have an accurate promoter sequence?



Are These Genes Crucial to Seed Development?



- HOX7D
 - is seed lethal, arresting development in the globular stage
 - is widely expressed
 - appears to impact the size of leaves and stems in mature plants
- MYB89
 - does not yet exhibit an observable phenotype

Acknowledgements