

What are the Functions of DCL1 and MYB33?

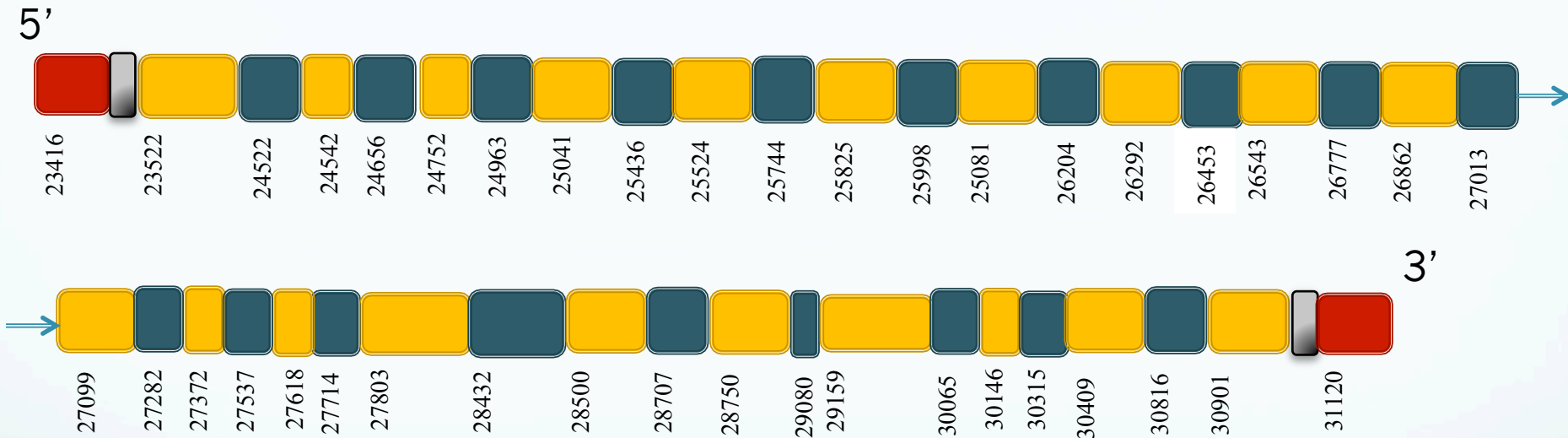
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HC70AL 2014

What is AT1G01040?

- Known as Dicer Like 1 (DCL1)
- Located in Chromosome 1
- Forward Orientation
- 7,704 bp
- 1910 AA
- Codes for a Dicer Homologous Protein
 - Involved in miRNA processing

What is the Structure of AT1G01040?

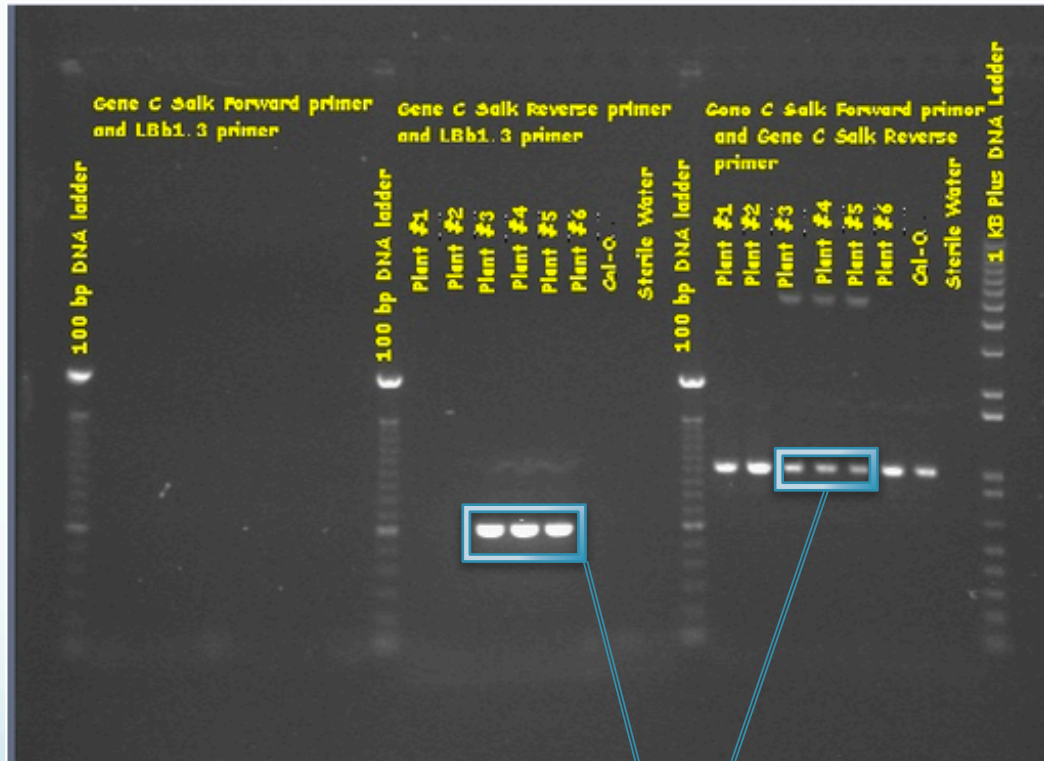
- 20 exons and 19 introns



Start Codon

Stop Codon

What are the Genotypes of my Plants?

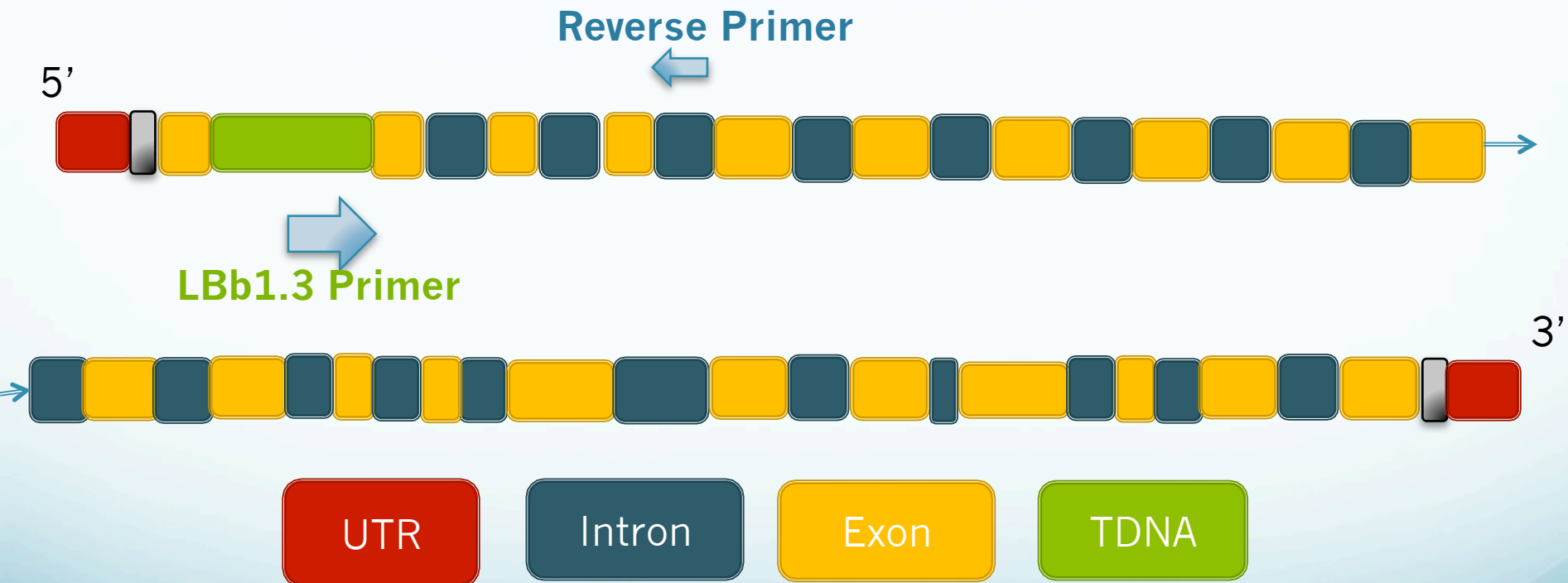


- Plants 3-5 are heterozygous for the TDNA insert.
- 5KB bands formed from the forward and reverse primers which made a product through the TDNA.

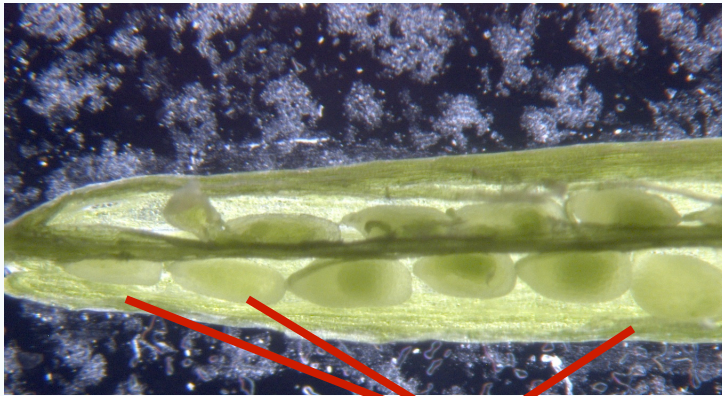
Heterozygous TDNA insert

Where is the TDNA inserted?

- TDNA inserted at nucleotide 23866 in Exon 1



What effect does the TDNA insert have?

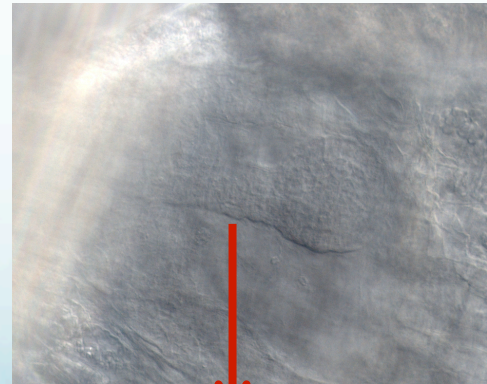


White Mutant Seeds

- Light Microscopy revealed a 1:3 ratio of white (mutant) seeds to mature green seeds.

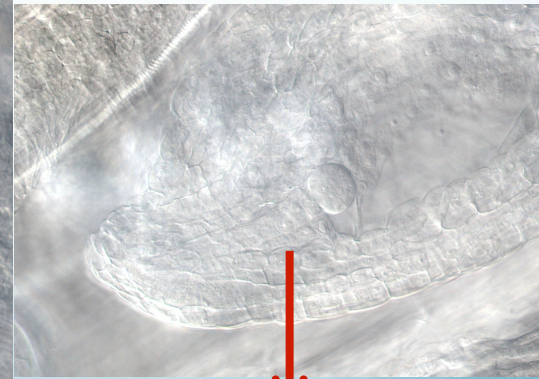
- Nomarski Microscopy reveals white (mutant) seeds to be in globular stage with abnormal suspensor.

Mutant Phenotype



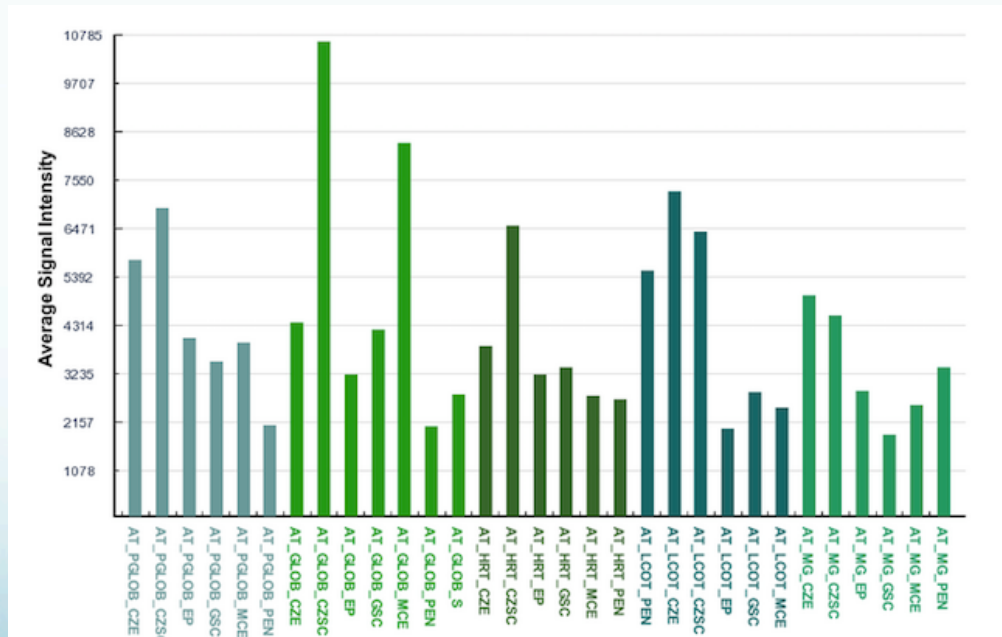
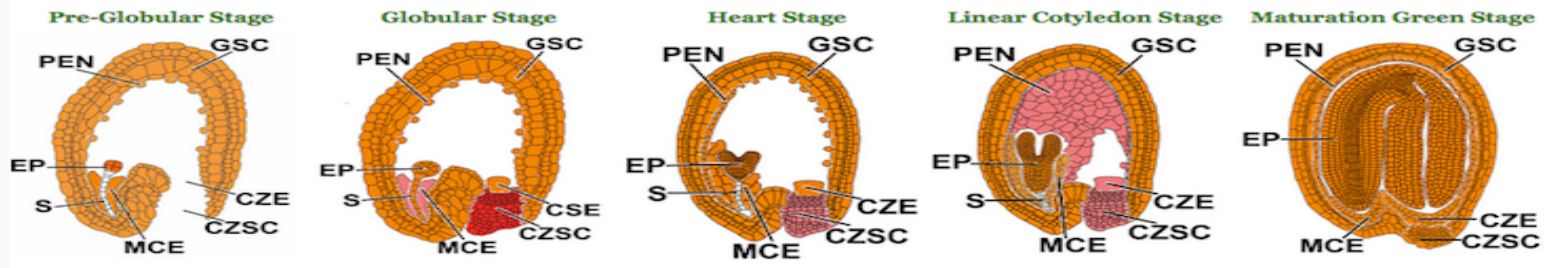
Two cell line suspensor with jagged edging

Wild Type Phenotype



Single cell line suspensor with smooth edging

When and Where is DCL1 expressed during Seed Development?

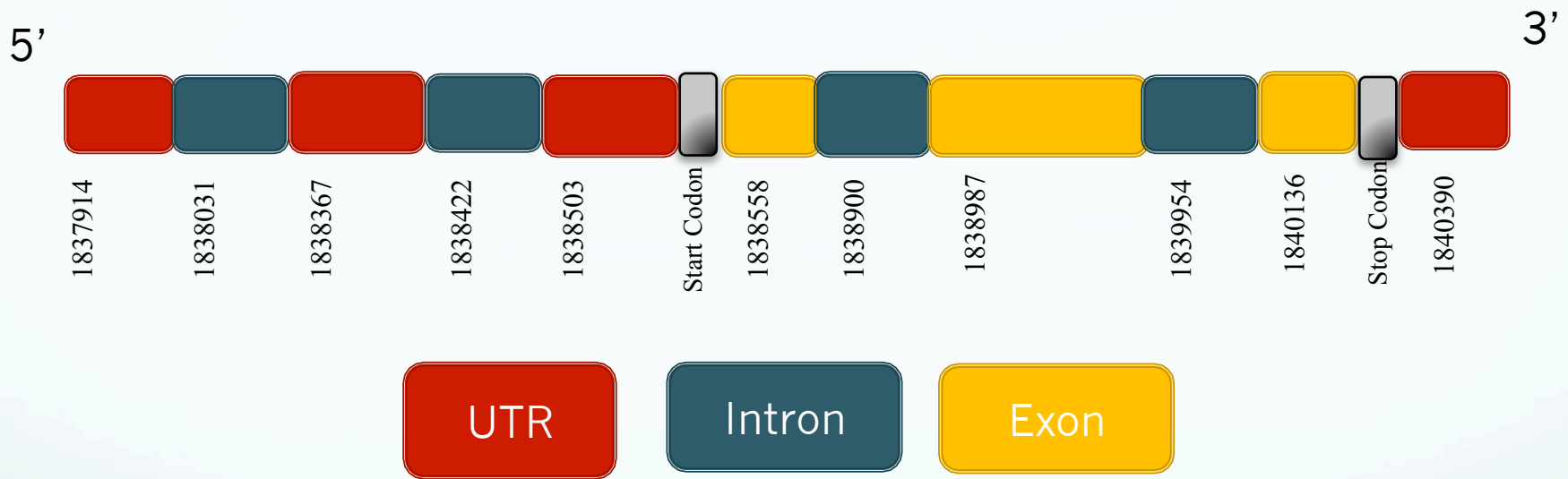


What is AT5G06100?

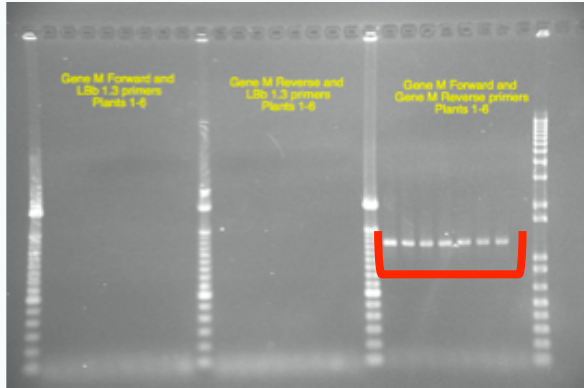
- Known as MYB33
- Located in Chromosome 5
- Forward Orientation
- 2,813 bp
- 520 AA
- Codes for a MYB33 Transcription Factor

What is the structure of AT5G06100?

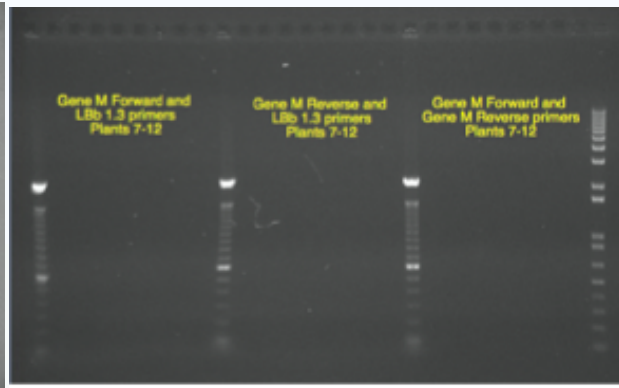
- 3 Exons and 4 Introns



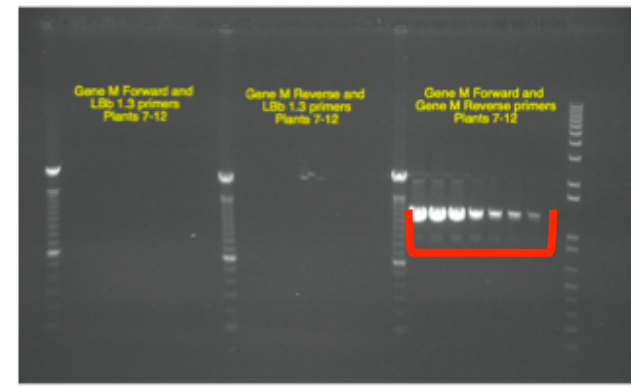
What are the Genotypes of my Plants?



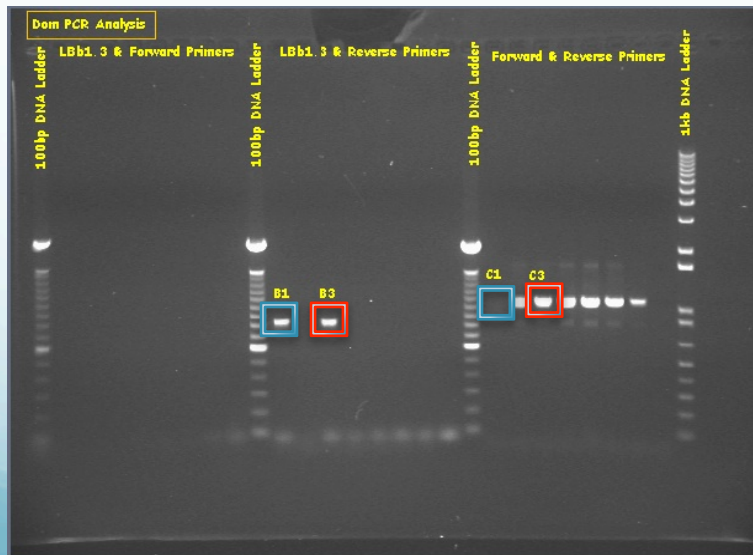
PCR Plants #1-6 Are Wild Type



PCR Plants #7-12 Inconclusive



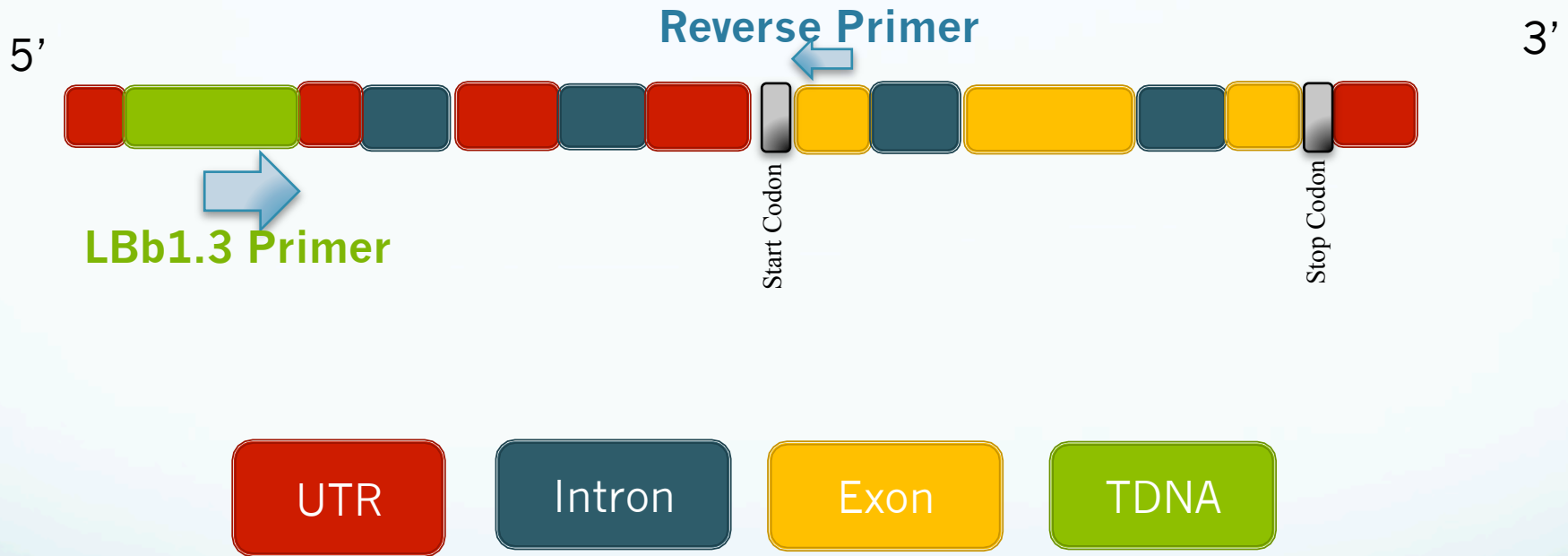
PCR Plants #7-12 Are Wild Type



- Due to limited time frame for more screening of plants, I switched from Plant M to Plant O.
- PCR Results from Saadi, D.
- Results reveal **Plant #1** is homozygous for TDNA insert and **Plant #3** is Heterozygous for TDNA insert.

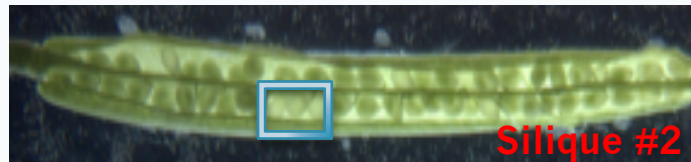
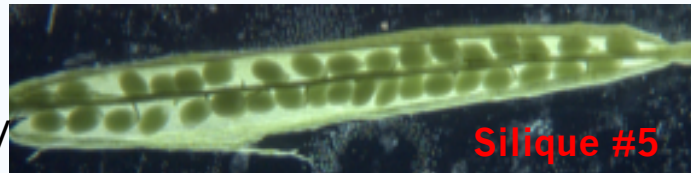
Where is the TDNA Inserted?

- TDNA inserted in 5' UTR at nucleotide 1838003



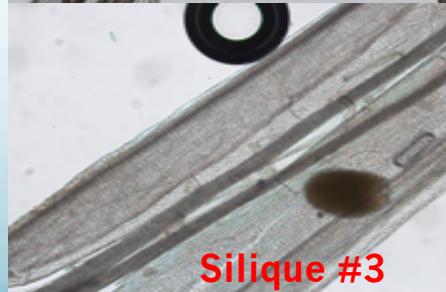
What Effect does the TDNA insert have?

- Light Microscopy reveals few phenotypic Differences



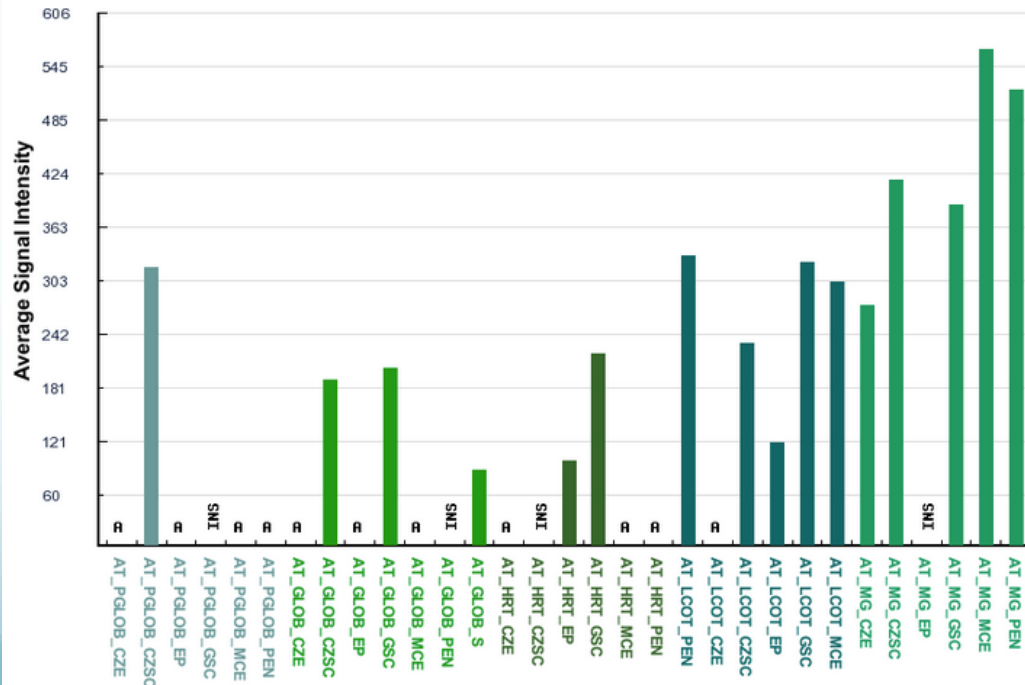
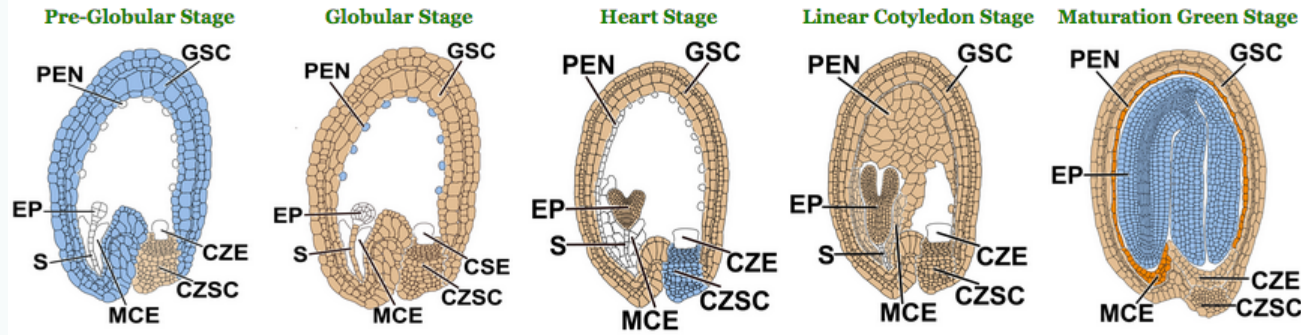
3 of the 10 white/light green seeds in a Mature Green Silique.

Sil. #	TDNA or WT	Length	Notes (seed count, abnormalities, etc.)
1	TDNA	1.5 cm	57 seeds total; 3 empty spots
2	TDNA	1.4 cm	53 seeds total; 10 white/ light green
3	TDNA	1.2 cm	34 seeds total; 6 aborted
4	WT	1.0 cm	26 seeds total; 3 empty spaces; few seeds
5	WT	1.1cm	36 seeds total; 1 empty space



- Nomarski reveals few phenotypic differences
- Seeds from Silique #5 show synchronized seeds in the mature stage.
- Seeds in Silique #2 also shows synchronized development but has several shriveled seeds.
- Silique # 3 also shows synchronized embryonic development in mature stage.

When and Where is MYB33 expressed during Seed Development?



Other Experiments with Double Mutant of MYB33 and MYB65 Show Male Sterility

- The knockout of this gene did not demonstrate seed lethality.
- Currently, it has been noted that the double mutations in MYB33 and MYB65 causes male sterility.
- Therefore more research can be on the causes of male sterility and this may provide insight into the function of these two genes.

Download Graphics Next Previous Descriptions

MYB65
Sequence ID: lc|154253 Length: 553 Number of Matches: 1

Range 1: 1 to 449 Next Match Previous Match

Score	Expect	Method	Identities	Positives	Gaps
526 bits(1356)	0.0	Compositional matrix adjust.	278/453(61%)	331/453(73%)	28/453(6%)
Query 1	MSYTTSDSD-----	HNESPAADDNGSDCRSRWDGHALKKGFWSSAEDDILIDYVNK	51		
Sbjct 1	MSYT+ +D HNESPA D + CRSR LKKGPR+S ED ILIDYV K	MSYTTATADSDDGMSHSHNESFAPDSISNGCRSRGKRSVLKKGFWSTEDGILIDYVKK	60		
Query 52	HGEGNWNAVQKHTSLFRCGKSCRLRWANHLRPNLKKGAFSQEEQLVIVELHAKMGNRRAR	111			
Sbjct 61	HGEGNWNAVQKHTSLRCGKSCRLRWANHLRPNLKKGAFSQEEQLVIVEMHAKMGNKWAQ	120			
Query 112	MAHLPGRTDNEIKNYWNTRIKRRQAGLPLYPPEMHEVALEWSQYAKSRVMGED--RR	169			
Sbjct 121	MAHLPGRTDNEIKNYWNTRIKRRQAGLPLYPPE++V+ L WS+EY KS ++ D RR	180			
Query 170	HQDFLQLGSCSNVFFDTLNF-TDMVPGTFDLADMTAYKNMGNCASSPRYENFMFTPTPS	228			
Sbjct 181	HQDFLQLG+ + NV FD LNF ++P DL+D+ A +G ASS RYE++M P +PS	240			
Query 229	SKRLWESLPPCCSSTIKQEFSSPQFRNTSPQITSKTCSFVPCDVEHPLYGNRHSF-	287			
Sbjct 241	K++WES ++P CSS IK EF SPE F+NT+ Q ++CS S PCDV+H Y N+HS	299			
Query 288	-VMIPDSHTPDGIVPYSKPLYGAVKLELPSFQYSETT-FDQWKKSSPPHSDLLDFDFT	345			
Sbjct 300	+M+PDSHT T G+ P SKPL+GAVKLELPSFQYSET+ FDQWK + SPHSDLLD D	359			
Query 346	YIQSPPPPTGGEESDLYSNFDTGLLDMLLLEAKIRNNSKNNLYRSCASTIPSDLGQVT	405			
Sbjct 360	YIQS PPP+ EESD +S+ DTGLLDMLL EAKI+ S K+L S S+	417			
Query 406	VSQ-----TKSEEFNLSKSLVHSEMT	429			
Sbjct 418	V+Q KS E+++S K +L SE+++	449			

Related Information