

Investigation of HISN2 and DREB Transcription Factor



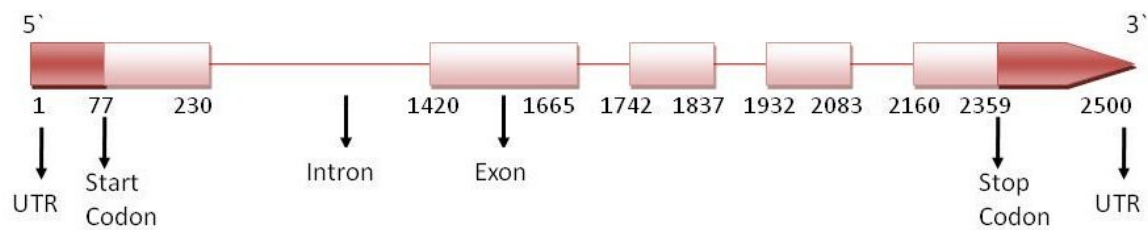
By Lauren Daoust

What Does HISN2 Code For?

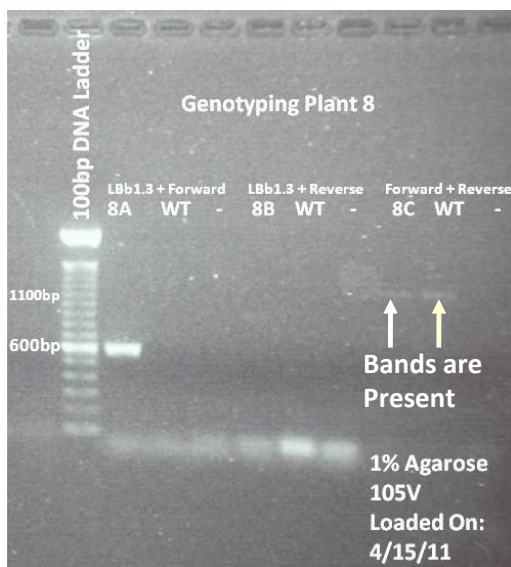
- It codes for a protein that is involved with the biosynthesis of Histidine, an essential amino acid for protein production and seed development
- HISN2 plays a large role in seed development during all embryonic stages

How is HSN2 Structured?

- 2500bp long
- Contains 5 exons and 4 introns
- Lies on the complement strand of chromosome 1
- Encodes a protein that is 281aa long

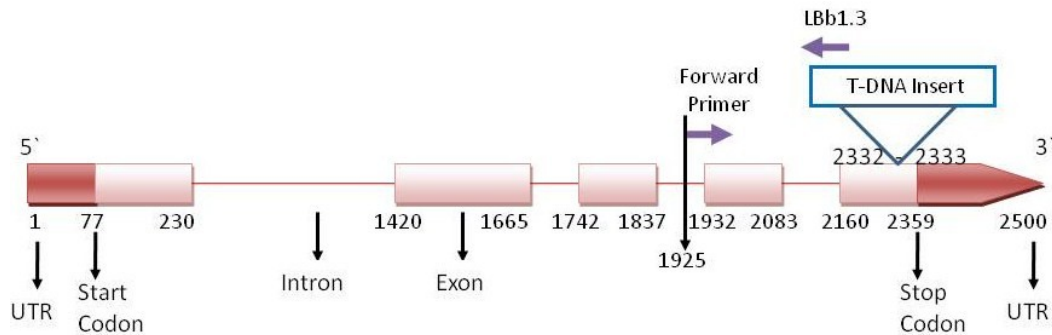


What are the Genotypes of the Plants Investigated?



- Only one plant had conclusive results
- Plant 8 was found to be heterozygous for the mutant allele

Where is the T-DNA Inserted?

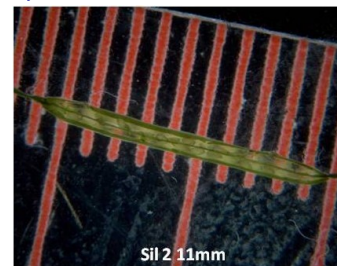


- It is located in the 5th exon
- A PCR Product of 580bp is made with the forward primer

What are the effects on seed development?

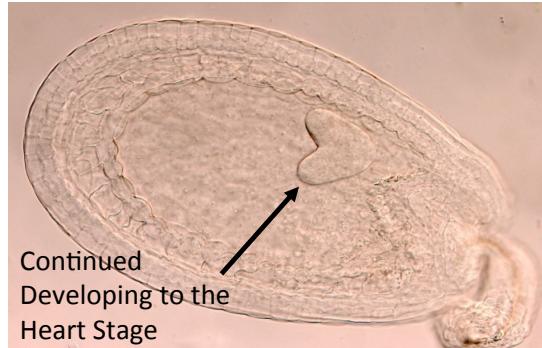
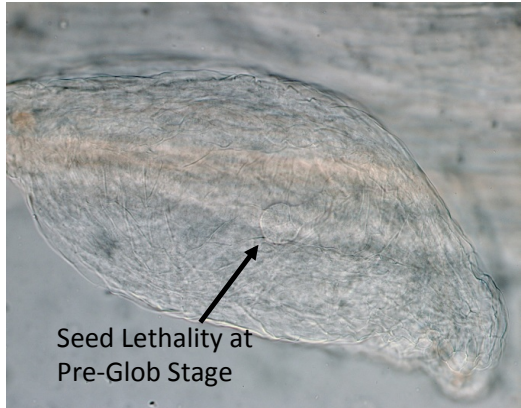
- A 3:1 Ratio of green to mutant seeds was found for 4 siliques studied

	Observed	Observed	Expected	Expected	
No. of Seed Phenotype Found:	Green	Mutant	Green	Mutant	Chi-Squared Test
Silique1	34	9	32.25	10.75	0.3799
Silique2	26	12	28.5	9.5	0.871
Silique3	38	8	34.5	11.5	1.42
Silique4	25	11	27	9	0.592



Is Seed Lethality Present?

- A H1SN2 Mutation is expected to cause seed lethality during the pre-globular stage
- As expected, this was observed when seeds from plant 8 were examined using the Nomarski Microscope

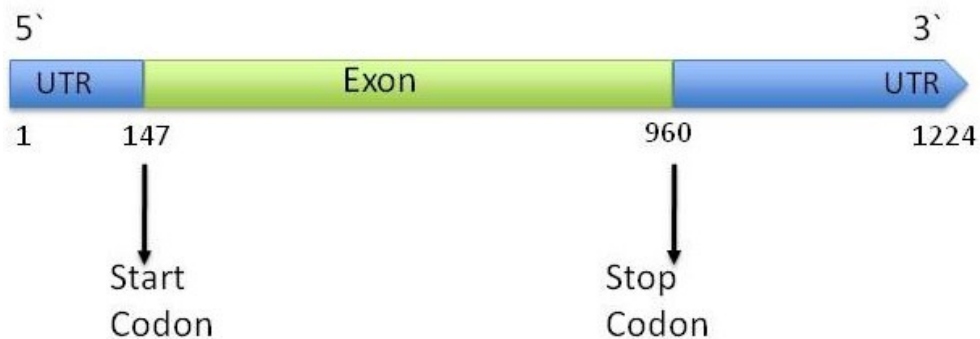


What is the Function of DREB Transcription Factor?

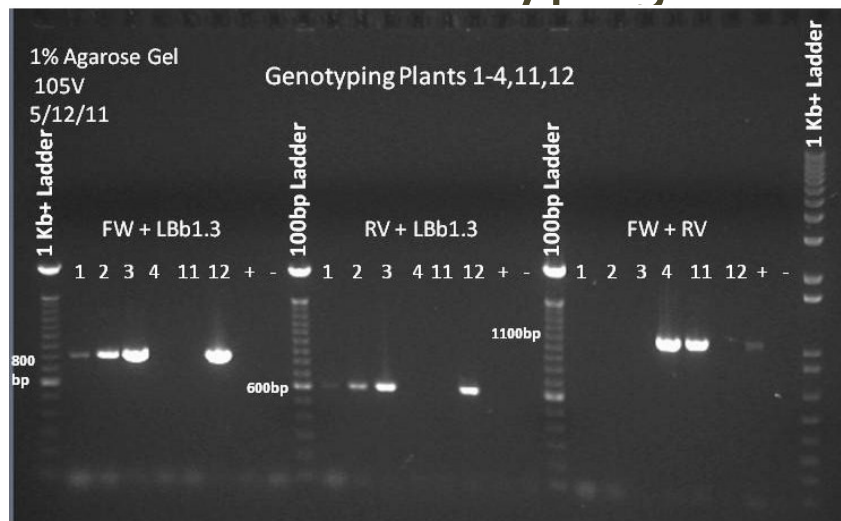
- It is a member of the AP2 Transcription Factor Family as well as the A-6 DREB (Dehydration Response Element Binding factor) subfamily
- As a member of DREB, it binds to the promoters of genes that are activated in response to stressors
- Expression is high in the chalazal seed coat during the linear and maturation cotyledon stages

What is its Structure?

- 1224bp long
- 1 exon, 0 introns
- Lies on the complement strand of chromosome 4
- Encodes a protein that is 272aa long

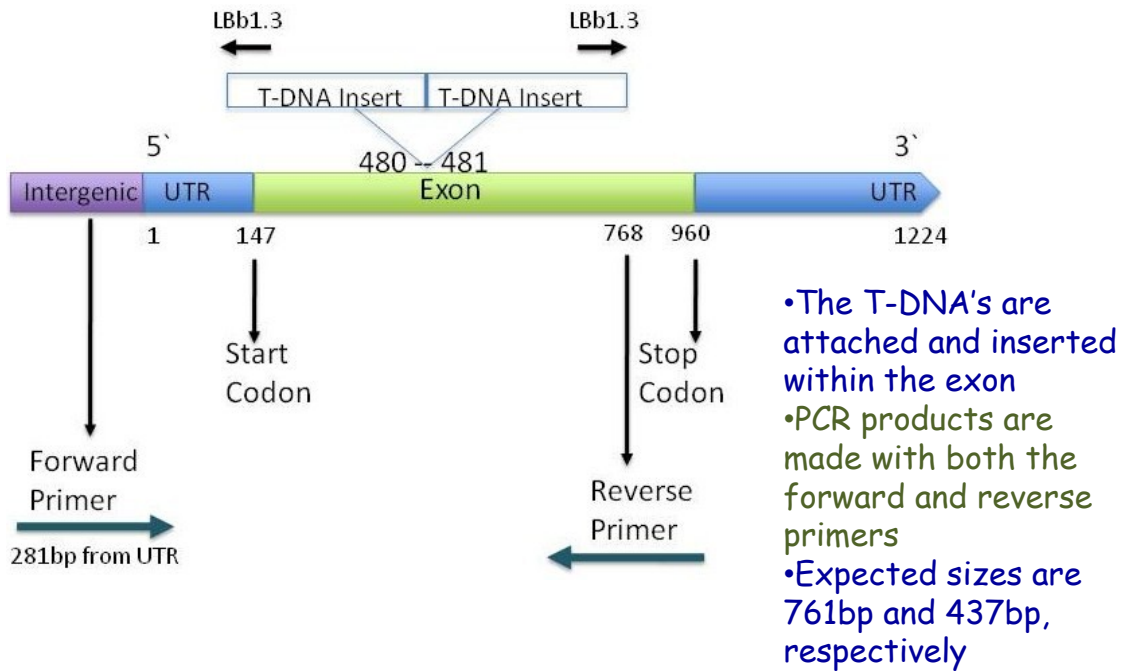


What are the Genotyping Results?



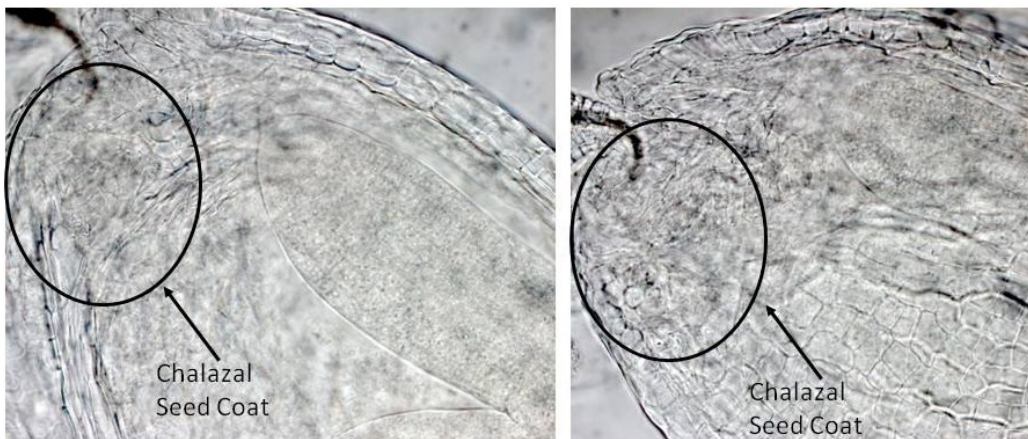
- Plants 1,2,3,12 were found to be homozygous for the mutant allele, with two T-DNA inserts present
- Plants 4 and 11 were found to be wild-type

Where are the T-DNA's Inserted?



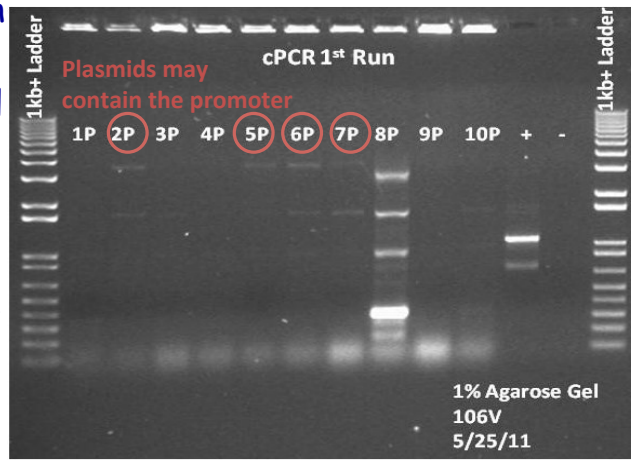
Is there an Observable Phenotype?

- There was no 3:1 ratio of green to mutant seeds found, all seeds had at least developed past the heart stage so no seed lethality
- No observable differences between the wild-type seeds and homozygous mutant seeds were found during the linear and maturation cotyledon stages



Where is DREB Transcription Factor Expressed?

- Promoter Cloning will help determine where and when the gene is expressed
- I have only done the initial steps of recombining the promoter with the pENTR/TOPO Plasmid and Selecting bacteria that contain these recombinant plasmids after insertion
- Some colonies were found to have the recombinant plasmids, but more tests will need to be done to further prove this hypothesis



Final Conclusions

- HISN2
 - Mutation Results in Seed Lethality during the Pre-Glob stage
 - Involved in the biosynthesis of histidine, an essential amino acid for protein production and seed development
 - Disruption of this biological process will result in no production of histidine, so proteins cannot be produced so the seed cannot develop and therefore dies at an early stage
- DREB Transcription Factor
 - Causes no seed lethality or any mutant phenotype, the seeds continue to develop normally
 - Possible that another transcription factor gene codes for a similar protein allowing normal function
 - Also possible that the gene does not function under normal conditions, but only when the plant is under stress so no phenotype would be seen when the plant is grown under normal conditions

What Further Experiments can be done?

- The role of histidine in seed development
- HSN2's exact role in the biosynthesis of histidine
- Continuation of promoter cloning using GUS and GFP genes to identify gene expression
- Grow knock-out plants under stress and observe phenotype
- What other genes exist that might accommodate for the loss of function in DREB Transcription Factor since no observable mutant phenotype was present

Thank you
Dr. Goldberg, Kelli,
HC70AL TA's and
Everyone in the
Goldberg Lab For
an Amazing
Experience!!!!!!

