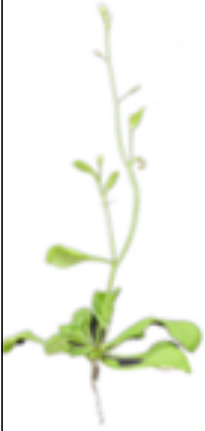


What are the functions of TanMei Gene and Homeodomain-like Superfamily Protein Gene?



Reece Fenning
June 2nd 2011

What is the function of TANMEI gene?

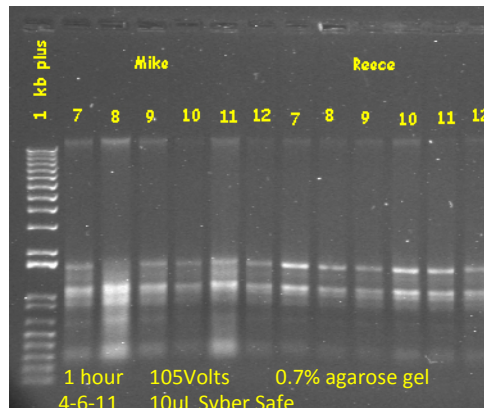
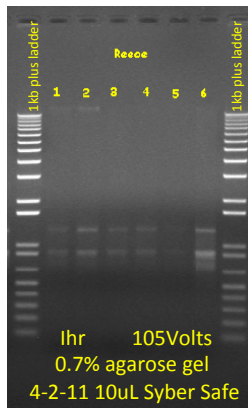
- Causes seed lethality when gene is knocked out
 - Jagged bumpy edges for Torpedo staged embryo
- Involved in later stages of seed development
 - Shoot and Root development
 - Hypocotyls fail to elongate (part of stem of an embryo)
- Knockouts are intolerant to desiccation
- Build up of Anthocyanin
 - Tan colored seeds
 - Red/Purple pigment congregates at the tip of a torpedo staged embryo

What Methods were used to Study TANMEI Gene?

- Sowing Seeds
- Nucleic Acid Isolation
- Polymerase Chain Reaction Genotyping
- Sequencing
- Light Microscopy
- Nomarski Optics

What are my Nucleic Acid Isolation Results?

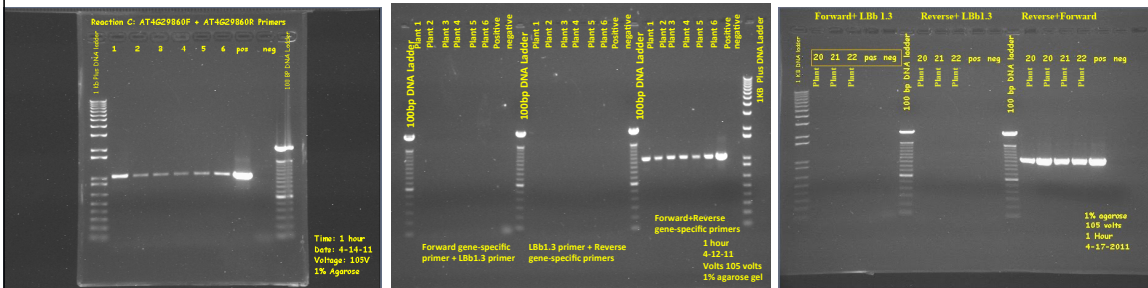
Nucleic acids were isolated from 22 different plants in order to be used for polymerase chain reactions



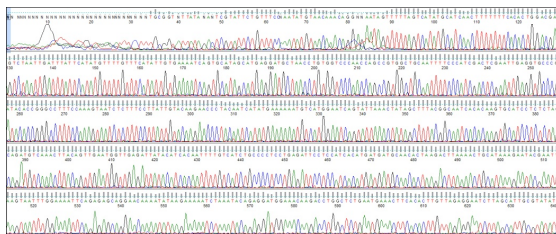
Plant Number	Nucleic Acid Concentration (ng/uL)
Plant 1	22.4
Plant 2	25.5
Plant 3	14.5
Plant 4	13.2
Plant 5	9.1
Plant 6	26.4
Plant 7	29.03
Plant 8	21.32
Plant 9	20.1
Plant 10	39.48
Plant 11	25.66
Plant 12	24.57
Plant 13	90.59
Plant 14	65.39
Plant 15	108.57
Plant 16	58.73
Plant 17	42.7
Plant 18	31.66
Plant 19	64.25
Plant 20	61.12
Plant 21	131.85
Plant 22	22.86

What are my Genotyping Results?

- Gene specific forward and reverse primers produced a band for plants 1-13 and 20-22.
 - The band size was 1,191 base pairs.
 - No bands were ever produced using any T-DNA primers for all plants.

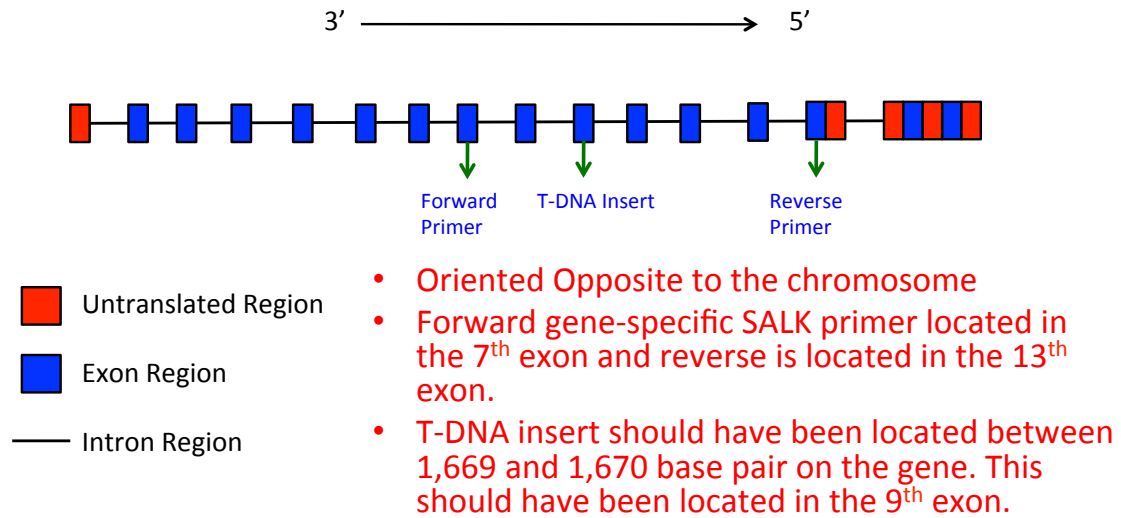


What are my Sequencing Results?

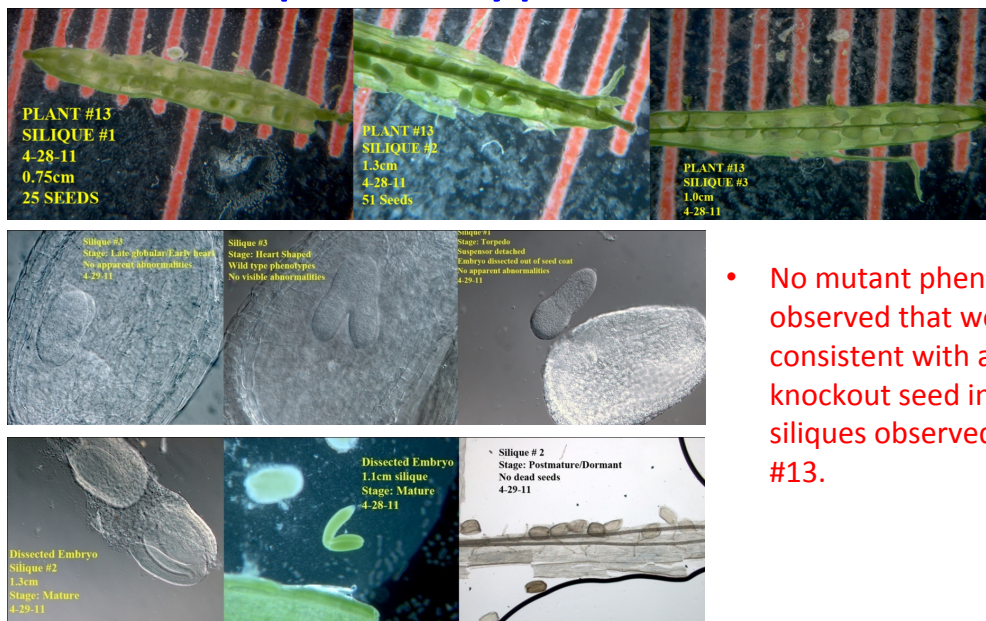


- Sequencing results of the first 640 nucleotide base pairs using Finch TV
 - Most nucleotides are predicted with high confidence demonstrated by the peaks.
- Top hit on the TAIR was AT4G29860 or TanMei
 - E-value of 0.00
 - Alignment 933/952
 - Amino acid length 386
 - From nucleotide number 2254-1303 on the gene

What is the Structure of TANMEI Gene?



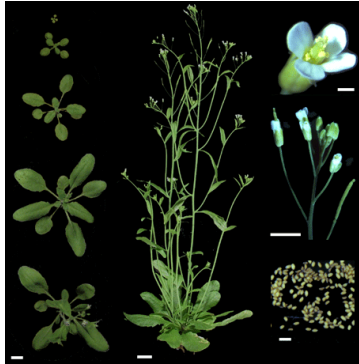
What phenotypes did I observe?



- No mutant phenotype was observed that would be consistent with a TanMei knockout seed in any siliques observed for plant #13.

Future Experiments

- Sow new seeds and repeat process to find heterozygous plants.
 - Observe phenotypes of plants



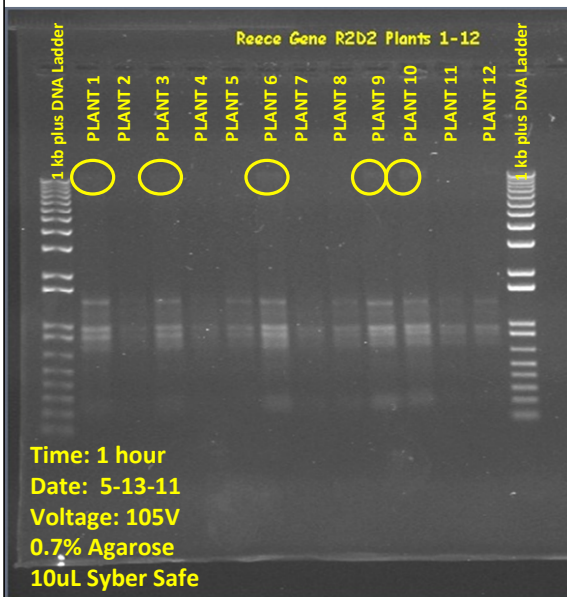
What is the Function of Homeodomain-like Gene?

- The homeodomain-like superfamily protein binds to DNA
- Transcription factor activity
- Expressed in thirteen different growth stages
 - Expressed during pre-globular and cotyledon stages during seed development
 - Preglobular stage expressed in the Chalazal Seed Coat
 - Cotyledon Linear stage expressed in the general seed coat
 - Cotyledon Mature Green stage expressed in the chalazal endosperm and seed coat

What methods were used to study the Homeodomain-like gene.

- Sowing Seeds
- Nucleic Acid Isolation
- Polymerase Chain Reaction Genotyping
- Sequencing
- Light Microscopy
- Promoter Cloning

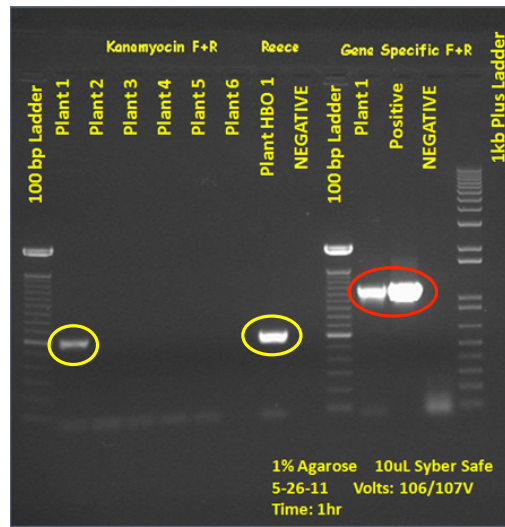
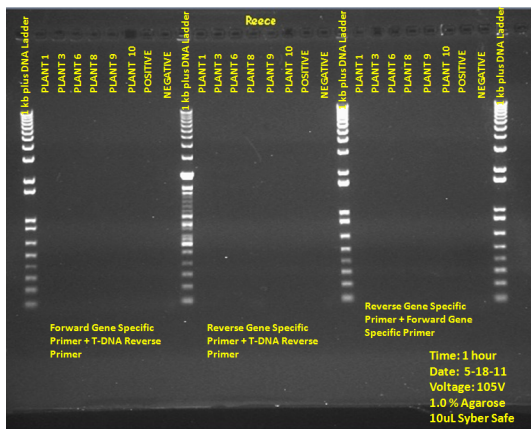
What are my nucleic acid isolation results?



- Plants 1,3,6,10 and 11 had the highest nucleotide concentrations and had faint bands on the top of the gel electrophoresis results.
 - Low nucleic acid concentrations can be explained by small leaves were extracted due to the plants being young.

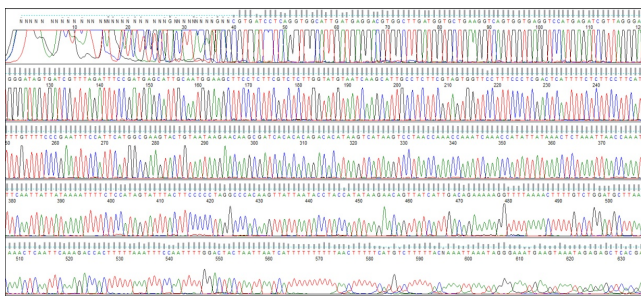
Plant Number	Nucleic Acid Concentration (ng/uL)
Plant 1	35.07
Plant 2	8.61
Plant 3	24.86
Plant 4	8.23
Plant 5	9.94
Plant 6	28.79
Plant 7	7.35
Plant 8	9.99
Plant 9	25
Plant 10	30.69
Plant 11	7.24
Plant 12	7.24

What are my genotyping results?



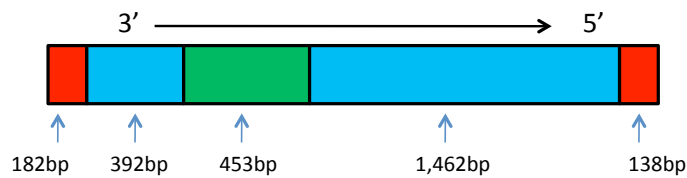
- Kanamycin forward and reverse primers produced a band in plant #1 and HBO plant #1 as a positive control.
 - Expected base pair size of 612bp matches results.
- Gene specific forward and reverse primers produced a band at 1,217bp for the positive control and plant #1 that corresponds with the expected results.

Sequencing Results



- Sequencing results of the first 630 nucleotide base pairs using Finch TV
 - Predicted nucleotide bases between 100 and 500 base pairs have high confidence values
- Top hit on the TAIR website AT1G28300
 - E-value 0.00
 - Alignment 2,767-2,244 nucleotide position on gene
 - 524/524
- Conflicts with primers that are located in AT5G28300
- Explanation: Some type of mix up occurred.
- Further Steps:
 - 1.) Sequence a different plant with primers
 - 2.) Call SALK
 - 3.) Use different primers
 - 4.) Isolate a different gene as a control test

What is the Structure of the Homeodomain-like Gene?



182bp ↑ Untranslated Region

392bp ↑ Exon Region

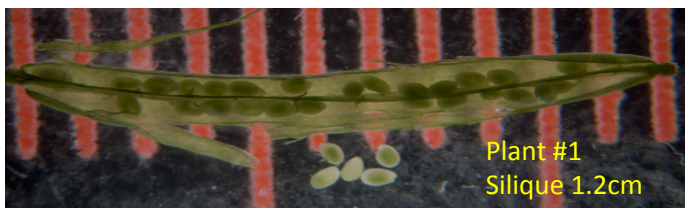
453bp ↑ Intron Region

1,462bp ↑

138bp ↑

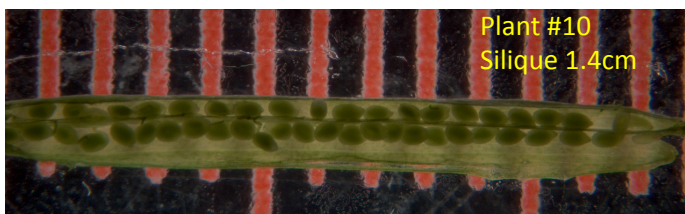
- **Gene Number: AT5G28300**
 - Oriented opposite to the chromosome
- T-DNA should have been located in the first intron between the 983 base pair and 984 base pair in the gene.
- Predicted amino acid length 619

What phenotypes did I observe?



Silique contains 32 seeds that had green seed coats and green embryos.

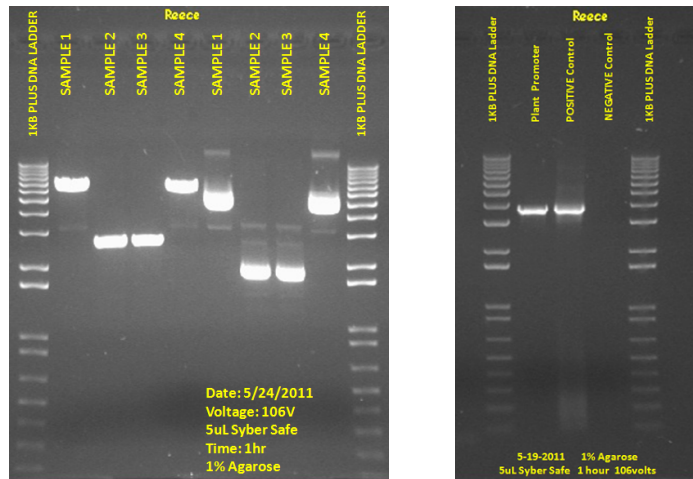
One seed was slightly less developed and did not have a visible green embryo



Silique contains 47 mature seeds that have dark green embryos with green seed coats.

- 40 Siliques observed
- 10 plants observed
- No mutant phenotypes observed using light microscopy
- Further experiment: Nomarski optics

Promoter Cloning



- Samples 1 and 4 have promoter sequences inserted in their pENTR vector.
- Promoter size 3,804 bp
- pENTR size 2580 bp

Future Experiments

- Continue with the Promoter Cloning experiment
 - Form a recombinant promoter pENTR vector with a vector containing GFP and GUS
 - Transform and screen *E.coli* cells
 - Transform agrobacteria and then transform Arabidopsis plant
 - select with herbicide.



THANK YOU SO
MUCH FOR
EVERYTHING THIS
QUARTER!

