Function of Genes in Arabidopsis Thaliana

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HC70AL GENE DISCOVERY LAB
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What Are We Studying?

- Purpose: to identify and study Arabidopsis plants with a specific gene knocked out
- Genes Studied:

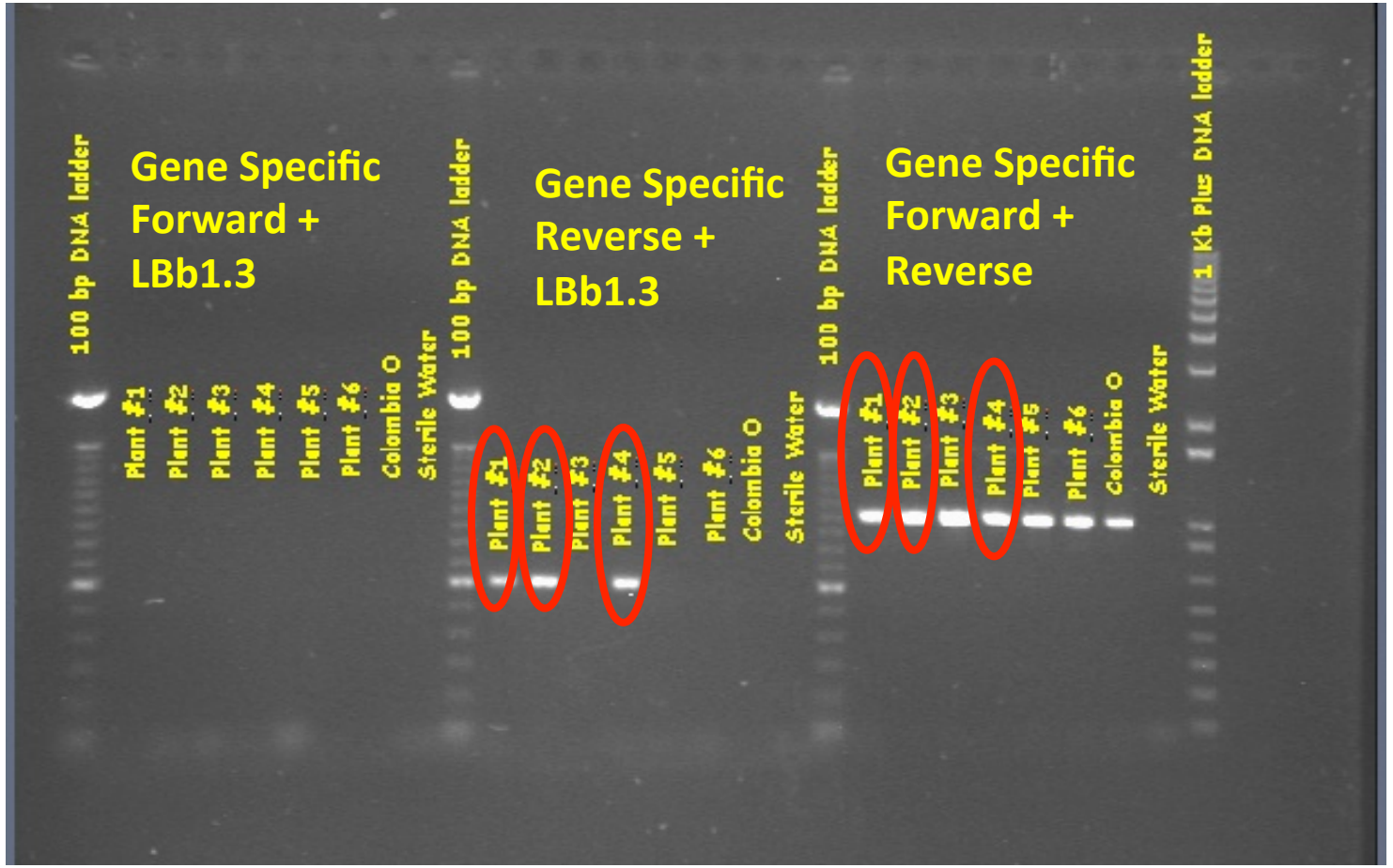
  AT2G43650 (Gene “D”)
  AT2G42660 (Gene “X”)
  AT5G58850 (Gene “P”)

Process

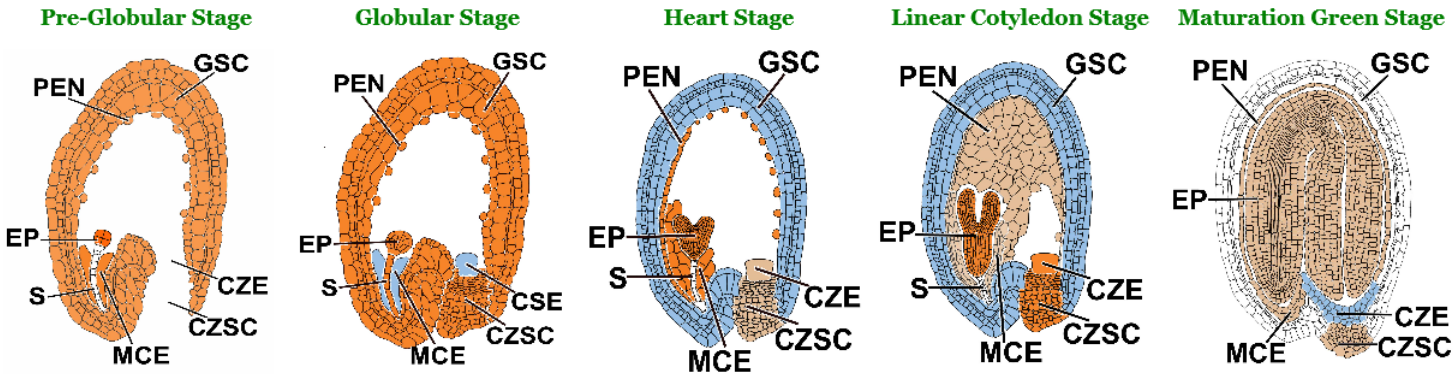
- Isolation of genomic DNA from 5 knockout line plants and 1 wildtype plant
- PCR analysis to identify which plant's genomes contained a tDNA insert
- Utilization of bioinformatics to identify which gene is interrupted and begin to understand its function
- Light and Nomarski Interference Contrast Microscopy to observe phenotypes of seeds

Gene 1 “D” : AT2G43650

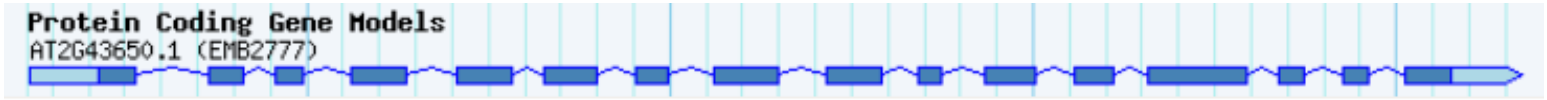
- Plants 1, 2, 4 heterozygous for tDNA insert
- Two sequencing reactions with LBb1.3 and the Reverse primer
- Tair database to identify gene

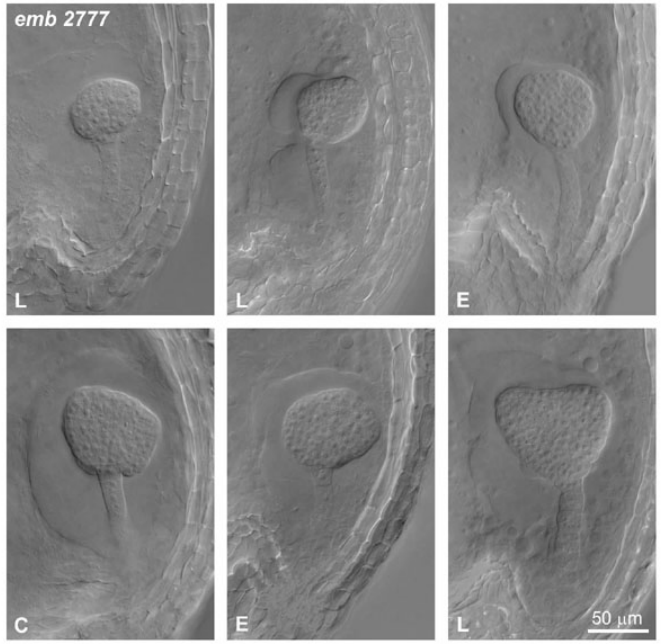


AT2G43650: What Do We Know?



Cartoon of gene expression throughout development (seedgenenetwork.net)

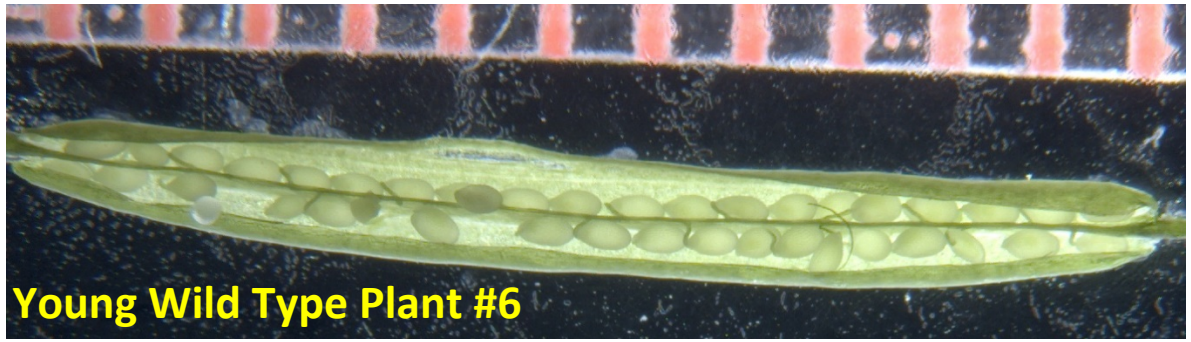


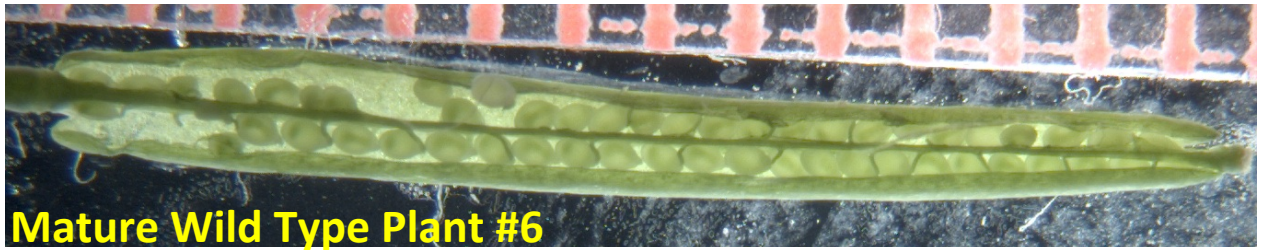


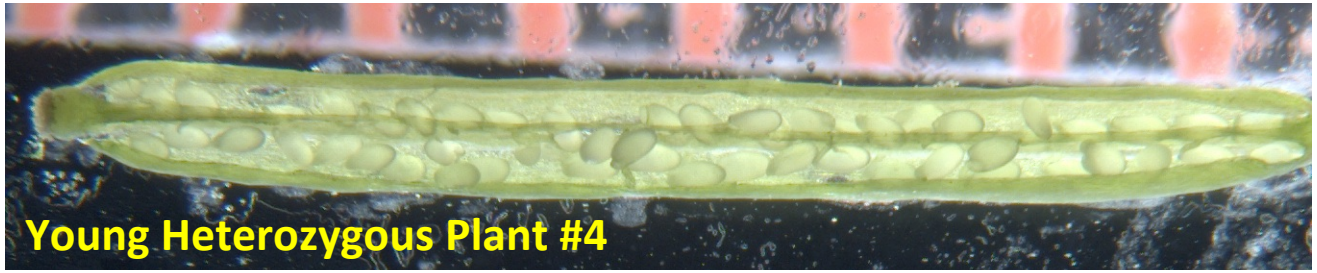
- "EMBRYO DEFECTIVE 2777" Protein Function: "Putative regulator of chromatin silencing"
- 4, 107 base pairs
- Expect disruption/mutation in the globular stage
- Expect white seeds

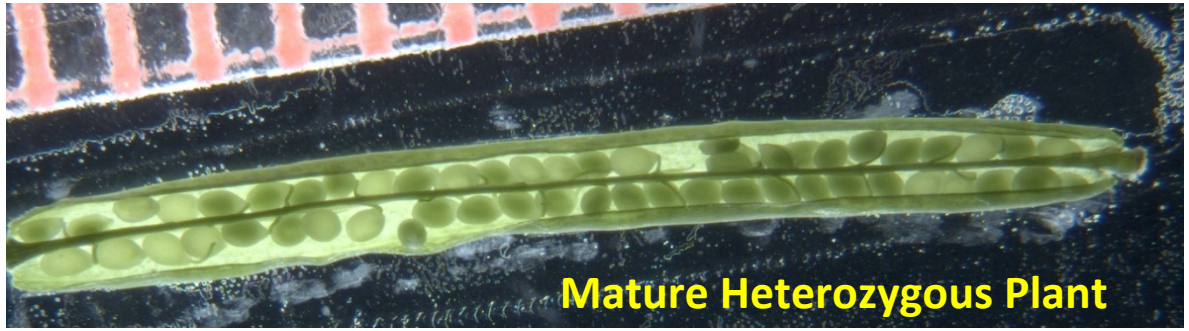
Images from Meinke/Salk mutation seedgene.org

Phenotype Analysis AT2G43650: Light Microscopy



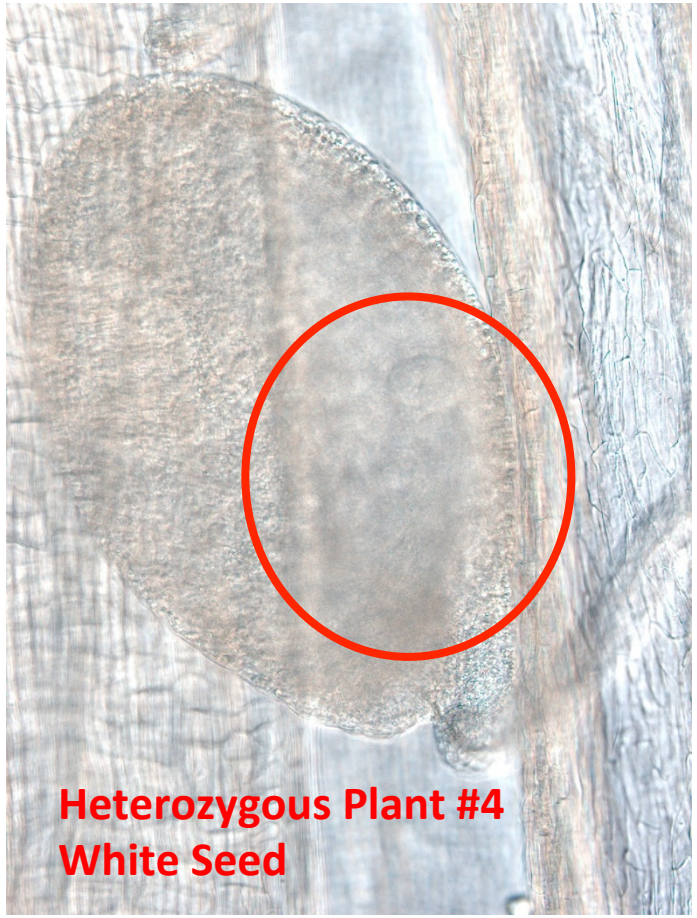


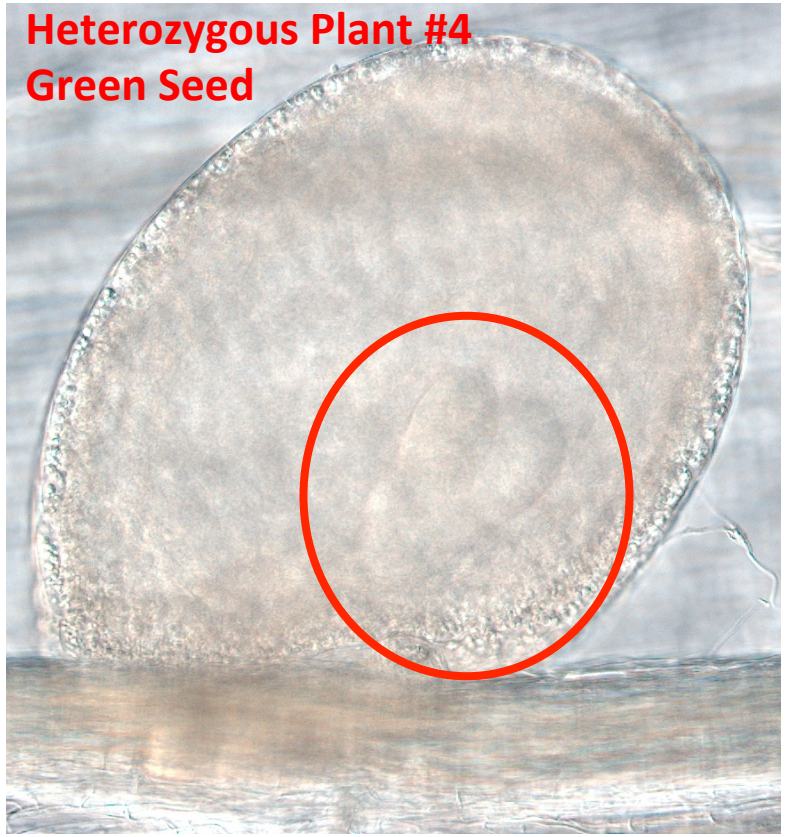


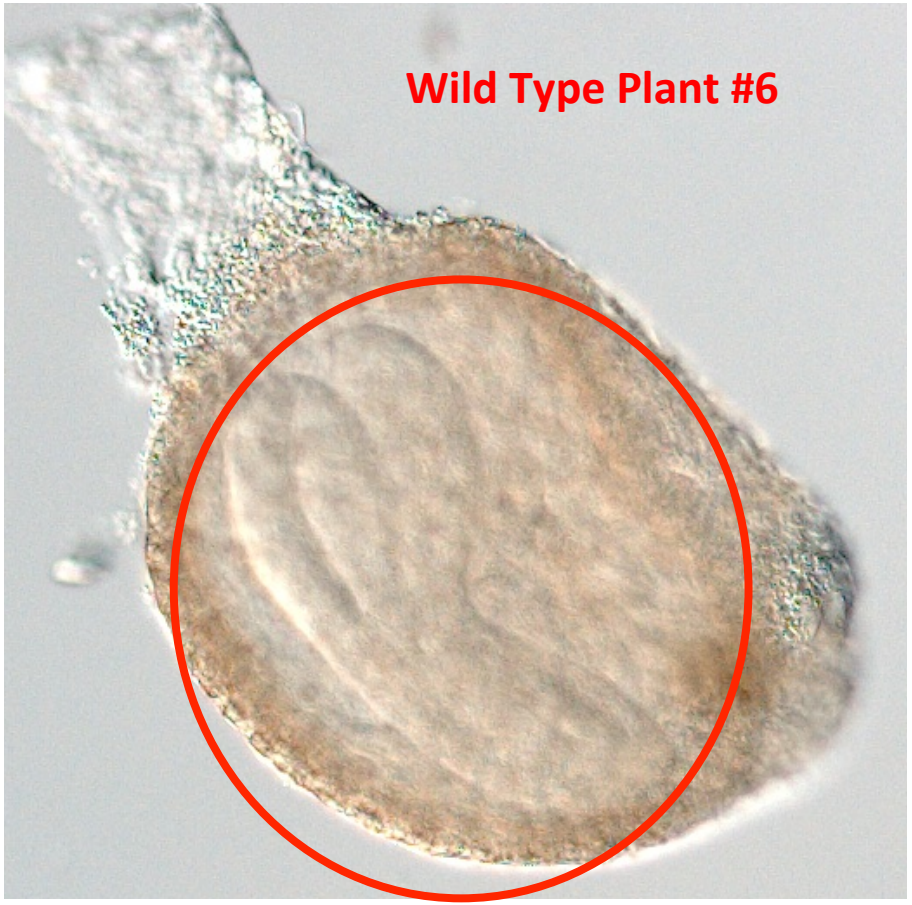


- No difference in younger stages
- Mature Heterozygous Plant shows 17 : 38 ratio of white to green seeds
- Chi Squared Value: 0.862 | P-value > 0.5

Phenotype Analysis AT243650: Nomarski

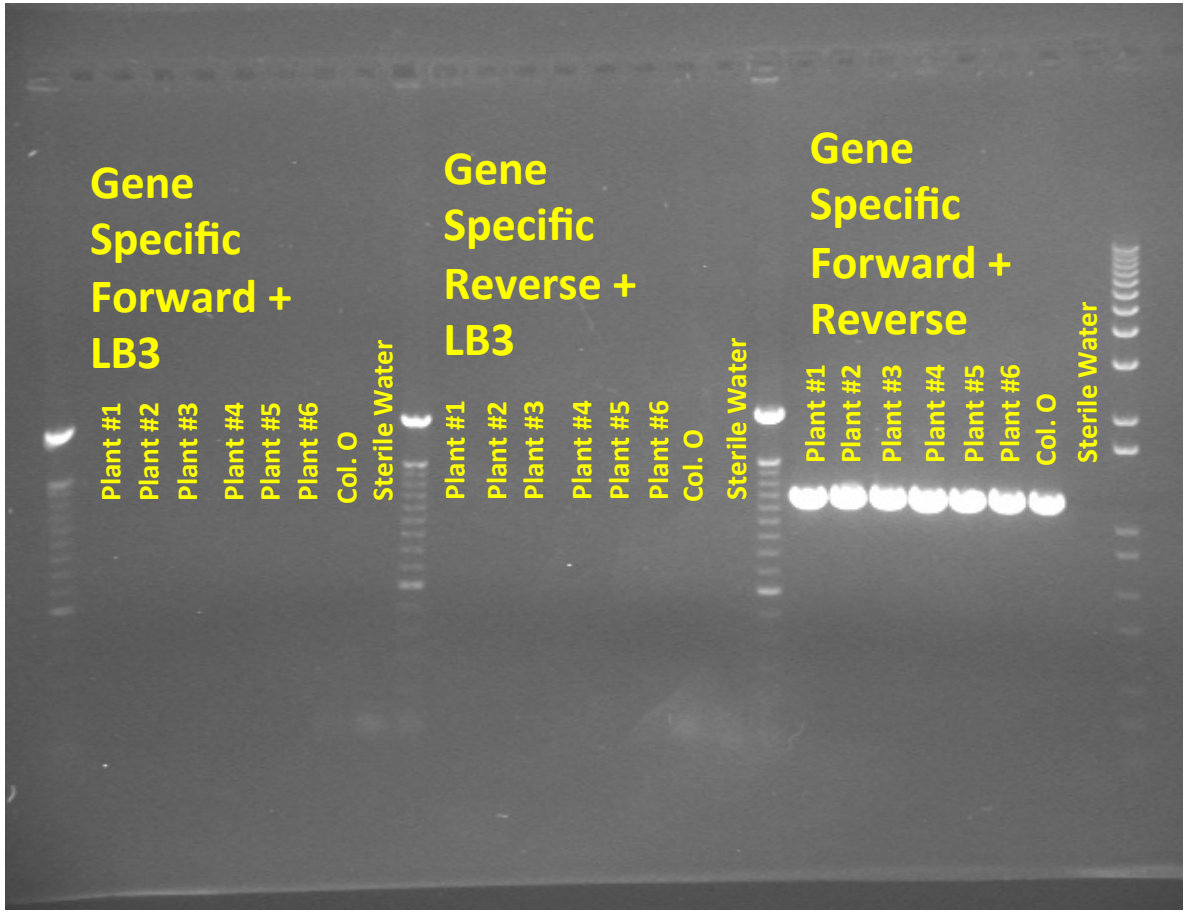


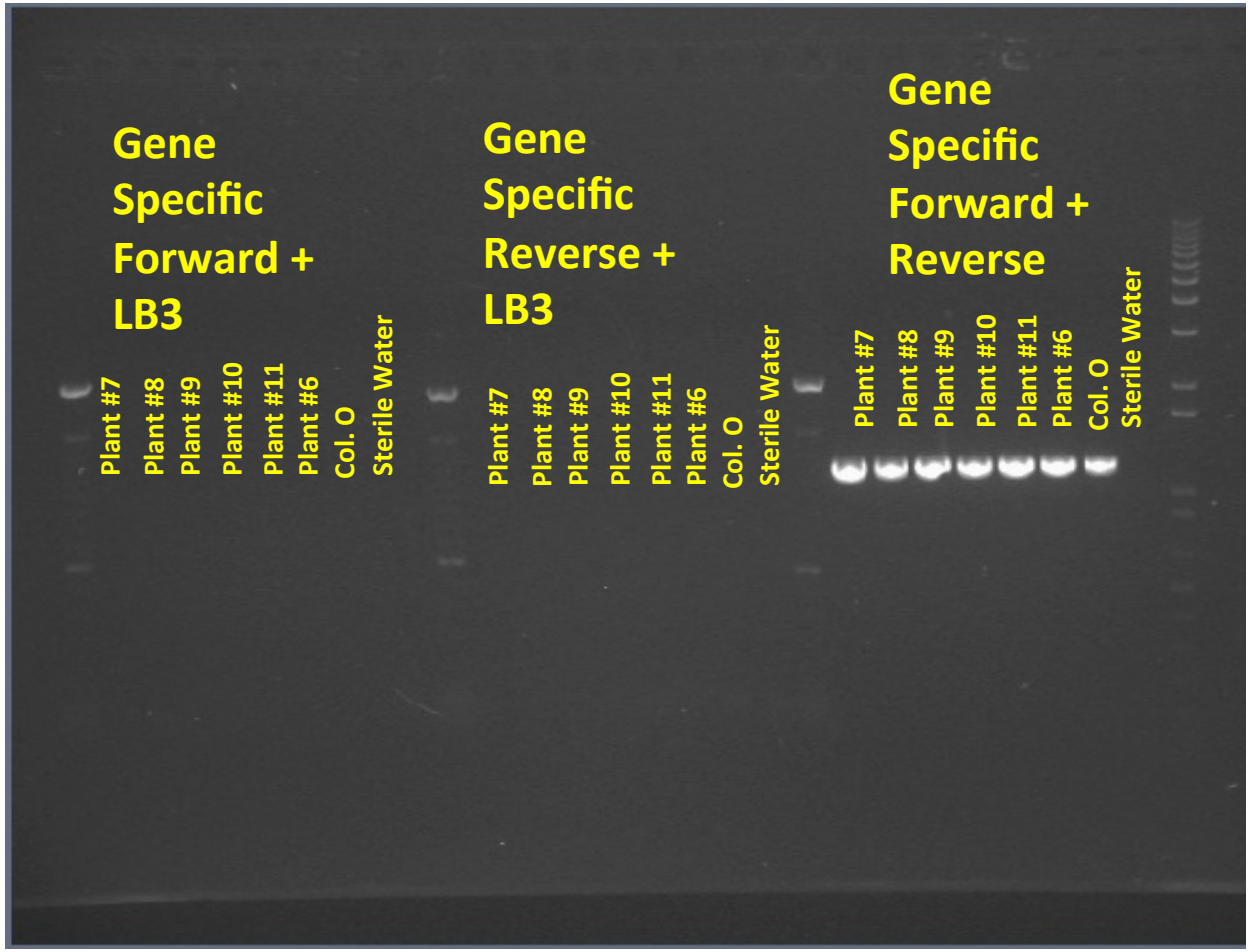




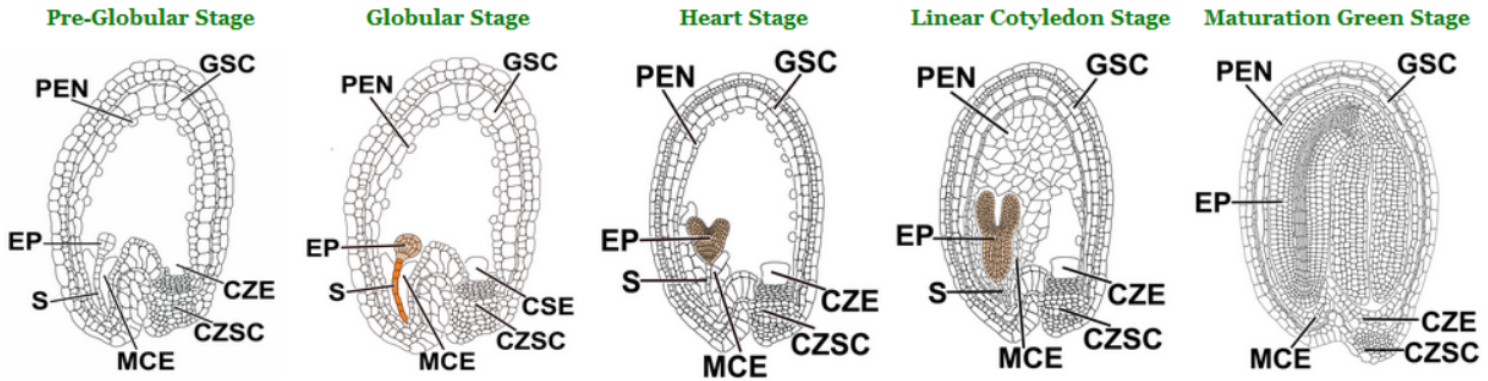
Phenotype Analysis AT2G43650: Nomarski

Gene 2 “X” : AT2G42660





AT2G42660: What Do We Know?

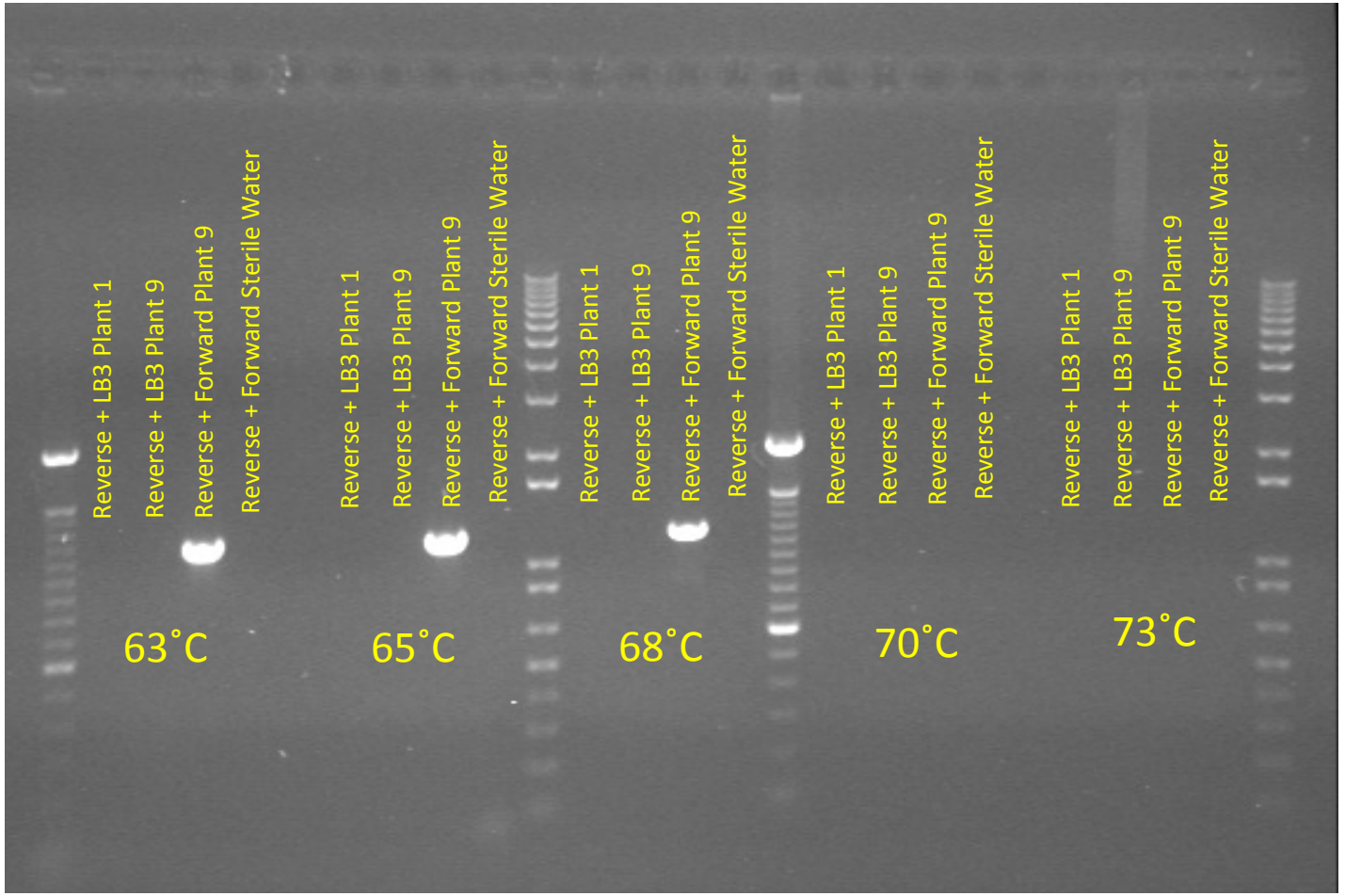


Cartoon of gene expression throughout development (seedgenenetwork.net)

- Myb-like protein and involved in regulation of transcription
- 1, 240 base pairs
- Thousands of other blast hits to bacteria, viruses, eukaryotes: maybe very important?
- If the suspensor doesn't work in the globular stage, the embryo proper cannot develop, so maybe look for seeds that didn't pass the Pre-Globular stage
- Alternatively, suspensor is not affected severely and seeds all develop normally

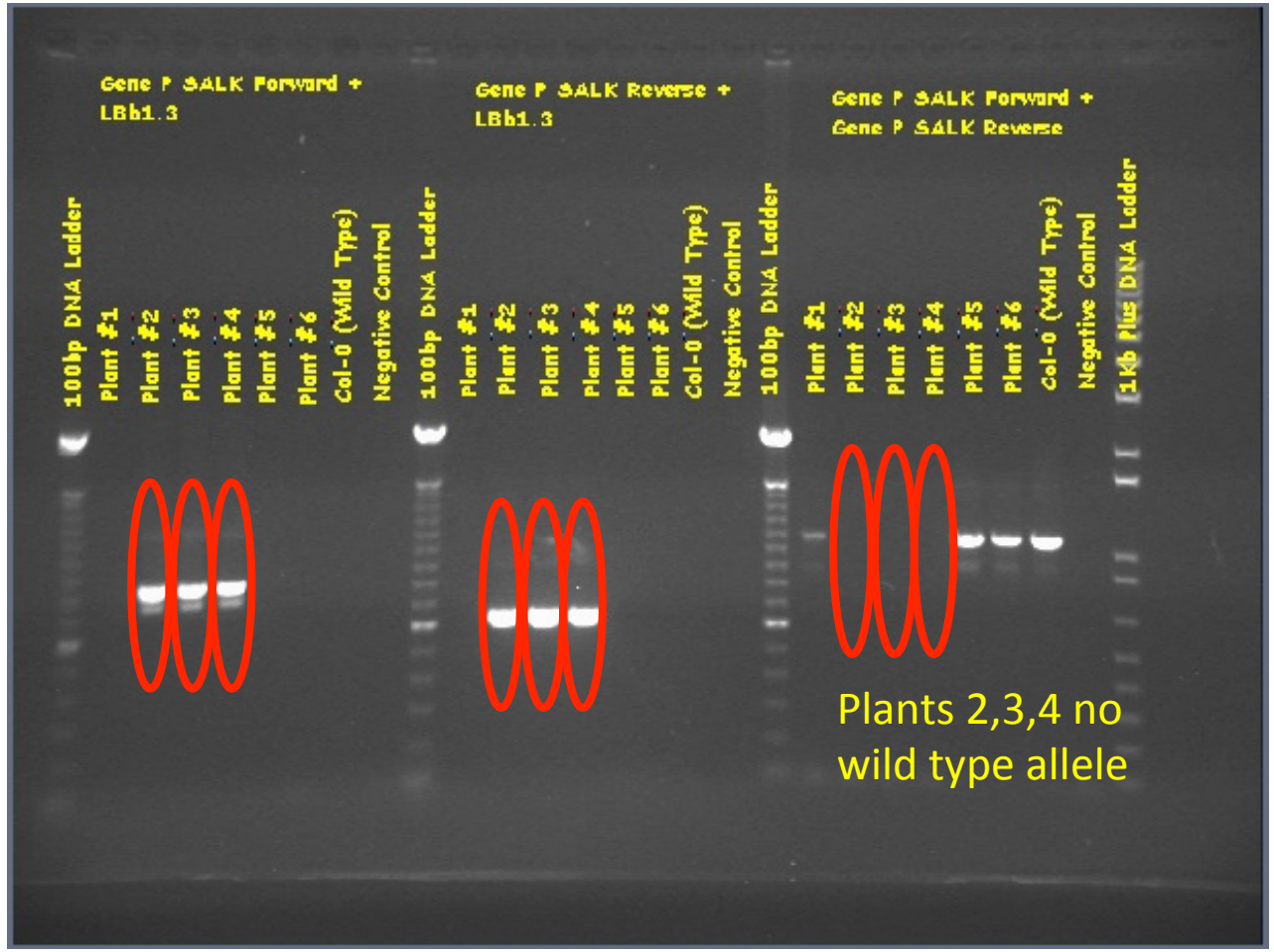
Gradient PCR To Test LB3

- If there were a tDNA insert, I would expect a 1,000 bp product with the Reverse and LB3 primer



Gene 2 “P” : AT5G58850

- Plants 2, 3, 4 homozygous for tDNA insert
- Sequencing reaction and Tair analysis



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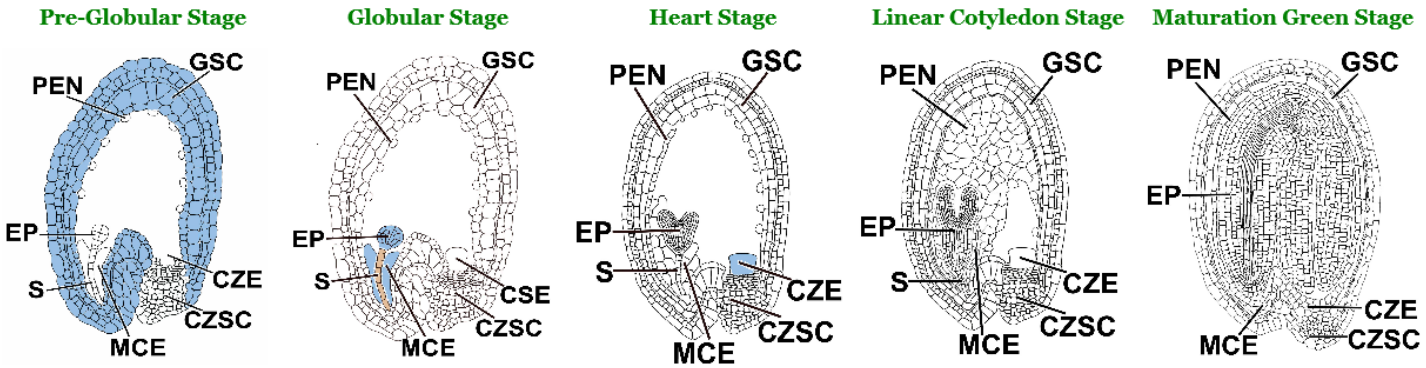

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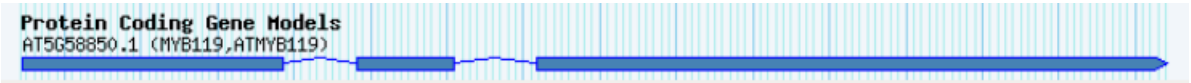
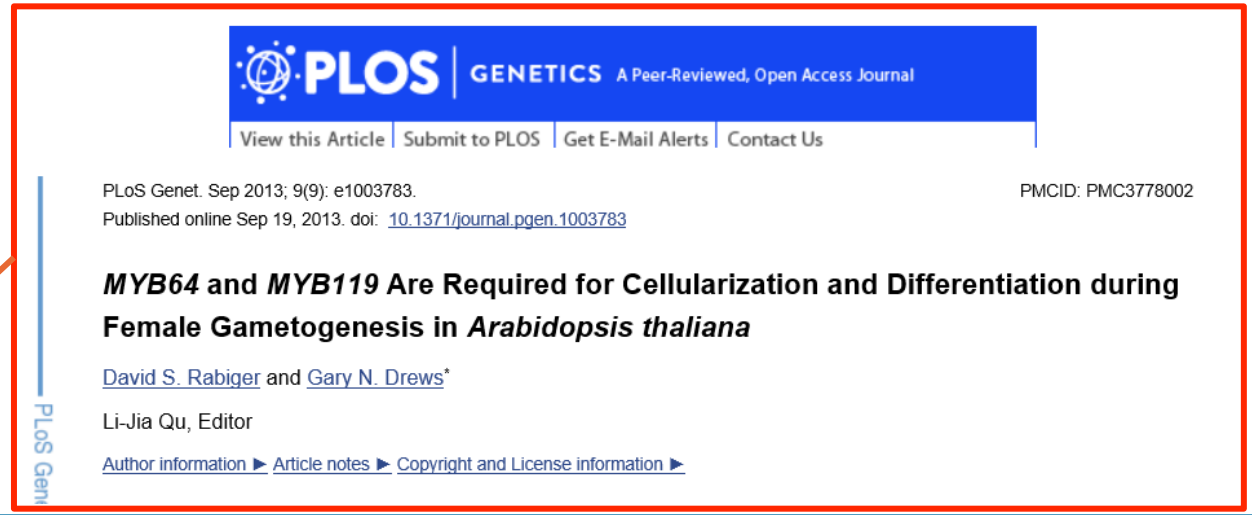
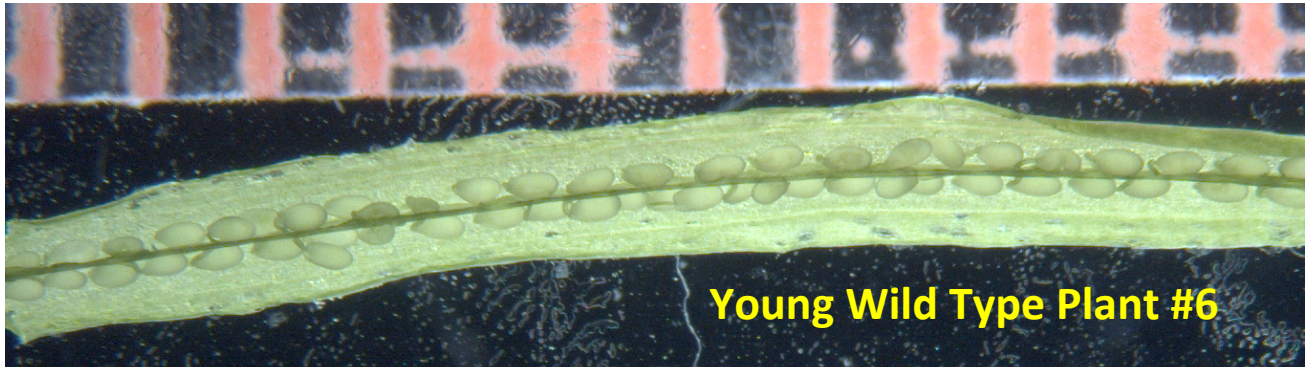


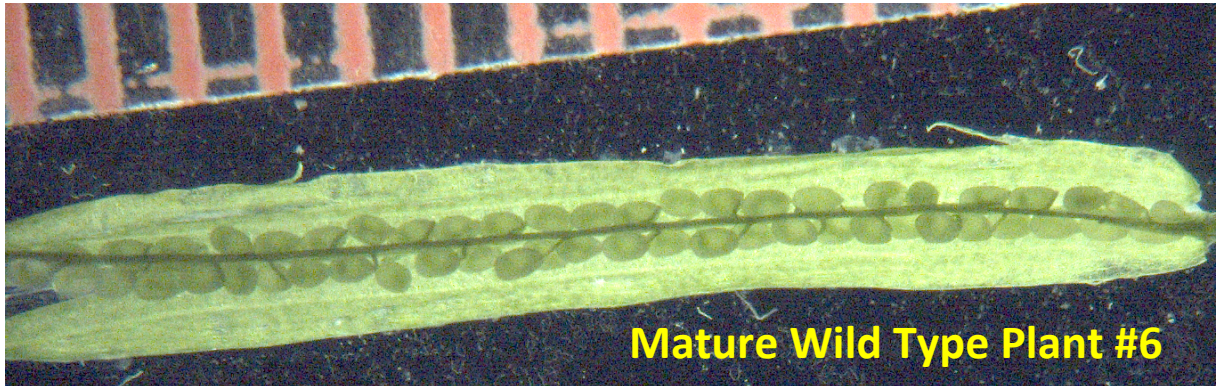
- Protein MYB119: "Encodes a putative transcription factor, member of the R2R3 factor gene family (MYB119)."
- 1, 496 base pairs
- Expect mutations only in suspensor
- Recent study suggests similar amino acid sequence between MYB119 and MYB64, and mutation only occurs when both are knocked out



Phenotype Analysis AT5G58850: Light Microscopy

- As expected, seeds developing normally



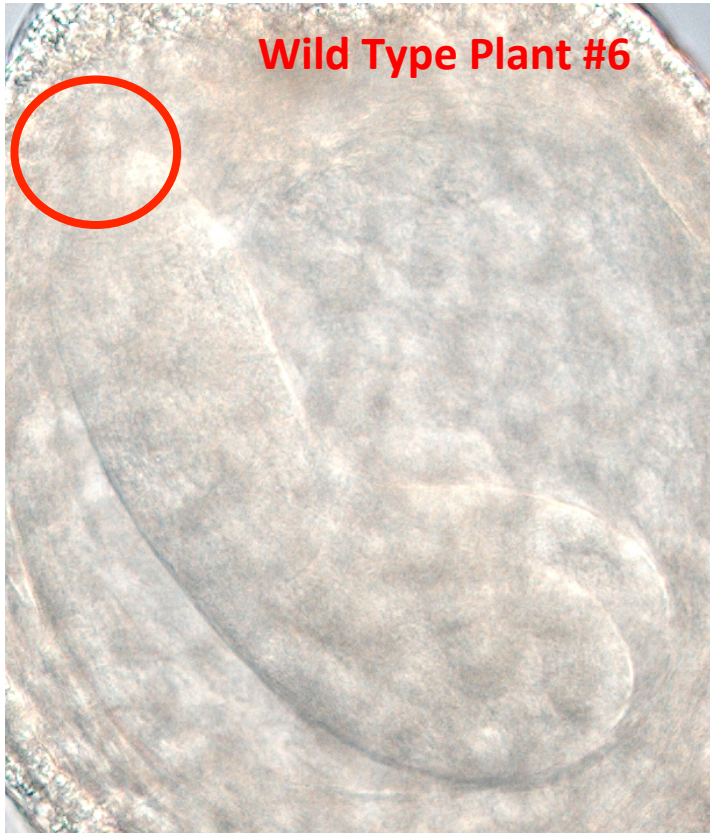


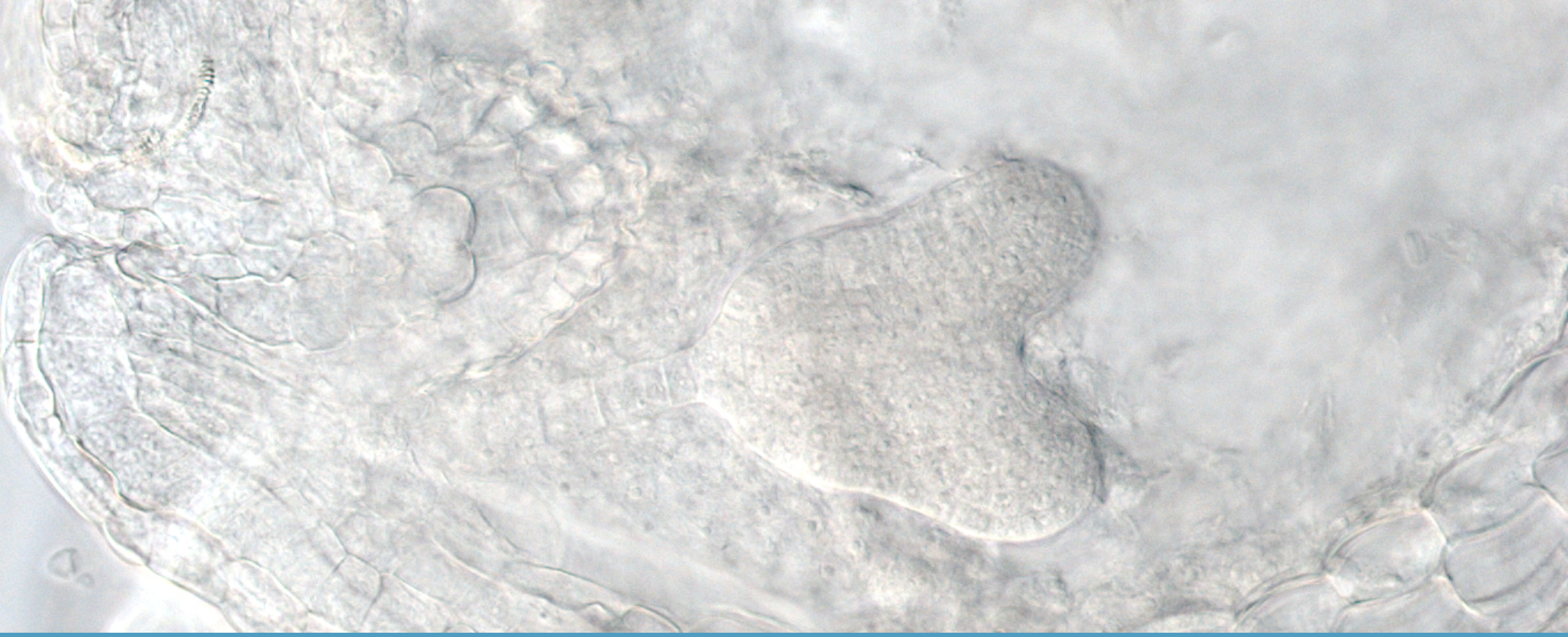
Phenotype Analysis AT5G58850: Nomarski

Homozygous Mutants Plant #3: Normal Suspensor Development

Wild Type Mutants Plant #6: Normal Suspensor Development

Phenotype Analysis AT5G58850: Nomarski





Thank you for an amazing 6 weeks!