

# HC 70AL Solo Presentation

Will Wolf

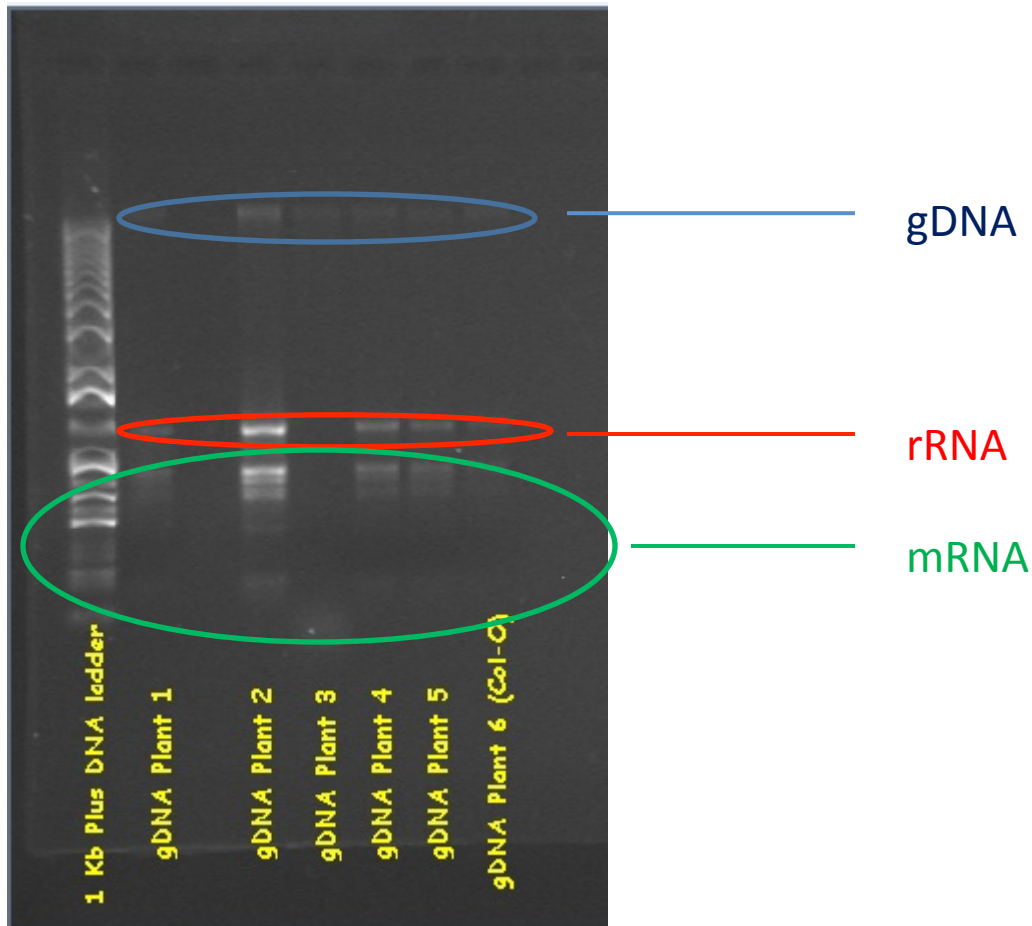
# AT3G60740

- Also known as TTN 1 (TITAN 1).
- Partially responsible for tubulin production and therefore integral to cellular division.
- Its inhibition is generally considered to be fatal.

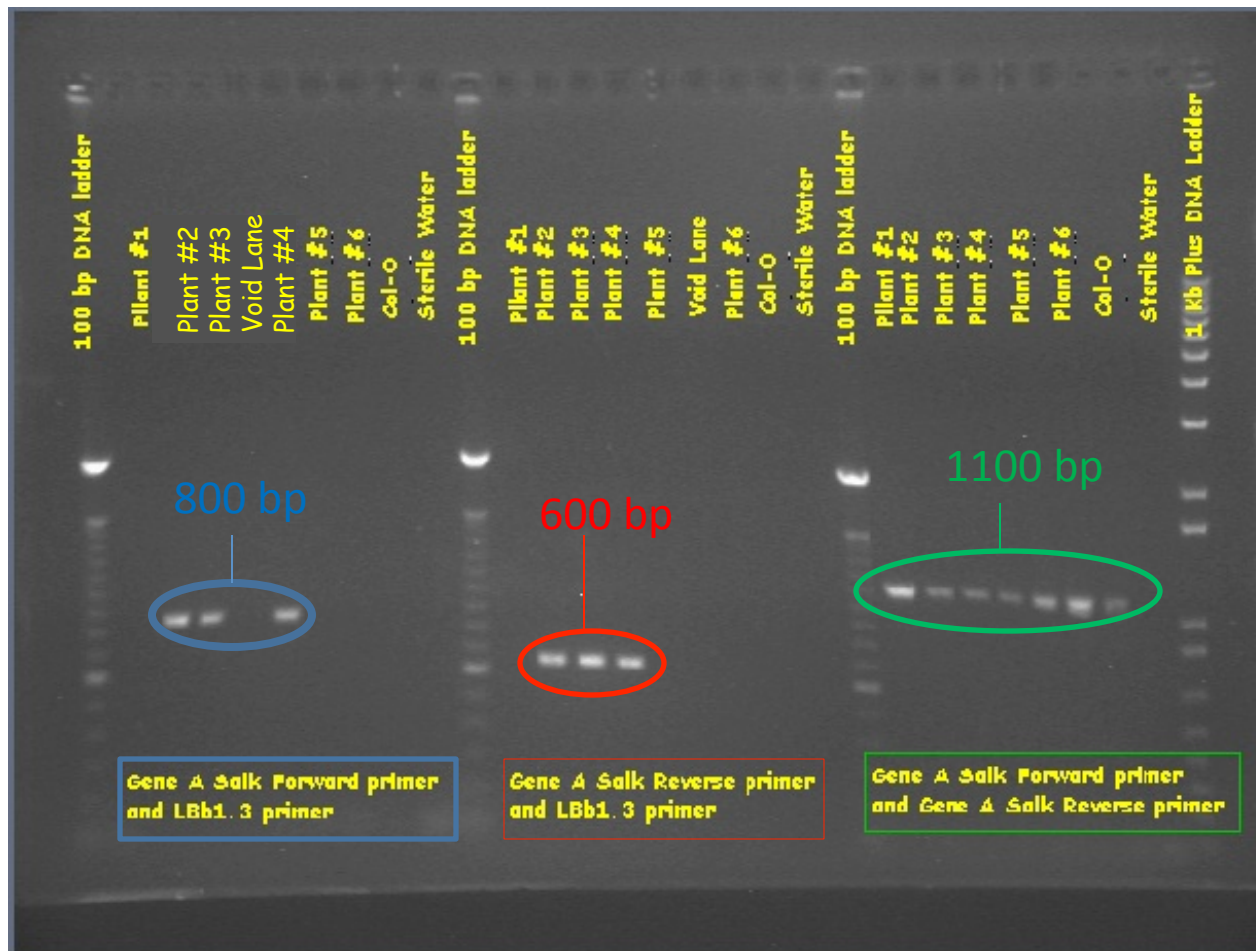
# Experiment Overview

- *Arabidopsis Thaliana* is modified via a T-DNA insert from *Agrobacterium*.
- gDNA is extracted and checked for quality and concentration.
- PCR products produced from gene and T-DNA specific primers are used to determine the presence of mutants.
- The mutant gene is sequenced to confirm T-DNA insertion accuracy.
- The phenotype of mutant plants are visually observed after they reach sexual maturity.

# Testing the Quality of gDNA



# Screening for Mutants

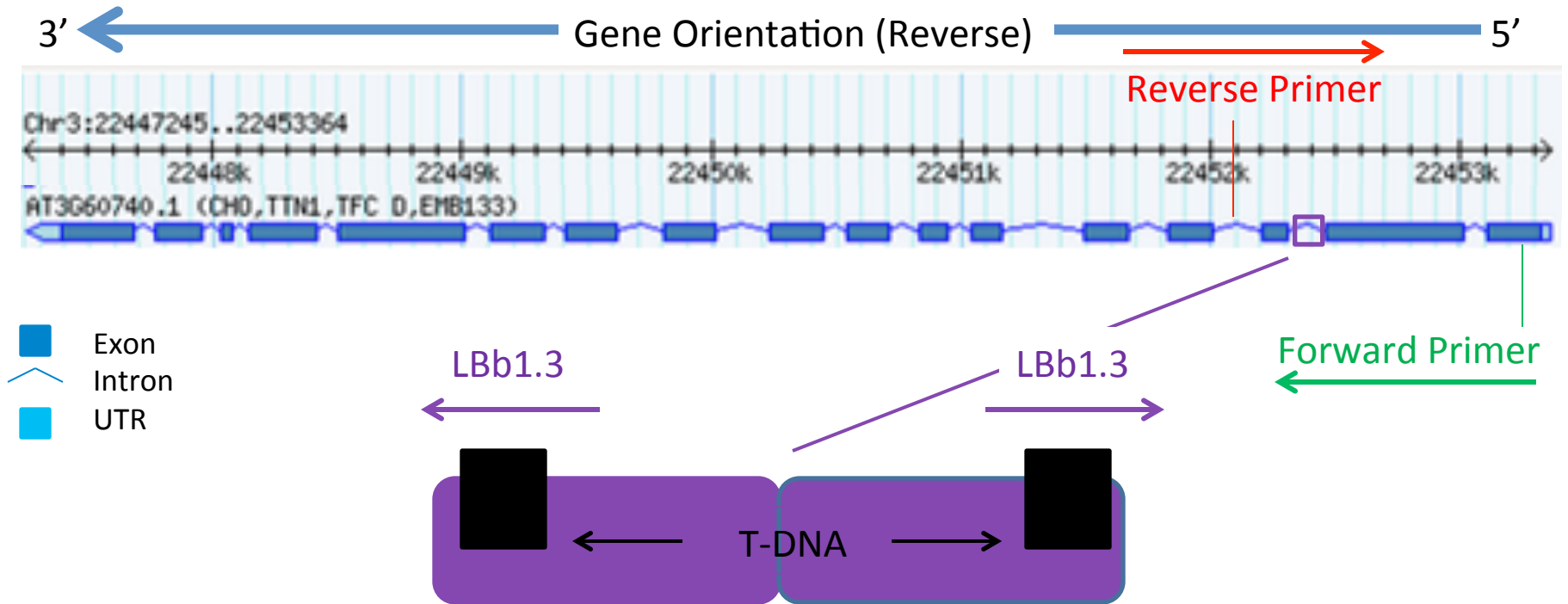


Plants 2,3, and 4 produce bands with both **Forward + LBb1.3** and **Reverse + LBb1.3** primers.

In addition **ALL** plants produce bands with **Forward + Reverse** primers

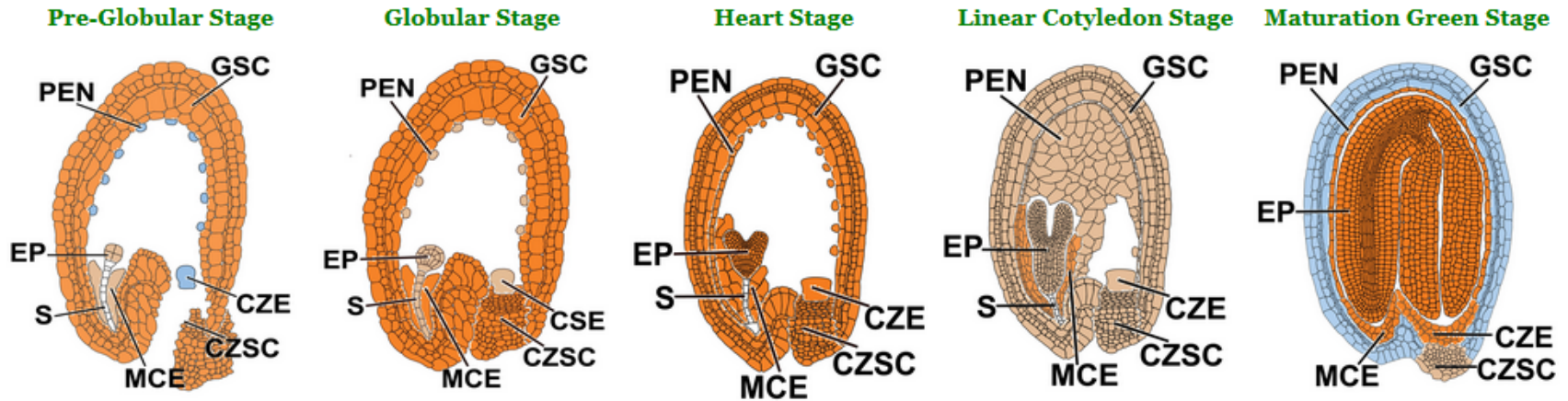
This suggests that Plants 2,3, and 4 are **heterozygous** mutants with a T-DNA insert that is both **forward** and **reverse oriented!**

# Gene Structure



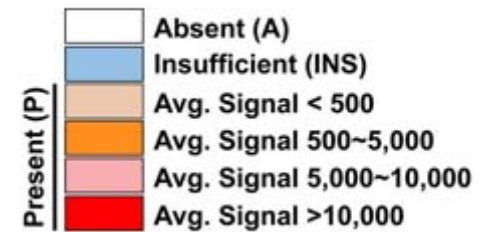
T-DNA inserted at nucleotide 22452525, 32 bp away from expected location of 22452590

# TTN 1 Expression Patterns



Tissue: CZE - Chalazal Endosperm; CZSC - Chalazal Seed Coat; EP - Embryo Proper; GSC - General Seed Coat; MCE - Micropylar Endosperm; PEN - Peripheral Endosperm; S - Suspensor

TTN 1 is known to be expressed in most seed tissues during all phases of growth, so where can we expect to see a mutant phenotype?



TTN 1 is important in tubulin production, a protein vital to cell division.

The embryo is one of the first organs to see cell division, so we will look there first for a phenotype!

# Effects of TTN 1 Inhibition

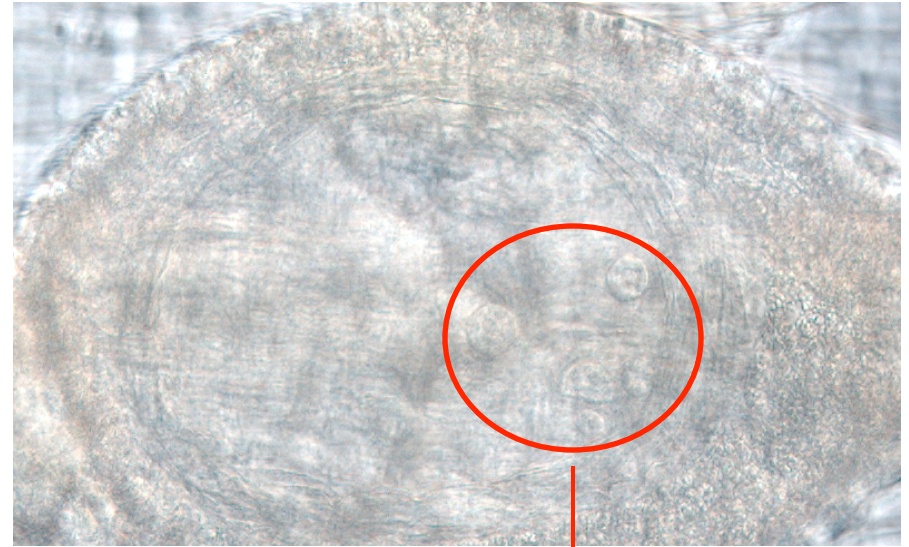
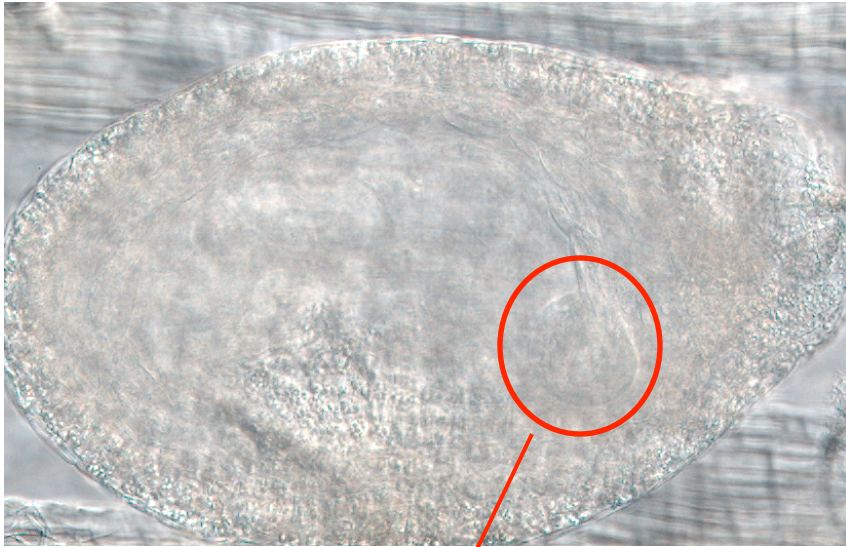


Mutant seeds observed in a roughly 3:1 ratio as expected.

TTN deficient seeds appear white and disfigured above.



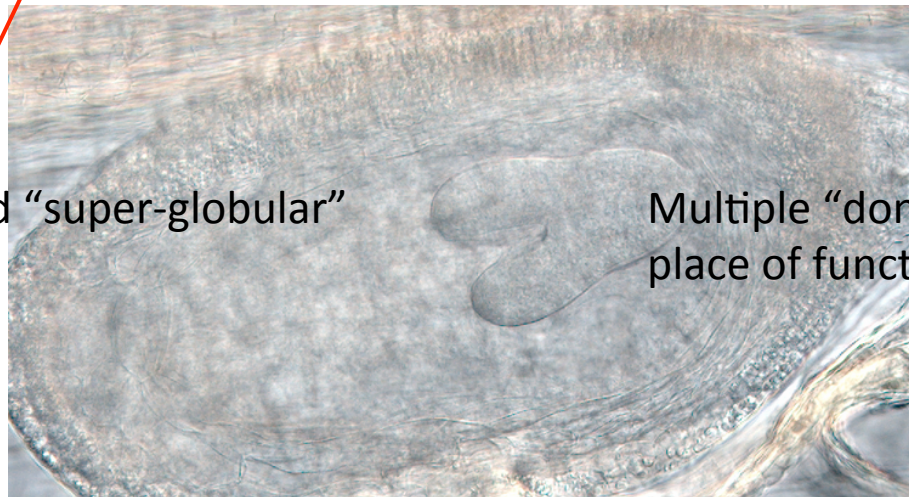
# Effects of TTN 1 Inhibition



Mutants above contrasted with normal seed below from the same heterozygous silique

Undifferentiated "super-globular" embryo

Multiple "donut-like" objects in place of functional embryo



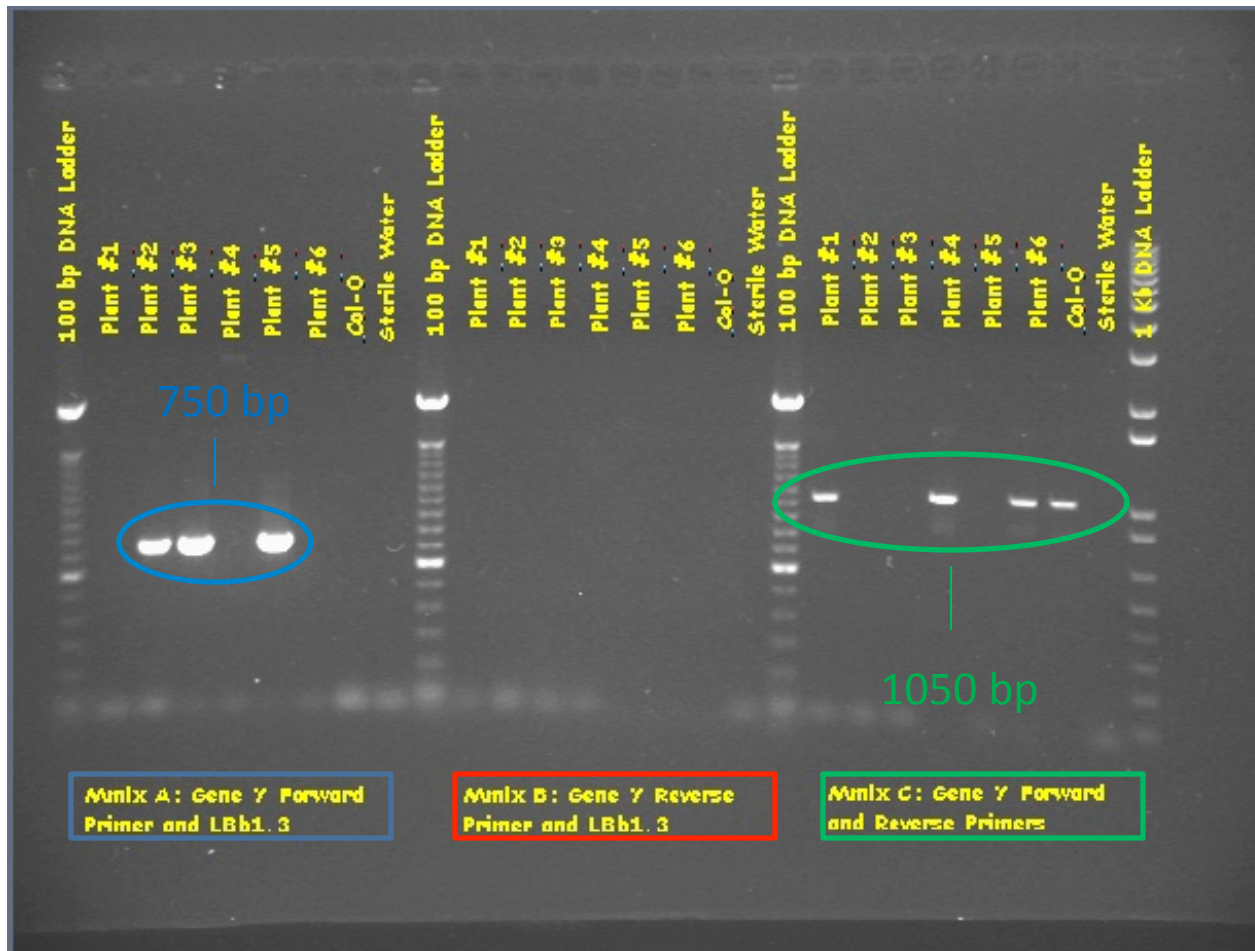
# AT2G33710

- One of at least 7-members of the ERF B-4 subfamily of ERF/AP2 transcription factors
- Functions known to be some-what redundant
- Expressed almost exclusively in the micropylar and chalazal endosperm

# Experiment Overview

- *Arabidopsis Thaliana* is modified via a T-DNA insert from *Agrobacterium*.
- gDNA is extracted and checked for quality and concentration.
- PCR products produced from gene and T-DNA specific primers are used to determine the presence of mutants.
- The mutant gene is sequenced to confirm T-DNA insertion accuracy.
- The phenotype of mutant plants are visually observed after they reach sexual maturity.

# Screening for Mutants



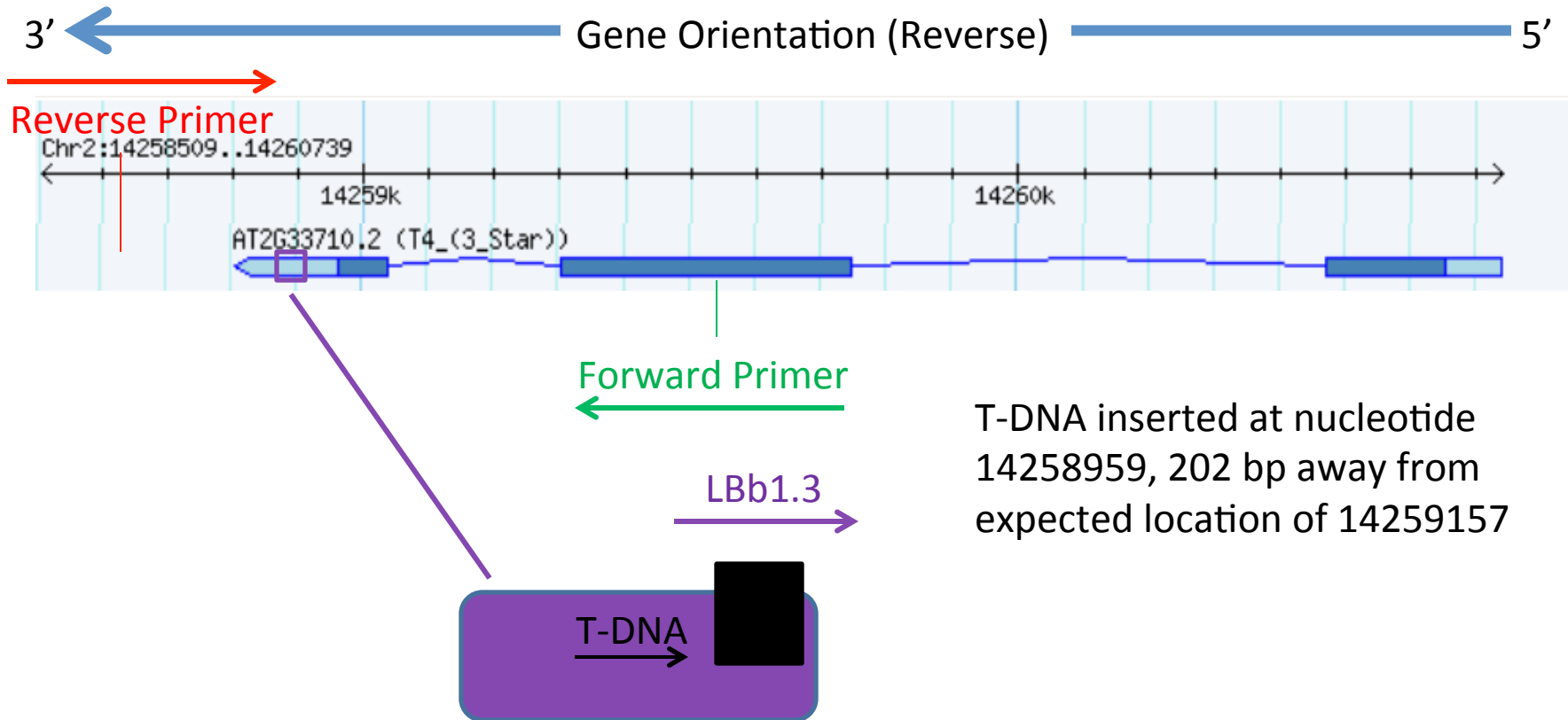
Plants 2,3, and 5 produce **thick** bands with **Forward + LBb 1.3** primers.

No bands are produced by **Reverse + LBb1.3** primers.

Only Plants 2,3, and 5 **DO NOT** produce bands with **Forward + Reverse** primers

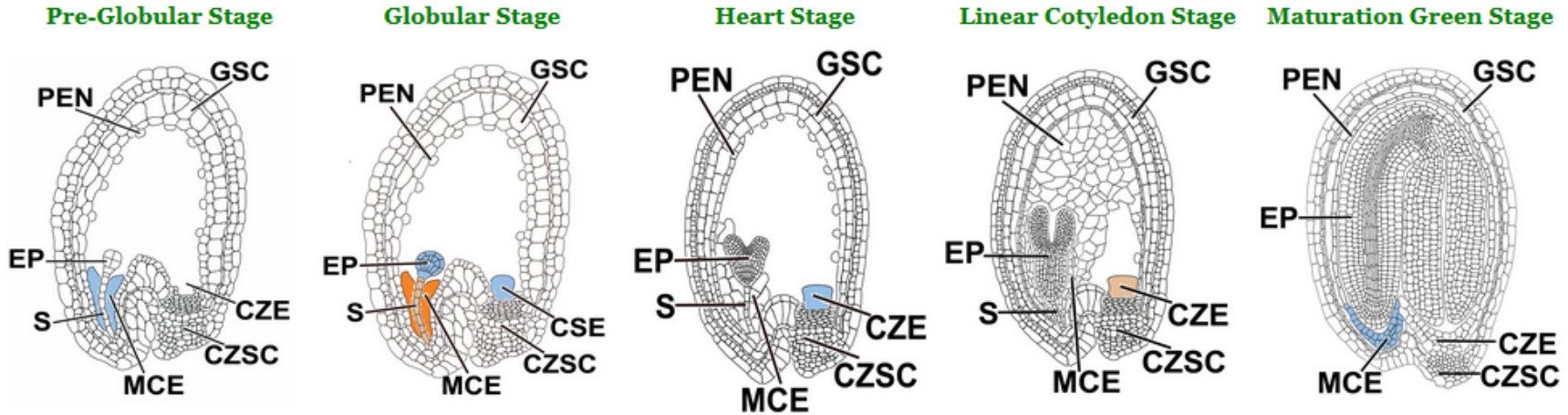
The presence of thick bands with **Mmix A**, absence of any bands in **Mmix B**, and absence of Plants 2,3, and 5 in **Mmix C** suggest that Plants 2,3, and 5 are **homozygous** mutants!

# Gene Structure





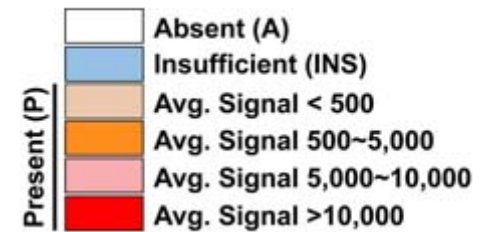
# AT2G33710 Expression Patterns



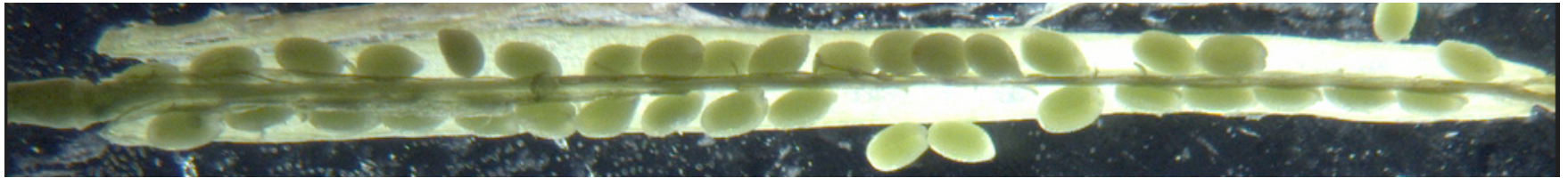
Tissue: CZE - Chalazal Endosperm; CZSC - Chalazal Seed Coat; EP - Embryo Proper; GSC - General Seed Coat; MCE - Micropylar Endosperm; PEN - Peripheral Endosperm; S - Suspensor

Gene is primarily expressed in the micropylar and chalazal endosperm.

This is where I would focus my observation later in the experiment.

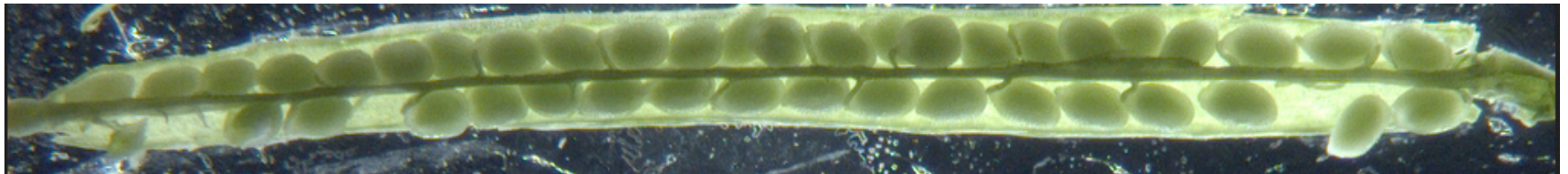


# Effects of AT2G33710 Inhibition



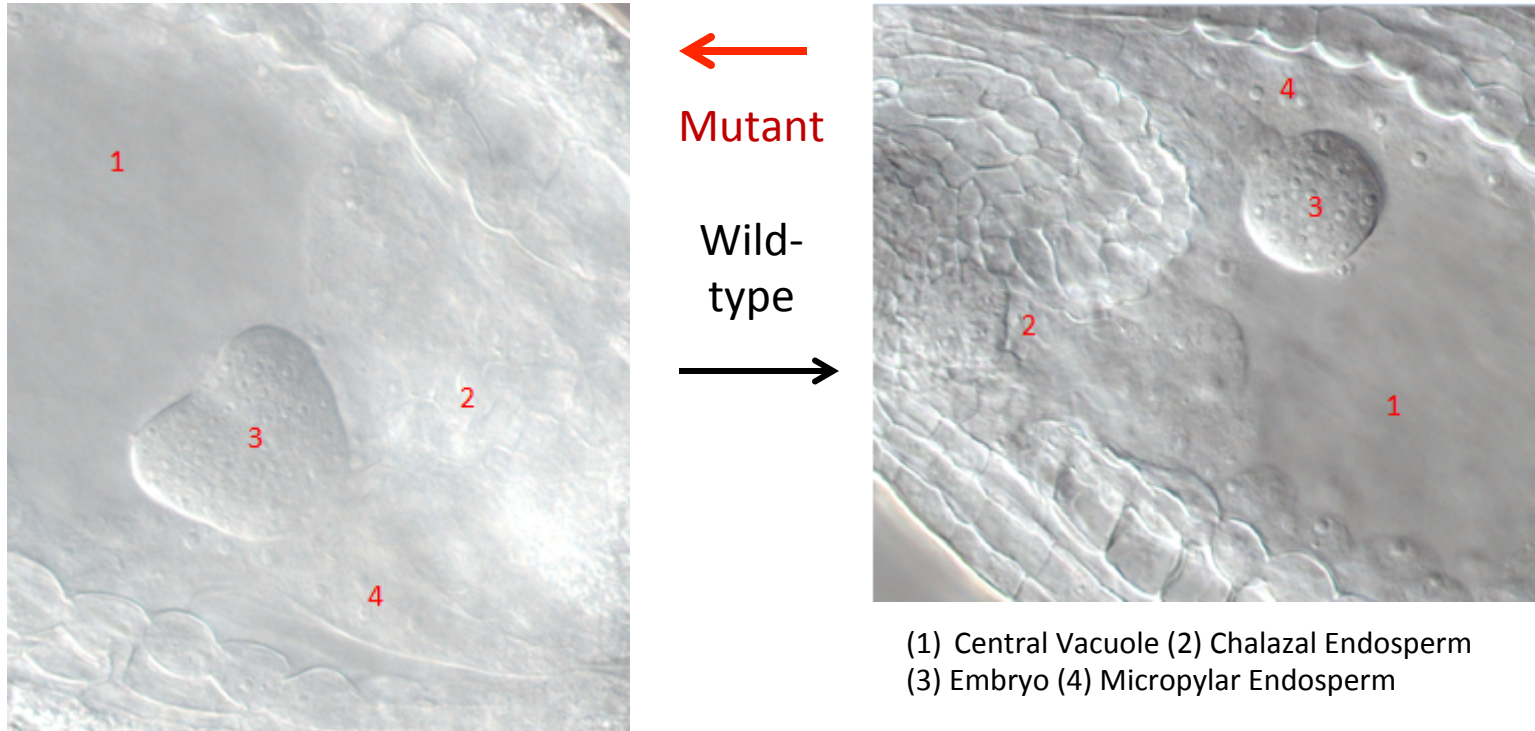
Mutant

Wild-Type



Under light microscopy there is no discernible difference between mutant and wild-type

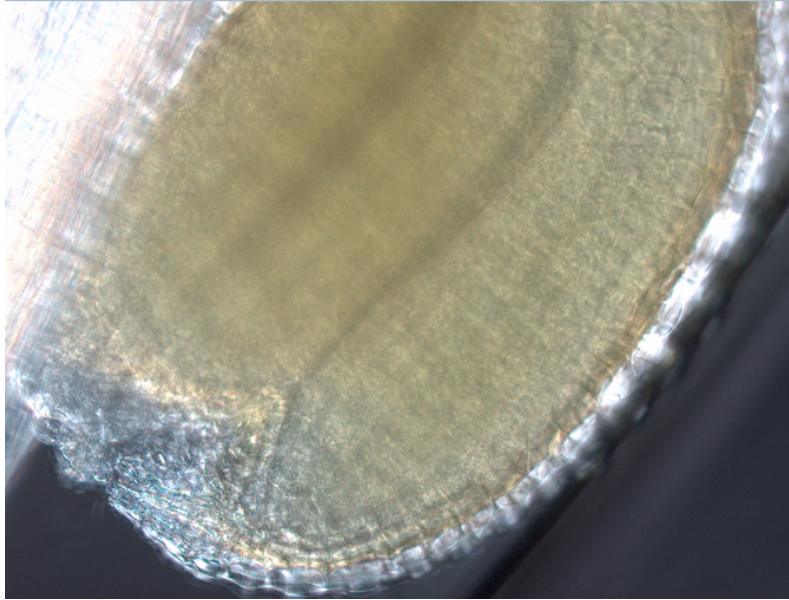
# Effects of AT2G33710 Inhibition at Early Stages of Development



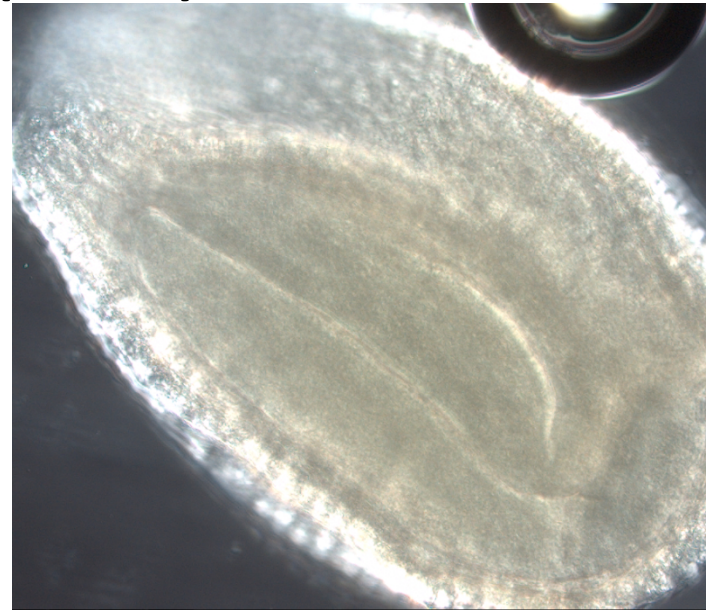
No discernible difference between mutant and wild-type seeds observed at the globular/heart stage



# Effects of AT2G33710 Inhibition at Mature Green (MG) Phase



Mutant



Wild-Type

Seed development appears normal in both mutant and wild-type seeds at MG phase

# So What's Next?

- Knock out of AT2G33710 yields no phenotype despite the homozygous nature of the mutation.
- Since AT2G33710 is part of a family with known redundancy, more members of its immediate ETF B-4 subfamily must be knocked out simultaneously to potentially generate a mutant phenotype.

# Thank You!

Special thanks to Mike and Kelli for putting up with all our shenanigans and preparing solutions that actually work!