Characterization of Gene Expression in Transgenic Plants with Modified Floral Gene Expression

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Oregon State University Undergraduate Summer Program

Why Poplar and Sweetgum?

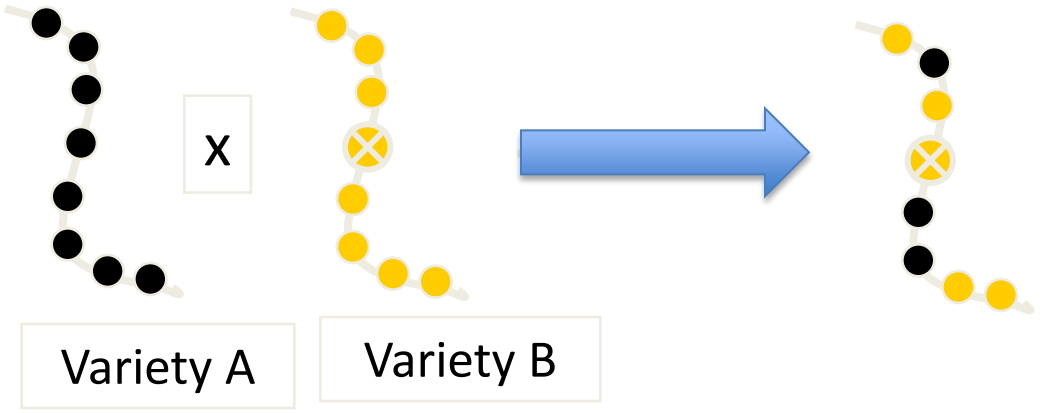
- Poplar trees
  - Wood
  - Paper
  - Biofuel source
- Sweetgum
  - Timber
    - Southeast US
  - Ornamental
    - Northwest US

Transgenic Plants

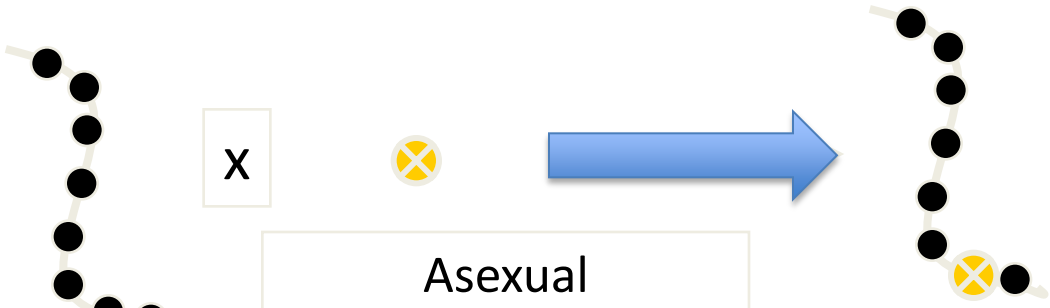
- Genetically modified through the use of recombinant DNA techniques
- Insertion of advantageous genes or modification of existing genes
- Increases diversity of plant characteristics available to plant breeders

Genetic Engineering Defined

Traditional Plant Breeding



Genetic Engineering



Containment

- Issue: the dispersal of transgenic material in the environment
- Option: produce trees that cannot produce viable pollen or seed
- Question: is developing an efficient, consistent method to create sterile trees possible?

Overview

- Sweetgum
  - Development of basic methods
- Poplar
  - Analysis of gene expression

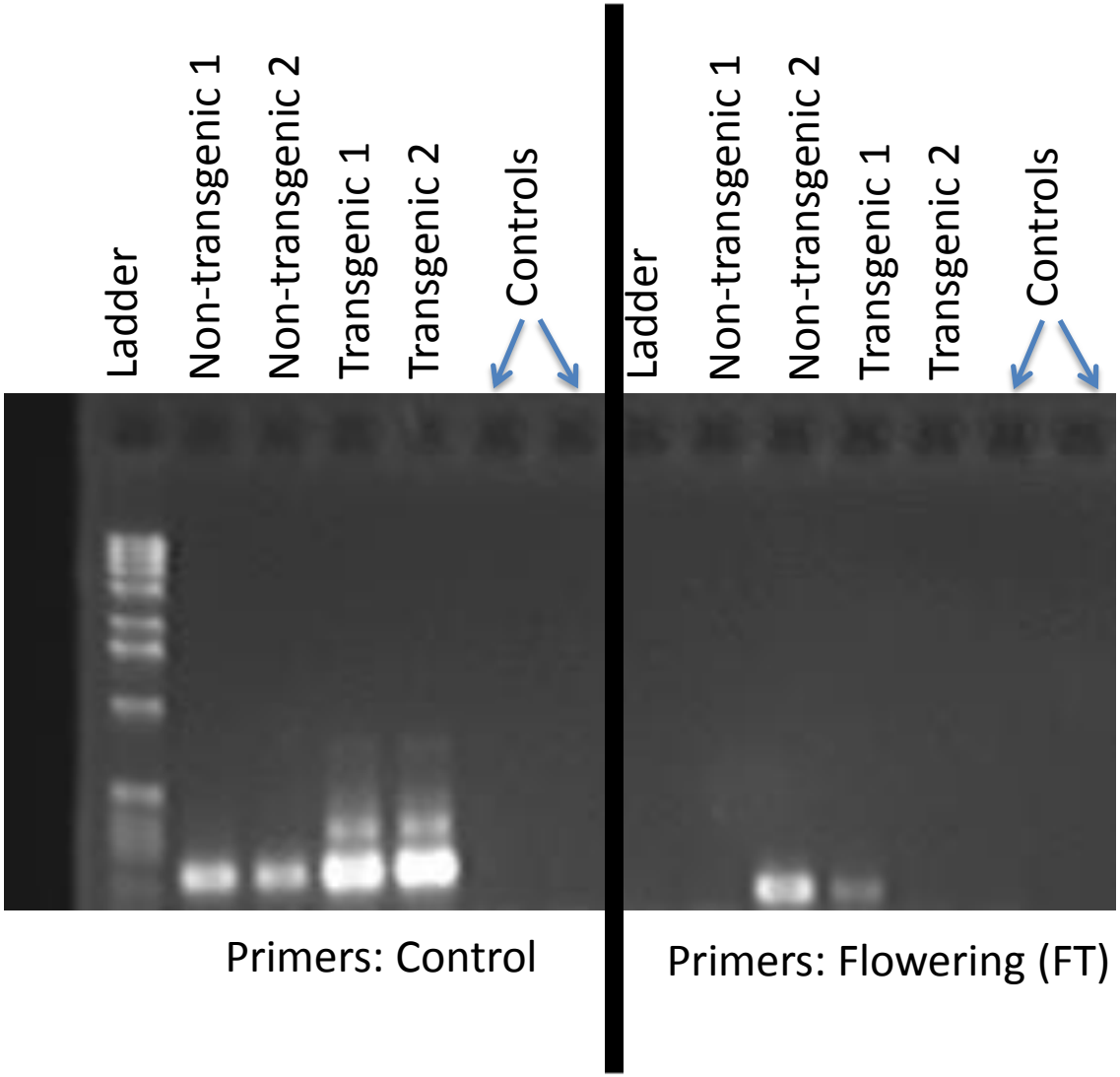
RNA Interference (RNAi)

- New technology
  - Nobel Prize in 2006
- Turns off specific genes
  - Allows one to target the genes that responsible for forming flowers
- RNAi breaks down the molecules that make flower proteins

General Procedure

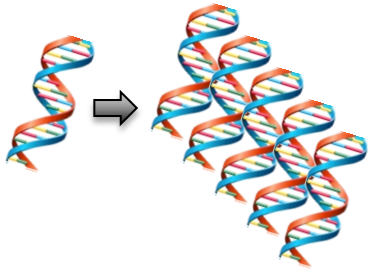
- RNA extraction
  - Qiagen RNeasy kit
- Complementary DNA (cDNA) synthesis
  - Reverse transcription
- Polymerase chain reaction (PCR) using gene-specific primers
- Gel electrophoresis to determine expression of inserted genes

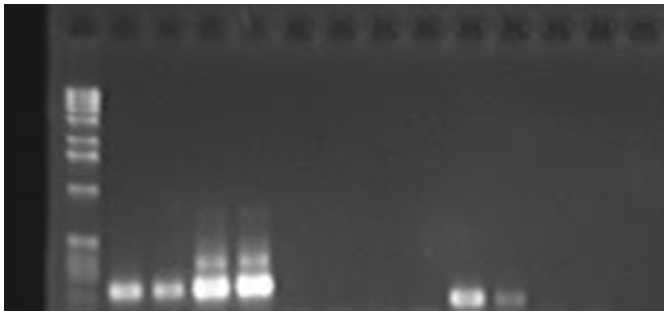
Gel electrophoresis



New Techniques

- gDNA Extraction
- RNA Extraction
- Spectrophotometry
- PCR with Gene-Specific Primers
- Gel Electrophoresis





Sweetgum Project Overview

- Identify trees where RNAi is effective
  - RNAi is used to turn off the flowering gene AGAMOUS
  - Analyze leaf tissues to determine gene expression

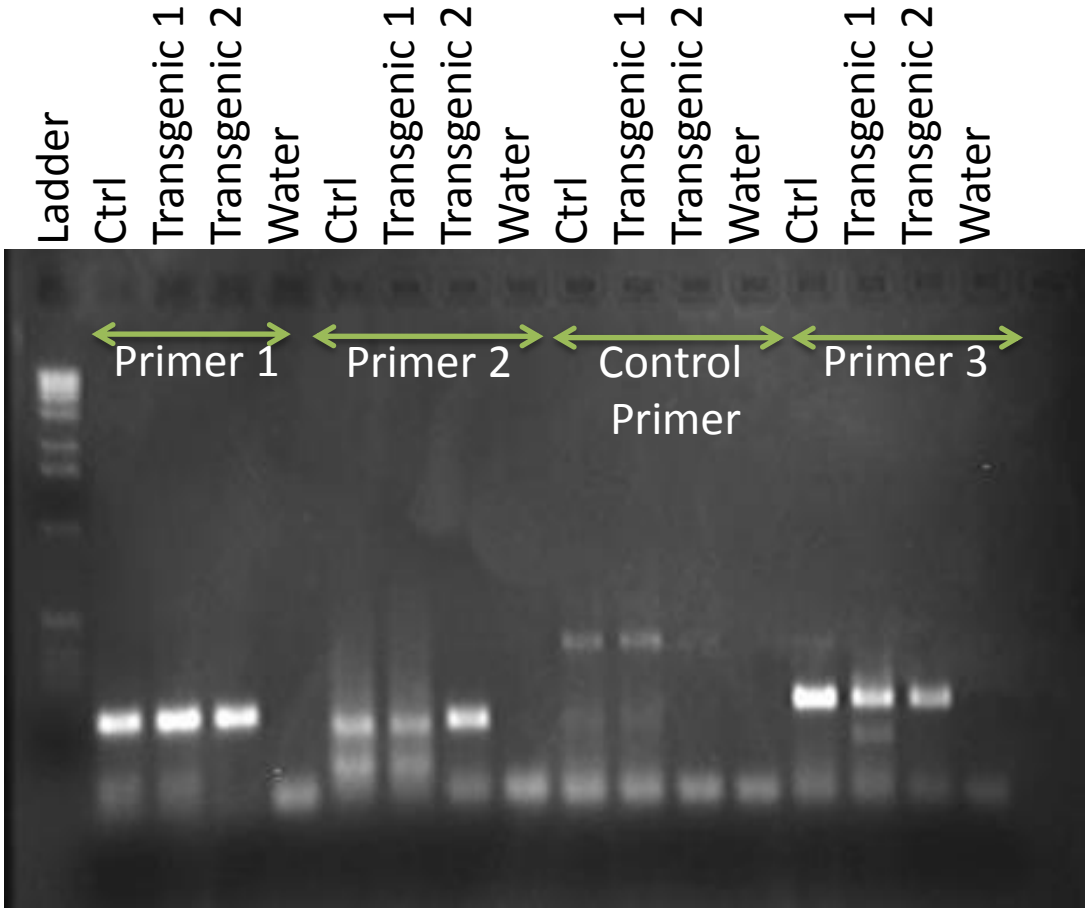
Sweetgum RNA Extraction

- Attempted to extract RNA using the BioRad sample kit
  - No bands present for gel electrophoresis
  - Minimal spectrophotometry nucleic acid levels
- Other RNA Extraction Methods
  - RNeasy kit
  - Zymogen kit
  - CTAB method (standard lab protocol)
    - All the above methods gave a low RNA yield
  - Modified CTAB method
    - Successful!

Sweetgum Primer Testing

- Tested primers for sweetgum gDNA samples
  - Control and transgenic samples
  - Primers specific to control and sterility genes

Gel Electrophoresis Results



Next Steps

- We now have an effective RNA extraction method, as well as primers
- The sweetgum samples have been collected from the field for further testing

Poplar Project Overview

- The lab uses RNAi to turn off 2 genes
  - *LEAFY* and *AGAMOUS*
  - Both genes contribute to flower formation

- The gene *HSP:FT* is added to help us study the effects of RNAi
  - The *Heat Shock Promotor* (*HSP*) gene allows us to turn on *FT* by turning up the heat
  - *FLOWERING LOCUS T (FT)* causes plants to flower early
  - Flowering would take years without HSP:FT

HSP:FT Poplar Trees during Heat Induction

Poplar Project Overview

- Determine the levels of *FLOWERING LOCUS T* (FT) gene required to induce flowering

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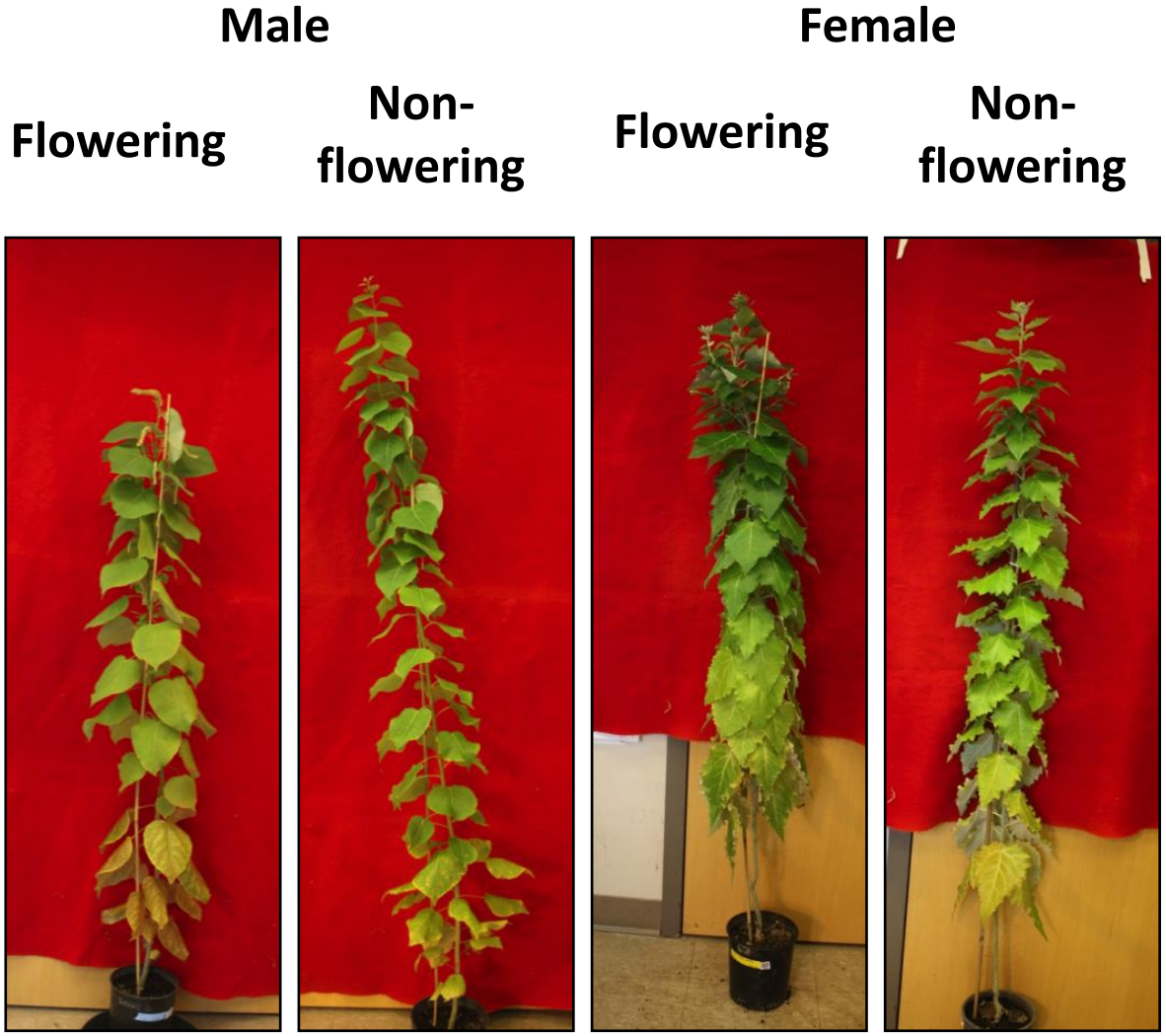
- Determine the levels of *FLOWERING LOCUS T* (FT) gene required to induce flowering

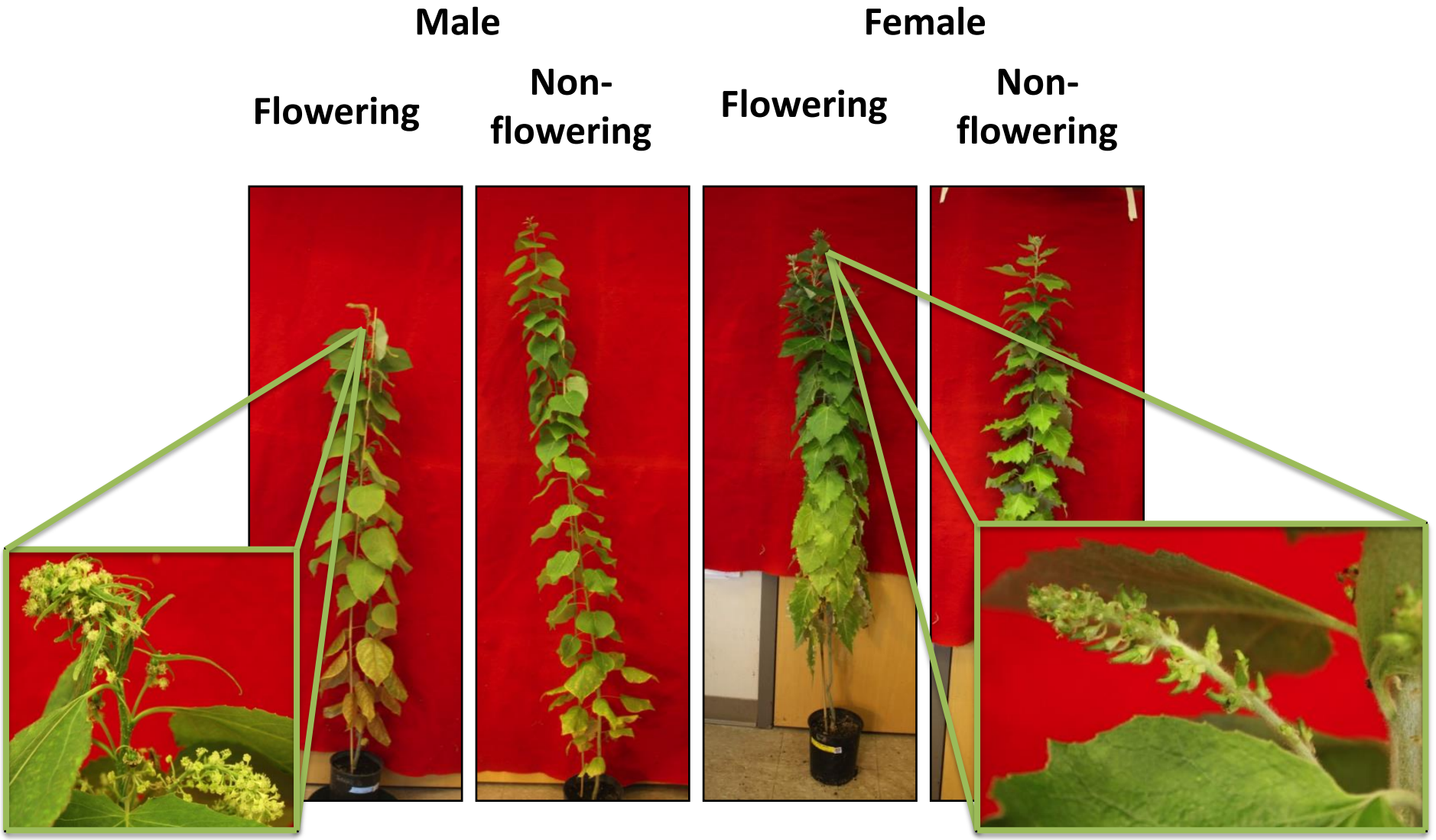
Male Flower

Female Flower

Male and Female Flowering and Non-flowering Poplars

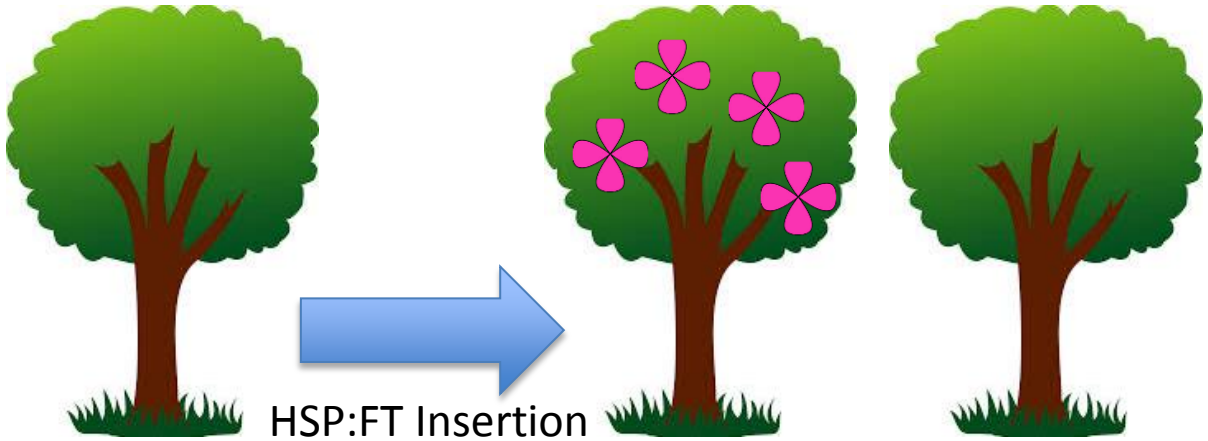


Male and Female Flowering and Non-flowering Poplars



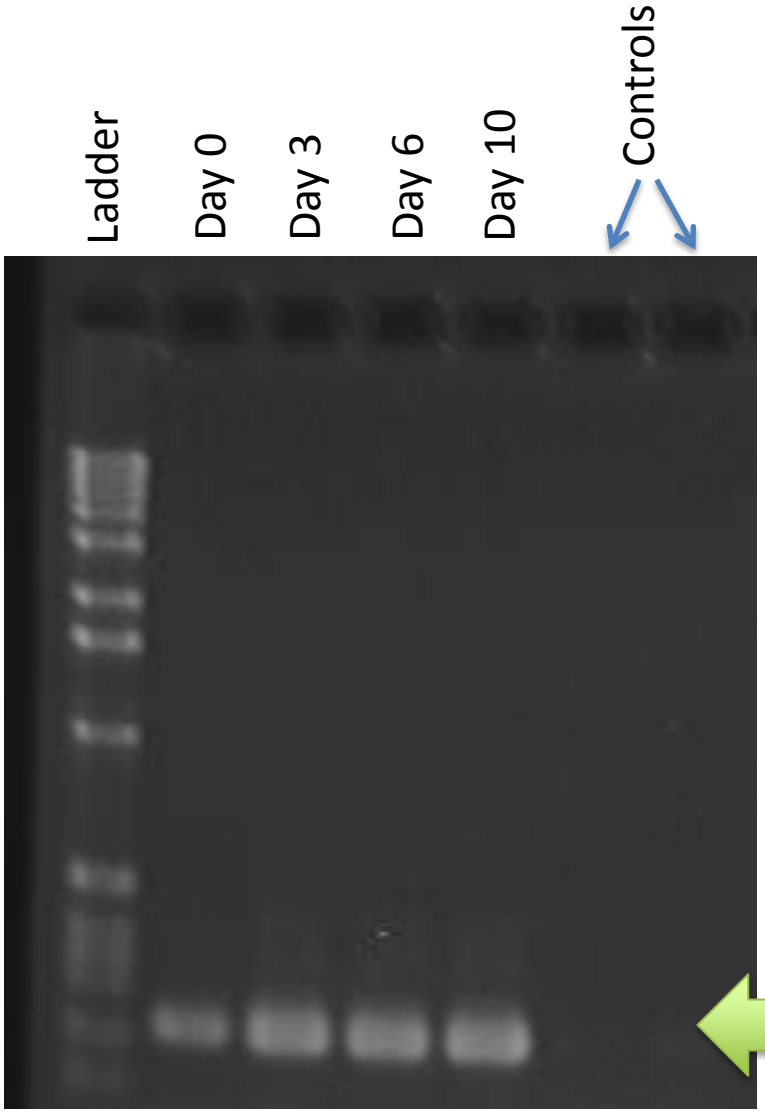
FT Threshold Study

- Not all plants with HSP:FT flower
  - Need plants that reliably make flowers so we will know when RNAi works
- Goal: determine if there is a threshold of FT needed for flowering
- 3 categories of sample



Poplar Non-HSP:FT Control

Over 10 Days of Heat Induction



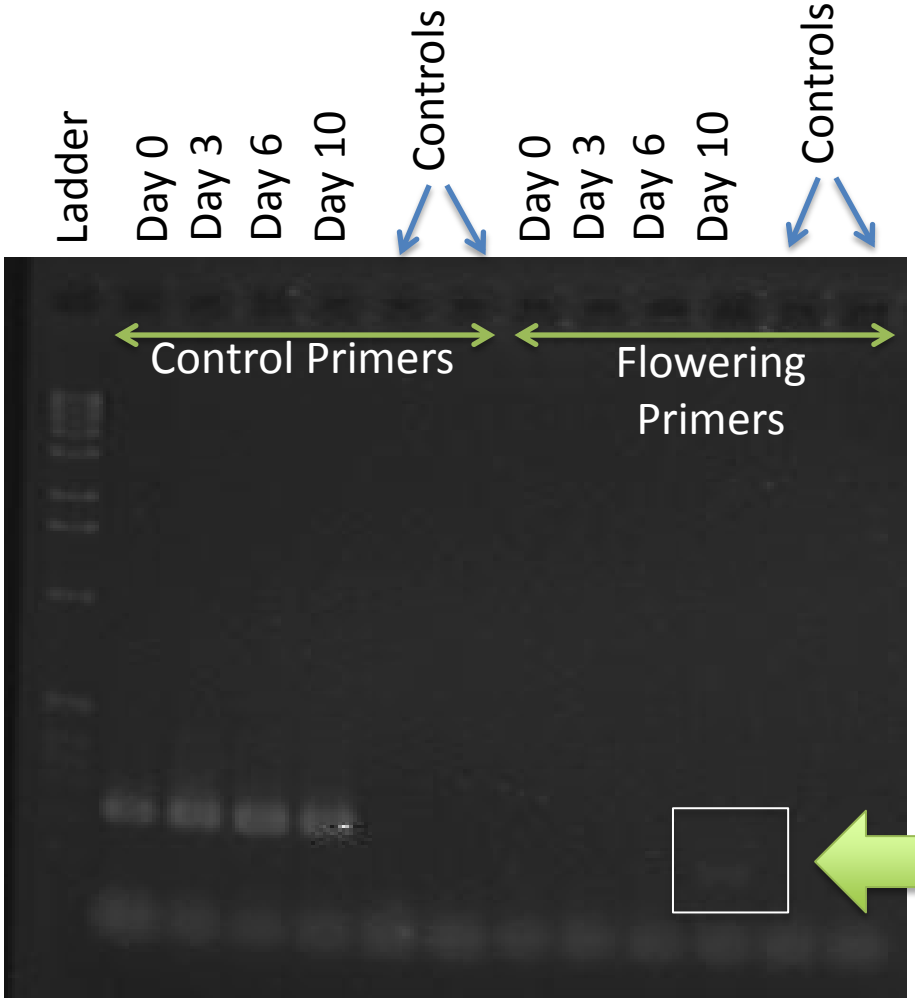
Control primers used

Control bands present

Poplar Flowering Group

HSP:FT Insertion

Over 10 Days of Heat Induction

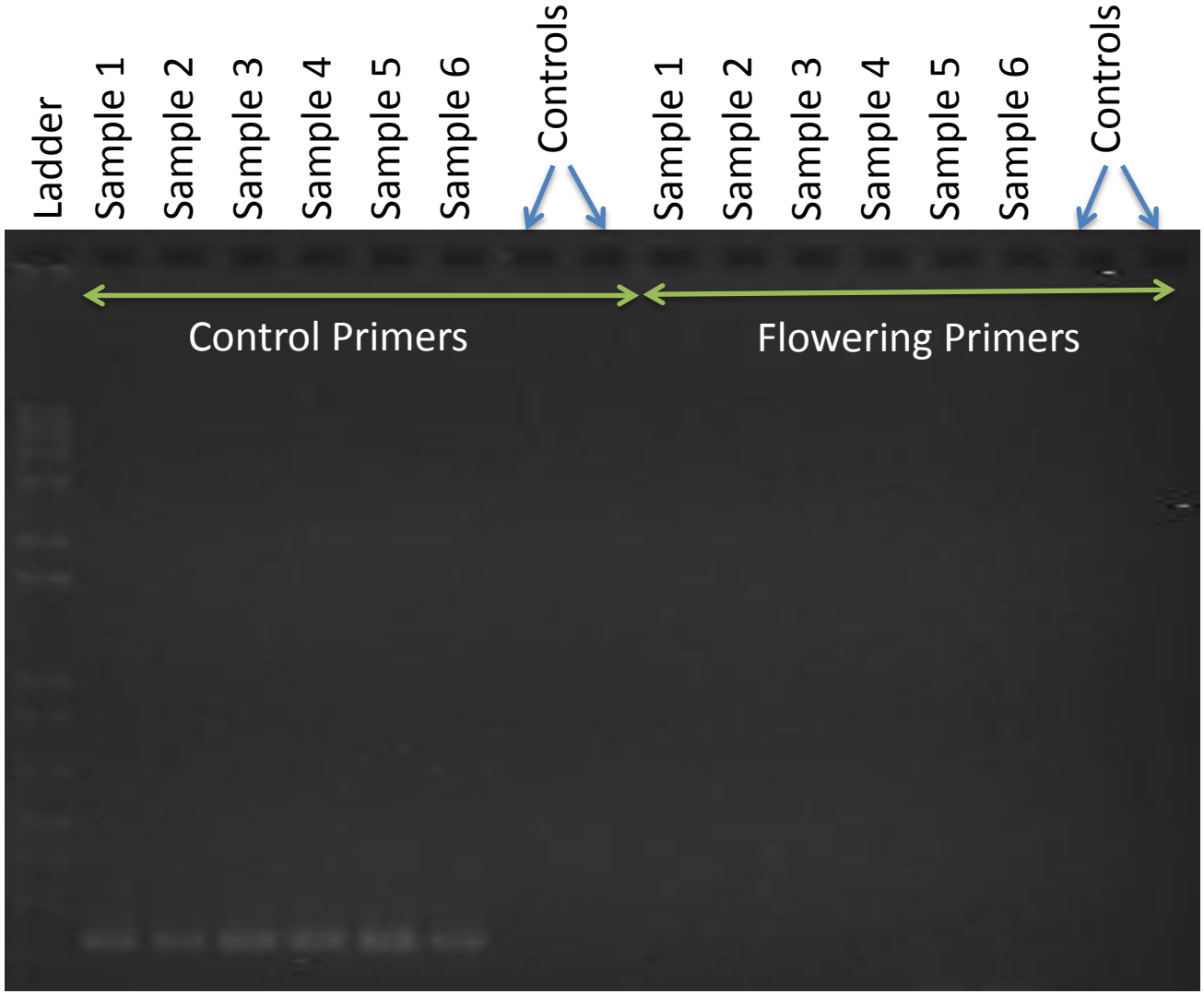


Faint band for Day 10 sample (Flowering primer)

Poplar Non-Flowering Group

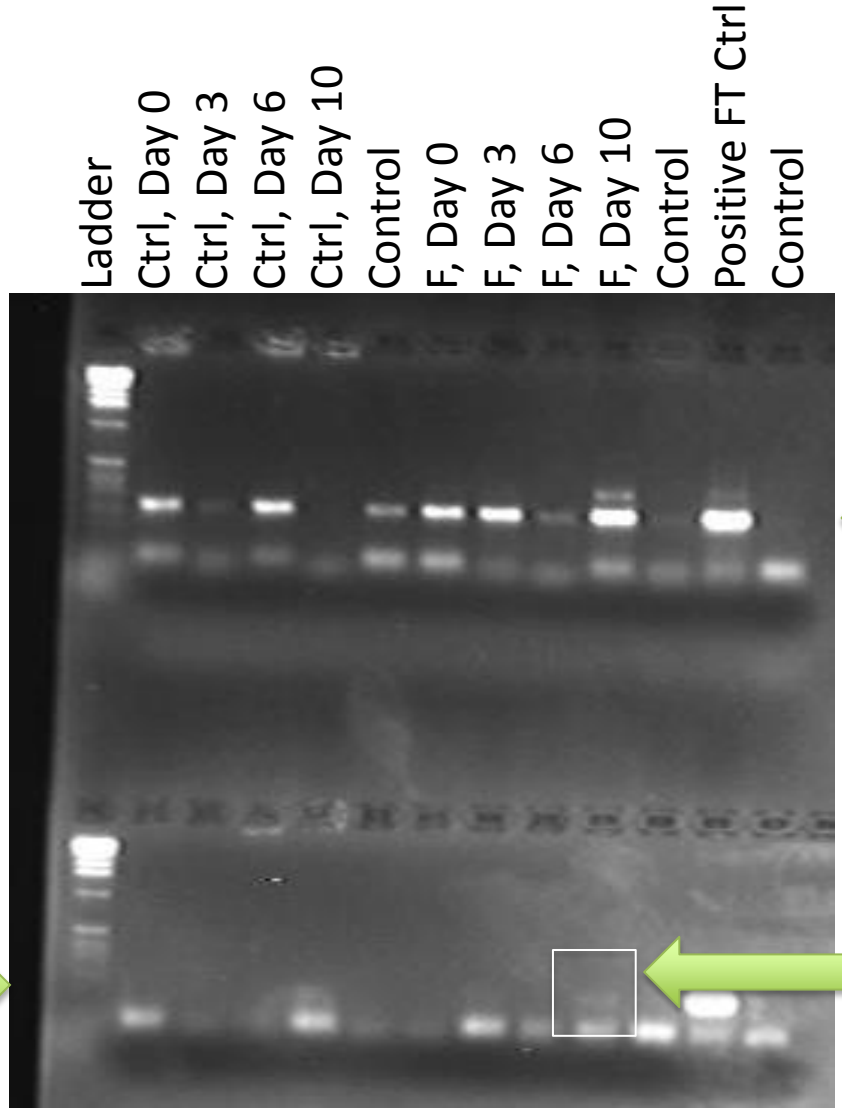
HSP:FT Insertion

Samples Taken After 10 Days of Heat Induction



Non-HSP:FT Control and Flowering Group Subsets

Positive Control Added



Control Primers

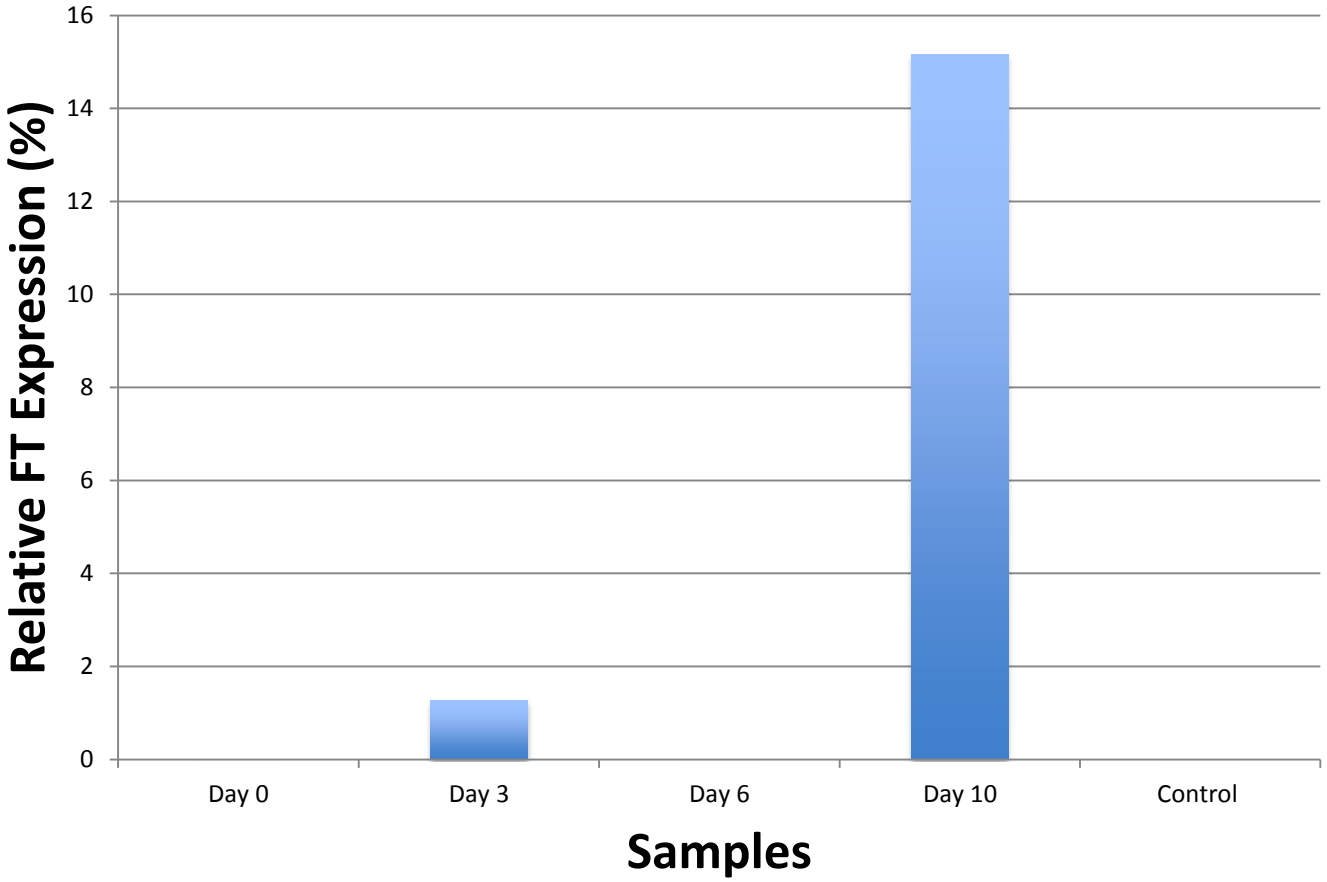
Flowering Primers

Faint band for Day 10 sample

Conclusion to Poplar Project

- *FT* expression present in flowering poplars only
  - Correlation between presence of *FT* and flowering
- Next Steps
  - There are many more samples to be tested for *FT* expression
  - If band is present for flowering primers, we can quantify the band intensity relative to the control bands

Relative FT Expression



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