# Scanning the transposon insertion line for HSFTF24

Samantha Renard, David Wang, Laura Gates, Ryan Sartor, Zhouxin Shen, Steven Briggs

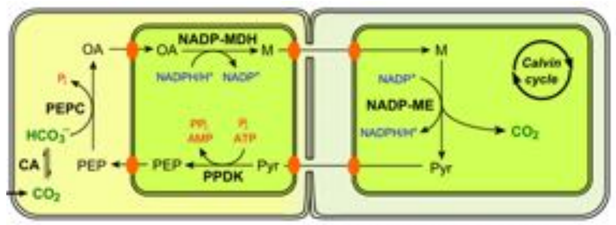
UCSD | Academic Connections

## Abstract

C4 Photosynthesis is more efficient than C3 photosynthesis, but the regulators of C4 photosynthesis remain unknown. The Briggs Lab generated a gene regulatory network (GRN) that lead to a prediction that a protein, heat shock transcription factor 24 (HSTF24), was important for C4 photosynthesis. To test this prediction, seeds with the transposon inserted into their genome to knock out HSFTF24 were obtained. In this experiment, those seeds were screened for their transposon and determined wild type, heterozygous, or homozygous mutant. Seeds were first grown into full maize plants, then genomic DNA was extracted and PCR analysis was performed. One of these plants had a visible mutant phenotype, and this plant was hypothesized to be a homozygous mutant, but after extensive screening, was determined to be a heterozygous plant.

## Introduction

C3 photosynthesis is the process that occurs in plant cells and uses the RuBiSCO enzyme to complete the calvin cycle, which creates glucose or sugar molecules from carbon. In photorespiration, the RuBiSCO enzymes attempt to make glucose from oxygen when carbon levels in the cell are low, which can be caused by high temperatures. Photorespiration is not only futile, but also wasteful to the plant's energy, nitrogen, and carbon resources. To counteract the RuBiSCO's efficiency problems during photorespiration, most plants mass-produce the enzyme. But maize, more commonly known as corn, is not most plants.




From: Raghavendra et al., 2010

Instead, maize uses C4 photosynthesis, where carbon dioxide is introduced to a mesophyll cell at night and processed by a bundle sheath cell during the day. This process is shown in **figure 1.** The RuBiSCO enzyme is held in the bundle sheath cell, where there is a heavy concentration of carbon, resulting in the absence of photorespiration. By collecting the carbon dioxide at night through the plant's pores, maize can withstand relatively high temperatures and dry environments. These factors and advantages of C4 photosynthesis have lead to maize becoming the third most-produced crop in the world (after wheat and rice), and of these staple crops, has the highest tolerance for hot and dry climates. The genes responsible for C4 photosynthesis in maize (as opposed to C3) have been proposed, but the expression and key regulators of such genes remain unknown.

To predict the regulators of C4 photosynthesis, the Briggs lab built a gene regulatory network, or GRN, of all transcription factors. Transcription factors ("TFs") are proteins that regulate and control the rates of expression of a given gene by binding to it. By making a GRN, a prediction of regulator interactions with gene expression, a subset of TFs was predicted to regulate the photosynthesis/C4 genes. To test the predicted GRN relationships, the lab obtained maize seeds with transposon lines inserted in them for some of these regulators. Transposons disrupt a given regulator by inserting ~1500 bps somewhere in the gene, which can produce frameshift mutations, but are not guaranteed prevent expression. By using simple mendelian punnett squares, the predicted outcome of the line of maize seeds should have been ¼ wild type (gene regulator intact), ½ heterozygous, and ¼ homozygous mutant.

The goal of this experiment was to screen a group of maize plants with transposon insertion line for heat shock factor 24, and  To screen the maize crops, each sample had to go through DNA extraction, PCR (gene amplification), and placed into agarose gel to scan for the presence of the insertion.

## Transposon insertional mutagenesis of HSFTF24

To determine if HSFTF24 is a regulator of C4 photosynthesis, we obtained transposon-inserted mutants from the UniformMu population for HSFTF24. The transposon includes a DNA element on both ends called tandem inverted repeats (TIR). By performing the PCR, or polymerase chain reaction, with a TIR primer, the PCR can detect then amplify the insertion.

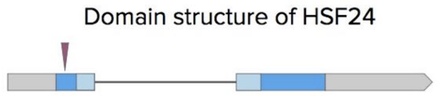



**Fig 2: Gene model diagram of HSFTF24.** The transposon was inserted upstream, where the arrow is. Light blue region represents DNA binding domain, which is separated by the intron, shown as a line. The dark blue regions are the exons of the gene, and light grey regions show untranslated regions.

The transposon was inserted upstream of the first DNA binding domain, with the intent of causing a frameshift mutation on the DNA binding region. By inserting the transposon upstream, it also was more likely to cause a frameshift effect on the exons, which would encode for the HSFTF24 protein the plant would require.

## Phenotype Results


**Fig 3A**: Abnormal plant exhibits albino and green sectors on leaves, shows leaf curling, and plant's dwarf phenotype. Leaves and curling documented in **Figs 3E** and **3F**. **Fig 3B**: Normal sibling to the abnormal plant, normal leaf phenotype shown in **Figs 3C** and **3D.**

Twelve plants that were predicted to have the transposon line in them were obtained and planted. Because C14-7's leaves showed variegation and the plant as a whole showed dwarfism, C14-7 was hypothesized to be the homozygous mutant. In Figure 3, the phenotype of C14-7 is compared to the phenotype of a wildtype.

## Co-segregation analysis of phenotype with HSF24 transposon insertion

To determine if the variegated phenotype was due to the presence of the transposon in HSF24, we extracted genomic DNA and genotyped the plants using polymerase chain reaction PCR assay. Nine out of the twelve plants identified contained transposon insertion.

By amplifying with a gene specific primer with a transposon specific primer **[Fig 4**], the only plants that showed a band were the ones that had a transposon inserted into them. When two gene specific primers that flank where the transposon is were used in tandem to determine if the plant was heterozygous or homozygous mutant.

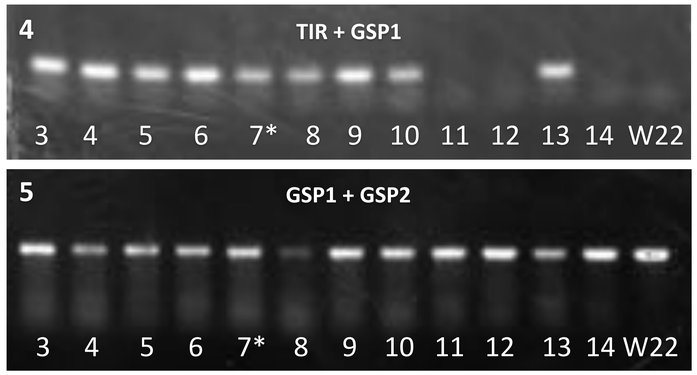
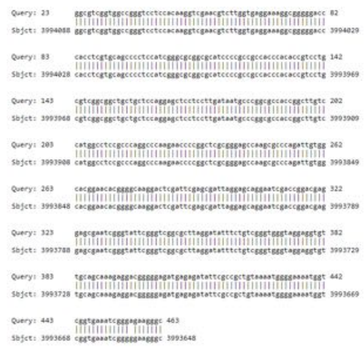



**Fig 4**: **TIR + GSP1.**  **Fig 5**: **GSP1 + GSP2.** C14-7 is starred because it was hypothesized to have been the homozygous mutant.

## Sequencing analysis of gene specific amplicon



When testing the gene specific primers, all plants were shown to have a band, so the gene-specific amplicon needed to be sequenced to make sure they were not non-specific. To do this, the band was extracted from the agarose gel and submitted for sequencing using both gene specific primers. The gene sequence, when BLASTed against the maize W22 genome, showed that the amplicon aligned perfectly with HSFTF24, and there was no other perfect alignment anywhere else in the genome.

## Discussion and Conclusions

The plant with the mutant phenotype was only a heterozygous, and no homozygous mutants were found. These two results suggest that phenotype does not co-segregate with the transposon. The phenotype of the C14-7 plant might be due to background transposons or a random mutation completely unrelated to transposon–genesis. In the future, it would be interesting to observe the progeny of plant and it's sibilitings, to see if the phenotype occurs again in the next generation.

## References and Acknowledgements

Thank you to Briggs Lab for hosting me, and I am grateful for Research Scholars at Academic Connections for allowing me to have this opportunity. Special thanks to Laura Gates for being an amazing mentor.

1. Huynh-Thu, V.A., Irrthum A., Wehenkel L., Geurts P., 2010. Inferring Regulatory Networks From Expression Data Using Tree Based Methods. PLoS One 5:9
2. McCarty D.R., Suzuki M., Hunter C., Collins J., Avigne W.T., Koch K.E., 2013. Genetic and molecular analyses of UniformMu transposon insertion lines. Methods Mol. Bio. 1057:157-66
3. Walley J.W., Sartor, R.C., Zhouxin S., Schmitz, R.J., Wu K.J., Urich M.A., Nery J.R., Smith, L.G., Schnable J.C., Ecker, J.R., Briggs, S.P. 2016. Intergration of Omic networks in a developmental atlas of maize. Science 353:6301.