BIT 161B : Experiment 5
11 June 2018

Insertional T-DNA mutagenesis for Identifying NHX genes in Arabidopsis Thaliana

Aditya Nirgun 999723427

Abstract

The transformation of gene sequences by an agrobacterium vector is utilized in this investigation of NHX 5 and 6 gene expression. The combination of phenotypic analysis with controlled mutagenesis allows for rapid experimentation with specific gene sequences anywhere in the genome given that the sequence base pairs can be identified and used to fabricate code-specific primers within T-DNA constructs. In this experiment, the roles of PCR and gel electrophoresis were necessary to confirm the successful transformation by the insertional T-DNA. Although the transformation appeared to take 100% effect in the phenotypic analysis, the genetic confirmation failed and it stood to ultimately invalidate our experimental conclusions unless further verification is performed on the subjects at the DNA level.

Introduction and Objectives

An empirical understanding is often necessary to couple with the bioinformatics approach in the process of determining gene function. This experimental evidence is acquired by inducing mutations in experimental subjects on the genetic level and observing the subsequent changes in phenotype. Agrobacterium tumefaciens is, again, used as a delivery vehicle for inserting customized and targeted vectors into the subjects. In this experiment, we are inserting t-DNA constructs into Arabidopsis Thaliana with NHX-targeting primers based on our designs from Experiment 1. These constructs are intended to knock out the NHX genes of interest so that we can make valuable inferences about their function.

The mutants are verified for successful knockout by analyzing the gel electrophoresis result which should represent the presence of, or lack thereof, the appropriate T-DNA tags in the corresponding sequences in each mutant.

Methods

The procedure diverged from that which is printed in the lab manual in several places. The differing treatment conditions for the Arabidopsis germination tests are not stated explicitly in the lab manual. The condition treatments varied by temperature.

On Day 2, we obtained two leaves per whole seedling for freezing and lysing, with 3 grinding beads in the microcentrifuge tube instead of the suggested two.

On Day 4, the 1% Agarose TAE solution was initially comprised of 75mL of TAE, 0.75 grams of Agarose, 1 uL of Gel Red, 15 uL of PCR product, 3 uL of 6X loading dye. From this solution, 50mL was used as agarose gel for the electrophoresis process.

Our electrophoresis well placements and subsequent results were organized differently from the reference template provided. One untreated control and three insertional mutants of Arabidopsis were the plant individuals from which our genomic DNA samples were sourced. Of the insertional mutants, one had NHX 5 knocked out, the other had NHX 6 knocked out, and the last had both NHX 5 and NHX 6 knocked out. For each of these plants, the samples underwent PCR with four different primers. The four wells were loaded with genomes of the controls and mutants and each set of four wells and a ladder corresponded to a type of primer. One primer targeted the presence of the unmodified NHX 5 sequence, one primer targeted the presence of the T-DNA tag indicating an induced knockout mutation in the NHX 5 sequence, one primer targeted the presence of the unmodified NHX 6 sequence, and one primer targeted the tag for NHX 6 mutation. The PCR process replicated the DNA fragments corresponding to the primer targets creating differentially sized aggregations that are visible in gel electrophoresis. Results

Figure 1. Morphological variation between the treatment and control under different conditions.

The above image is of the Arabidopsis Thaliana control plants (located in the top three rows) under different conditions. The bottom three rows are the insertional mutants under the same treatments. There is a clear visual difference in overall size between the subjects where the insertional mutants are proportionally smaller in the growth of leaves and stems. These observations are presumably evidence for the protein sequestering effects of NHX that were affected by the gene knockout.

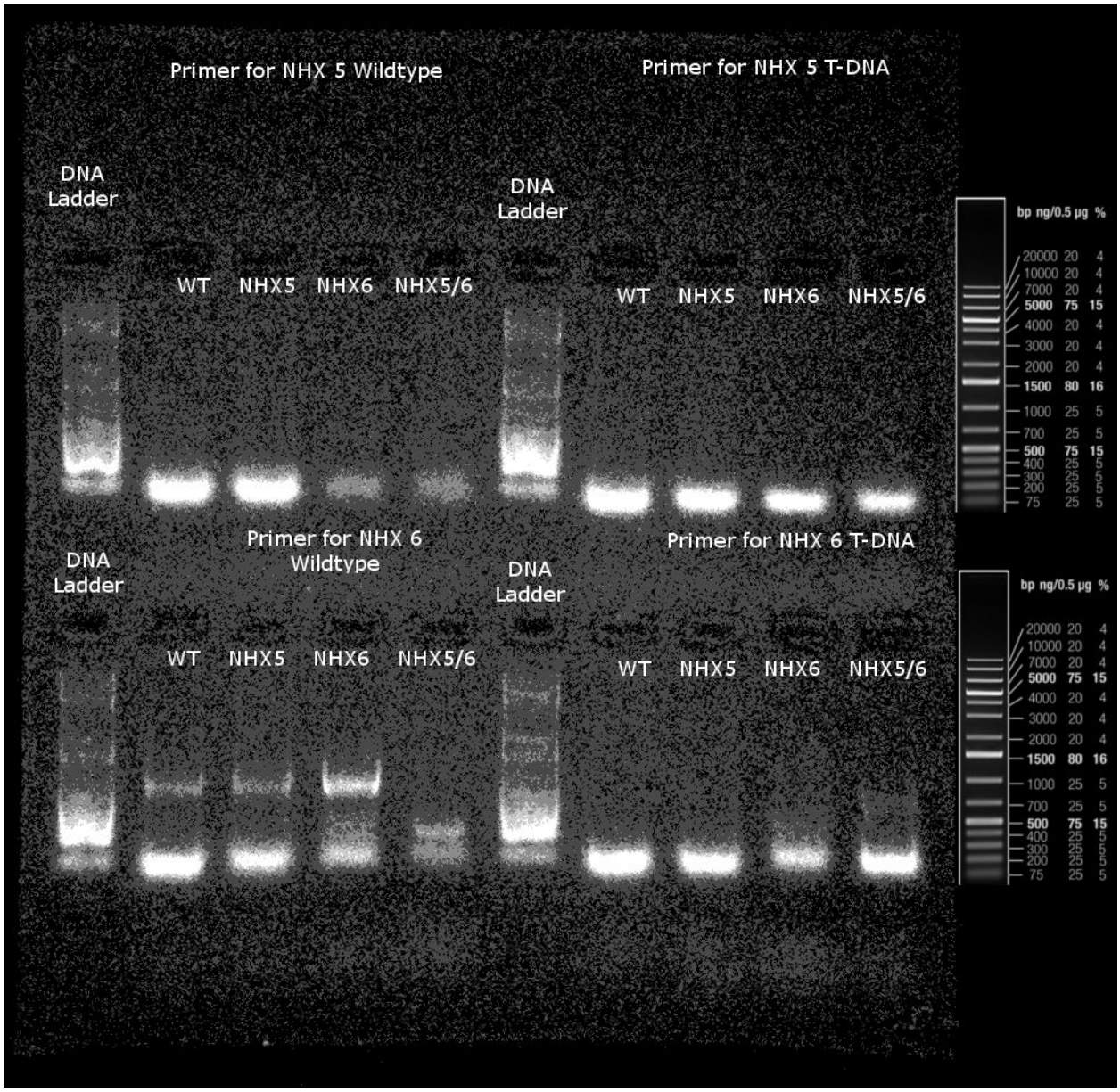

Figure 2. Gel electrophoresis of the genomic DNA for each plant subject treated with each primer captured on June 4, 2018 at 3:13 PM

The photo above contains the results of our gel electrophoresis. Unmodified NHX 5 sequences were not detected in the electrophoresis result for the genome of any of our test subjects. Similarly, no TDNA tags for the NHX 5 knockout mutations were found in our genomes. The unmodified NHX 6 gene sequence was detected in every genome with a lesser amount of PCR product detected in the NHX 5/6 double knockout genome. The tag for the NHX 6 knockout mutant was detected in the NHX 5/6 double knockout mutant. Our ladders were not properly run in the gel so their ability to indicate fragment size is diminished. The provided ladder image resized to match our gel run indicates a size of 1000bp for the NHX 6 sequence except in the case of the double knockout subject where the fragment was 200 to 300 bp. The NHX 6 mutation tag detected in the double knockout was roughly 700bp.

Conclusions

The lack NHX 5 sequences detected in our genomes indicates an experimental error in the construction or incorporation of the primer into our master mix for NHX wild-type or a failure in the loading or running of the gel electrophoresis. The lack of NHX 5 and 6 TDNA detection can be attributed to anything from a failure in agrobacterium transformation, failure in gene knockout on the insertional level, improper PCR execution, or failure of the gel electrophoresis. It is possible that the insertion did not take place at all for the NHX 5 TDNA construct. If the insertion for NHX 6 did not take place we would not have observed the fragment in the double knockout, assuming that fragment is not an error in the PCR or gel electrophoresis process. The primer for the unmodified NHX 6 sequence replicated a sequence for every single subject. While the presence of wild-type NHX 6 was expected in the wild-type and NHX 5 knockout treatments, this result does indicate that none of the NHX 6 knockouts were effective.

Overall, our gel electrophoresis failed to produce an expected result in any of the treatments. This lack of consistency does little to inform us about the success of the TDNA insertions and it is likely that there was an experimental error during the PCR process since there was no detection of expected fragments in the wild-type controls. It is also apparent from the image result that we did not let the gel run for long enough, possibly obscuring or completely diminishing our ability to visually detect a smaller PCR fragment.

References

Bennett, Alan. “EXPERIMENT 5: Functional Genomics.” Lab Write-up. UC Davis. May 21, 2018