



# Identifying Mutant Genes and Their Effects in Maize Circadian Rhythms

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## ABSTRACT

Circadian clock controls daily changes of photosynthetic activities, cell growth, and expression of several genes. The rhythms of this clock are set by cues from the environment<sup>1</sup>. In plants, the circadian clock maintains approximately 24-hour rhythms in a large number of key plant processes. In the studies of mutants in maize, three important genes to the general functioning of the maize circadian clock were identified: *timing of CAB expression 1* (TOC1), *zeitlupe* (ZTL), and *Arabidopsis gigantea* (GI). GI is a component of the core circadian oscillator. ZTL indirectly controls the clock's pace through its effect on TOC1 degradation while TOC1 was one of the main components in controlling the clock's pace<sup>2</sup>.

This project is interested in the reverse genetics of maize mutants in circadian clock genes and focuses on the continued development of transposon mutants in several genes that encode potential circadian clock components. During the summer of 2012, transposon mutants will be identified among several hundred maize plants by PCR genotyping. Molecular procedures including DNA extraction, PCR, and DNA sequencing were used to determine the precise locations of the mutations being mapped. This work is critical for future studies of the maize circadian rhythm and ultimately to the improvement of the agronomic traits of maize and, potentially, other crop species as well.



## INTRODUCTION

Corn is by far the largest component of global coarse-grain trade, accounting for about three-quarters of total volume in recent years. The United States is the world's largest producer and exporter of corn with around 80 million acres of land planted to corn<sup>1</sup>. Research in maize genetics have increased crop yield and sweetness, along with other crop qualities in order to meet the increasing needs of the world today.

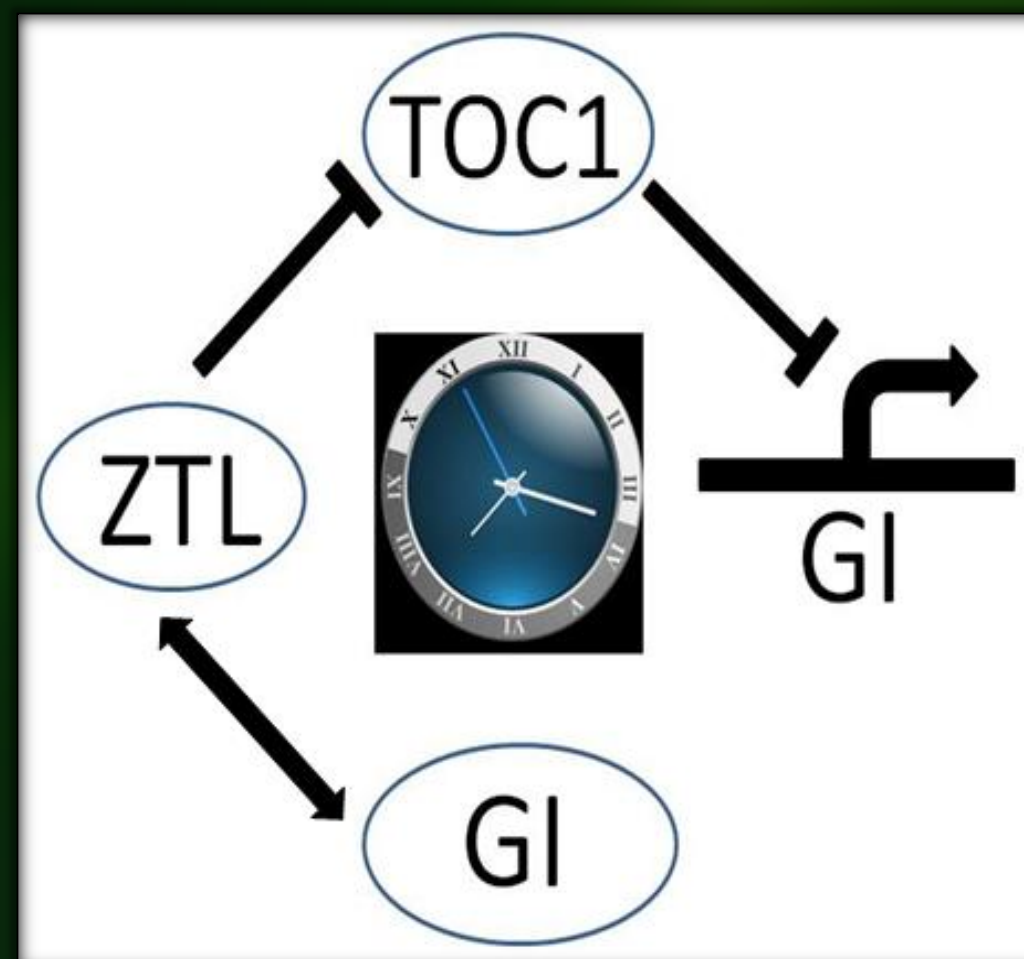


Figure 1 – Circadian clock genes

By using DNA extractions in PCR to identify plant genotypes with an electrophorated gel under UV light, we have identified maize Mutator (Mu) transposable element insertions<sup>2</sup> in GI, TOC1, and ZTL. Sequencing of the Mu elements confirms that for each gene at least one of these elements interrupts an exon in the target gene. Furthermore, analysis of transcript levels in plants with the Mu alleles confirms each exon-located insertion reduces gene expression. These mutants will be useful tools to study the contribution of the maize circadian clock to important agronomic traits.

The regulatory relationship between GI, ZTL, and TOC1 keep a plant's circadian clock running on a 24-hour schedule. ZTL is a blue-light clock-specific photoreceptor and F-box protein that promotes the degradation of the TOC1 transcription factor. TOC1 represses expression of GI, as well as other core circadian clock genes. GI complexes with ZTL block its interaction with TOC1, which leads to greater TOC1 accumulation. The balance between TOC1 accumulation and its degradation is the key to setting the period of the circadian clock.

## METHODS

### DNA Extraction

Corn tissue collected from leaves was mixed with 600μL extraction buffer and crushed in a mixer mill with metal beads to disrupt their cells.

150μL of ice cold KAC was added and centrifuged at max speed for 15 minutes to precipitate proteins.

A pipette was used to carefully transfer 400μL of supernatant into a new tube which contained 280 μL of isopropanol and centrifuged again. DNA was precipitated by the isopropanol into a white nucleic acid pellet and washed with 70% ethanol after pouring off the supernatant. This step was repeated two more times with 95% and 100% ethanol respectively to remove impurities before placed in a vacuum centrifuge to dry.

Finally, 50 μL of sterile water was added to dissolve DNA to prepare for quality testing in a NanoDrop spectrophotometer.

Once the DNAs have been extracted and cleaned up, the PCR setup can begin.

**Polymerase Chain Reaction (PCR)** is a method that amplifies a specific DNA sequence into a vast number of copies.

A PCR requires several components and reagents below.

- Sterile water and 10X ExTaq buffer to provide a suitable chemical environment for reaction.
- A mixture of dNTP, which includes essentially free nucleotides to synthesis for new DNA strands.
- Both the forward primer and reverse primer are short pieces of single-stranded DNA that are complementary to the target sequences. One primer attaches to the top strand at one end of the segment, and the other primer attaches to the bottom strand at the other end.
- Taq – hTaq 2X is a DNA polymerase that will synthesize new strands of DNA complementary to the target sequence.
- A genomic DNA template likely containing the DNA target sequence to be amplified.

### PCR process

- **Denaturation step** proceed at 95°C. The hydrogen bonds between double stranded DNA were broken and the DNA double helix was separated into two single strands.
  - **Annealing step:** At 50-65°C, the two primers were allowed to attach where their complementary sequences are located on the single stranded DNA.
  - **Extension step:** At 70-74°C, the Taq catalysed the duplication of the target sequence. The free nucleotides were used to form new double-stranded DNA fragments.
- The temperature cycling process was then repeated 30-40 times, doubling of the number of copies of the target sequence at each cycle.

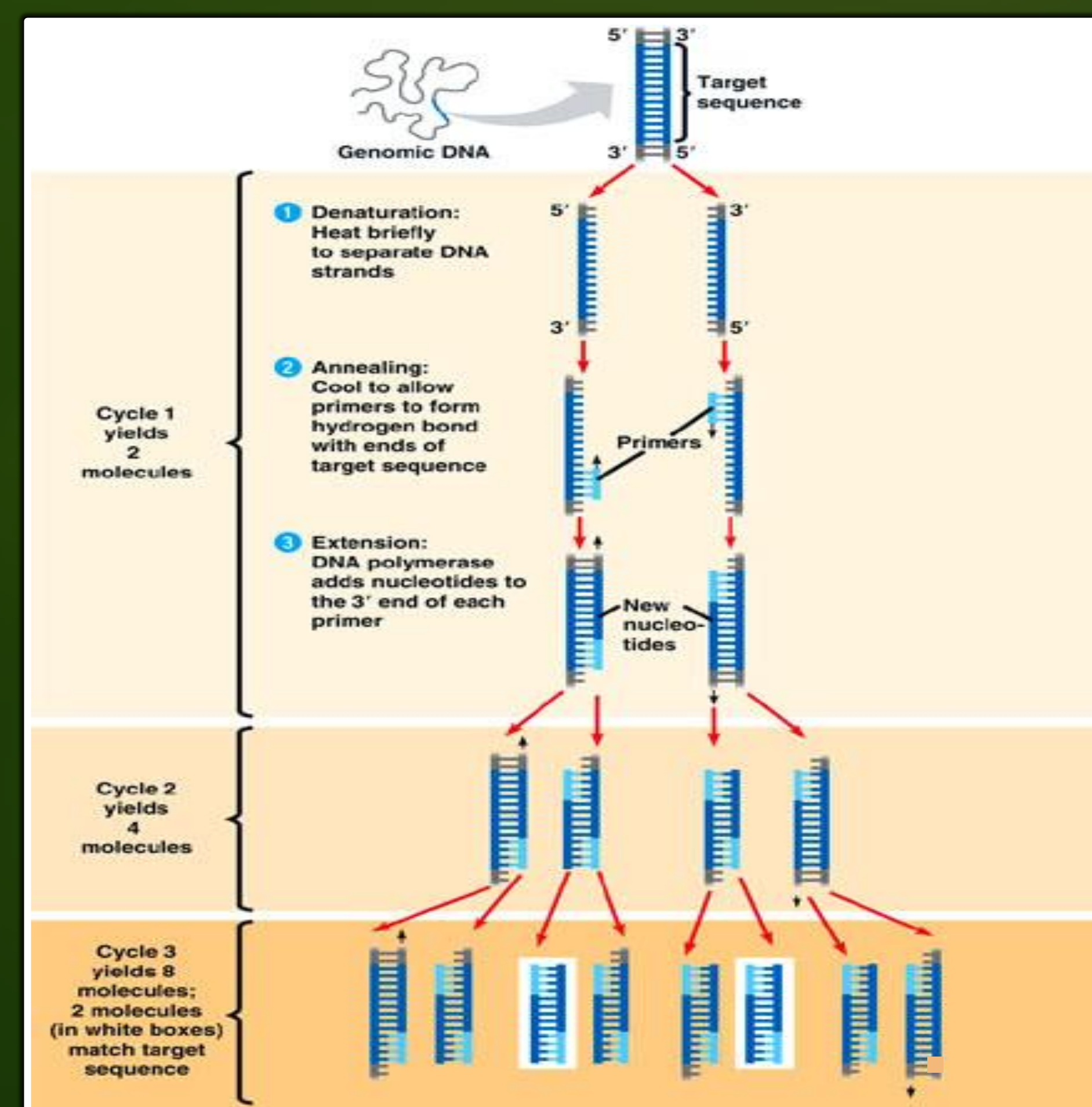


Figure 2 – PCR process

### Agarose Gel Electrophoresis

1% agarose gel included 1 gram agarose in 100mL 1x TAE (Tris-Acetate-EDTA). 5 mg/ml ethidium bromide was followed by ratio 1:20. Then, the dyed PCR products were loaded in a gel seated in a buffered solution within a chamber between two electrodes at 110V for 35 minutes. Finally, the mutant corn plants were identified by the presence of ethidium bromide in the gel mixture and illuminating any DNA presented under UV light.

## RESULTS

Primers #1305 and #698 were used to determine mutants in plants of UFMu-01896 (TOC1b), where primer 1305 targets a specific location on one of the maize chromosomes and primer 698 targets the sequence of the transposon mutation if it is present in the DNA. Comparing the resulting product size to a 2-Log DNA Ladder, the size of DNA bands were approximately at 0.5 Kilobases indicated that plants 17, 19, 23, 24, 29, and 30 are mutants. Conversely, the lack of PCR bands indicate that plants 16, 18, 20, 21, and 25 through 28 do not carry the mutation.

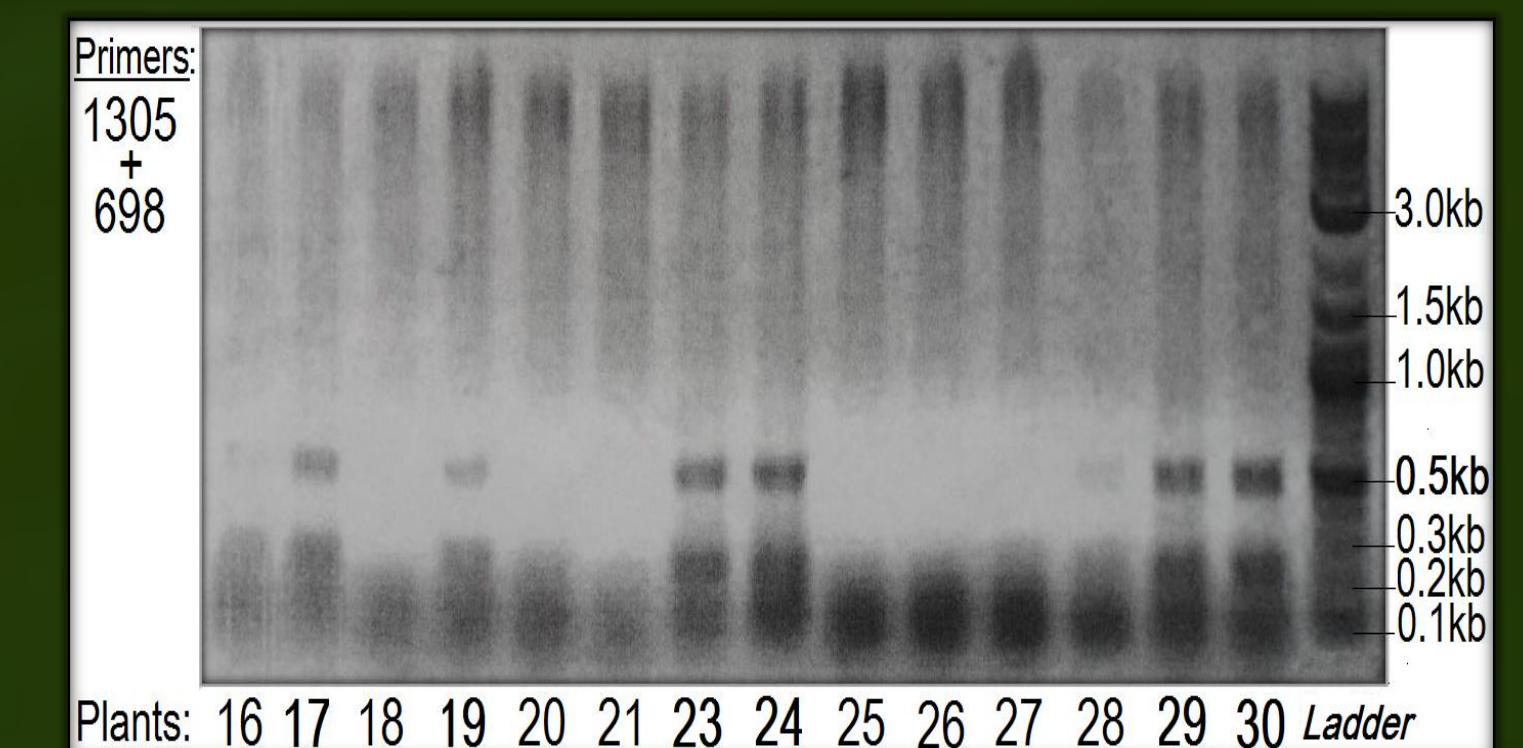


Figure 3 – PCR product on agarose gel

Through direct sequencing of the purified PCR product out of the electrophoresis gel (Figure 4.1), it can be seen that the PCR product sequence aligns with the TOC1b sequence perfectly (Figure 4.2), which is located in Chromosome 5 as shown here (Figure 4.3).

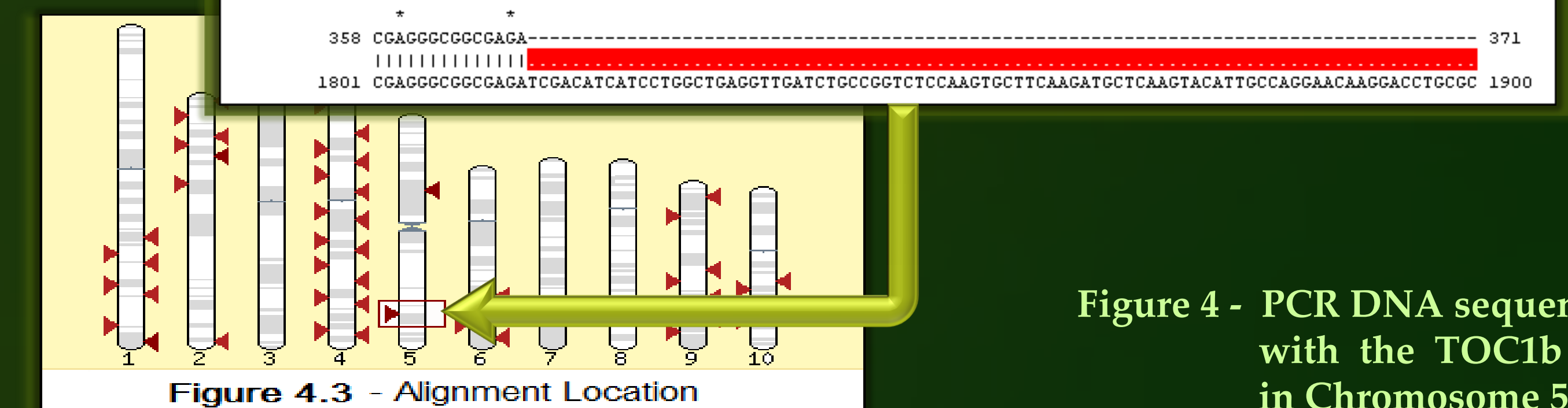
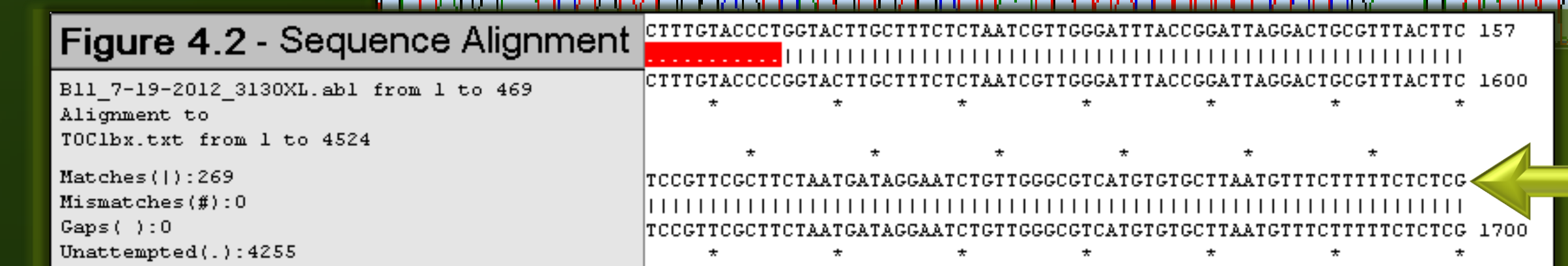
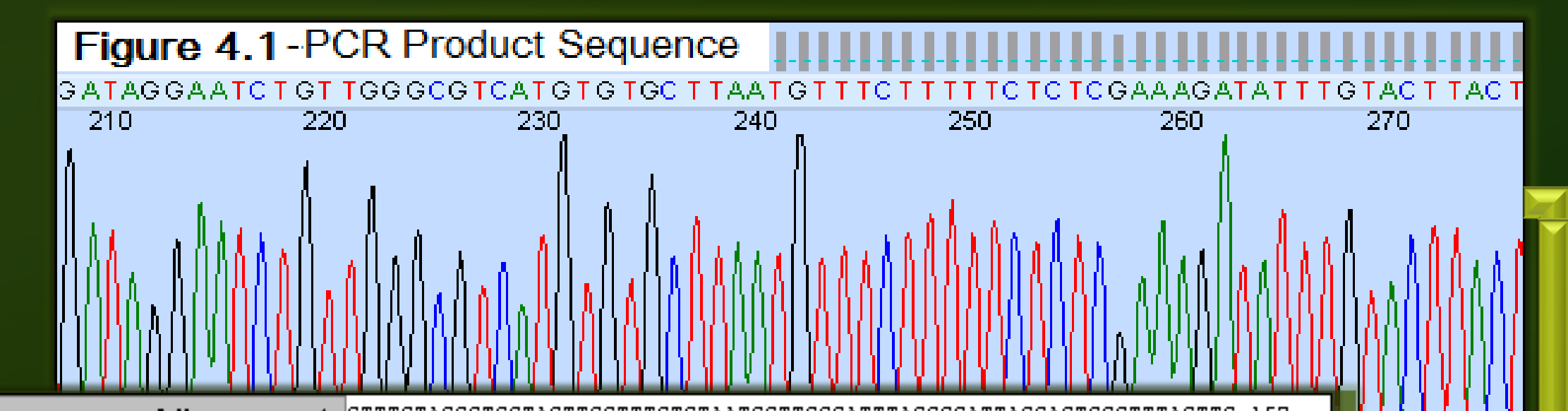


Figure 4 - PCR DNA sequence aligns with the TOC1b sequence in Chromosome 5.

## CONCLUSIONS

- ✱ The presence of unique transposon insertions in the maize TOC1b gene were confirmed with PCR.
- ✱ The precise location of a transposon insertion was determined using direct sequencing.
- ✱ Plants harboring the mutation will be crossed out to normal plants to remove unwanted transposons.
- ✱ Succeeding generations of plants with this mutation will be studied for changes in flowering time.
- ✱ Succeeding generations of these mutant plants will be monitored for changes in TOC1b gene expression.

## REFERENCES AND ACKNOWLEDGEMENTS

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