

# Detection of *abs1* in *Zea Mays*



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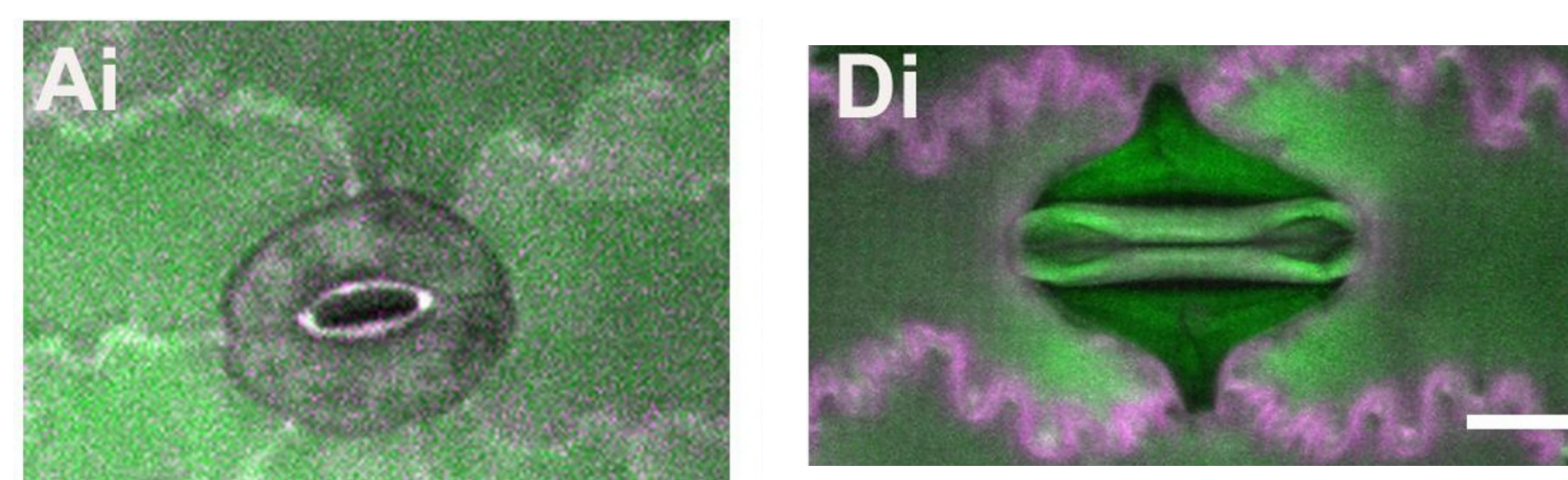
## INTRODUCTION

### What are stomata?

Stomata are pores in the leaf's surface that take up CO<sub>2</sub> for cellular processes. They are also a major source of water loss for plants. Stomata consist of two guard cells surrounding the pore (stoma) that facilitate its opening and closing.

### Why are corn stomata important?

Corn, grasses, and other grain crops including wheat and rice, have stomata that contain four cells: two guard cells and two subsidiary cells. These subsidiary cells flank guard cells and aid in ion exchanges. Until recently, stomata have mostly been studied in species that only have guard cells.



Pictured above is an open stomata. Because it is open, the guard cells are turgid to allow for ion exchange. This does not contain subsidiary cells, only guard cells. Image derived from Gray et al (2020).

Pictured above is a closed stomata with flaccid guard cells that do not allow for the movement of ions into the stoma. This cell contains both guard cells and subsidiary cells. Image derived from Gray et al (2020).

## METHODS

- abs1* mutant was obtained from the stock center totaling 24 seeds; 6 mutants
  - Compare phenotypes of *bzu2* and *abs1*
  - Impressions taken on leaves using super glue and examined under the microscope

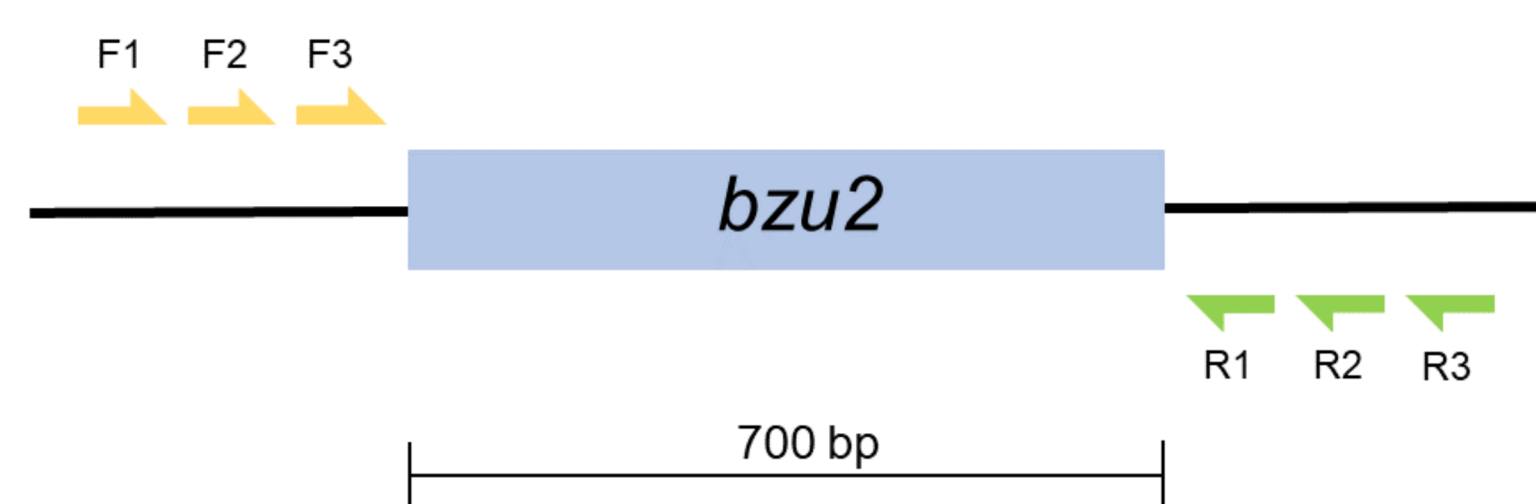
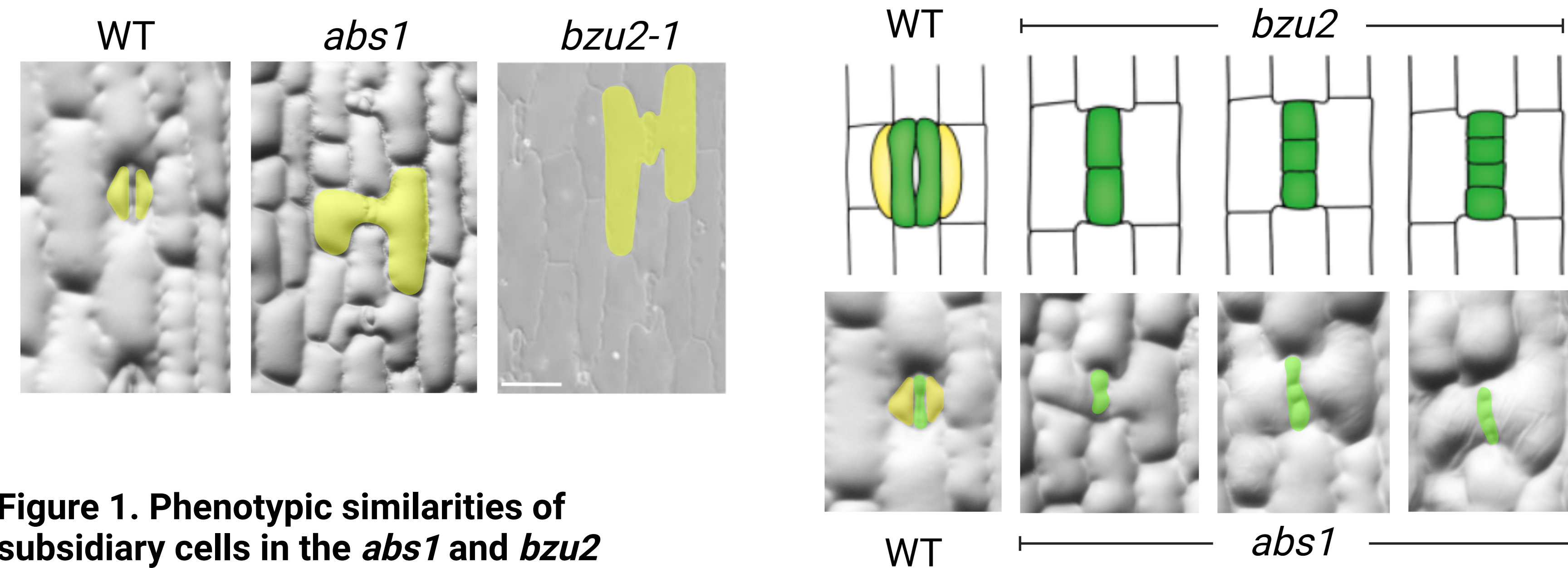


Illustration of the *bzu2* gene including primer sets. This gene is a single exon with an 85% GC content. Forward primers are labeled in yellow and reverse primers in green.

### 2. PCR design and optimization

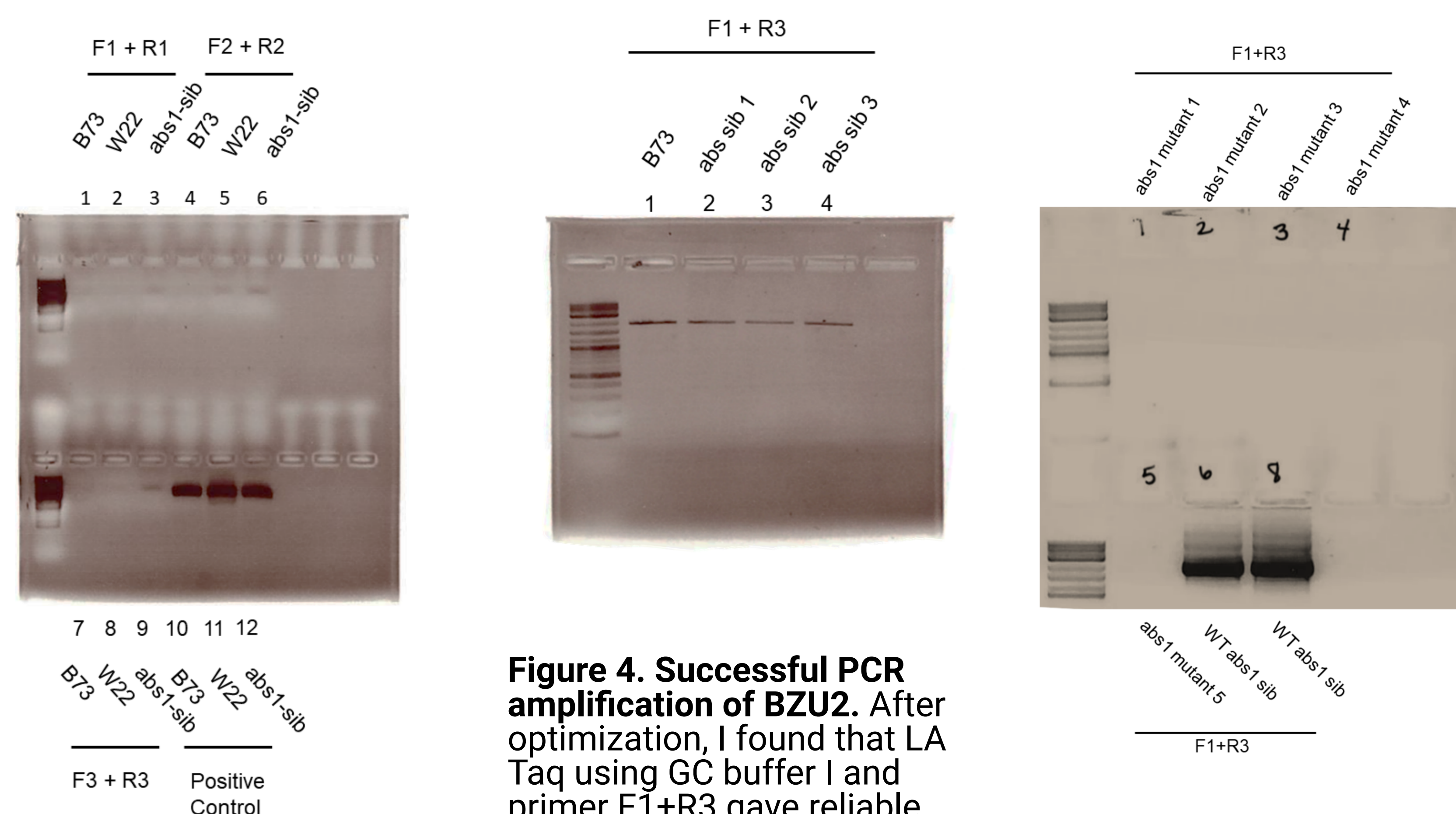
- Primers were designed to span the entire gene, which is a single exon with an 85% GC content
- PCR was optimized using different annealing temperatures, additives/buffers, and Taqs

## IS *abs1* THE SAME GENE AS *bzu2*?



**Figure 1. Phenotypic similarities of subsidiary cells in the *abs1* and *bzu2* mutants.** Highlighted in yellow are subsidiary cells, where WT displays normal cells. Both mutants express abnormal subsidiary cells compared to wildtype cells. *bzu2-1* image from Wang et al (2019).

**Figure 2. Both *bzu2* and *abs1* mutants have multi-celled stomata.** Highlighted in yellow are subsidiary cells, and in green are aborted stomatal cells. Both mutants lack subsidiary cells and contain defective stomata that split into two, three, and four cells. Illustration derived from Wang et al (2019).



**Figure 3. PCR prior to optimization.** Three primer pairs and a positive control pair were tested using Q5 polymerase and DMSO additive. Three different DNA samples from different maize lines were used. No product was obtained using our primers, however, the controls indicate that the DNA quality and PCR mixture was good.

**Figure 4. Successful PCR amplification of BZU2.** After optimization, I found that LA Taq using GC buffer I and primer F1+R3 gave reliable product in WT siblings and *abs1* mutants.

**Figure 5. PCR using *abs1* mutants.** With the primer pair F1+R3, I successfully tested *abs1* mutants with two WT siblings. The WT siblings act as a positive control to demonstrate that the PCR was successful in amplifying the DNA.

## CONCLUSION

In order for this research to be done, fieldwork was required. Over the course of the summer, I spent my time working in both the lab and the field. This included planting our mutants, collecting leaves for impressions and DNA extractions, shootbagging, and cross pollinating. This coming fall, we will harvest the ears of the maize plants we pollinated this summer.



Upper field in South Deerfield, MA, late July



Pollination of maize crop in August 2022



Growing maize in the lower field in South Deerfield, MA



Maize with shoot bags over growing ears to protect from pollen contamination

## ACKNOWLEDGEMENTS

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## References

- Gray, A., Liu, L., & Facette, M. (2020). Flanking support: how subsidiary cells contribute to stomatal form and function. *Frontiers in Plant Science*, 11, 881.
- Wang H, Guo S, Qiao X, Guo J, Li Z, Zhou Y, et al. (2019) BZU2/ZmMUTE controls symmetrical division of guard mother cell and specifies neighbor cell fate in maize.