# Effect of ZAG4 in Paramutation of *Zea mays*

Julia Won[1], Yacob Gebreab[1], Kathryn Koirtyohann[1], Dr. Jason Lynn[2], and Dr. Karen McGinnis[1]

[1]Florida State University, [2]Cold Spring Harbor Laboratory

Department of Biological Science
Florida State University
Tallahassee, FL 32306

## Background

**Epigenetics** = change in gene expression without altering gene sequence

**Paramutation** happens when one allele on a diploid locus epigenetically changes the gene expression of the other allele. In *Zea mays,* the phenotypic effects of paramutation in the *b1* locus can be seen in stem pigmentation.

- Homozygous *B'* = light stems
- Homozygous *B-I* = dark stems
- Heterozygous *B'/B-I* = light stems

Paramutation also exists in mice, *Arabidopsis*, flies, worms, and humans. Therefore, understanding more about the mechanism of paramutation in *Zea mays* will help us understand how paramutation operates in other organisms.

| Gene | Paramutagenic allele (silenced by RdDM pathway) | Phenotype | Paramutable allele (transcribed but can be silenced) | Phenotype |
|---|---|---|---|---|
| *b1* | *B'* | Light stems | *B-I* | Dark stems |

**Table 1:** Effects of gene silencing

An example of epigenetics is paramutation in maize *Zea mays.*

- The *booster1* (*b1*) gene controls how much anthocyanin is expressed in maize stems.
- The *b1* hepta tandem repeat (*b1TR*) DNA sequence is found 100 kb upstream to the *b1* gene.
- Proteins interacting with *b1TR* helps it enhance or silence the *b1* locus.

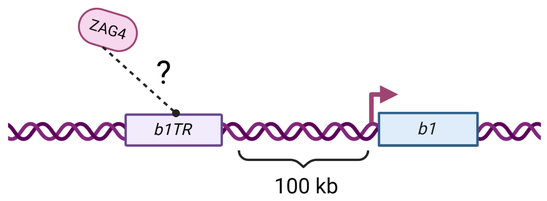



**Figure 1:** DNA model of ZAG4 possible interaction with *b1TR.* Created with BioRender.com

Single Locus Immunoprecipitation Proteomics (SLIP) was used to find these *b1TR*-binding proteins. One of the proteins found was ZAG4, which is involved in plant flowering and orthologous to the better-known *Arabidopsis* AGL5 protein. An yeast 1 hybrid assay will be done to confirm if ZAG4 binds to *b1TR*.

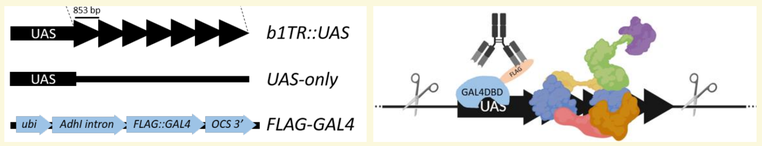
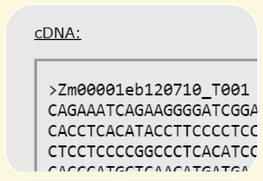
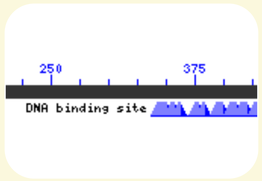



**Figure 2:** Left diagram illustrates the three transgenes used in SLIP. Right diagram shows the chromatin immunoprecipitation portion of SLIP. Modified from Lynn, 2020

## Methods

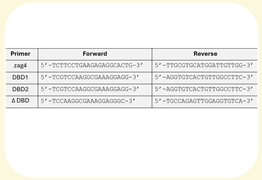



1. Found *zag4* gene model in MaizeGDB



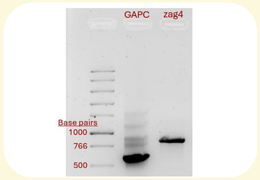

2. Found DNA-binding domain with NCBI conserved domain search


3. Aligned with better-known *Arabidopsis agl5* gene model

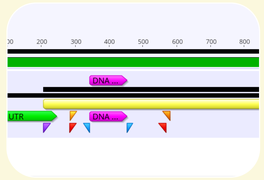


| Primer | Forward | Reverse |
|---|---|---|
| zag4 | 5'-TCTTCCTGAAGAGAGGCACTG-3' | 5'-TTGCGTGCATGGATTGTTGG-3' |
| DBD1 | 5'-TCGTCCAAGGCGAAAGGAGG-3' | 5'-AGGTGTCACTGTTGGCCTTC-3' |
| DBD2 | 5'-TCGTCCAAGGCGAAAGGAGC-3' | 5'-AGGTGTCACTGTTGGCCTTG-3' |
| Δ DBD | 5'-TCCAAGGCGAAAGGAGGC-3' | 5'-TGCCAGAGTTGGAGGTGTCA-3' |

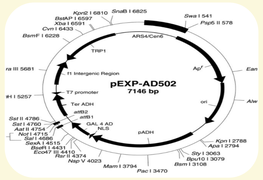
4. Designed *zag4* primers using NCBI primer blast



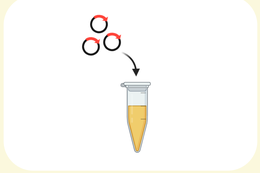

5. Amplified *zag4* from cDNA with primers



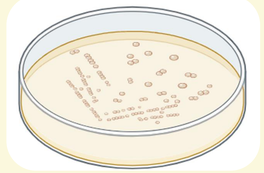

6. Inserted *zag4* into TOPO-TA clone




7. Inserted *zag4* clone into pEXP-AD502 vector, the prey plasmid



8. Transform yeast with prey and bait plasmids



9. Do beta-galactosidase assay

## Results

- Alignment of ZAG4 with AGL5 indicated that the *zag4* gene sequence encoded for the full-length protein (see step 3).
- PCR for the *zag4* gene resulted in the expected 868 bp product (see gel in step 5).
- TOPO-TA cloning with the *zag4* gene was successful (see step 6).
- The prey plasmid for the yeast 1 hybrid has been successfully made (see Figure 3).

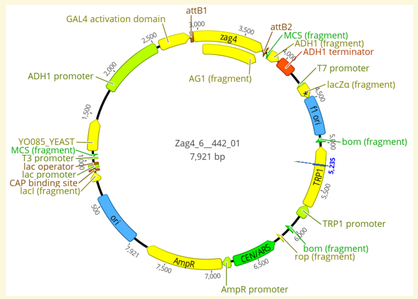



**Figure 3:** Prey plasmid containing *zag4* fused to the GAL4 activation domain

## Discussion

For the yeast 1 hybrid assay, future steps will be to transform baker's yeast *Saccharomyces cerevisiae* RTY300 strain with the bait plasmid containing *b1TR* and the prey plasmid containing ZAG4. A positive control prey plasmid with a protein known to bind to the *b1TR* will be used. A negative control prey plasmid will also be constructed using ZAG4 without the DNA-binding domain.

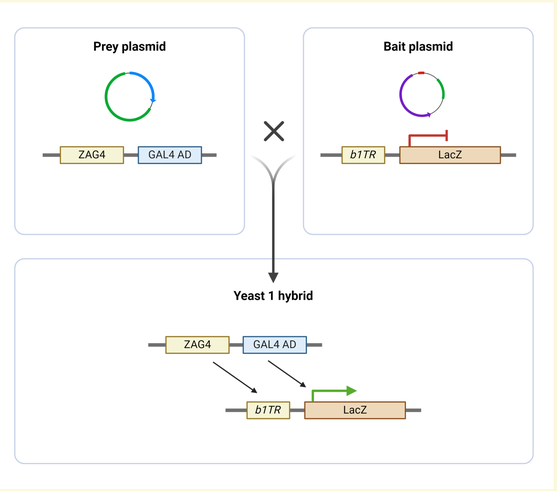



**Figure 4:** Prey and bait plasmids for the yeast 1 hybrid assay. Created with BioRender.com

## Acknowledgments

I would like to thank everyone who has guided and supported me throughout this project: Dr. McGinnis for funding and generating ideas for this project; Yacob Gebreab for assisting with each step of this project; Kathryn Koirtyohann for directing and teaching all the lab techniques; and Dr. Jason Lynn for doing preliminary research on DNA-protein interactions. Thank you to Dr. Washburn and Cheryl Pye of the Molecular Cloning Facility for their excellent work in making clones and plasmids. And a special thank you to my colleague Jaliyah Edwards for laying the basis for this project with her research on DDT4.

## References

Dorweiler, J.E., Carey, C.C., Kubo, K.M., Hollick, J.B., Kermicle, J.L., & Chandler, V.L. (2000). Mediator of paramutation1 is required for establishment and maintenance of paramutation at multiple maize loci. *The Plant Cell*, *12*(11), 2101–2118. https://doi.org/10.1105/tpc.12.11.2101

Hollick, J.B. (2016). Paramutation and related phenomena in diverse species. *Nature Reviews Genetics*, *18*(1), 5–23. https://doi.org/10.1038/nrg.2016.115

Lynn, J.S. &. McGinnis, K.M. (2020). The Epigenetic Influences on Chromatin-Interacting Proteins and Transcriptional Responses to Stress in Maize. *DigiNole.* https://purl.lib.fsu.edu/diginole/2020_Summer_Fall_Lynn_fsu_0071E_16031