Jaliyah Edwards
Dr. Karen McGinnis and
Kathryn Koirtyohann
Florida State University
Department of Cell and Molecular Biology

DETERMINING THE ROLE OF DDT4 IN PARAMUTATION OF THE B1 TANDEM REPEAT IN *ZEA MAYS*

INTRODUCTION

Sometimes phenotypes display inheritance patterns other than what is expected for typical Mendelian inheritance. Paramutation is one mechanism that can change the inheritance pattern of two alleles in a heterozygous diploid individual and is observed when two alleles at the same locus influence the other to invoke a heritable change in expression. When paramutation occurs in a heterozygous individual, one allele converts the other, making that individual homozygous for the paramutagenic allele. The *booster 1 (b1)* locus in maize codes for a blue pigment and is widely studied for the paramutagenic effect of the *B'* allele on the *B-I* allele. Paramutation at *b1* is determined by a non-coding DNA tandem repeat sequence called *b1TR*. In attempts to understand how this repeat functions, a protein called DDT4 was identified that is thought to interact with the *b1TR* sequence. The focus of this research is to confirm whether DDT4 is a chromatin-binding protein to the *b1TR* sequence via a yeast-1 hybrid assay. Identifying components of this paramutation pathway is a worthy undertaking because each piece of the puzzle helps us understand the overall biochemical processes and relationships in a plant species as important as maize. Ultimately, this research will guide our understanding and exploration of more obscure non-Mendelian genetic inheritance and expression, which increases our ability to apply genetic practices.

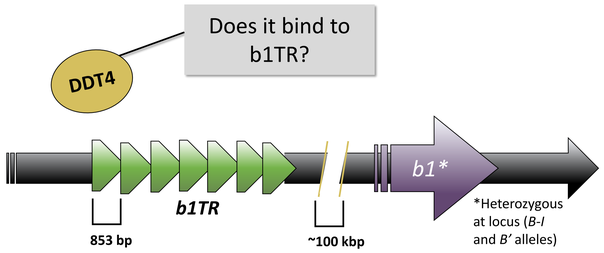


Figure 1: Representation of b1TR in relation to b1 locus with possibility of DDT4 interaction.

ABSTRACT

In *Zea mays*, the booster 1 gene locus (b1) tandem repeat (b1TR) interacts with a network of chromatin binding proteins to influence b1 expression. Prior research suggests DDT4 may be one of these interacting proteins, and we are currently testing the DNA binding ability of the protein to this sequence by performing a Yeast-1 hybrid assay. We are using the commonly used plasmid vectors pLacZi and pEXP-AD502 as backbones for our bait and prey constructs respectively. The DDT4 yeast construct will be built via Gibson assembly, sequenced via Primordium, and purified via gel purification. We will have three positive control prey plasmids (PVC mieszko, cibur, and stojgniew). The *Saccharomyces cerevisiae* (bakers' yeast) RTY300 strain will be transformed with these plasmids through our collaboration with the Yu Lab, which specializes in yeast research. The presence or absence of positive yeast colonies after the hybrid assay will be used to analyze DDT4's binding abilities and relationship to the b1TR. The results of this experiment will contribute to the growing data the scientific community has on the complex RNA-directed epigenetic processes in plants.

MATERIALS AND METHODS

Cloning and Proliferation of Plasmids

- Bacterial Transformations of the plasmids to clone more for project

Sequencing

- Sent plasmids to be fully sequenced by Primordium Labs. Created detailed plasmid maps.

Assembly of DDT4 prey construct

- Restriction digest and Gibson Assembly to insert the DDT4 clone in the prey vector

Yeast-1 Hybrid Assay

- Transform both constructs into yeast RYT330. Plate on TRP and allow the opportunity for positive colonies to form. PCR positive colonies if present to confirm transformation and genotype.

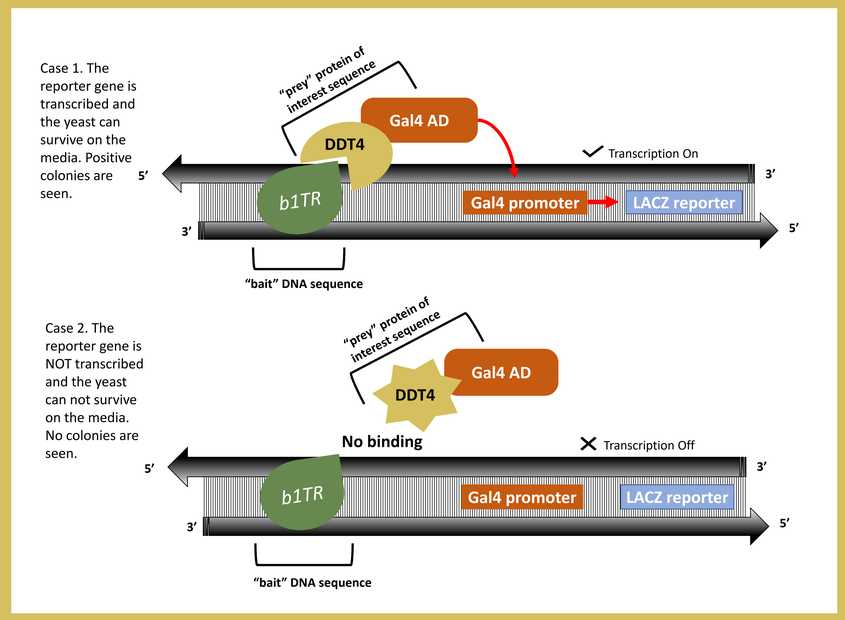


Figure 2: Visual representation of binding and nonbinding of prey and bait plasmids for a Yeast-one hybrid assay.

PRELIMINARY RESULTS

Bacterial transformations for the plasmids were completed with efficient DNA yield. Sequencing and subsequent annotating of plasmids was completed (*Figure 3 & 4*). Successful restriction digest of DDT4 was accomplished.

Figure 3

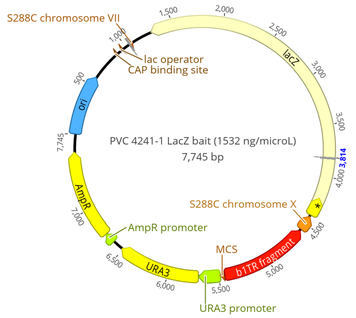
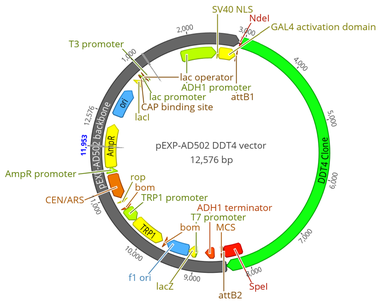


Figure 4



Figures 3 & 4: Plasmid maps for Yeast-one hybrid. Bait construct with *b1TR* DNA sequence in pLacZi vector (*fig. 3*). Prey construct with DDT4 protein of interest in pEXP-AD502 vector (*fig. 4*). *Maps created via Geneious Prime software after Primordium sequencing.*

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