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Natalie Wayne Colby College

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# Characterization of the Wheat Grain PKABA1-Interacting Protein TaWD40

Natalie Wayne

Department of Biology, Colby College, Waterville, ME 04901

#### **Abstract**

Abscisic acid (ABA)-mediated gene expression is a critical component of plant responses to this important hormone, which affects plant growth, development, and responses to environmental stresses. Plant responses to ABA are mediated by a number of factors including PKABA1, an ABA-induced protein kinase involved in ABA-suppressed gene expression in cereal grains, and TaWD40, which has previously been shown to physically interact with PKABA1. A full-length 1.9 kb TaWD40 cDNA, CK210682, was sequenced as part of this project. Based on the deduced protein sequence, it is thought that TaWD40 may belong to the family of E3 ubiquitin ligases, possibly targeting PKABA1 for destruction. Construction of expression plasmids for overproduction of the TaWD40 polypeptide in E. coli is currently underway. The TaWD40 cDNA has been successfully amplified from the source plasmid and inserted into an intermediate plasmid, pCR2.1 The TaWD40 cDNA is currently being cloned from the pCR2.1 intermediate plasmid into two different expression vectors, pRSET-A and pMAL-c2x, for future protein production and purification.

### Sequencing of TaWD40

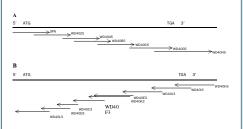
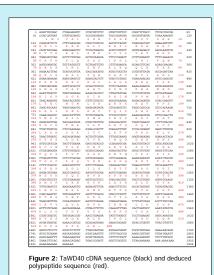


Figure 1: Diagram of the TaWD40 cDNA, CK21068. Start (ATG) and stop (TGA) codons are indicated. A. Primers used to sequence cDNA beginning at 5' end. B. Primers used to sequence cDNA beginning at 3' end.



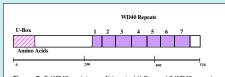
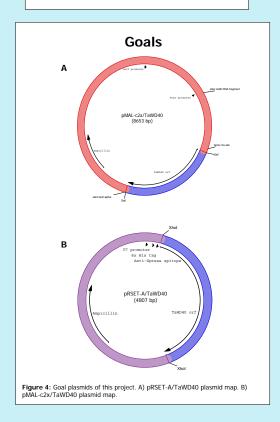


Figure 3: TaWD40 contains an N-terminal U-Box and 7 WD40 repeats. The presence of the U-box and the WD40 repeats suggest that TaWD40 may belong to the family of E3 ubiquitin ligases.



#### Results

· Successful amplification of TaWD40 cDNA.



• Successful preparation of intermediate plasmid: pCr2.1/TaWD40 for pMAL-c2x plasmid preparation.



• Large-scale digestions and purifications of pCR2.1/TaWD40 and pMAL-c2x plasmids with Sal I.





 Large-scale digestions and purifications of pCR2.1/TaWD40 and pRSET-A plasmids with Xho I.



#### **Future Work**

- Plasmid construction for pMAL-c2x/TaWD40 plasmid.
- · Plasmid construction for pRSET-A/TaWD40 plasmid.
- · Large-scale preparation and purification of TaWD40.
- Protein expression studies of TaWD40.

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