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Cloning and recombinant expression of two *Arabidopsis* 14-3-3 binding proteins expressed during seed development

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As a participant in cellular signaling, the 14-3-3 binding protein is essential to cellular function in both plants and animals. Two Arabidopsis thaliana 14-3-3 genes from phylogenetically different families were studied: the GF14-chi and GF14-231, 14-3-3 epsilon which were previously shown to be expressed in developing Arabidopsis seed. One of the principal functions of these proteins is to mediate signals by binding specific client proteins. One strategy to characterize these interacting client proteins is to use purified recombinant 14-3-3 proteins as bait in pull-down assays. The objective of this thesis was to create the expression constructs and purify the recombinant protein. To obtain a cloned gene, isolation of plasmid DNA was followed by PCR amplification. After a ligation reaction and transformation into E.coli Top 10, products were digested. The digested products were sent for sequencing. The GF14-231, 14-3-3 epsilon was successfully cloned. To express the 14-3-3 protein in E.coli, the construct was transformed in BL21 Star (DE3) One Shot cells. After transformation, IPTG was added to induce protein expression. Recombinant 14-3-3 protein was then purified by Ni-NTA column chromatography and analyzed by SDS-PAGE analysis. After obtaining purified protein an antibody will be developed. Future work will include a similar analysis of the GF14-chi.

This project was completed to fulfill a Capstone requirement.