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SMP1-3 Insight into Seed Longevity

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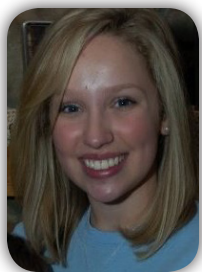
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Taylor Lloyd

SMP1-3 Insight into Seed Longevity

With seeds representing over 70% of our food, they occupy a fascinating and significant platform for research. This particular lab and project deal directly with working to further understand how seeds perceive the correct conditions allowing completion of germination and how they cope with harsh physiological conditions during quiescence (surviving at 5% moisture content fresh weight). During extended quiescence, select proteins in the seed accumulate isoaspartate damage, thus the functionality of the protein is reduced or diminished causing an inability to complete germination. Within this lab, a phage display cDNA library of mature, dehydrated or germinating seed of the model plant *Arabidopsis thaliana* was constructed and a biopan with recombinant PROTEIN ISOASPARTYL METHYLTRANSFERASE (rAtPIMT1) conducted. Specific proteins that were more susceptible to isoaspartate accumulation were identified, one of which was At3g12960, the “SEED MATURATION PROTEIN (SMP).”

This project begins with the identification of a phenotype for a mutation in this gene obtained from the Cold Spring Harbor Laboratories. The phenotype was discovered to be a determining factor for secondary seed dormancy. With wild type (WT) seeds, the normal response to heat shock is a “secondary dormancy” which causes the seed to wait four or five days once put under ideal conditions before it completes germination. It was found, however, that the mutant of this particular gene, *smp1-3*, did not display this “suspicion” to the new conditions and would complete germination almost immediately once transferred to ideal conditions. Thus, we could identify the role of this gene by observing the behavior of the plant lacking the functional SMP.

In spite of this logic it must be proven whether the gene that produces this protein is indeed non-functional in the *smp1-3* mutant. To do this we used the properties of the inserted transposon DNA from maize. The engineered transposon inserted, Maize AC, displays kanamycin resistance; therefore we tested the seeds on (Murashige and Skoog, MS) kanamycin 50 plates to insure the presence of the transposon in the seeds. Following this test we proceeded with a series of PCR reactions processed through agarose gel, Ethidium Bromide Electrophoresis to test the DNA to determine where the transposon was inserted in the genome – before, after, or within the gene of interest (GOI). It would be ideal if we could find that the transposon is directly inserted in the coding region (CDS) of the intronless GOI before proceeding to the next step. From the PCR we were able to determine that the trans-

poson was indeed inserted in the CDS if the GOI and that the orientation was 5' to 3'. We were not however able to predict another feature of this insertion until we moved on and tested cDNA.

To first obtain the cDNA, we extracted RNA from the leaves of the WT and *smp1-3* (the mutant plant). We made the assumption that SMP would still be present in sufficient amounts in mature leaves when it is a factor promoting seed dormancy. This RNA was then treated to remove DNA also extracted through our protocol and then Reverse Transcribed (RT) to produce single stranded cDNA that could be tested as with the genomic DNA used previously. However, the unique property of the cDNA is that because it is made directly from the mRNA of the plant, which is transcribed from the gene, we can observe the efficiency of mRNA production. While we were preparing the cDNA, we used genomic DNA from both the WT and mutant, and sent it for sequencing in order to identify the exact insertion site of the transposon. We then examined the sequence for where known plant genomic DNA turns into the known sequence of the Maize AC transposon.

Once the cDNA was prepared, it was tested with β -TUBULIN primers as a control for cDNA concentration as well as SMP specific primers, including a combination of 5' UTR and 3' UTR primers and a primer designed to span the insertion site of the transposon. Results correlating to the primer designed to span the insertion site surprised us because this should have been the best location to demonstrate an inability of the *smp1-3* to produce SMP mRNA (and hence cDNA), yet we saw amplification of the back end of the cDNA.

We returned our focus to the sequence found earlier and re-evaluated the insertion site. It was then observed that the transposon had duplicated the eight nucleotides before the insertion site, and then again after the insertion site. Therefore our test on the insertion site failed because the primer was able to “sit down” (anneal) after the inserted transposon and amplify the back end. Another fascinating element was that this back-end amplicon, demonstrated through the PCR, was much greater in quantity than that observed in the WT because the cell recognized that it was not producing protein from that mRNA and continued to signal to the nucleus to produce more of that particular mRNA, causing over expression in the mutant.

Once the duplication of the DNA was understood, a final test of the cDNA proved that there was an interruption in our GOI at the known site that prevented the function of the SMP gene, a knockdown or knockout of the gene. We are now confident in our observation that the phenotype of this mutant of SMP is directly correlated with the knockout of this gene, causing the lack of suspicion in our mutant as compared to the WT with functioning SEED MATURATION PROTEIN.