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Starch Phosphates: A Means to Manipulate Biofuel Production

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As a result of an intensive search through expressed sequence tags (EST) databases I have obtained an EST of LSF2. Through my search of the EST databases, I have determined that LSF1 Chlamydomonas doesn't exist. Since there was no Cr LSF1 I went ahead only with the Cr LSF2.

Once the Cr LSF2 EST arrived from the EST supplier I designed primers to PCR the gene from the cDNA. I used the primers to PCR the gene out of the cDNA and then ran the PCR product on a TAE DNA gel electrophoresis. On the DNA gel I made sure the PCR product was the right size then I cut out the band and purified the gel band using standard molecular biology protocols and then measured the concentration of the purified DNA.

When the Cr LSF2 gene was obtained I needed to insert it into a vector in order to transform it into bacteria cells. As a result, I ligated the gene into the pet28 vector using standard molecular biology ligation protocols. It took several tries to insert the gene into pet28 because some genes are easier to ligate than others, but the Cr LSF2 gene was inserted into the pet28 vector.

Once the gene was in the vector, I transformed the vector into E. coli BL21 cells. A small scale induction test was performed to determine if the protein expressed well from the pet28 vector. The test showed that the protein did not induce well from pet28 and a different vector needs to be used.

Throughout the summer I have learned a lot about the Chlamydomonas glucan phosphatase LSF2 and the Gentry Lab will use the data I have collected this summer to continue to study Chlamydomonas glucan phosphatases.