In the summer of 2003, I participated in a research project to construct an expression plasmid capable of expressing CG3075 gene in E.coli. CG3075 gene encodes the "C" subunit of the trimeric NF-transcription factor, composed of three subunits, A, B and C. NF-Y is thought to control the transcription of the ci gene in Drosophila. Ci encodes a transcription factor that is a key factor in Hedgehog signaling. Hh signaling causes an alternation of ci protein from a repressor form to an activator form. In the presence of Hh signaling, ci protein will be an activator form, and then will go into the nucleus to turn on the Hh target genes. If Hh signaling is absent, ci protein will serve as a repressor form, going into the nucleus to turn off the Hh target genes. Regardless of Hh signaling, ci protein will go into the nucleus and regulate the Hh target genes. That CG3075, involved in regulation of ci transcription, is indicated by ectopic expression of CG3075 in the wing imaginal disc. When CG3075 is ectopically activated in the wing disc, ci RNA levels are drastically reduced. We hypothesize that overexpression of the "C" subunit of NF-Y (encoded by CG3075) causes the oversaturation of dimeric forms of NF-Y subunits to the exclusion of the trimeric NF-Y protein. The lack of trimeric NF-Y protein results in the failure of ci transcription. My project was to make CG3075 protein for in vitro study. CG3075 protein will be used as an immunochemistry to study CG3075 activities in development. The protein will also be used to study biochemistry of NF-Y. The goal was to express two forms of proteins, full length CG3075 protein and full length CG3075 protein with GST tag. Different vectors are used for different proteins; pET vector was used for the former and pGEX was used for the latter. The latter got GST from pGEX. Genetic engineering and cloning techniques were used to construct the CG3075 expression construct. We have engineered specific restriction sites (Bam, Nde and EcoR1) into a PCR fragment following amplification. Using specific restriction enzymes, we cut both the PCR product and the appropriate vector. The digested PCR was ligated into a plasmid (vector). A successful ligation product will carry an inducible promoter and CG3075 coding region. The CG3075 expression plasmid was then transformed into E-coli. To verify that the correct protein can be synthesized, the CG3075 gene was induced from the inducible promoter on the plasmid. Proteins, synthesized following induction, were characterized by SDS-PAGE electrophoresis to determine if a protein of the correct, predicated size was synthesized following induction. In this project, the CG3075 gene was inserted into pGEX and pET vectors. None of the inserts in the pET vectors were capable of synthesizing a correct CG3075 protein. Four successful ligants with GST-fusion tag were successfully generated in the pGEX vector.