# Cloning in Three Segments of the GLU1 Gene of *Arabidopsis thaliana*

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## Introduction

Serine Hydroxymethyltransferase (SHMT) is an enzyme that catalyzes the reversible transfer of a hydroxymethyl group from serine to tetrahydrofolate. This process is the largest supplier of one-carbon units to cells. This mechanism in plants is unique and differs from similar pathways in other organisms. The hydroxymethyl group transfer is an important part of one-carbon metabolism, a process which produces precursors for purines, pyrimidines, proteins and other important metabolites and is vital to plant survival. When studied *in vitro*, SHM1, an SHMT found in the mitochondria and involved in photorespiration, is capable of transferring the hydroxymethyl group without requiring additional proteins. When experiments are performed *in vivo*, however, SHM1 activity requires ferredoxin-dependent glutamine oxoglutarate aminotransferase (fd-GOGAT). A direct physical interaction between SHM1 and fd-GOGAT is suspected during the one-carbon metabolic process, but the nature of the interaction is not clearly understood. The goal of this experiment is to express fd-GOGAT in a Rosetta strain of *E. coli* to allow further exploration of the physical relationship between SHM1 and fd-GOGAT.

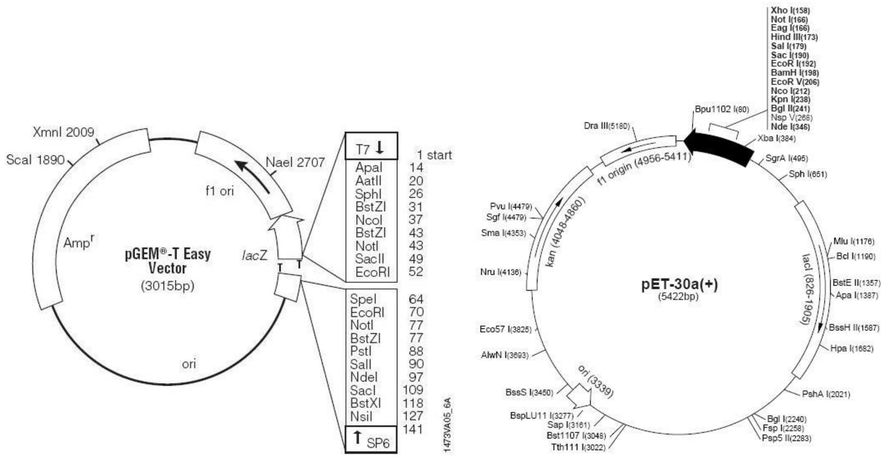
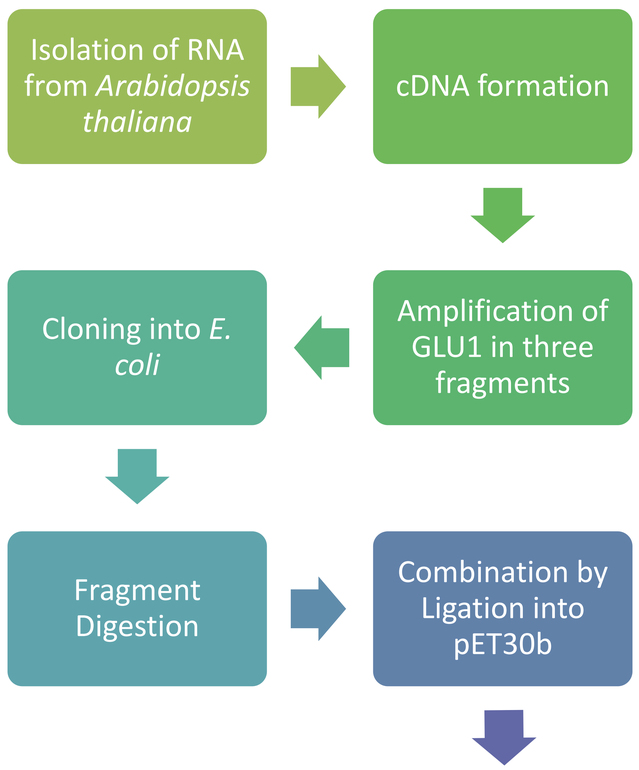



**Figure 1a.** pGEM-T Easy Vector. Includes a lac-operon sequence that allows for blue/white screening of colonies. LacZ codes for part of a β-galactosidase tetramer, while the competent cells code for another portion. When both are ligated together, active β-galactosidase is formed. β-galactosidase digests Xgal, and additive on the agar plate, and causes the bacteria to grow blue. If an insertion has been properly made, the two gene fragments cannot form β-galactosidase, and the colony grows white.

**Figure 1b.** pET-30 vector. Includes an inducible T7 promoter which allows for an inducible high expression of the inserted GLU1 gene.

## Experimental Overview




## Methods

The first step was to isolate the mRNA from *Arabidopsis thaliana* to make cDNA by flash freezing young Arabidopsis leaves, grinding them into a powder, and using the Qiagen RNeasy kit to extract RNA from the powder. An Omniscript RT Kit was used to make cDNA from the RNA template. The cDNA was used in three PCR reactions. Because the GLU1 gene is long (5 kbp), three sets of primers were designed to break the gene into fragments, the first 1.2 kbp long, the second 2.1 kbp long, and the third 2.0 kbp long. The PCR fragments were then gel purified, and treated with Taq polymerase to add an Adenine tail that aids the uptake of the fragment into pGEM vector (Figure 1a). Each fragment was then ligated into the pGEM vector and transformed into *E. coli*, and a sample of each underwent colony PCR and gel purification. The fragments were sequenced to ensure the correct transformation had taken place. The three contructs will be digested and ligated together into a pET30 vector (Figure 1b).

## Results

Cloning the fragments into *E. coli* proved to be more challenging than anticipated. Initially, the first and final gene fragments were successfully cloned into the pGem vector in *E. coli* cells (see Figure 2a & b).
The middle section, which was also the largest fragment at 2.1 kbp, was much more challenging to insert. A variety of adaptations were made to enhance the insertion of the vector into the competent cells, including adjusting ligation temperature and duration, insert:vector ratios, brands of competent cells, and antibiotics used in plating.

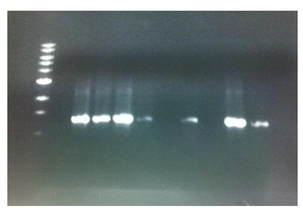


**Figure 2a.** The first GLU1 gene fragment. Seven of 12 samples had proper fragment length of about 1 kbp.

**Figure 2b.** The third segment colony PCR gel. Only one of the 14 white colonies contained the proper sized segment (far right) at 2 kbp.

## Future Experiments

Once all of the fragments have been successfully cloned into pGEM T easy vector, the three fragments will be digested with restriction enzymes and ligated together in pET30b to reform the entire GLU1 gene. GLU1 will then be transformed into a Rosetta strain , a special strain of *E. coli* that enhances gene expression of a protein. The expressed fd-GOGAT will then be isolated from the bacteria and used in various assays with SHM1 to further characterize the physical relationship between the two proteins.

## Acknowledgements

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## Literature cited

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