



Cloning and Expression of GST-Tagged *Drosophila* Gid8 Protein

Vanessa He

Princeton High School, Princeton, NJ, USA

Abstract

The cloning and expression of specific proteins are classical approaches for biochemical characterization of protein function and regulation. This project aims to clone, express, and purify Gid8 protein, a conserved subunit of the GID (Glucose-Induced Degradation-deficient) complex, in *Drosophila Melanogaster*. Although Gid8 is highly conserved in eukaryotes, its molecular functions remain poorly understood. The Gid8 coding sequence was amplified by Polymerase Chain Reaction (PCR) and inserted into the pGEX4T1 plasmid vector to generate a construct encoding Glutathione S-Transferase (GST)-Gid8 fusion proteins. The recombinant plasmid was subsequently transformed into *E. coli* Rosetta cells, and protein expression was induced by the addition of isopropyl beta-D-1-thiogalactopyranoside (IPTG). The GST-tagged Gid8 protein was then isolated using glutathione agarose affinity resin and analyzed by SDS-PAGE with Coomassie staining and Western blotting. These experiments demonstrated the successful cloning, expression, affinity purification, and detection of GST-Gid8, providing a foundation for future biochemical analyses of Gid8 and the GID complex in *Drosophila*.

Keywords: Gid8, GST fusion protein, pGEX4T1, recombinant protein expression, SDS-PAGE, Coomassie staining, Western blot

Introduction

Proteins are essential in our cells: they perform diverse functions that include tissue maintenance and repair, metabolic regulation, and intracellular signaling. One fundamental approach to study the function of a specific protein is through cloning and expressing its corresponding gene. By inserting a gene of interest into a bacterial expression vector, recombinant protein can be produced in large quantities in bacterial cells. To isolate the target protein from other bacterial proteins, affinity tags are commonly utilized. Glutathione S-transferase (GST) is a widely used affinity tag for recombinant protein purification. When a target protein is fused to GST, the fusion protein can bind specifically to glutathione-conjugated resin, allowing it to be separated from many other bacterial proteins and purified for subsequent biochemical analyses¹. The expression of the fusion protein can then be validated by Western blotting using an anti-GST antibody.

In this study, *Drosophila melanogaster* was chosen as the model organism because of its short life cycle, well-characterized genetic system, and extensive conservation of genes and biological pathways with humans. Approximately 60% of human genes have conserved counterparts in *Drosophila*, including 75% of human disease-causing genes^{2,3} and 90% of genes implicated in human cancer³. Among the conserved pathways is the Glucose-Induced Degradation-deficient (GID) complex, a multiprotein E3 ubiquitin ligase that recognizes specific substrate proteins and targets them for degradation through the ubiquitin-proteasome system⁴. For the GID complex to work, the ubiquitin system involves three major enzymes: E1 enzyme activates floating ubiquitin molecules by priming it, E1 then passes the activated ubiquitin to E2 carrier enzyme, and E3 ubiquitin ligases holds the specific substrate protein and transfers the ubiquitin from E2 to the target. When a specific protein needs to be destroyed, the GID complex attaches polyubiquitin chains for recognition and degradation by the proteasome. The proteasome subsequently degrades these proteins into smaller peptides while releasing ubiquitin for reuse.

The focus of this study is *Gid8*, a conserved subunit of the GID complex. *Gid8* is thought to bridge *Gid1* subunit and the LisH domain-containing subunit⁵. Linking these subunits to one another allows for the complex to function properly. However, the molecular functions and regulation of *Gid8* remain poorly understood. Producing GST-tagged *Gid8* provides a valuable tool for determining its biochemical properties, protein-protein interactions, and functional contributions to the GID complex. Therefore, the goal of this project was to clone the *Drosophila* *Gid8* gene into the pGEX4T1 expression vector, express the GST-*Gid8* fusion protein in bacterial cells, purify it using glutathione agarose affinity resin, and confirm its expression and purification by Coomassie-stained SDS-PAGE and Western blotting. This work establishes a foundation for future biochemical studies to elucidate the molecular functions of *Gid8* and the GID complex in *Drosophila*.

Materials and Methods

Gene Cloning

The *Gid8* DNA template was obtained from *Drosophila* Genomics Resource Center (DGRC). Gene-specific primers were designed to amplify the coding sequence of *Gid8*. To facilitate the cloning into GST expression vector pGEX4T1^{6,7} (**Figure 1**), restriction enzyme sequences were designed at the 5' ends of the primers. Specifically, a *Bam*HI site was added to the forward primer and a *Not*I site to the reverse primer, allowing the amplified *Gid8* fragment to be inserted into the vector in the correct orientation. The expected PCR product was approximately 678 bp. PCR products were analyzed by agarose gel electrophoresis to verify the size of the target fragment before downstream cloning procedures.

Figure 1. pGEX4T1 vector map

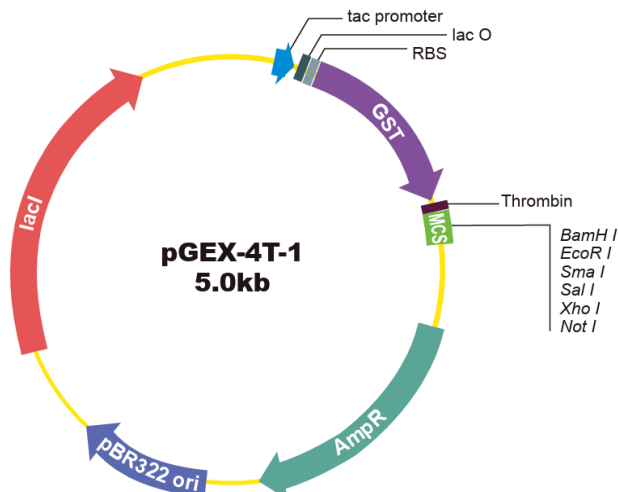


Table 1. Primer sequences used for amplification of *Gid8* and cloning into expression vector

Gene	Primer	Sequence
Gid8	Gid8-BamHI-5'	ATATGGATCCATGAGCTACAACGAGAAATC
Gid8	Gid8-NotI-3'	TTAAGCGGCCGCTTCTTGGGCTCCAGGTGCG

Protein Expression

Following successful cloning of the *Gid8* coding sequence into the pGEX4T1 vector, the recombinant plasmids were transformed into competent *E. coli* Rosetta cells. Transformed cells were cultured in the presence of ampicillin to select bacteria carrying the recombinant plasmids. Protein expression was induced with the addition of 0.4 mM IPTG. To optimize protein yield, induction was performed under two conditions: overnight incubation at 22°C or incubation at 30°C for 4 hours.

Table 2. Protein expression conditions

Parameter	Condition
Host strain	Rosetta
Antibiotic selection	Ampicillin
Growth temperature before induction	37°C
Induction condition	0.4 mM IPTG; 22°C, overnight or 30°C, 4 hours

Protein Purification

Following induction, bacterial cultures were harvested by centrifugation, and bacterial pellets were lysed by sonication. The lysates were incubated with glutathione agarose resin to capture the GST-tagged Gid8 fusion protein. The resin was washed with a GST wash buffer to remove nonspecifically bound proteins. In this experiment, the purified proteins were not eluted with free glutathione; instead, SDS sample loading buffer was added directly to the resin to release the bound proteins for subsequent SDS-PAGE gel analysis.

Table 3. Purification materials and workflow elements.

Step	Material or method
Lysis	Sonication
Affinity resin	Glutathione agarose
Wash buffers	GST Wash I and GST Wash II
Elution or sample release	SDS loading buffer directly from beads

SDS-PAGE Analysis

Protein samples collected during the expression and purification procedures were separated by SDS-PAGE. A prestained protein molecular weight ladder was included to estimate molecular weight. The gel was stained with Coomassie Blue to visualize total protein and assess the expression of the GST fusion proteins.

Western Blot Analysis

Protein samples separated by SDS-PAGE were transferred to a membrane for Western blot analysis. The membrane was incubated with a rabbit anti-GST primary antibody, followed by the anti-rabbit secondary antibody. GST-tagged Gid8 was detected as a band at the expected molecular weights of approximately 54 kDa, corresponding to the combined molecular mass of GST tag and Gid8 protein.

Experimental Results

The overall experimental procedures used for cloning, expression, and purification of GST-Gid8 is summarized in **Figure 2**.

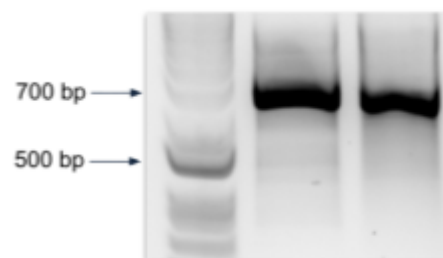
Figure 2. Experimental overview



PCR Amplification and Cloning

PCR amplification of the *Gid8* coding sequence was performed with an initial denaturation step at 98°C for 1 min, followed by 35 cycles of denaturation at 98°C for 10 seconds, annealing at 65°C for 15 seconds, and extension at 72°C for 30 seconds. Agarose gel electrophoresis revealed a PCR product of the expected size, approximately 678 bp for *Gid8*, as shown in **Figure 3**.

Figure 3. PCR Amplification of *Gid8* Gene



This DNA fragment was then digested with BamHI and NotI. The pGEX4T1 vector was digested with BamHI and NotI and treated with Calf Intestinal Alkaline Phosphatase (CIP1) enzymes to reduce vector self-ligation. The digested *Gid8* fragment was then ligated into the digested pGEX4T1 vector. After ligation, the recombinant plasmids were transformed into competent *E. coli* Rosetta cells. Transformants were selected on ampicillin-containing agar plates. Successful cloning was confirmed by selecting bacterial colonies, culturing the bacteria, purifying the plasmids, and performing restriction enzyme digestion. The resulting DNA fragments were consistent with the expected sizes of the vector and *Gid8* insert, confirming successful insertion of the *Gid8* coding sequence into pGEX4T1.

Expression of recombinant GST-*Gid8*

Following IPTG induction, recombinant protein expression was evaluated by SDS-PAGE and Coomassie staining. As shown in **Figure 4**, IPTG-induced samples showed an increase in the intensity of a protein band at the expected molecular weight of GST-*Gid8* compared with uninduced controls.

Purification using glutathione agarose resin

Bacterial cells were lysed by sonication, and insoluble cellular debris was removed by centrifugation. The clarified lysate was incubated with glutathione-Sepharose beads (Thermo Fisher: #16100) to capture the GST-tagged *Gid8* fusion proteins. The resin was then collected by centrifugation and washed with GST Wash Buffer I and GST Wash Buffer II to remove nonspecifically bound proteins and other contaminants. Finally, the GST-bound proteins were maintained in a storage buffer for subsequent analysis or purification.

Figure 4. Induction and Detection of GST-GID8 Protein by Coomassie Staining

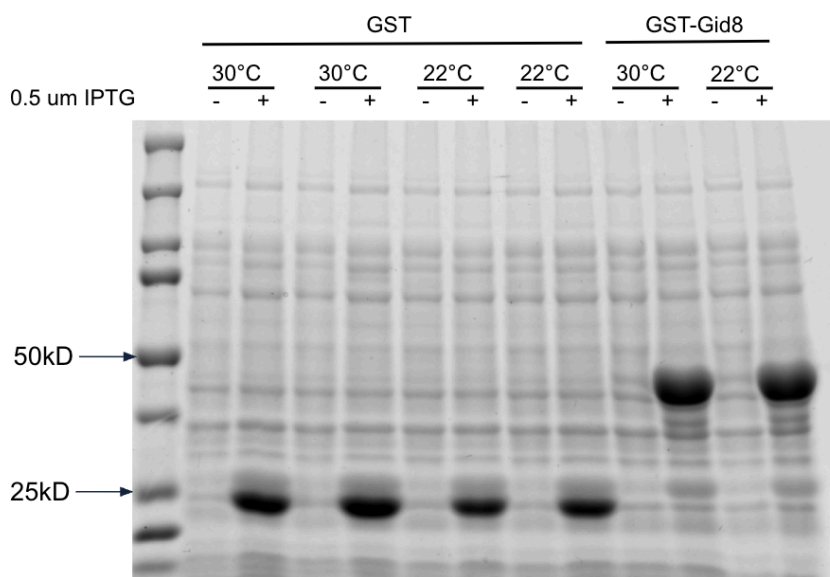


Table 4. Components of solutions (per 1L)

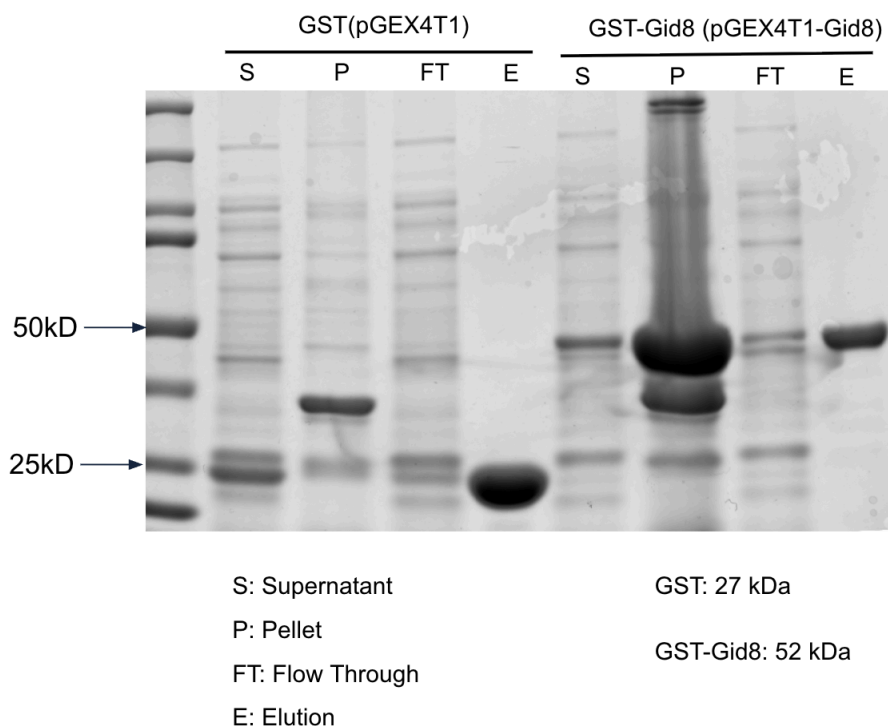
Components	GST Extraction buffer	GST Wash I	GST Wash II
1M HEPES (pH=7.6)	20 mL (20 mM)	20 mL (20 mM)	20 mL (20 mM)
5M NaCl	100 mL (0.5M)	200 mL (1.0M)	0
2M KCl	0	0	50 mL (100mM)
0.5M EDTA	1 mL (0.5 μ M)	1 mL (0.5 μ M)	200 μ L (0.1 μ M)
Glycerol	100 mL (10%)	100 mL (10%)	100 mL (10%)
NP-40	5 mL (0.5%)	10 mL (1%)	0.2 mL (0.02%)

Coomassie Staining

Protein samples from supernatant, pellet, flow-through, and elution fractions were analyzed by SDS-PAGE followed by Coomassie Blue staining. GST has an expected molecular weight of approximately 26 kDa, and Gid8 is around 28 kDa; therefore, the expected molecular weight of the GST-Gid8 fusion protein is approximately 54 kDa. For sample sets that came from cells with GST vector expression, a band of smaller molecular weight at 26 KD can be detected in the supernatant and elution buffers which is consistent with the size of GST. Similarly, a band at ~50 kD can be detected in the pellet and elution sample; a faint band can be seen in the supernatant, but cannot be seen in the flow through. This indicates that most of the Gid8-GST

was pulled down by the resin in the pellet, only a small portion of it was still retained in the supernatant. Gid8-GST was not released into flowthrough during washing steps, but was released into an elution buffer. Coomassie-stained SDS-PAGE provided visual evidence of GST-Gid8 expression and enrichment and allowed comparison between protein profiles among the different fractions collected during purification.

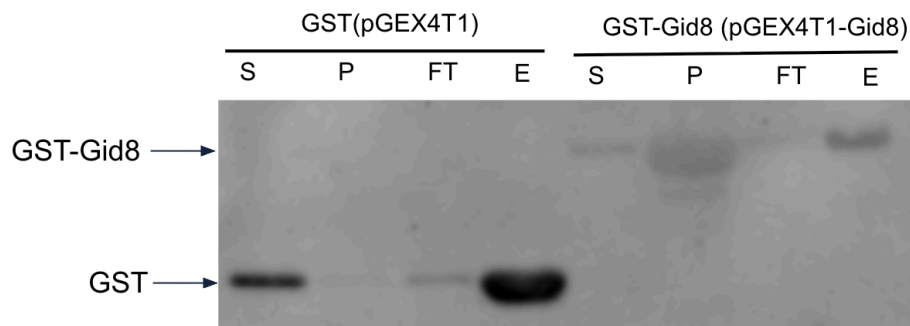
Figure 5. Purification and Detection of GST-GID8 Protein by Coomassie Staining



Western Blot Confirmation

Western blot analysis was performed using an anti-GST antibody to confirm the GST-tagged recombinant protein. The antibody detected a GST-reactive protein band at ~54kDa, consistent with the expected molecular weight of the GST-Gid8 fusion protein. This result supported the conclusion that the protein enriched during affinity purification was the intended recombinant fusion protein.

Figure 6. Purification and detection of GST-GID8 protein by Western Blot



Discussion

This project succeeded in cloning, expressing, and purifying *D. melanogaster* Gid8 as a GST-tagged recombinant protein. The initial ~678 bp PCR product validated the success in amplifying the gene *Gid8* in *Drosophila melanogaster*; an ~52-54 kDa GST-tagged fusion protein band appeared strong on Coomassie-stained SDS-PAGE under induced conditions; and the target protein was verified via anti-GST Western blotting. By providing a reliable protocol in isolating high-purity Gid8, this project provides a foundational framework in future studies of Gid8, enabling future manipulation, structural modeling, and downstream assays to deepen the understanding of the protein's interactions in the GID E3 ubiquitin ligase networks.

The experimental results across each stage of the workflow confirmed the successful expression and affinity enrichment of the fusion protein. After ligation into pGEX4T1 and IPTG induction, Coomassie-stained SDS-PAGE showed strong expression of the target protein at both 22°C and 30°C (Figure 4), proving the efficacy of pGEX4t1 expression in Rosetta host cells. This was seen in the prominent band marked at the expected weight of ~52-54 kDa. Since the control unfused GST is ~26 kDa, Gid8 must have a length of ~26 to yield the total GST-Gid weight of ~52-54 kDa. However, a second Coomassie staining of downstream purification fractions revealed significant variations (Figure 5). A substantial portion of GST-Gid8 was present in the insoluble pellet fraction instead of the remaining soluble. This showed that the resin had high affinity to GST, and it pulled down GST-Gid8 as an insoluble pellet after incubation. There was also the unexpected presence of bands near ~26 kDa across the fractions which suggests that the bridge connecting GST tag with Gid8 was exposed for a small fraction of the recombinant GST-Gid8 proteins and broke during cell lysis, resulting in a free GST tag floating. Additionally, a fraction of the remaining protein was detected in the supernatant, indicating the incomplete adsorption of glutathione Sepharose resin. Subsequent anti-GST Western blot analysis confirmed that the eluted band was at ~52 kDa and was the

GST-tagged Gid8 fusion protein, demonstrating successful expression and purification of fusion Gid8 proteins

Despite encouraging outcomes, many technical and methodological limitations were identified during the experiments. First, the agarose gel analysis showed a relatively faint DNA ladder signal, which in turn limited the absolute certainty of molecular weight quantification of the Gid8 protein. Second, while the restriction enzyme digestion successfully integrated cloning into pGEX4T1, sequencing was not performed to confirm the complete insertion sequence. Consequently, there could be potential point mutations that were introduced during PCR amplification that can't be ruled out. Third, during purification, there was a significant amount of GST-Gid8 that was absorbed into the bacterial pellet fraction. This can be further improved by optimizing the incubation conditions. Lastly, because this study focused primarily on cloning and isolating, the biological activity and binding ability of the purified recombinant protein were not directly tested through functional assays.

To strengthen and further mitigate these shortcomings, future research directions should be pursued. First, a Sanger sequencing should be carried out to check for mutations in the final protein elution. A Bradford or BCA assay should also be performed to accurately determine the specific concentration of Gid8 as the gel can only reveal its presence and approximate size. To optimize protein solubility and eliminate variations in the purified fractions. Finally, since this project stopped after the fusion of GST and Gid8, the next step is to further isolate Gid8 in order to study the protein's behavior on its own. Within the pGEX4T1 vector, there lies specific amino acids between the GST and Gid8. This allows the utilization of thrombin to recognize that specific sequence and cut it. This ultimate step will allow the study of Gid8 in nature. As a result, the pure protein can be used in numerous experiments in deepening the understanding of its interactions with its neighboring proteins (like Gid4 and Gid1) in the GID complex. Furthermore, it can be used to identify novel Gid8-interacting proteins using immunoprecipitation followed by mass-spectrometry analysis.

Conclusion

The *Gid8* coding sequence was successfully cloned into the pGEX4T1 GST expression vector and expressed in bacterial cells. GST affinity purification enriched the recombinant GST-Gid8 fusion protein, and Coomassie staining and anti-GST Western blotting confirmed the presence of the expected protein. These results establish a foundation for future functional studies to elucidate the molecular functions of Gid8.

Thanks to Dr. Mengmeng Liu and Dr. Jun-Yuan Ji from the Department of Biochemistry and Molecular Biology at the Tulane University School of Medicine in project design, experimental work, and manuscript preparation.

References

1. Smith, D. B., and Johnson, K. S. (1988) Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. **Gene** **67**, 31–40.
2. Banerjee, S., Benji, S., Liberow, S., and Steinhauer, J. (2020) Using *Drosophila melanogaster* To Discover Human Disease Genes: An Educational Primer for Use with “Amyotrophic Lateral Sclerosis Modifiers in *Drosophila* Reveal the Phospholipase D Pathway as a Potential Therapeutic Target”. **Genetics** **216**, 633–641.
3. Fortini, M. E., Skupski, M. P., Boguski, M. S., and Hariharan, I. K. (2000) A Survey of Human Disease Gene Counterparts in the *Drosophila* Genome. **J. Cell Biol.** **150**, 23–30.
4. Maitland, M. E., Finch, J. N., and Melnycky, M. J. (2022) Structural and Functional Insights into GID/CTLH E3 Ligase Complexes. *Int. J. Mol. Sci.* **23**, 5863.
5. Sherpa, D., Chrustowicz, J., and Schulman, B. A. (2025) The many faces of the GID/CTLH E3 ligase complex. **Biochem. Soc. Trans.** **53**, 1351–1362.
6. Smith, D. B., and Johnson, K. S. (1988) Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. **Gene** **67**, 31–40.
7. <https://www.genscript.com/gsfiles/vector%2dmap/bacteria/pGEX%2d4T%2d1%2epdf?=20231215?1768664770>