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# Understanding Protein Trap – A *Drosophila* Model

## Introduction

Drosophila melanogaster, also known as the fruit fly, has been widely used as a genetic model organism for over a century. One unique feature of Drosophila genetics is the convention of naming genes based on their phenotypic effects. For example, the "white" mutation causes white eyes(Morgan 1910), and the "groucho" mutation causes bushy eyebrows above the eyes, reminiscent of the comedian Groucho Marx(Lindsley et al. 1992). Other examples of genes with entertaining names include "tribbles," "wingless," "Notch," and "hedgehog." (Jennings 2011). Many Drosophila genes have subsequently been identified in other organisms, and the fly names have been adopted or adapted. However, if a related gene has been characterized in other organisms before a mutant allele is isolated in Drosophila, the established name is adopted. All Drosophila genes have been given a "CG number" during genome annotation, and there are still many uncharacterized genes with no known mutations or orthologs (Tweedie et al. 2009).

RNA interference (RNAi) is a technique used to reduce the expression of individual genes in Drosophila cells by expressing double-stranded RNA corresponding to that gene's sequence (Carthew 2001). The Vienna Drosophila RNAi Center maintains a collection of transgenic fly lines carrying an inducible UAS-RNAi construct against a single protein-coding gene. This collection currently includes over 22,000 transgenic fly lines, which provide knockdowns for over 88% of Drosophila genes (Dietzl et al. 2007). These transgenic RNAi constructs can be driven in a tissue-specific manner using the "GAL4 system," which provides a simple yet powerful strategy to study the role of individual genes in diverse biological processes (Brand and Perrimon 1993). Other resources include collections of transgenic lines that allow inducible overexpression of individual genes and transgenic lines expressing green fluorescent protein (GFP) or β-galactosidase (LacZ) in specific tissues or in the pattern of specific genes. These resources have greatly facilitated the

study of gene function in Drosophila and have contributed to our understanding of numerous biological processes (Tweedie et al. 2009).

Over the course of the past six weeks, I have conducted research involving six stocks with the aim of characterizing the protein trap and investigating the characteristics of these genes in Drosophila wing development. This work has provided me with invaluable insights into the fluorescence expression and characterization of these tagged genes in the wing discs and other discs of the larvae. Through the course of this research, I have gained a deeper understanding of the effects of gene mutation and the importance of these conserved genes. This experience has been highly informative and has significantly enhanced my knowledge and understanding of this area of research.

**Methodology**

Freshly made solutions:

1. 1X PBS (pH 7.4)
2. 25% glucose solution

I conducted protein trap studies using transgenic larvae expressing several proteins tagged GFP (Table 1). I collected ten third instar larvae of each and submerged them in ice-cold 1X PBS solution. Using no. 5 Dumont forceps, I dissected each larva and inverted them inside-out, following the dissection protocol outlined in (Srivastava 2013). I carefully extracted individual larval tissues - wing discs, salivary gland, Alimentary canal, and fat body, and mounted them as described in the slide. Finally, I observed the mounted slides under a Confocal microscope to visualize GFP expression in the aforementioned tissues.

**Result**

**Stock #80113**

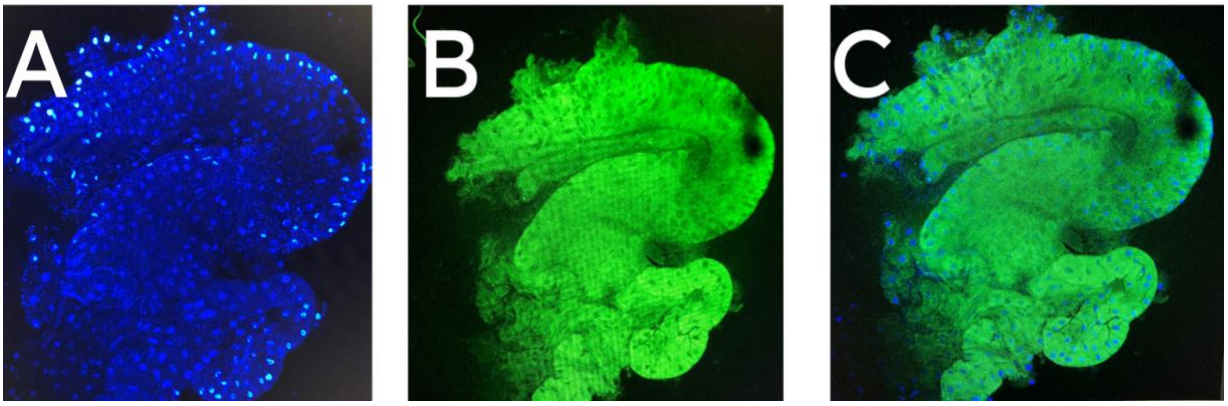



**Figure 1: Expression pattern of EGFP-TBH in the salivary gland of *Drosophila melanogaster*. A–C.** Expression of GFP-tagged TBH in salivary gland of third instar larvae. Green channel represents expression of GFP while blue channel represents nucleus stained with DAPI.

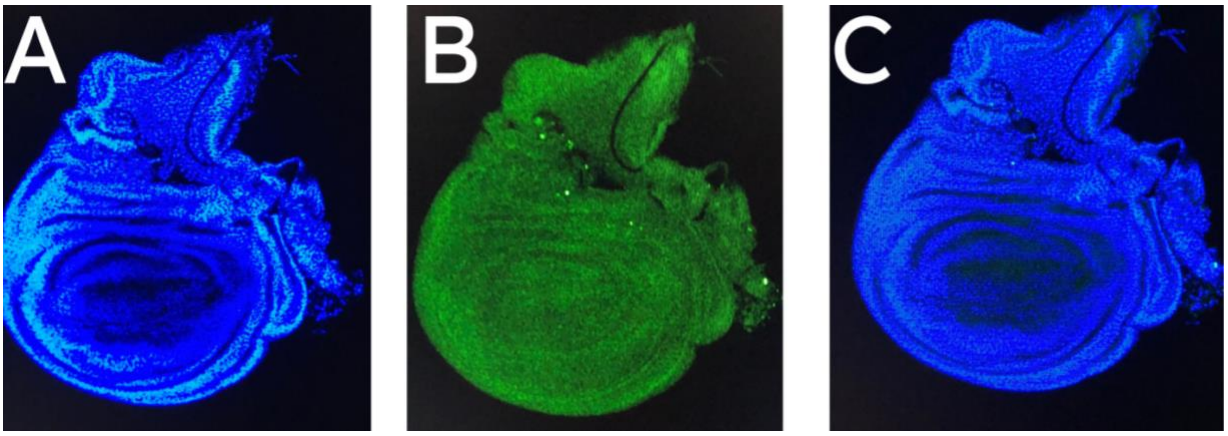


**Figure 2: Expression pattern of EGFP-TBH in the Wing Disc of *Drosophila melanogaster*. A–C.** Expression of GFP-tagged TBH in wing disc of third instar larvae. Green channel represents expression of GFP while blue channel represents nucleus stained with DAPI.

**Stock #80114**

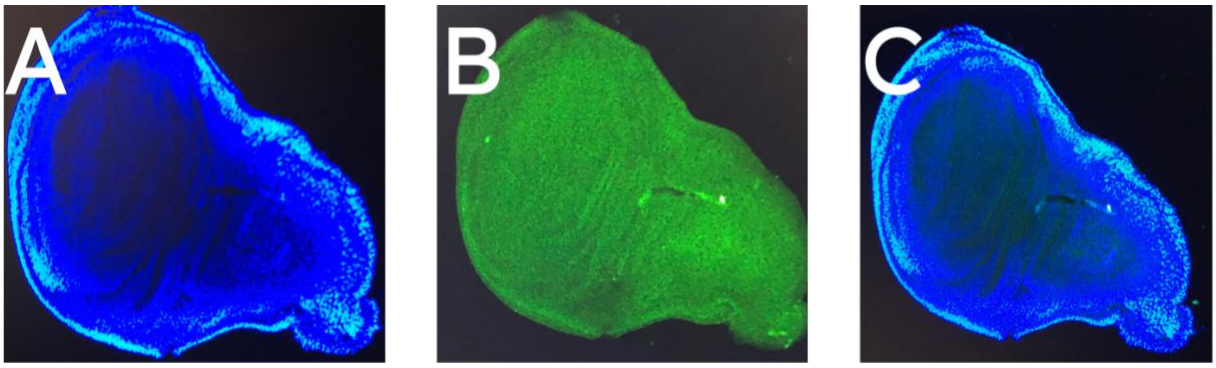



**Figure 3: Expression pattern of EGFP- CG17841 in the Wing Disc of *Drosophila melanogaster*. A–C.** Expression of GFP-tagged CG17841in wing disc of third instar larvae. Green channel represents expression of GFP while blue channel represents nucleus stained with DAPI.

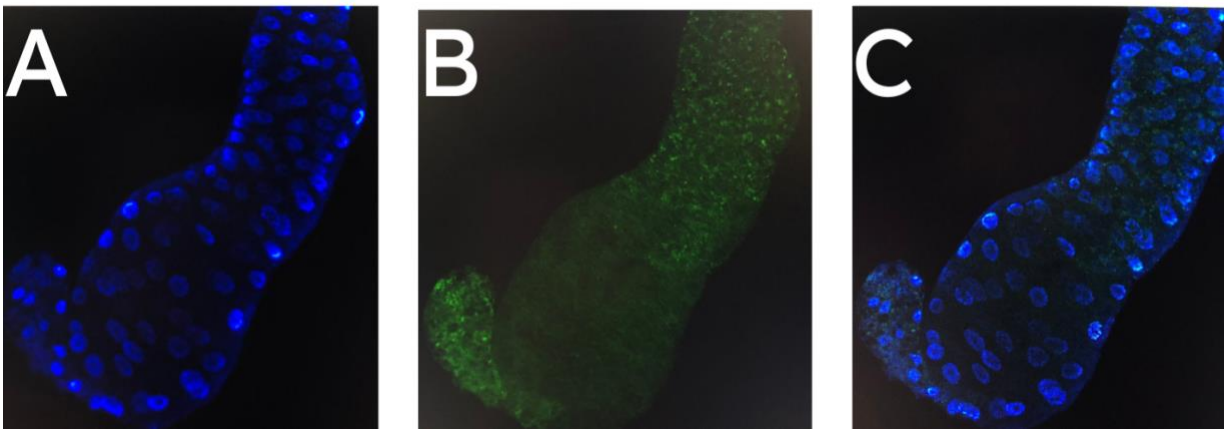



**Figure 4: Expression pattern of EGFP- CG17841 in the Alimentary Canal of *Drosophila melanogaster*. A–C.** Expression of GFP-tagged CG17841in alimentary canal of third instar

larvae. Green channel represents expression of GFP while blue channel represents nucleus stained with DAPI.

**Stock #80115**

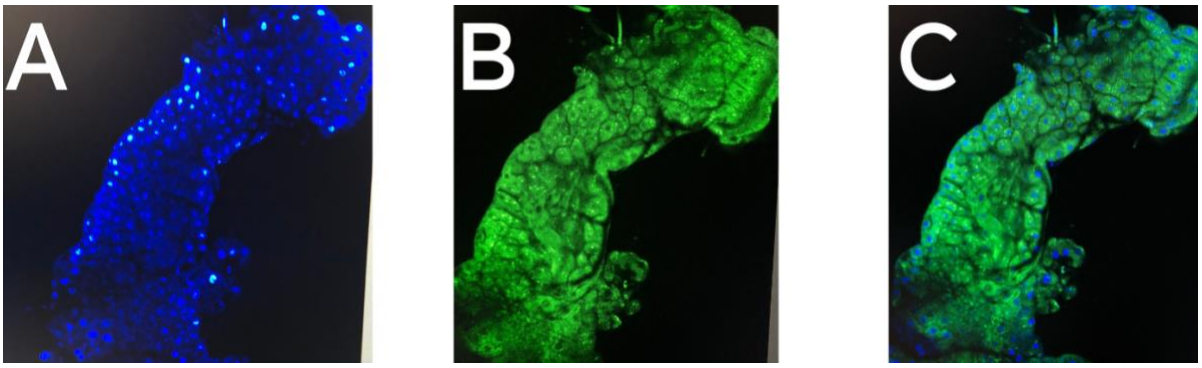



**Figure 5: Expression pattern of EGFP- CG6293 in the Alimentary Canal of** *Drosophila melanogaste***r. A–C.** Expression of GFP-tagged CG6293 in alimentary canal of third instar larvae. Green channel represents expression of GFP while blue channel represents nucleus stained with DAPI.

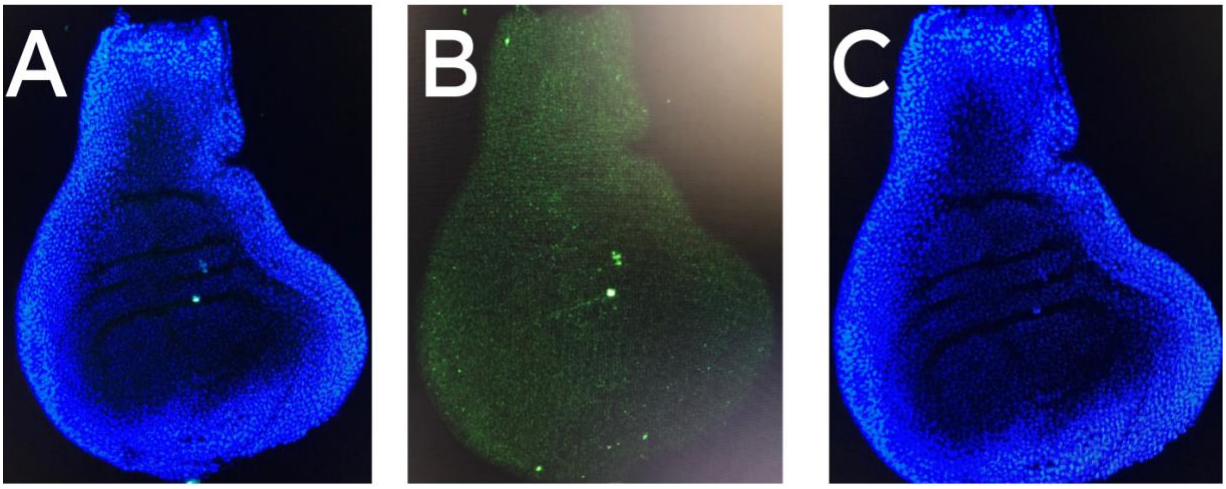


**Figure 6: Expression pattern of EGFP- CG6293 in the Wing Disc of** *Drosophila melanogaster***. A–C.** Expression of GFP-tagged CG6293 in wing disc of third instar larvae. Green channel represents expression of GFP while blue channel represents nucleus stained with DAPI.

**Stock #80117**

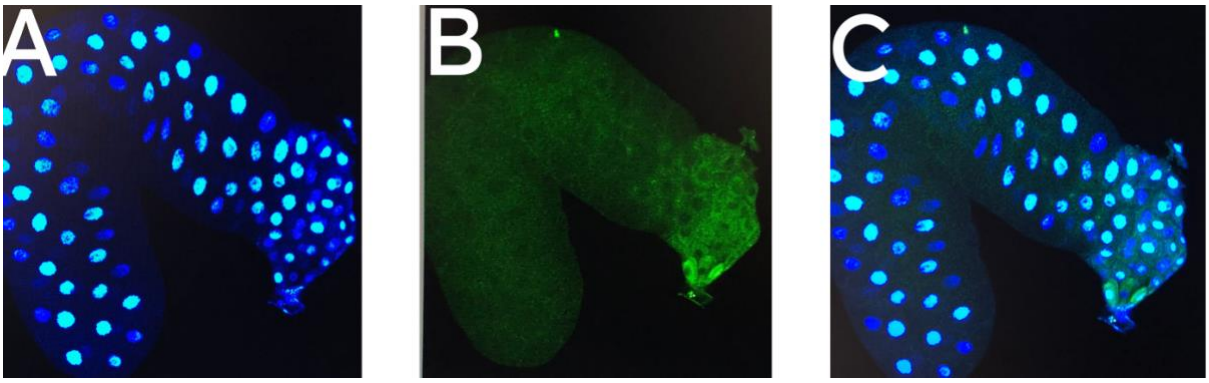



**Figure 7: Expression pattern of EGFP- trp-1 in the Alimentary Canal of** *Drosophila melanogaste***r. A–C.** Expression of GFP-tagged trp-1 in alimentary canal of third instar larvae. Green channel represents expression of GFP while blue channel represents nucleus stained with DAPI.

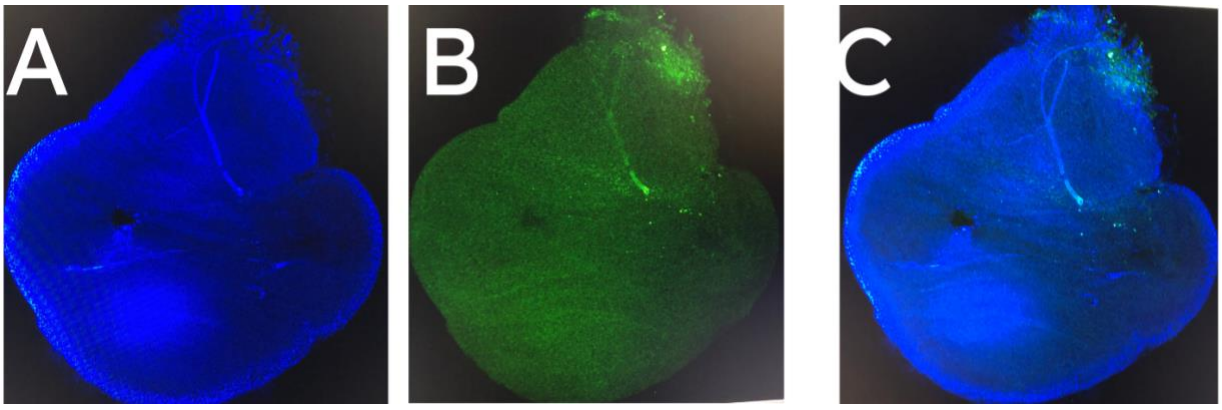



**Figure 8: Expression pattern of EGFP- trp-1 in the Wing Disc of** *Drosophila melanogaster***. A–C.** Expression of GFP-tagged trp-1 in wing disc of third instar larvae. Green channel represents expression of GFP while blue channel represents nucleus stained with DAPI

**Stock #81126**

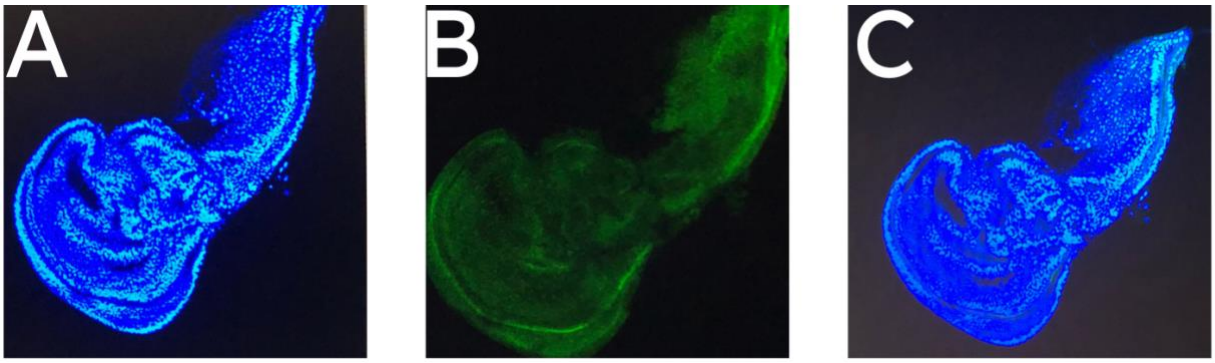



**Figure 9: Expression pattern of EGFP- Kibra in the Wing Disc of** *Drosophila melanogaste**r***.
**A–C.** Expression of GFP-tagged Kibra in wing disc of third instar larvae. Green channel represents expression of GFP while blue channel represents nucleus stained with DAPI.

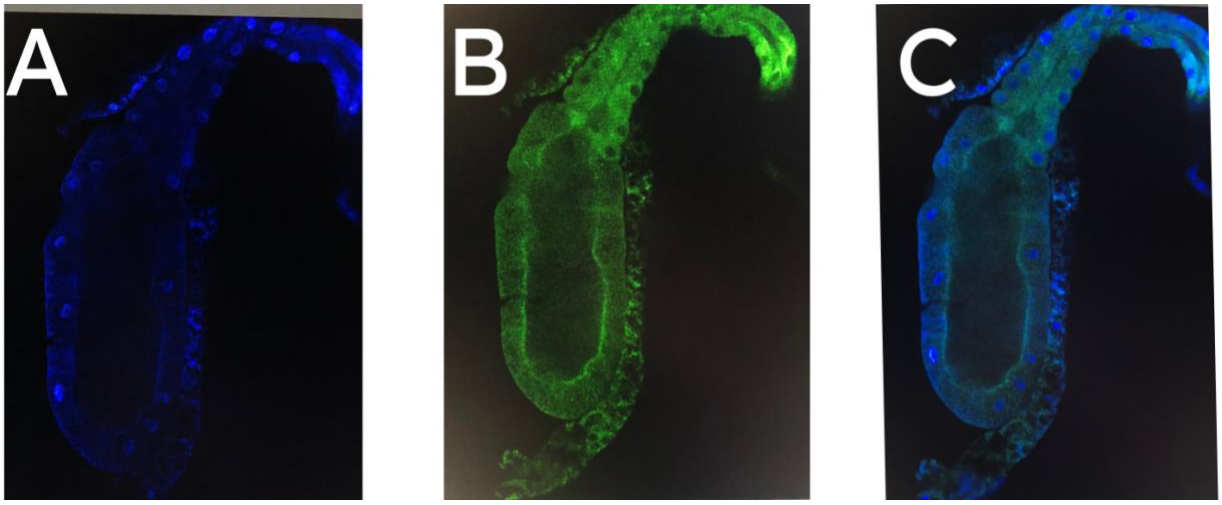


**Figure 10: Expression pattern of EGFP- CG6293 in the Alimentary Canal of** *Drosophila melanogaster***. A–C.** Expression of GFP-tagged Kibra in alimentary canal of third instar larvae. Green channel represents expression of GFP while blue channel represents nucleus stained with DAPI.

**Stock #81127**

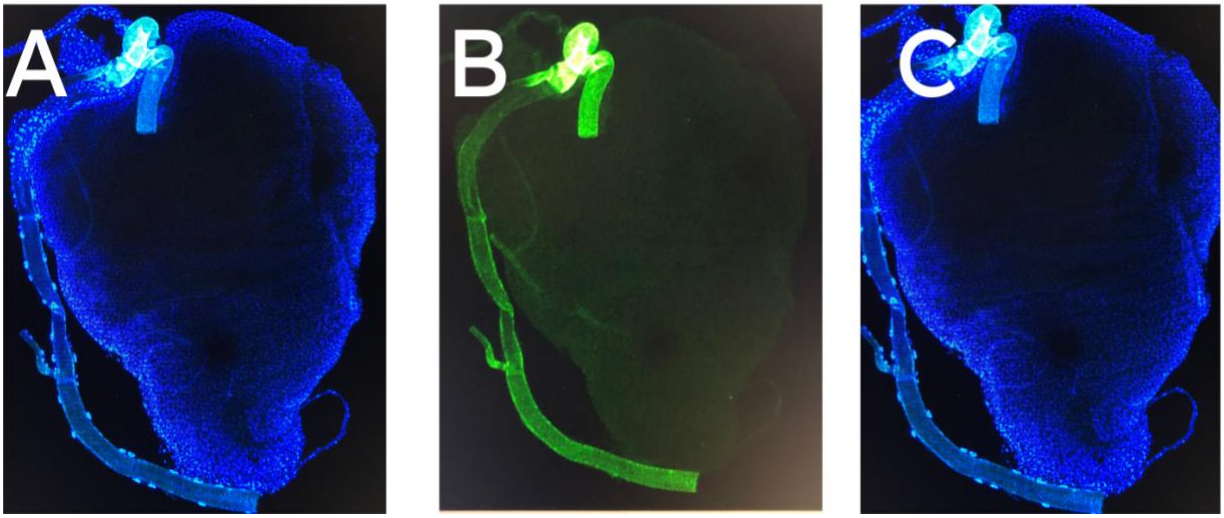



**Figure 11: Expression pattern of EGFP- 5-HT2B in the Wing Disc of** *Drosophila melanogaste***r. A–C.** Expression of GFP-tagged 5-HT2B in wing disc of third instar larvae. Green channel represents expression of GFP while blue channel represents nucleus stained with DAPI.

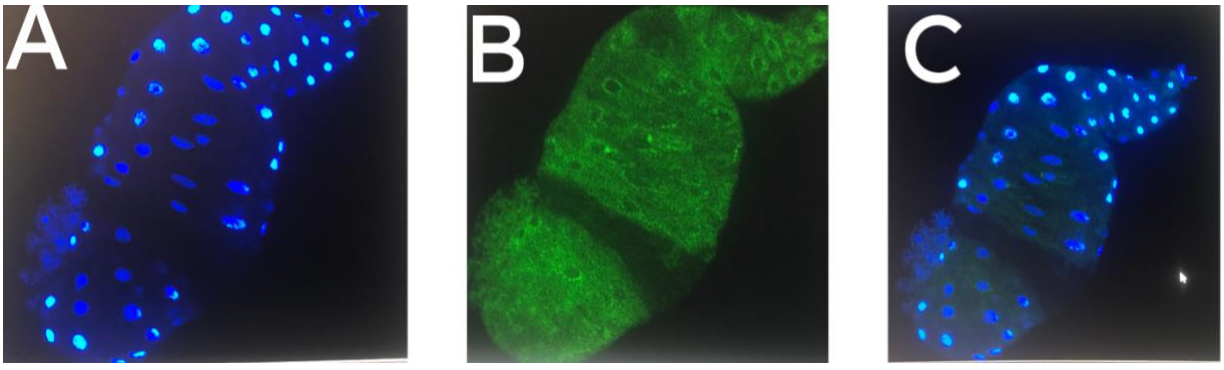


**Figure 12: Expression pattern of EGFP- 5-HT2B in the Alimentary Canal of** *Drosophila melanogaster***. A–C.** Expression of GFP-tagged 5-HT2B in alimentary canal of third instar larvae. Green channel represents expression of GFP while blue channel represents nucleus stained with DAPI

**Discussion**

From the result above. The expression of the tagged gene can be visualized. There are other mutations that are associated to individual genes and thes are briefly summarized in the table 1 and 2.

| Stock Number | 80113 | 80114 | 80115 |
|---|---|---|---|
| Gene Tagged | Tbh (Tyramine β hydroxylase) tagged with EGFP | CG17841 tagged with EGFP | CG6293 |
| Associated Gene | 1. Dmel\w<br>2. Dmel\y<br>3. Tbh | 1. Dmel\w<br>2. Dmel\y<br>3. Dmel\CG17841 | 1. Dmel\Kr (Kruppel)<br>2. Dmel\wg (wingless)<br>3. Dmel\CG17841 |
| Types of Gene | Protein Coding Gene | Protein Coding Gene:<br>Predicted to be involved in lipid homeostasis | to be<br>involved in L-ascorbic acid transmembrane transport. |
| Protein Size | 670AA | 429 AA | 573 AA |
| Domain Conserved/ Human Othorlogs | Hsap\DBH<br>Hsap\MOXD1 | TLCD3A (TLC domain containing 3A)<br>TLCD3B (TLC domain containing 3B) | Orthologous to human SLC23A1 (solute carrier family 23 member 1)<br>SLC23A2 (solute carrier family 23 member 2) |
| Gene Functions | 1. Converts tyramine into octopamine,<br>a neurotransmitter, involved in ovulation and locomotion.<br><br>2. Functions in an amine-mediated Bacc-dependent signaling pathway that negatively regulates acute ethanol sensitivity | It is expressed in several structures, including adult head; embryonic/larval oenocyte; and gut section.<br><br>Human ortholog(s) of this gene implicated in cone-rod dystrophy. | It is a extracellular matrix structural constituent. |
| Mutations | Tβh-null flies lack octopamine but<br>can survive until adulthood. External appearance is normal, but female Tβh-null flies are unable to reproduce. Even though they mate, they are sterile because they retain fully developed eggs. | Unknown | Unknown |

**Table 1:** Summary of stock 80113, 80114 and 80115.

| Stock Number | 80117 | 81126 | 81127 |
|---|---|---|---|
| Gene Tagged | trp-l tagged with EGFP | kibra tagged with EGFP | 5-HT2B tagged with EGFP |
| Associated Gene | 1. Dmel\w<br>2. Dmel\y<br>3. Dmel\trpl | 1. Dmel\Sb<br>2. Dmel\ser<br>3. Dmel\TM3<br>4. Dmel\kibra | 1. Dmel\Tb<br>2. Dmel\y<br>3. Dmel\TM6B |
| Types of Gene | Predicted to be involved in L-ascorbic acid transmembrane transport. | Protein Coding Gene | Protein Coding Gene |
| Protein Size | 379 AA | 1288 AA | 617AA |
| Conserved/ Human Othorlogs | Hsap\TRPC4<br>Hsap\TRPC5 | Hsap\WWC1<br>Hsap\WWC2 | Hsap\HTR2A<br>Hsap\HTR2B |
| Gene Functions | Transient receptor potential-like (trpl) encodes a non-selective plasma membrane cation channel with modest Ca[2+] permeability. It is highly eye-enriched and contributes to the electrical response to light in photoreceptors. | 1. Regulator of the Hippo/SWH (Sav/Wts/Hpo) signaling pathway, a signaling pathway that plays a pivotal role in organ size control and tumor suppression by restricting proliferation and promoting apoptosis. | 1. They bind and transmit the signal from the neurotransmitter 5-HT (serotonin).<br><br>2. It also enables G protein-coupled serotonin receptor activity |
| Mutations | Flies with the trp-l mutation have impaired vision and reduced sensitivity to light, as the loss of TRP protein prevents the opening of the ion channels required for phototransduction | Overexpression of KIBRA leads to the stimulation of Yes-associated protein (YAP) phosphorylation, which is an effector of the Hippo pathway. On the other hand, when KIBRA is depleted using RNA interference, there is a | Elimination of the 5HT2 gene from the neurons leads to loss of sleep homeostasis. |

**Table 2:** Summary of stock 80117, 81126 and 81129.

## Discussion

Based on the results obtained, it appears that all stocks analyzed demonstrate some level of expression in the tissue studied, indicating that the trapped proteins are expressed ubiquitously throughout various Drosophila third instar larval tissues. Although no published research on the function of these proteins in wing development was found, it is evident from the mutations of these genes that they play a significant role in Drosophila development.

## Conclusion

Overall, this report highlights the importance of Drosophila melanogaster as a model organism in genetic research. The RNAi technique and protein trap studies have greatly facilitated the study of gene function and provided valuable insights into the effects of gene mutation. The research conducted using these techniques has contributed significantly to our understanding of numerous biological processes. Therefore, this review emphasizes the continued importance of Drosophila in genetic research and encourages further studies using these techniques.

## References

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