

# 1.1-fold expression difference in *spen*, a gene involved in neural development, between *D. Melanogaster* male and female heads

Vogel, M.<sup>1</sup>, Lee, J.<sup>1</sup>, Gong, S.<sup>2,3</sup>, Bozinovic K.<sup>2,3</sup>

<sup>1</sup>Torrey Pines High School, <sup>2</sup>Boz Life Science Research and Teaching Institute, <sup>3</sup>University of California San Diego Extension

## Summary

- Under unstressed conditions, is *spen* expressed differently between male and female *Drosophila melanogaster* (fruit flies)?
- qRT-PCR was performed on RNA extracted from male and female fruit fly heads.
- Results indicate a 1.1 fold higher expression of *spen* in male fruits flies than in females.

## Abstract

This study quantified the expression of *spen* in male and female fruit fly heads under controlled conditions. *spen* is a gene involved in guiding axon growth during the development of neurons. qRT-PCR results showed a 1.1 fold difference between tissue-specific expression of *spen* in male fly heads compared to female fly heads. This result indicates that there is no significant difference in expression of *spen* between male and female fly heads.

## Introduction

### Objective of the Experiment

Quantify expression differences in *spen* between male and female fruit flies' heads.

### Significance of project

Gender differences can be an important factor in developing new medicines and their proper use<sup>1</sup>. Flies are a useful model organism for human disease studies because about 75% of the genes related to human disease have homologs in flies<sup>2</sup>. We studied gene expression of *spen* in heads to better understand differences between males and females under unstressed conditions.

### Gene function and significance

#### *spen*

- Present in fruit flies with a homolog in humans
- Function: axon guidance during neural development
- Significance:
  - Involved in neural development, essential to forming neural pathways. The protein product guides the process of forming synapses.<sup>3</sup>

### Model organism

#### *Drosophila melanogaster*

Working with fruit flies facilitates the study due to their:

- Short life cycle
- Large number of offspring
- Distinct differences between male and female flies<sup>4</sup>



**Figure 1: Male fruit fly (*Drosophila melanogaster*)**  
Male-specific characteristics circled in red. Image taken with light microscope at 20X magnification.



**Figure 2: Female fruit fly (*Drosophila melanogaster*)**  
Female-specific characteristics circled in red. Image taken with light microscope at 20X magnification.

## Methodology

Fruit Fly Head Dissection

RNA Extraction

Bioinformatics

qRT-PCR

Data Analysis

## Materials and Methods

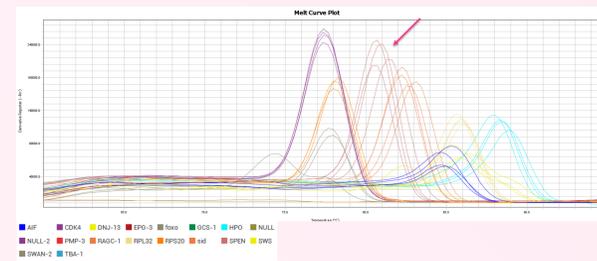
- RNA extraction completed with QIAGEN RNeasy Plus Mini Kit
- qRT-PCR primers designed with cDNA pulled from NCBI database Primer-BLAST program
- $\Delta\Delta Ct$  analysis on qRT-PCR data used to generate heatmap and bar graph
- Unpaired t-tests were performed to determine significance ( $p < 0.05$ )

## Materials and Methods

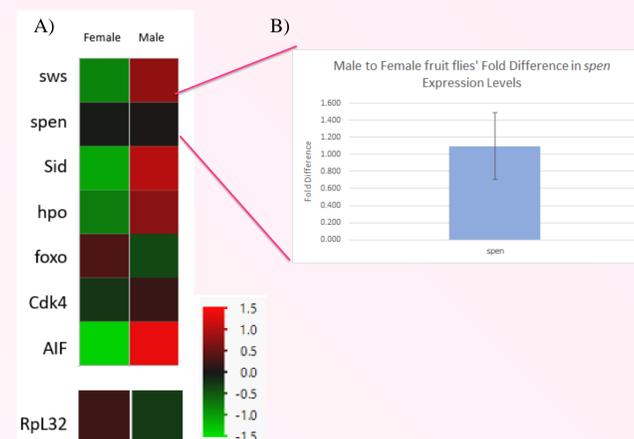
<i>spen</i>	RefSeq	Forward Primer [5' -> 3']	CTCAACGGTTCGCGTCGATAC
	NM_079979.5	Reverse Primer [5' -> 3']	TTTTAACAACCATTGGAGCTGCTTT

**Table 1. *spen* gene qPCR primer sequence.** The primers were designed using the Primer-BLAST tool from NCBI.

## Results



**Figure 3: Melt curve plot for qPCR for all fruit fly genes tested.** This figure shows various melting temperatures for the four dsDNA products of the qRT-PCR *spen* gene (indicated by the arrow)



**Figure 4. Heat map of the gene expression levels in all fruit fly genes tested.** A) The gene RpL32 was used as a control. The heatmap was produced with JMP Pro. The brighter green color demonstrates lower expression levels of the gene, while a brighter red color demonstrates higher expression levels of the gene. *spen* is similarly expressed between genders. B) The  $\Delta\Delta Ct$  analysis shows that there is a 1.1-fold difference when comparing the expression level of *spen* in male to female. In order to be considered significant, the value must reach 2-fold or below 0.5-fold.

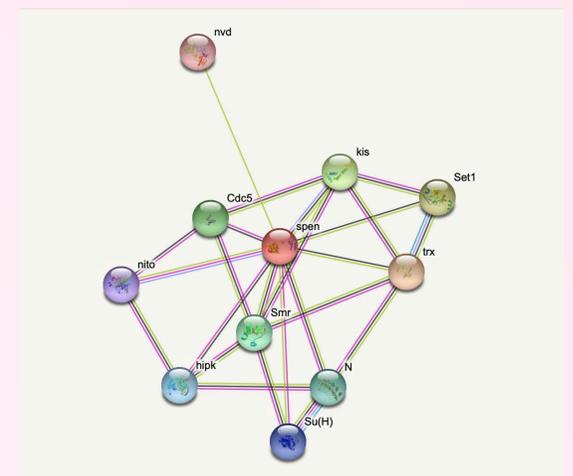
## Conclusion

- qRT-PCR results were analyzed and show a 1.1 fold difference in expression of *spen* between male and female fruit fly heads.
- The 1.1 fold difference in expression of *spen* was not considered as significant ( $p = 0.8982$ ).
- Our results suggest that *spen*'s involvement in neural development may not be sexually dimorphic.

### Study limitations

- Individual differences were not considered; 30 fly heads were pooled for each sample.

## Future directions



**Figure 5. STRING-predicted protein interactions.** *spen* interacts with proteins involved in neuronal regulation or development.

- Find correlations between gender-specific gene expression levels in fruit flies and humans.
- Exposing fruit flies to stressors such as heat or starvation and repeating the experiment to quantify gender-based gene expression differences.
- Using the information from STRING, experiment on different proteins that interact with *spen*.

